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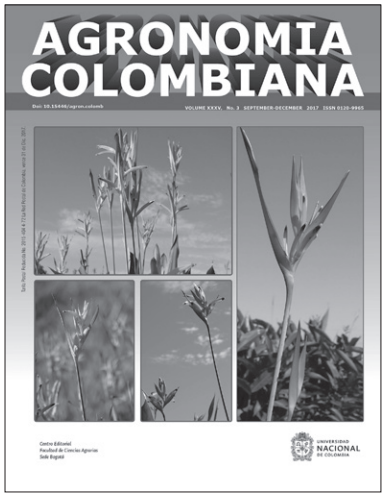

Our cover:
Genetic divergence of Heliconiaceae species in the Central West Brazil region.
Article on pages: 342-349

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AGRONOMIA COLOMBIANA

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Abrimos este nuevo número de la revista *Agronomía Colombiana*, con una distinción especial a una de las personas que hizo de este proyecto editorial un importante y significativo medio de difusión científico-técnico del sector agrícola del país. Este reconocimiento y admiración es para el profesor Gerhard Fischer, quien durante más de 10 años estuvo a cargo de la dirección de la revista *Agronomía Colombiana*; pero ¿Quién es Gerhard Fischer?

El profesor Fischer nació en 1950 en el pequeño pueblo de Lieblos, Alemania. Inicialmente, realizó sus estudios de pregrado en horticultura en la Universidad para las Ciencias Aplicadas de Wiesbaden, posteriormente realizó su maestría en la Universidad Técnica de Berlín, para la cual desarrolló la tesis "Efecto de la temperatura del suelo y de la poda sobre el crecimiento y la producción de dos variedades de la *Ipomoea batata*, provenientes de Papúa-Nueva Guinea". Sus estudios de doctorado en Ciencias Agrarias los realizó en la Universidad de Humboldt, Berlín, en la cual enfocó su tesis en "El efecto de la altitud tropical y de la temperatura radicular sobre el crecimiento, desarrollo y calidad de la uchuva (*Physalis peruviana* L.)".

Ahora bien, su interés en el área de la horticultura comenzó desde que era muy pequeño, ya que creció en una familia de floricultores. Sus padres le inculcaron el trabajo por las plantas ornamentales y es allí donde comienza su experiencia como ingeniero hortícola de la empresa de Flores Fischer en Gründau (Alemania). Cuando era adolescente se dedicó al fútbol, incluso estuvo cerca de ser un jugador profesional. Jugó en Alemania en el equipo de su pueblo cerca de Frankfurt, pero tuvo que dejar todo atrás cuando decidió iniciar su carrera en horticultura y con ella vinieron las prácticas en varias fincas y en una escuela profesional especializada. "Esto me acompañó toda la vida", afirma Fischer, quien dice que todo lo que hizo en práctica fue lo que le ayudó hoy en día a cumplir varias de sus metas, pues **"la teoría es la mitad del camino, pero la práctica es lo que te hace fuerte".**

Gracias a este trabajo se pudo consolidar como Subdirector de la Asociación Federal de Empresas Hortícolas del Estado de Hessen (Frankfurt) durante algunos años. De manera simultánea también fue instructor en varios Institutos Técnicos de Horticultura en el área de Floricultura y director de la Feria Nacional de Horticultura Frankfurt.

Llegó a Colombia por primera vez en 1986 a la Universidad Pedagógica y Tecnológica de Colombia (UPTC) en Tunja, para gestionar un proyecto encomendado por la Universidad Técnica de Berlín. Para aprender a hablar español, Fischer tomó antes cursos del idioma castellano en España. Después volvió por segunda vez a Tunja en 1988, para ser profesor visitante de pregrado, dictando las materias: "Fruticultura del clima frío" y "Fruticultura del clima cálido". Incluso para poder dictar dichas clases, tuvo que preparar con anterioridad el texto en español para luego leerlo en clase. Por esos años, también, contrajo matrimonio con la pareja que le dio el apoyo requerido para

permanecer en el país, entre otras cosas, a perfeccionar el idioma español y con quien tiene dos hijos.

En 1989, pasó a ser coordinador en posgrados de la Especialización en Frutales de Clima Frío de la UPTC, hasta 1994. En el año siguiente volvió a Alemania a culminar su tesis doctoral sobre la uchuva, fruta que conoció en la huerta de la UPTC la cual se constituyó en el objeto de su interés para investigar y mejorar su cultivo, hasta el día de hoy.

En 1996 regresó a Colombia y fue allí donde ingresó a la Universidad Nacional de Colombia, sede Bogotá, mediante una convocatoria de investigadores internacionales, la cual buscó durante 5 años, 125 investigadores, en cuyo concurso fue seleccionado. A partir de allí se desempeñó como profesor en la Facultad de Agronomía. La primera materia de pregrado que impartió fue: "Manejo de frutales caducifolios", a partir de esta se desglosa una lista de materias las cuales coordinó o participó, como: "Fisiología de frutales", "Manejo de frutales de clima frío", "Manejo de frutales tropicales", "Producción de frutales", "Fisiología de cultivos", "Sistemas de producción", "Propagación vegetal" y "Ciclo productivo - área frutales". En el posgrado también tuvo la oportunidad de dictar: "Fisiología de cultivos", "Fisiología avanzada en frutales", "Fruticultura avanzada" y "Seminario avance de tesis". Durante los años 2000 a 2002 y 2010 a 2012 fue director de la Escuela de Posgrados de la Facultad de Agronomía.

Algunas de las actividades que más recuerda como investigador son: jefe del vivero UPTC en Paipa; líder del proyecto Sena-Unal con uchuva y pitahaya, y coinvestigador en varios proyectos financiados por el Ministerio de Agricultura, Colciencias, Asohofrucol y el Corredor Tecnológico. Todo lo anterior y gracias a la dirección de más de 70 trabajos de grado, tesis de maestría y doctorado, sus publicaciones y libros, hizo que recibiera de Colciencias la mención "Investigador emérito", además reconocimientos de la Sociedad Internacional de Ciencias Hortícolas, Sociedad Colombiana de Ciencias Hortícolas, Academia Colombiana de Ciencias Exactas y varias distinciones, a nivel de la Facultad y de la Sede de la Universidad Nacional de Colombia.

Hasta este punto hemos reconocido toda su trayectoria docente e investigativa, pero ¿cuando llegó el profesor a la revista *Agronomía Colombiana*? pues bien, gracias a su labor como profesor y su anterior experiencia en el campo editorial, en el año 2004 tuvo la oportunidad de ser nombrado editor de la revista *Agronomía Colombiana,* en donde aunó esfuerzos para recuperar casi dos años de la revista, en los cuales se había dejado de publicar. En ese primer año de su gestión se lograron publicar hasta tres números y se perfeccionó el estilo científico-técnico y editorial que hoy demuestra la revista. Desde ese entonces, se publicaron mínimo dos números por año con al menos 10 artículos cada uno, y a partir de 2012 fueron tres números/año con 15 artículos cada uno. Hizo una pausa como editor durante los años en que fue nombrado director de la Escuela de Posgrados de la Facultad de Agronomía.

Nuevamente, en el año 2014 retoma la revista *Agronomía Colombiana* que mientras tanto había ingresado a la base de datos de SCOPUS, una base de datos bibliográficos de artículos de revistas científicas, reconocida a nivel mundial. Adicionalmente, en el año 2010 se decidió cambiar el idioma oficial de la revista al inglés, esto con el fin de aumentar la visibilidad de los artículos. Fischer admite que comenzar a publicar en inglés no fue fácil para los autores colombianos; sin embargo, era una tarea necesaria, "para hacer que todo el mundo pueda leer estos artículos, no solo en Colombia o en los países de habla hispana". Además, esto contribuye a su vez en la acreditación de la Facultad de Ciencias Agrarias.

Su gusto por el mundo editorial no comenzó con *Agronomía Colombiana*. Sus primeros pasos en el campo editorial empezaron en la Asociación de Horticultura en Frankfurt en 1974.

En Colombia inició editando la revista *Agro-Desarrollo* de la UPTC en Tunja. Sin embargo, su experiencia no quedó allí. Fue editor asociado de la *Revista Brasileira de Fruticultura*, editor de la *Revista Colombiana de Ciencias Hortícola*s, hizo parte del comité editorial de la *Revista Corpoica* y perteneció al comité científico de varias revistas nacionales. Además, todavía es evaluador de artículos en más de 25 revistas científicas nacionales e internacionales.

Como editor, Fischer destaca la labor que no sólo realizan los autores, sino también el papel de los evaluadores y su propia labor de revisión y perfeccionamiento de manuscritos. Señala que para la publicación de un número de una revista científica, con arbitraje por pares, se necesitan muchas revisiones y así se pueden detectar los problemas que se deben corregir. Al respecto él dice: "para mí, editar una revista, es una obra, una creación (…), un texto no muere si es bien escrito y publicado". Es muy satisfactorio para Fischer que lo que hace o dirige pueda servir para el desarrollo del agro mismo y de la sociedad.

El primer artículo que publicó fue en 1988 en la revista Acta Horticulturae, como resultado de una presentación en un congreso en Florianópolis (Brasil) sobre los frutales caducifolios en Colombia. En la actualidad, sus números

en productividad científica son sobresalientes, particularmente para un investigador en ciencias agrarias. Como ejemplo, en el portal de ResearchGate cuenta con un listado de 249 documentos sobre investigaciones y presentaciones publicadas, con 175.000 lecturas y casi 2.000 citaciones, lo cual le otorga un índice H de 20, excluyendo autocitaciones. En sus trabajos aparecen más de 250 coautores diferentes, es decir el trabajo en grupo fue otro de sus lemas. Dijo en muchas ocasiones a sus estudiantes: "Al comienzo uno es un desconocido, pero el trabajo en equipo potencializa tus conocimientos y rendimientos".

Gerhard Fischer, proximamente, en febrero de 2018, recibirá la jubilación de parte de la Universidad Nacional de Colombia, dejando el cargo de Editor en jefe de la Revista *Agronomía Colombiana*. Sin embargo, no cesa su actividad editorial, pues trabaja en la edición de la *Revista Colombiana de Ciencias Hortícolas*. Siempre ha sido un apasionado por el mundo de la edición tanto de revistas como de libros y afirma que "cuando se investiga algo y no se publica, se archiva, no sirve para nadie, por eso hay que publicar". De su pensamiento no desaparece la importancia de difundir conocimientos a través de la publicación de los mismos, algo que según su criterio muchos profesores deberían priorizar y así mismo inculcar esto a sus estudiantes. Dentro de sus proyectos a futuro contempla terminar un libro sobre fisiología de frutales, el cual empezó hace muchos años y por supuesto dedicarse a su familia agradeciendo especialmente el apoyo en todos estos años a su esposa Pilar. Justamente con su familia ahora disfruta su cabaña en Villa de Leyva. Asegura que todavía le gusta practicar el fútbol, aunque en un nivel diferente, y ahora uno de sus grandes hobbies es coleccionar con su hijo las láminas del álbum del mundial de fútbol. Como pasatiempo personal colecciona literatura importante de frutales y de fisiología de todo el mundo.

A pesar de que lleva más de 25 años en nuestro país aún no cuenta con la nacionalidad colombiana, pues no quiere perder la ciudadanía alemana, pero afirma sin temor a dudas que: **"MI PATRIA AHORA ES COLOMBIA".**

El Centro Editorial de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia sede Bogotá, junto con el equipo de la revista *Agronomía Colombiana*, quieren con esta ficha rendirle un merecido homenaje a Gerhard Fischer, agradeciéndole los años y la dedicación que invirtió en hacer de *Agronomía Colombiana* una de las más importantes publicaciones seriales, de tipo científico y enfocada en el agro, en Colombia.

Mauricio Parra Quijano
Editor en jefe
Revista Agronomía Colombiana

Recopilación biográfica y corrección de estilo:
DAYANA MELISA GARCÍA BELTRÁN y VALENTINA MARTÍN ROA

Agronomic evaluation and clonal selection of ginger genotypes (*Zingiber officinale* Roscoe) in Brazil

Evaluación agronómica y selección clonal de genotipos de jengibre (*Zingiber officinale* Roscoe) en Brasil

Eleonora Zambrano Blanco[1]* and José Baldin Pinheiro[1]

ABSTRACT

The analysis of the genetic diversity of ginger based on agronomic traits is essential to know its performance and to design breeding programs. In this study, we analyzed the phenotypic variability of 61 accessions of the ginger germplasm collection of the "Luiz de Queiroz" College of Agriculture at the University of Sao Paulo (ESALQ/USP) in a complete randomized block design with four replications. An analysis of variance test was performed and genetic parameters such as heritability, genetic variance, environmental variance, genetic-environmental variation ratio (CVg/CVe) and genetic correlations were estimated. There were highly significant differences ($P \leq 0.01$) among the accessions for all the agronomic traits analyzed. The CVg/CVe ratio (>1), along with the high heritability (>80%), showed a significant contribution of genetic factors on the phenotypic expression of plant height, rhizome thickness and yield traits, favoring the clonal selection of genotypes. Accessions Gen-29, Gen-29, Gen-32, Gen-36, Gen-37, Gen-40, Gen-41, Gen-42, Gen-50 were selected due to the best agronomic performance when compared to the rest of the germplasm. The results obtained may be useful in future breeding programs in Brazil.

Key words: agronomic performance, germplasm, genetic parameters, selection, phenotypic variability.

RESUMEN

El análisis de la variabilidad genética del jengibre (*Zingiber officinale*) con base en características de importancia agronómica es esencial para conocer su potencial productivo y direccionar correctamente programas de mejoramiento genético. Este estudio evaluó la variabilidad fenotípica de 61 accesiones del banco de germoplasma de jengibre de la Escuela Superior de Agricultura Luiz de Queiroz/Universidad de Sao Paulo (ESALQ/USP), utilizándose un diseño experimental de bloques completos al azar (BCA) con cuatro repeticiones. Para orientar el proceso de selección se realizó un análisis de varianza y se estimaron algunos parámetros genéticos tales como heredabilidad, varianza genética, varianza ambiental, relación entre el coeficiente de variación genético y ambiental (CV_g/CV_e) y correlaciones genéticas. Se presentaron diferencias altamente significativas ($P \leq 0{,}001$) entre las accesiones para todas las características agronómicas analizadas; la relación CV_g/Cv_e (>1), en conjunto con la alta heredabilidad (>80%), mostró que hubo una importante contribución de los factores genéticos en la expresión fenotípica de los caracteres altura de planta, espesor del rizoma y rendimiento, favoreciendo la selección clonal de genotipos.Las accesiones Gen-05, Gen-29, Gen-31, Gen-32, Gen-36, Gen-37, Gen-40, Gen-41, Gen-42, Gen-43, Gen-44 y Gen-50 fueron seleccionadas por presentar el mejor desempeño agronómico al ser comparadas con el resto del germoplasma. Los resultados obtenidos en este estudio podrán ser útiles en futuros programas de mejoramiento genético y promoverán la producción de este cultivo en Brasil.

Palabras clave: desempeño agronómico, germoplasma, parámetros genéticos, selección, variabilidad fenotípica.

Introduction

The *Zingiber* genre is an important member of the family *Zingiberaceae* due to its medicinal and seasoning properties. Among them, *Zingiber officinale* (cultivated ginger) is the most remarkable specie and including two subspecies known as: *Z. officinale* var *rubra* e *Z. officinale* var *rubrum* (Muda *et al*., 2004). The *Zingiber officinale* specie was morphologically described by Silvestrini *et al.* (1996) as an herbaceous and perennial plant with erected stems, formed by many dystic leaves, zygomorphic flowers, hermaphroditic, and yellow to green color. In addition, it has vegetative propagation by an articulated septant rhizomes, fleshy, rough epidermis and brown color. This species exhibits self-incompatibility and a high sterility specifically from chromosomic origin as a result either of translocations and

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inversions (Adaniya, 2001; Adaniya and Shirai, 2001) or derived from the bounding of bivalent counterparts with irregular segregation of genomic complements which leads to the formation of sterile gametes which decreases the production of viable seeds (Das *et al*., 1999). Consequently, it is possible that the cultivated ginger is a sterile hybrid which originally comes from the crossing between two distant species and it was able to survive because vegetative propagation is a successful survival strategy (Peter *et al.,* 2007).

The agronomic evaluation is essential for plant breeders in order to know the level of variability present in the germplasm collections aiming to promote their use in breeding programs (Cruz *et al.,* 2004). Therefore, an important part of the breeding program success is about the existence of genetic variability in the targeted population (Cruz *et al*., 2011). In case that the agronomic trait variability exists, it could therefore be possible to obtain genetic progress during recombination and selection processes or even determine a favorable situation for the genotypes clonal selection in the case of vegetative propagation species such as ginger. Both characterization and agronomic evaluation of ginger has been studied worldwide, mainly in those countries where ginger is highly valued such as India, China, Japan, etc. Many studies have detected different levels of variability (Wicaksana *et al*., 2011; Yeh *et al*., 2012; Chongtham *et al*., 2013; Jatoi and Watanabe, 2013; Wang *et al*., 2014) and provided relevant information about the association degree among plant characteristics and its yield as well as their combined effect on genetic variability.

Knowledge regarding the association degree among different agronomic traits is important in plant breeding since it supports genetic selection. If two characters have shown a favorable genetic correlation, then is possible to get progress for one of them by indirect selection of the other one (Cruz *et al*., 2004). There is a consensus in the literature that the phenotypic characteristics such as: plant height, leaf area, length and width of the leaves, and the number of tillers per plant, have shown direct and positive effects on the yield of ginger (Sing *et al*., 2001; Manhomandas *et al*., 2000; Abraham and Latha, 2003; Lincy *et al*., 2008; Aragaw *et al*., 2011). Studies including multiple regression analysis of morphological characteristics have shown that the yield per plant at 120 d after planting may be predicted by considering the following characteristics: plant height, number of leaves, and the width of the last leaf completely opened (Ratnambal *et al*., 1982; Rattan, 1989; Rai *et al*., 1999).

Different commercial ginger varieties (local and genetic improved) with high potential yields and quality attributes, have been used extensively by farmers from India and China. Variations on growth habits, fiber and essential oils contents, flavor and yield have been observed. Most of them are named considering either their origin or domestication (Sasikumar *et al*., 1996). Ginger is widely grown plant in both Southwest and Southern regions of Brazil. Nonetheless there is a lack of knowledge concerning the level of genetic variability and agronomic potential of this crop. The most commercialized ginger cultivar is the "Gigante" variety because of the better fit to the local market requirements. However, some regional clones are also cultivated despite of reduced rhizome sizes and these are not well accepted in the local market (Elpo and Negrelle, 2004).

In order to study the genetic diversity of ginger and promote its cultivation and commercialization, the Department of Genetics of the "Luiz de Queiroz" College of Agriculture, University of São Paulo, Brazil (ESALQ/USP) created a germplasm collection with accessions coming from different Brazilian states and some introduced accessions from Colombia. The aim of this study was to evaluate agronomic characteristics of the available germplasm in order to verify the existent genetic variability level in the cultivars, and by doing this, to select the best genotypes based on their agronomic performance for future plant breeding studies.

Material and methods

Planting and experimental design

Sixty-one accessions from the ginger germplasm collection of the Department of Genetics (ESALQ/USP) were planted and evaluated during 2013/2014 agricultural year in the Anhembi`s experimental station which is located in Piracicaba (São Paulo/Brazil) (Tab. 1).

Accessions were arranged in a complete randomized block design with four replications. The experimental unit was represented by plots of 1.0 m^2 with planting distance of 1.0 m between rows, and 0.3 m between plants, in total 10 plants per plot. Chemical fertilization was made 8 d before planting with 4-14-8 (N, P, K fertilizer at 100 g m^{-1}) following by three more fertilizations done along of the crop period with ammonium sulfate (23 g m^{-1}), potassium chloride (14 g m^{-1}) and triple superphosphate (64 g m^{-1}).

TABLE 1. Description of 61 Brazilian accessions of ginger (*Zingiber officinale*) kept in the germplasm collection of Department of Genetics, ESALQ/USP.

Id.	Accession name	Common name	Biological status	Country of origin[1]	Germplasm source
1	Gen-03	Variedad blanca	Landrace	Colombia	Genebank
2	Gen-04	Variedad amarilla	Landrace	Colombia	Genebank
3	Gen-05	Variedad jamaiquina	Landrace	Colombia	Genebank
4	Gen-07	Col-clone-europeo	Landrace	Colombia	Genebank
5	Gen-08	Jengibre	Landrace	SE-Brazil	Garden
6	Gen-10	Jengibre	Clonal selection	SP-Brazil	Market
7	Gen-11	Jengibre	Clonal selection	SP-Brazil	Market
8	Gen-12	Jengibre	Landrace	SP-Brazil	Garden
9	Gen-13	Jengibre	Clonal selection	AM-Brazil	Market
10	Gen-14	Jengibre	Clonal selection	ES-Brazil	Market
11	Gen-15	Jengibre	Clonal selection	SE-Brazil	Market
12	Gen-16	Jengibre	Clonal selection	SE-Brazil	Market
13	Gen-17	Jengibre	Clonal selection	SC-Brazil	Market
14	Gen-18	Jengibre	Clonal selection	SC-Brazil	Market
15	Gen-19	Jengibre	Clonal selection	AM-Brazil	Market
16	Gen-20	Jengibre	Clonal selection	SC-Brazil	Garden
17	Gen-21	Gigante	Clonal selection	SC-Brazil	Market
18	Gen-22	Jengibre	Landrace	SC-Brazil	Garden
19	Gen-23	Havai	Clonal selection	SP-Brazil	Field
20	Gen-24	Jengibre	Landrace	SP-Brazil	Garden
21	Gen-25	Jengibre	Clonal selection	SP-Brazil	Field
22	Gen-27	Jengibre	Clonal selection	SP-Brazil	Field
23	Gen-28	Gigante	Clonal selection	SP-Brazil	Field
24	Gen-29	Gigante	Clonal selection	SP-Brazil	Field
25	Gen-30	Havai	Clonal selection	SP-Brazil	Field
26	Gen-31	Jengibre	Clonal selection	SP-Brazil	Field
27	Gen-32	Dominica	Clonal selection	SP-Brazil	Field
28	Gen-33	Chinesse	Clonal selection	SP-Brazil	Field
29	Gen-34	Gigante	Clonal selection	SP-Brazil	Field
30	Gen-35	Gigante	Clonal selection	SP-Brazil	Field
31	Gen-36	Gigante	Clonal selection	SP-Brazil	Field
32	Gen-37	Gigante	Clonal selection	SP-Brazil	Field
33	Gen-38	jengibre	Clonal selection	SP-Brazil	Field
34	Gen-39	Jengibre	Clonal selection	SP-Brazil	Field
35	Gen-40	Jengibre	Clonal selection	SP-Brazil	Field
36	Gen-41	Gigante	Clonal selection	SP-Brazil	Field
37	Gen-42	Jengibre	Clonal selection	SP-Brazil	Field
38	Gen-43	Jengibre	Clonal selection	SP-Brazil	Field
39	Gen-44	Jengibre	Clonal selection	SP-Brazil	Field
40	Gen-45	Jengibre	Clonal selection	SP-Brazil	Field
41	Gen-46	Gigante	Clonal selection	PR-Brazil	Field
42	Gen-47	Paulista	Clonal selection	PR-Brazil	Field
43	Gen-48	Havai	Clonal selection	PR-Brazil	Field

Continue

Id.	Accession name	Common name	Biological status	Country of origin[1]	Germplasm source
44	Gen-49	Gigante	Clonal selection	PR-Brazil	Field
45	Gen-50	Havai	Clonal selection	PR-Brazil	Field
46	Gen-51	Gigante	Clonal selection	ES-Brazil	Field
47	Gen-52	Gigante	Clonal selection	ES-Brazil	Field
48	Gen-53	Gigante	Clonal selection	ES-Brazil	Field
49	Gen-54	Jengibre	Landrace	ES-Brazil	Garden
50	Gen-55	Jengibre Incaper	Clonal selection	ES-Brazil	Field
51	Gen-56	Gigante	Clonal selection	ES-Brazil	Field
52	Gen-57	Gigante	Clonal selection	ES-Brazil	Field
53	Gen-58	Gigante	Clonal selection	ES-Brazil	Field
54	Gen-59	Gigante	Clonal selection	ES-Brazil	Field
55	Gen-60	Gigante	Clonal selection	ES-Brazil	Field
56	Gen-61	Gigante	Clonal selection	ES-Brazil	Field
57	Gen-62	Gigante	Clonal selection	ES-Brazil	Field
58	Gen-63	Jengibre Blanco	Clonal selection	SP-Brazil	Field
59	Gen-64	Jengibre Amarillo	Clonal selection	SP-Brazil	Field
60	Gen-65	Caipira	Landrace	SP-Brazil	Garden
61	Gen-66	Jengibre Azul	Clonal selection	SP-Brazil	Field

[1] Abbreviation of the names of the origin region of the accessions: SE: Sergipe; SP: São Paulo; AM: Amazonas; ES: Espirito Santo; SC: Santa Catarina; PR: Paraná; Id: Identification of the accessions.

Agronomic traits

Agronomic evaluation of the accessions was made according to phenotypic descriptors as suggested by Ahmad (2008). In this study, seven quantitative traits were evaluated as shown in Tab. 2. Mean values from 4 plants per plot were used for statistical analysis with the exception of the plot performance (PP), which was evaluated for the whole plot.

TABLE 2. Agronomic traits evaluated in ginger germplasm (*Z. officinale*).

Characteristic	Method and time of evaluation
Plant height (PH)	Measure from the soil surface to the end of the last leaf (cm), at maximum vegetative growth.
Leaf length (LL)	Measure from the base to the tip of the leaf (cm), at maximum vegetative growth.
Leaf width (LW)	Measured in three different parts of the leaf (cm), in the maximum vegetative growth.
Number of leaves per tiller (NLT)	Number of leaves per tiller after flowering.
Number of tillers per plant (NTP)	Number of tillers per plant after flowering
Rhizome thickness (RT)	Measured with pachymeter in 3 different rhizomes in postharvest (mm)
Yield per plot (YP)	Rhizomes weight of the plot (kg m^{-2}), corrected for the final number of plants per plot (7,184 plants) by the covarianza method.

Statistical analysis

Descriptive statistics (mean, rank and coefficient of variation) were calculated. The Shapiro-Wilk Test was used to verify the normality of the data ($P \leq 0.05$) and analysis of variance was performed to detect differences between the accessions. Once significance level was confirmed between the accessions, a Scott-Knott means comparison test was performed. To verify the association degree among traits, both genotypic and phenotypic correlations were calculated. The following genetic parameters were calculated based on the mean values obtained from the experimental plots:

- Phenotypic variance (σ_p^2): $\frac{GMS}{r}$
- Environmental variance (σ_e^2): $\frac{RMS}{r}$
- Genotypic variance (σ_g^2): $\frac{GMS - RMS}{r}$
- Heritability (h^2): σ_g^2 / σ_p^2
- Genetic coefficient of variation (CV_g, %): $\frac{(100\sqrt{\sigma_g^2})}{\mu}$
- Genetic-environmental variation ratio (CV_g/CV_e): $\sqrt{\frac{\sigma_e^2}{\sigma_g^2}}$

Where:

GMS = genotypes mean square
RMS = residual mean square
r = number of experimental replications
μ = average mean for evaluated trait

The experimental data were analyzed by SAS (version 9.3; SAS Institute Inc., Cary, NC) and GENES software (Cruz, 2013).

Results

Shapiro-Wilk test which was performed to determine the normality of the residuals, confirmed that the seven traits followed a normal distribution. An example for NTP trait is presented (Fig. 1). Analysis of variance showed that there are highly significant differences ($P \leq 0.01$) among phenotypic means of the accessions for all of analyzed agronomical traits (PH, LL, LW, YP, RT, NTP and NLT) indicating that phenotypic variability exist (Tab. 3).

The mean comparison analysis for PH revealed that most of the accessions (54.10%) presented phenotypic values above of the average mean (>60 cm) being classified as high phenotypes and therefore gathered in one group (identified by letter a). The rest of the germplasm accessions showed an average plant height (between 54 and 59 cm; letter b), and low plant height (<54 cm; letter c) (Tab. 4).

Slightly less than a half of the accessions displayed longer leaves (>20 cm) and wider (>2.30 mm) compared to mean values for the other accessions. Similarly 66% of the accessions showed high density of foliage reaching up to 18.38 leaves per tiller whilst for the rest of the accessions, between 11.88 and 14.51 leaves per tiller were counted. The Gen-18 accession showed the lowest NLT (10) as presented in Tab. 4.

Approximately 35% of the accessions showed the highest YP ranging from 4.50 to 7.20 kg m^{-2}. In 20 accessions (32.79%), the NTP produced the greatest number of tillers (≥16) whilst in little bit more than a half (54.10%) of the

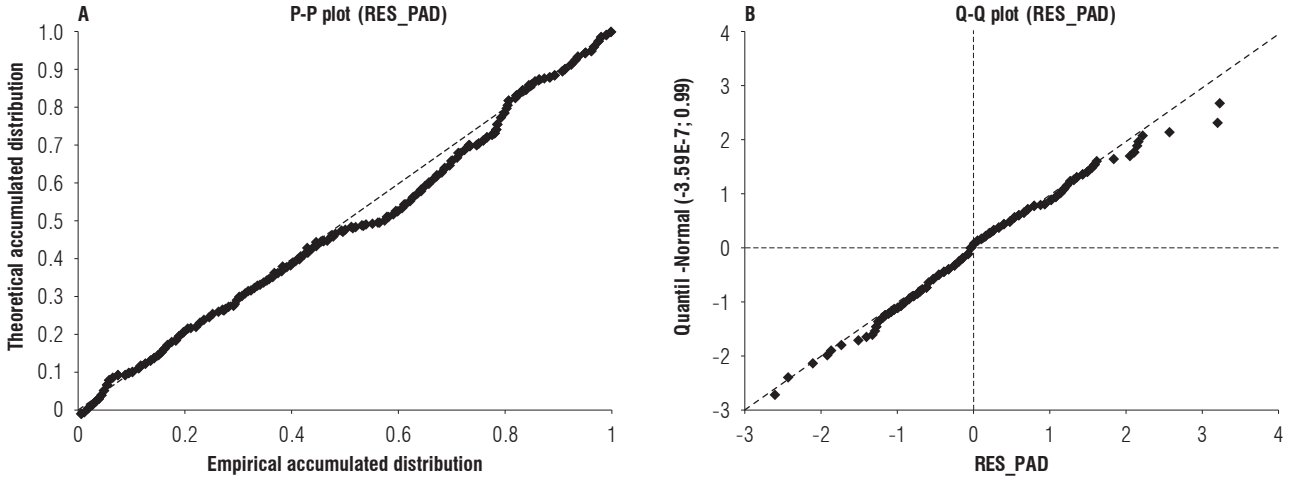

FIGURE 1. Normal distribution chart of standardized residuals for the NTP trait evaluated in 61 ginger accessions. Normality test Shapiro-Wilk = 0.078 ($P \leq 0.05$).

TABLE 3. Summary of the analysis of variance of the agronomic traits evaluated in 61 accessions of ginger (*Z. officinale*).

FV	GL	MS						
		PH	LL	LW	YP	RT	NTP	NLT
Blocks	3	749,93	11,96	0,66	7,25	6,23	62,32	36,23
Accessions	60	137,14**	7,89**	0,11**	12,17**	104,08**	55,77**	8,41**
Residue	180	25,49	1,98	0,03	1,13	7,24	15,02	3,15
Mean		59,41	20,68	2,29	3,83	30,67	14,49	14,93
CV (%)		8,50	6,80	7,28	27,78	8,77	26,75	11,89
Máx.		68,92	24,69	2,75	7,15	41,88	24,20	18,38
(accesión)		(Gen-10)	(Gen-17)	(Gen-17)	(Gen-42)	(Gen-33)	(Gen-42)	(Gen-33)
Mín.		46.38	15.72	1.68	1.04	17.98	6.15	8.87
(accesión)		(Gen-24)	(Gen-18)	(Gen-24)	(Gen-22)	(Gen-24)	(Gen-04)	(Gen-18)

**: Significant to 1% (F test); PH: plant height (cm); LL: leaf length (cm); LW: leaf width (mm); YP: yield per plot (kg m^{-2}); RT: rhizome thickness (mm); NTP: number of tillers per plant; NLT: number of leaves per tiller; Max: maximum value; Min: minimum value; CV: coefficient of variation; FV: sources of variation; GL: degrees of freedom; MS: mean square.

accessions, this trait ranged from 11 to 15.50 tillers per plant. Ten tillers per plant were observed for the rest of the accessions (13.11%). Regarding to RT trait, 27.87 % of the accessions were characterized by large (33.89 to 41.88 mm); intermediate (28.73 to 33.59 mm); and small rhizomes (17.97 to 28.01 mm) respectively (Tab. 4).

Genetic parameters for evaluated traits are shown in Tab. 5. The CV_g/Cv_e ratio displayed greater values than 1.0 and high heritability values (>80%) indicating an advantageous genotype selection towards PH, RT, and YP. These results indicate that an important contribution of the genetic factors exist in the final expression of these agronomic traits. This most likely favors clonal selection of promissory genotypes.

The significance for the phenotypic (above of diagonal) and genotypic correlations (bellow of diagonal) among tested traits is shown in Table 6. Positive and highly significant correlations ($P \leq 0.01$) were detected among evaluated traits, except for LW and RT which correlations with NTP and NLT were non-significant. In general, YP showed higher correlations with PH (r = 0.69), NTP (r = 0.63), and RT (r = 0.54) compared with the other traits. High correlation between PH and LL was also detected (r = 0.75). Conversely, LW was higher correlated with LL and RT (r = 0.67 and 0.61, respectively). Finally, moderate correlations were observed between NLT and PH (r = 0.523), LL (r = 0.455), YP (r = 0.483) and NTP (r = 0.474).

TABLE 4. Means comparison analysis (Scott and Knott) for the seven agronomic traits evaluated in 61 accessions of ginger (*Z. officinale*).

Accession	PH	LL	LW	YP	RT	NTP	NLT
Gen-03	63.67 a	21.39 a	2.35 a	3.05 c	23.13e	12.40 b	17.62 a
Gen-04	66.92 a	21.64 a	2.25 b	2.54 d	23.14e	6.15 c	12.87 b
Gen-05	60.67 a	21.34 a	2.23 b	6.84 a	21.22e	13.90 b	14.37 b
Gen-07	50.13 c	19.35 b	2.15 b	2.48 d	23.83e	11.88 b	15.44 a
Gen-08	60.00 a	20.58 b	2.27 b	4.56 b	25.92 d	20.13 a	16.63 a
Gen-10	68.92 a	22.04 a	2.24 b	2.54 d	23.61e	13.40 b	16.62 a
Gen-11	61.44 a	20.42 b	2.17 b	2.83 d	29.83 c	15.75 a	14.94 a
Gen-12	50.92 c	18.89 b	2.18 b	1.80 d	22.55e	14.65 b	14.62 a
Gen-13	63.41 a	21.78 a	2.35 a	3.27 c	35.49 b	13.84 b	15.51 a
Gen-14	58.19 b	21.58 a	2.37 a	3.21 c	32.07 c	15.00 b	15.56 a
Gen-15	48.67 c	20.41 b	2.48 a	2.36 d	26.82 d	7.57 c	13.54 b
Gen-16	68.17 a	23.14 a	2.30 a	5.23 b	35.34 b	20.15 a	15.12 a
Gen-17	66.42 a	24.69 a	2.75 a	2.93 c	27.50 d	22.90 a	14.87 a
Gen-18	49.67 c	15.72 c	2.19 b	2.53 d	31.50 c	8.40 c	8.87 c
Gen-19	53.17 c	20.09 b	2.35 a	2.73 d	28.73 c	15.90 a	15.12 a
Gen-20	61.56 a	20.84 a	2.22 b	3.06 c	33.59 c	11.94 b	15.19 a
Gen-21	54.44 b	20.11 b	2.22 b	2.52 d	32.33 c	11.45 b	14.51 b
Gen-22	52.41 c	20.32 b	1.93 c	1.04 d	21.81e	9.18 c	13.68 b
Gen-23	55.06 b	19.37 b	2.22 b	2.19 d	33.37 c	11.50 b	14.81 a
Gen-24	46.38 c	16.85 c	1.68 d	1.27 d	17.98f	13.20 b	14.74 a
Gen-25	51.00 c	19.89 b	2.34 a	2.06 d	29.00 c	13.25 b	14.00 b
Gen-27	51.38 c	18.50 b	2.18 b	1.18 d	26.95 d	8.56 c	11.88 b
Gen-28	61.31 a	20.46 b	2.23 b	3.53 c	34.86 b	13.00 b	13.44 b
Gen-29	64.06 a	21.53 a	2.32 a	6.12 a	32.38 c	19.94 a	15.06 a
Gen-30	54.75 b	20.38 b	2.51 a	2.73 d	35.45 b	13.44 b	12.63 b
Gen-31	67.13 a	22.53 a	2.43 a	7.04 a	35.66 b	19.13 a	15.88 a
Gen-32	63.75 a	21.64 a	2.41 a	6.57 a	37.53 b	14.19 b	15.69 a
Gen-33	64.19 a	22.10 a	2.59 a	5.59 b	41.88 a	12.00 b	18.38 a
Gen-34	58.44 b	19.74 b	2.48 a	5.62 b	37.59 b	14.94 b	16.56 a

Continue

Accession	PH	LL	LW	YP	RT	NTP	NLT
Gen-35	58.88 b	21.03 a	2.35 a	3.69 c	30.15 c	14.38 b	14.81 a
Gen-36	63.63 a	21.39 a	2.20 b	6.36 a	32.14 c	18.31 a	15.94 a
Gen-37	65.69 a	20.28 b	2.30 a	6.24 a	40.01 a	16.81 a	15.69 a
Gen-38	51.31 c	19.68 b	2.23 b	2.18 d	24.92 d	11.06 b	13.44 b
Gen-39	65.94 a	22.58 a	2.45 a	4.49 b	33.16 c	13.63 b	15.56 a
Gen-40	65.69 a	20.46 b	2.27 b	6.80 a	35.30 b	18.38 a	16.19 a
Gen-41	64.25 a	21.19 a	2.34 a	7.14 a	32.67 c	19.44 a	16.31 a
Gen-42	64.63 a	19.18 b	2.20 b	7.15 a	31.21 c	24.20 a	17.06 a
Gen-43	64.06 a	21.30 a	2.36 a	6.19 a	34.81 b	17.25 a	16.31 a
Gen-44	67.25 a	21.39 a	2.37 a	6.46 a	34.41 b	15.50 b	14.81 a
Gen-45	59.56 a	20.88 a	2.32 a	4.97 b	32.30 c	18.06 a	14.38 b
Gen-46	58.25 b	20.44 b	2.38 a	3.28 c	35.45 b	12.06 b	13.81 b
Gen-47	62.69 a	21.69 a	2.34 a	4.17 c	35.19 b	16.38 a	13.75 b
Gen-48	56.06 b	19.60 b	2.24 b	1.94 d	33.90 b	9.88 c	15.00 a
Gen-49	57.44 b	21.82 a	2.44 a	3.74 c	32.71 c	13.31 b	16.00 a
Gen-50	63.00 a	22.07 a	2.46 a	6.01 a	34.98 b	13.56 b	16.00 a
Gen-51	61.38 a	22.44 a	2.35 a	4.61 b	35.30 b	18.00 a	14.38 b
Gen-52	61.31 a	21.29 a	2.32 a	4.16 c	31.66 c	17.69 a	14.81 a
Gen-53	61.38 a	20.14 b	2.15 b	4.23 c	29.27 c	14.88 b	15.50 a
Gen-54	56.21 b	19.90 b	2.01 c	2.13 d	22.62e	7.74 c	15.15 a
Gen-55	52.44 c	19.46 b	2.17 b	1.67 d	28.01 d	11.63 b	13.63 b
Gen-56	53.25 c	19.89 b	2.08 b	2.57 d	30.21 c	13.69 b	14.81 a
Gen-57	53.19 c	19.71 b	2.22 b	2.60 d	30.25 c	12.00 b	14.31 b
Gen-58	55.96 b	21.15 a	2.42 a	2.67 d	29.53 c	14.77 b	14.23 b
Gen-59	65.31 a	20.56 b	2.33 a	3.83 c	30.69 c	19.94 a	15.69 a
Gen-60	55.75 b	20.59 b	2.28 a	2.72 d	32.03 c	10.50 c	14.06 b
Gen-61	57.50 b	20.05 b	2.34 a	3.67 c	30.28 c	14.19 b	12.94 b
Gen-62	54.92 b	20.18 b	2.21 b	3.09 c	31.62 c	15.04 b	14.17 b
Gen-63	66.63 a	22.06 a	2.44 a	5.70 b	33.02 c	18.13 a	16.50 a
Gen-64	67.56 a	21.95 a	2.45 a	5.25 b	33.52 c	15.06 b	15.69 a
Gen-65	53.19 c	18.75 b	1.88 c	2.47 d	18.31f	17.81 a	15.94 a
Gen-66	63.50 a	21.19 a	2.31 a	2.22 d	30.17 c	12.63 b	15.69 a

Values with the same letter are not significantly different ($P \leq 0.05$) and belong to the same group.

TABLE 5. Estimation of the genetic parameters for the agronomic traits evaluated in 61 accessions of ginger (*Z. officinale*).

Charateristic	σ_p^2	σ_e^2	σ_g^2	h^2 (%)	CV_g (%)	CV_g/CV_e
PH	34.28	6.37	27.91	81.41	8.89	1.05
LL	1.98	0.49	1.48	74.93	5.88	0.86
LW	0.03	0.01	0.02	74.38	6.20	0.85
YP	3.04	0.28	2.76	90.69	43.35	1.56
RT	26.02	1.81	24.21	93.05	16.04	1.83
NTP	13.94	3.75	10.19	73.07	22.04	0.82
NLT	2.10	0.79	1.32	62.54	7.68	0.65

TABLE 6. Phenotypic (above the diagonal) and genotypic correlations (below the diagonal) between the agronomic traits evaluated in 61 accessions of ginger (*Z. officinale*).

Characteristic	PH	LL	LW	YP	RT	NTP	NLT
PH	1	0.748**	0.476**	0.697**	0.449**	0.518**	0.523**
LL	0.788**	1	0.671**	0.443**	0.350**	0.386**	0.455**
LW	0.518**	0.705**	1	0.401**	0.606**	0.246^{ns}	0.189^{ns}
YP	0.730**	0.483**	0.457**	1	0.543**	0.626**	0.483**
RT	0.472**	0.376**	0.696**	0.564**	1	0.245^{ns}	0.166^{ns}
NTP	0.591**	0.446**	0.299^{ns}	0.697**	0.285^{ns}	1	0.474**
NLT	0.598**	0.558**	0.240^{ns}	0.566**	0.189^{ns}	0.657	1

**: Significant to 1%; ns: not significant.

Discussion

Variability and genotypes selection

A well known method to determine the genetic variability of crops is by the analysis of agro-morphological traits (phenotypic characteristics) which are relevant to establish their productive performance to improve the success of crop breeding programs (Mohammadi and Prasanna, 2003). In the present study, the evaluation of 61 accessions of a ginger germplasm bank (ESALQ/USP) indicates that there was moderate genetic variability for the evaluated agronomic traits.

The variables that contributed more to the germplasm variability were yield per plot, rhizome thickness, number of tillers per plant and lift width. Moderate genetic variability in ginger was also reported recently in accessions grown in Burkina Faso for 13 quantitative traits (Nandkangre *et al*., 2016). In that study, the analysis of variance highlighted the presence of some discriminatory traits such as number of leaves per tiller, leave length, plant height, and both length and width of the rhizome. Similar results were reported two decades ago by Ravindran *et al*. (1994) in India and more recently by Jatoi and Watanabe (2013) in Japan who found moderate variability for plant height, number of leaves per plant, yield per plant and associated traits. The number of tillers per plant and yield per plant were the most divergent traits among the evaluated accessions. The results of the present study are consistent based on the fact that the genetic variability in plant species with an exclusive vegetative propagation system such as ginger tends to be either moderated or limited, unless that the evaluated genotypes come from geographic areas with constant agroecology conditions. The long history of ginger domestication and its adaptation to several eco-geographic niches has been the main evolutionary driving force of this plant, therefore moderate to low variability exist among collected or planted accessions in nearby areas with similar environmental conditions. Conversely, large variation is observed when the accessions come from areas with contrasting eco-geographic conditions because of being influenced by different selection pressures (Ravindran and Nirmal-Babu, 2005; Bosseti *et al.,* 2011).

Phenotypic variability found among accessions is the result of the interaction of genetic and environmental factors. So, agronomic traits such as plant height, yield per plant/plot, etc., usually have polygenic inheritance due to be controlled by several genes highly influenced by the environment (Ceballos, 2000; Vallejo and Estrada, 2002). However, unlike this consensus, genetic parameters such as heritability, CV_g/CV_e ratio, etc., indicate that environmental variance had little influence on the final expression of the yield of ginger, despite of it is one of the traits with greatest contribution to the phenotypic variability of the analyzed accessions. These results suggest that phenotypic differences observed during the evaluated agricultural period were more related to genetic than environmental variables, thus is possible to perform clonal selection for ginger plant yield as recently done by Ravishanker *et al.* (2014) using 25 Indian *Z. officinale* genotypes. In the present study, 35% of the germplasm showed a higher yield related to the mean value and the following accessions are highlighted: Gen-05, Gen-29, Gen-31, Gen-32, Gen-36, Gen-37, Gen-40, Gen-41, Gen-42, Gen-43, Gen-44, and Gen-50 (yield per plot above 6.0 kg $m^{2\,-1}$). Most of the accessions originally come from the most highly productive ginger region in the São Paulo state ("Tapiraí" and "Piedade" municipalities), and they could be selected once the existent phenotypic variability indicates, in a considerable extent, their genetic potential. One of the advantages of clonal selection is that enables the capture of genetic superiority of the cultivars perpetuating it throughout the generations. Clonal propagation permits the fixation of favorable genetic combinations maintaining a high level of heterozygosity for one or more characteristics of agronomic interest (Mckey *et al.,* 2010; Bisognin, 2011).

Genetic variance, heritability and CV_g/CV_e ratio, among other genetic parameters were studied in 90 Indian ginger genotypes (Islam *et al.,* 2008). Similarly to the results obtained in the present study, the authors found little environmental effect on the phenotypic expression of the associated yield traits with high heritability values (>90%) for both height and yield per plant. In a study conducted in Ethiopia, genetic variability of 36 ginger accessions was evaluated. High heritability values (>90%) were reported for yield per plant, plant height, rhizome thickness, among other traits (Aragaw *et al.,* 2011). Most of the accessions evaluated in the present study are commercial cultivars or varieties which are widely adapted to the agro-ecological areas where they are cultivated. This broad adaptation capacity in addition to the favorable conditions for plant growth and crop management may contribute to the high heritability of the agronomic traits evaluated. High heritability values for these traits have been reported in *Curcuma Longa* (Singh *et al*., 2003; Chattopadhyay *et al*., 2004; Sasikumar, 2005), an specie characterized by its vegetative propagation and it is also a member of the *Zingiberaceae* family.

Genetic correlations

The study on the nature and magnitude of the existent relationships among traits is crucial for breeding purposes. In case that two characters present favorable genetic correlation is possible to obtain genetic gains for one of them by the indirect selection of the second one (Vencovsky and Barriga, 1992). As confirmed recently in the studies by Kumar *et al*. (2016) and Ravishanker *et al.* (2013); in the present study, yield per plant showed a positive and highly significant correlation with plant height, number of tillers per plant and rhizome thickness. Also, the magnitude of genetic correlations was more expressive than for phenotypic correlations (Tab. 6). These findings confirm the hypothesis towards that the variability found among the analyzed accessions is mainly genetic. In such scenario, may be plausible the early selection of superior genotypes for agronomic traits such as: plant height, number of tillers per plant, rhizome thickness, etc., and it leads to expressive productive gains taking into account that in some cases indirect selection based on correlated response could lead to faster progress compared with direct selection on the desired trait (Cruz *et al*., 2004).

Conclusions

There was moderate genetic variability among 61 accessions of the ESALQ/USP ginger germplasm collection. Among the studied agronomic traits, yield per plot, rhizome thickness, number of tillers per plant, and leaf width were the most variable characters. The present study identified the following accessions as the most promissory genotypes based on their agronomic performance: Gen-05, Gen-29, Gen-31, Gen-32, Gen-36, Gen-37, Gen-40, Gen-41, Gen-42, Gen-43, Gen-44 and Gen-50 because of that they were selected as a potential clones. However, it is important to emphasize that genetic superiority and adaptation of these accessions must be confirmed by conducting new agronomic studies at different environments and seasons in order to validate the obtained results in this study. So far, ginger phenotypic variability in Brazil has not been studied, for that reason the results of the present study may be useful in future ginger plant breeding programs and also to promote its cultivation and further study at national level.

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Genetic divergence of Heliconiaceae species in the Central West Brazil region

Divergencia genética de las especies de Heliconiaceae en la región Centro Oeste de Brasil

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ABSTRACT

The purpose of this study was to describe morphological traits and estimate genetic divergence and parameters between accessions of the genus *Heliconia* sp. from different municipalities in the state of Mato Grosso, Brazil. A set of 25 traits, 15 quantitative and 10 qualitative were evaluated. The genetic divergence was estimated based on Mahalanobis' distance, with the clustering methods known as Unweighted Pair Group Method using Arithmetic Averages (UPGMA). Genetic variability was observed for all assessed quantitative traits and the accessions were grouped in different classes. The traits with highest relative contribution to variability were longevity of flower stems and inflorescence length. The results indicated the existence of genetic variability among accessions of the *Heliconia* sp. germplasm bank, which can be used in breeding programs.

Key words: morphological description, pre-breeding, tropical ornamentals.

RESUMEN

El objetivo de este estudio fue describir los rasgos morfológicos, estimar la divergencia genética y los parámetros entre accesiones del género *Heliconia* sp. en diferentes municipios del estado de Mato Grosso, Brasil. Se evaluó un conjunto de 25 rasgos, 15 cuantitativos y 10 cualitativos. La divergencia genética se estimó sobre la base de la distancia de Mahalanobis, con los métodos de agrupación Unweighted Pair Group Method utilizando promedios aritméticos (UPGMA). Se observó variabilidad genética para todos los rasgos cuantitativos evaluados y las accesiones se agruparon en diferentes clases. Los rasgos con mayor contribución relativa a la variabilidad fueron la longevidad de los tallos de las flores y la longitud de las inflorescencias. Los resultados indicaron la existencia de variabilidad genética entre las accesiones del banco de germoplasma de *Heliconia sp*, que pueden ser explotadas en programas de mejoramiento de la especie.

Palabras clave: descripción morfológica, pre-reproducción, plantas ornamentales tropicales.

Introduction

The family *Heliconiaceae* consists of 182 neotropical species distributed in Central and South America. Twenty-nine species are found in Brazil, five of which are endemic (Braga, 2014). *Heliconiaceae* are distributed in the Central-West, Northern, Northeastern, and Southeastern regions of the country. Nine species of the genus inhabit the state of Mato Grosso, i.e., *Heliconia psittacorum, H. rostrata, H. episcopalis, H. marginata, H. subulata, H. acuminate, H. hirsuta, H. densiflora,* and *H. stricta;* the last two were first recorded in Mato Grosso (Braga, 2014).

The state of Mato Grosso lies in the so-called Ecological Transition Zone (ETZ) between the biomes savanna (Cerrado) and rainforest (Amazon region). The ETZ is a complex morphoclimatic domain at the North of the Cerrado and Southwest of the Amazon, where savannas and tropical forests coexist under similar environmental conditions (Furley *et al*., 1992).

Heliconiaceae species have herbaceous plants used in floriculture as ornamental plants, grown under full sun or partial shade, or as cut flowers, because the terminal inflorescences with intense colors of different shapes and sizes are greatly appreciated for event decoration and floral arrangements. Only few and recent studies addressed the agronomic potential of ornamental interest species. In the forest region Zona da Mata of Pernambuco, some studies analyzed genetic parameters of seven genotypes of *H. psittacorum* (Costa, 2007; Rocha *et al.,* 2010; Araujo *et al*., 2015).

Due to the large natural variability in *Heliconia* sp. populations and the potential of these species as ornamental, research on breeding, agronomic and genetic characterization should be intensified. A study target of breeding programs is to collect accessions in genebanks to develop genotypes with traits of economic interest that meet the demands of the ornamental market (Rocha *et al.,* 2010). Moreover, the genetic divergence and parameters can be

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analyzed to estimate genetic gains and determine the most appropriate breeding method (Cruz *et al.,* 2012).

This study addressed the morphological description and the estimation of genetic parameters and divergence of *H. psittacorum* and *H. densiflora* accessions, with a view to evaluate the genetic variability in the state of Mato Grosso, identifying possible parents to breed future hybrids with ornamental potential.

Material and methods

The germplasm collection of *Heliconia*s (BAG) of the State University of Mato Grosso (UNEMAT) was established in March 2014, in an experimental field in the municipality of Tangará da Serra, MT (14º39' S and 57º25' W; elevation 321 m a.s.l.). The climate is tropical, with well-defined dry and rainy seasons and annual means varying from 1,300 to 2,000 mm rainfall and 16-36°C temperature range (Martins *et al.,* 2010). The soil, with a flat to slightly wavy relief, is classified as Latossolo vermelho distrófico (Embrapa, 2006).

The area for planting of seedlings was harrowed and limed according to soil analysis. Fertilization at planting consisted of 50 g monoamonic phosphate (MAP) per planting hole and topdressing of monthly applications of 50 g urea and 20 g potassium chloride per hole and of 50g MAP every 6 months.

Four accessions of *H. densiflora* and 14 of *H. psittacorum,* from 13 municipalities in the state of Mato Grosso, were described (Tab. 1). The clumps were divided and four rhizomes planted, at a spacing of 3.0 m between rows and 1.5 m between plants in full sun. When needed, irrigation was applied three times a week. The experiment was conducted as described by Costa *et al*. (2007), in a randomized block design with 18 treatments (accessions), four blocks (replications) with one rhizome per plot.

Qualitative and quantitative morphological traits were evaluated around 400 d after planting, when the plants were fully established. Twenty-five descriptors were used, of which 15 were quantitative and 10 qualitative. The quantitative descriptors were: LL (cm) leaf length; LW (cm) leaf width; NLS (n) number of leaves on the flower stem; NIC (n) number of inflorescences per clump; SW (g) shoot weight (leaves and floral stem); FSW (g) flower stem weight without leaves, flower peduncle and inflorescence; FSL (cm) flower stem length; FSD (cm) flower stem diameter, measured 20 cm below the inflorescence; IL (cm) inflorescence length; LI (cm) inflorescence width; NFI (n) number of flowers per inflorescence; NBI (n) number of bracts per inflorescence; BL (cm) bract length; BD (cm) bract depth; and SL (days) longevity of the flower stem. The qualitative

TABLE 1. Accessions of *Heliconia densiflora* and *H. psittacorum* collected in different municipalities in the state of Mato Grosso, Brazil.

Accession	Species	Municipality	Latitude	Longitude	Altitude m a.s.l.
1	*H. densiflora*	Alta Floresta	9°51'05"	56°12'31"	281
2	*H. densiflora*	Alta Floresta	9°51'47"	56°12'04"	271
3	*H. densiflora*	Alta Floresta	9°52'43"	56°9'22"	281
4	*H. densiflora*	Carlinda	10°10'8"	55°48'53"	299
5	*H. psittacorum*	Nova Canaã	10°36'44"	55°42'05"	265
6	*H. psittacorum*	Colíder	10°46'55"	55°27'00"	310
7	*H. psittacorum*	Matupá	10°12'26"	54°57'39"	260
8	*H. psittacorum*	Guarantã Norte	9°46'02"	54°53'55"	348
9	*H. psittacorum*	Guarantã Norte	9°44'26"	54°53'16"	336
10	*H. psittacorum*	Peixoto Azevedo	10°16'9"	55°01'15"	324
11	*H. psittacorum*	Terra Nova do Norte	10°44'5"	55°08'43"	295
12	*H. psittacorum*	Santo Afonso	14°35'9"	57°10'56"	494
13	*H. psittacorum*	Nova Marilândia	14°21'5"	57°02'01"	355
14	*H. psittacorum*	Tangará da Serra	14°42'2"	57°47'31"	204
15	*H. psittacorum*	Barra do Bugres	15°07'6"	57°04'34"	156
16	*H. psittacorum*	Porto Estrela	15°18'1"	57°10'11"	168
17	*H. psittacorum*	Porto Estrela	15°24'2"	57°11'51"	148
18	*H. psittacorum*	Porto Estrela	15°35'37"	57°11'51"	155

descriptors evaluated in the inflorescences and leaves were: inflorescence growth, hairiness, waxiness, bract and flower color; arrangement of bracts (flat or spiral); hairiness and waxiness of the flower stem (pseudostem + floral peduncle + inflorescence) and leaves; firmness of the flower stem (bracts + rachis), (Costa *et al*. 2007).

Flower stems with two to three open bracts were cut between 7:00 and 8:00 h, twice a week, for 1 month. The stalks were stored in water recipients and transported in buckets to the postharvest laboratory. The inflorescences were cleaned (removing the flowers from within the bracts), washed, and cut to a standardized stem length of 80 cm). Quantitative and qualitative traits of five flower stems per clump were assessed, with four replications.

The flower stalks were placed in containers with water, which was exchanged every 2 d and maintained at 19ºC (cold room). The postharvest shelf-life (days) was evaluated every 2 d, for 21 d and the stems discarded when the bracts darkened on the inflorescences.

For the quantitative descriptors, analysis of variance was performed, the Scott Knott grouping test was applied and genetic parameters associated with efects of genetic and environmental nature, were estimated according to Cruz *et al*. (2012). The values of the genotypic determination coefficient (H^2) were expressed as the proportion of phenotypic variance caused by the genetic variability among treatment means (Cruz, 2006), and it can be used when the effect of genotypes is fixed, as in the case of this study.

To quantify the genetic divergence among accessions, Mahalanobis' generalized distance (D^2) was used for quantitative variables, while the simple match distance was used for the qualitative descriptors. The relative importance of variables was also evaluated by the methodology of Singh (1981). For the simultaneous analysis of qualitative and quantitative variables, the Gower's General Coefficient of Similarity was performed (1971).

The statistical analysis was performed using GENES software (Cruz, 2014). The accessions were grouped by the hierarchical method Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA) and validated by the cophenetic correlation coefficient using the software MEGA, version 5 (Kumar *et al*., 2009). The Pearson correlation between the distance matrices was also performed and the significance determined by the Mantel test (10,000 permutations). All Mantel tests performed here were conducted using the R packages *vegan* (Oksanen *et al*., 2012).

Results and Discussion

All H^2 values exceeded 87%, except for NIC (54.28%) and NFI (67.55%), which were considered low (Tab. 2). For the H^2 values, the variation index (I_v) was considered. According to Vencovsky and Barriga (1992), an I_v higher than one (1.0) indicates good conditions for selection gains by simple breeding methods, such as mass selection.

TABLE 2. Estimates of the genetic parameters of four accessions of *Heliconia densiflora* and 14 accessions of *H. psittacorum*.

Trait	Parameters					
	σ_f^2	σ_e^2	$\hat{\Phi}_g$	H^2 (%)	CV_g (%)	I_v
NIC	10.14	4.63	5.50	54.28	22.02	0.54
FSL	0.06	0.003	0.05	94.54	19.40	2.08
SW	2,068.08	178.20	1,889.88	91.38	35.30	1.62
FSW	997.75	86.61	911.13	91.31	34.04	1.62
FSD	0.49	0.06	0.43	87.74	13.88	1.33
NBI	1.29	0.08	1.21	93.80	21.32	1.94
BD	5.56	0.60	4.96	89.14	22.31	1.43
BL	10.81	0.39	10.41	96.31	26.71	2.55
IL	12.42	0.48	11.93	96.07	25.11	2.47
IW	6.75	0.70	6.04	89.53	26.13	1.46
NFI	26.30	8.53	17.77	67.55	20.58	0.72
NLS	4.75	0.10	4.64	97.73	27.91	3.28
LW	2.51	0.24	2.26	90.23	14.38	1.51
LL	50.78	1.82	48.96	96.41	20.94	2.59
SL	4.41	0.023	4.39	99.47	26.19	6.88

NIC: number of inflorescences per plant; FSL: flower stem length; SW: flower stem weight with leaf; FSW: fresh stem weight without leaf; FSD: diameter of the flower stem; NBI: number of bracts per inflorescence; BD: bract depth; BL: bract length; IL: inflorescence length; IW: inflorescence width; NFI: number of flowers per inflorescence; NLS: number of leaves on the flower stem; LW: leaf width; LL: leaf length (LL); SL: stem longevity.

The selection gain for the traits NIC and NFI may be minimized, since the H^2 value is low and I_v <1.0. The results also suggest the existence of genetic variability among accessions of *Heliconia* sp. for the studied traits, indicating favorable genetic values for breeding programs. For the 15 evaluated quantitative traits, several morphological groups were identified (Tab. 3)

Analyzing agronomic traits of *H. psittacorum* genotypes under full sun and partial shade, Costa *et al*. (2007) found H^2 varying from 82.25 to 97.33%, except for the trait days to inflorescence cut (20.02%). Similar results were reported by Rocha *et al*. (2010), in a study with *H. psittacorum* cultivars and interspecific hybrids, where I_v values between 0.21 and 1.85 were recorded for seven traits (days until inflorescence sprouting, period until stem harvest, cycle, stem weight without leaves, stem diameter, inflorescence length, number of open bracts per inflorescence).

TABLE 3. Relative contribution of 15 quantitative traits to genetic variability detection, based on Mahalanobis' generalized distance between 18 accessions of *Heliconia densiflora* and *H. psittacorum.*

Evaluated traits	Diversity (Sj)	Contribution (%)
Number of inflorescences per plant (NIC)	201.06	0.29
Flower stem length (FSL)	3,933.14	5.85
Flower stem weight (FSW)	820.57	1.22
Flower stem weight with leaf (SW)	1,502.58	2.23
Number of bracts per inflorescence (NBI)	3,376.23	5.02
Bract depth (BD)	1,080.07	1.60
Diameter of the flower stem (FSD)	707.27	1.05
Bract length (BL)	3,908.13	5.81
Inflorescence length (IL)	15,402.00	22.93
Inflorescence width (IW)	1,974.05	2.93
Number of flowers per inflorescence (NFI)	90.21	0.13
Number of leaves on the flower stem (NLS)	4,906.55	7.30
Leaf width (LW)	3,555.69	5.29
Leaf length (LL)	6,376.53	9.49
Stem longevity (SL)	19,335.58	28.78

The inflorescence traits such as length, stem length, fresh weight, and durability are of great commercial interest, due to promising ornamental potential. Inflorescences of *Heliconiaceae* can be classified in small (up to 10 cm), medium (10.1 to 30 cm), large (30.1 to 50 cm), and very large (>50 cm) (Castro *et al.,* 2007). Of the 18 accessions, 16 were classified as medium and two (accessions 5 and 16) as small (Tab. 4). The small to medium inflorescences are less difficult to handle and transport (Castro *et al.,* 2007). Genetic diversity studies of *H. psittacorum* and interspecific hybrids in the state of Pernambuco registered inflorescence lengths from 12.1 to 23.3 cm (Rocha *et al.,* 2010).

The traits FSL and FSD are important to ensure the quality and success of *Heliconia* species on the market. As standard length of floral stems of *Heliconiaceae*, Loges *et al.* (2005) suggested 80 cm. On the other hand, the higher the FSL, the greater the risk of stem breaking. Consequently, accessions with a FSL equal to or greater than 80 cm are recommended for breeding programs (Tab. 4).

TABLE 4. Mean values of 15 quantitative traits of *Heliconia densiflora* (accessions 1-4) and *H. psittacorum* (accessions 5-18).

Accession	Means of evaluated traits														
	IL	FSL	FSW	SL	SW	FSD	NIC	NBI	BD	BL	IW	LW	LL	NLS	NFI
	cm		G	days	g	Mm	un		mm	cm				un	
							H. densiflora								
1	15.6 c	88 e	60.3 c	6.5 d	82.8 c	5.1 b	8.7 b	3.9 d	12.3 a	14.2 b	4.7 c	9.1 c	33.1 c	6.0 d	17.4 b
2	23.2 a	124 c	108.5 b	6.5 d	146.7 b	5.4 b	13.2 a	4.6 c	8.5 c	21.1 a	6.4 c	11.2 b	45.3 a	5.9 d	17.5 b
3	17.0 b	93 e	56.25 c	6.6 d	73.8 c	5.2 b	8.2 b	3.9 d	8.7c	15.3 b	6.4 c	11.0 b	37.7 b	5.9 d	14.3 b
4	18.1 b	107 d	61.46 c	6.4 d	76.9 c	5.4 b	11.7a	3.9 d	12.6 a	15.1 b	4.7 c	12.1 a	31.0 d	4.8 d	16.0 b
							H. psittacorum								
5	8.3 e	135 b	87.96 c	6.5 d	127.2 b	6.3 a	7.0 b	4.3 c	5.3 d	7.3 d	9.3 b	12.3 a	13.3 f	11.3 a	10.3 b
6	12.7 d	180 a	155.0 a	6.6 d	203.5 a	4.5 c	8.25 b	5.6 b	9.0 c	11.1 c	12.8 a	11.8 a	37.9 b	10.7 a	18.1 b
7	12.7 d	141 b	112.5 b	6.6 d	169.4 b	5.0 b	13.0 a	6.3 a	6.9 d	10.0 c	9.3 b	12.3 a	34.7 c	11.6 a	26.0 a
8	12.6 d	127 c	94.4 b	8.5 b	136.3 b	4.6 c	9.0 b	6.2 a	6.9 d	10.5 c	10.4 b	11.1 b	33.5 c	8.6 b	16.0 b
9	12.1 d	144 b	111.6 b	7.7 c	148.2 b	5.4 b	11.0 a	4.4 c	10.4 d	9.6 d	9.1 b	9.5 c	44.2 a	6.1 d	25.8 a
10	12.0 d	143 b	115.8 b	7.7 c	162.8 b	4.7 c	8.75 b	6.8 a	8.9 c	10.6 c	10.2 b	11.2 b	34.6 c	9.1 b	21.1 a
11	11.3 d	148 b	143.3 a	7.8 c	211.8 a	4.6 c	7.25 b	5.7 b	10.7 b	10.3 c	12.5 a	12.8 a	38.1b	9.6 b	16.9 b
12	11.8 d	93 e	57.5 c	8.6 b	86.5 c	4.1 d	8.25 b	6.4 a	11.6 b	10.7 c	11.1 b	9.3 c	31.2 d	7.6 c	28.75
13	16.2 c	99 e	70.8 c	7.7 c	89.2 c	4.6 c	5.25 b	3.3 d	12.7 a	13.9 b	14.0 a	8.8 c	24.1 e	5.3 d	23.3 a
14	10.7 e	117 d	72.5 c	7.7 c	98.5 c	3.8 d	15.0 a	5.6 b	9.1 c	9.1 d	10.3 b	9.7 c	32.3 d	8.0 c	21.2 a
15	14.8 c	93 e	39.8 c	13.6 a	57.8 c	3.8 d	13.0 a	4.8 c	8.5 c	13.2 b	8.0 c	7.4 d	30.2 d	5.6 d	21.5 a
16	9.7 e	122 c	65.4 c	7.5 c	91.2 c	3.5 d	16.0 a	6.6 b	10.2 b	9.0 d	9.2 b	9.0 c	28.9 d	8.2 c	28.5 a
17	11.9 d	141 b	101.4 b	7.8 c	150.4 b	4.0 d	15.0 a	6.7a	13.5 a	10.9 c	11.1b	10.6 b	35.7 c	8.7 b	25.9 a
18	16.0 c	122 c	80.8 c	13.3 a	102.8 c	4.9 b	13.0 a	3.8 d	13.1 a	14.9 b	9.0 b	8.1 d	35.1 c	5.4 d	18.8 b

IL: inflorescence length; FSL: flower stem length; FSW: fresh stem weight without leaf; SL: stem longevity; SW: flower stem weight with leaf; FSD: diameter of the flower stem; NIC: number of inflorescences per plant; NBI: number of bracts per inflorescence; BD: bract depth; BL: bract length; IW: inflorescence width; LW: leaf width; LL: leaf length; NLS: number of leaves on the flower stem; NFI: number of flowers per inflorescence.

Means followed by the same letter in the column do not differ statistically by the Scott-Knott test at 5% probability.

The fresh weight of flower stems without leaf (FSW) ranged from 39.8 to 155.0 g (Tab. 4). This trait directly affects the stages management, preparation, packaging, and transport. The success of cut flowers on the market depends on the selection of accessions with a stem durability of more than 10 d. Long-lived stems can reach markets that are more distant by extending the shelf-life and maintaining the commercial quality (Castro *et al.,* 2007).

In the assessment of genetic diversity based on the 15 quantitative traits, three groups were determined using 255 as a cut distance (Fig. 1). Group I comprised the accessions 1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 14, 16, and 17, Group II accession 5 and Group III accessions 15 and 18. The isolation of the acession 5 of *H. psittacorum* in Group II was based on the traits of FSD and LL (Tab.4). The FSD expresses greater resistance of the flower stem against breaking by wind in the field, to transportation from the field to the location of cleaning, and in the subsequent selection stages (Albuquerque *et al.,* 2010).

The cophenetic correlation for the data was 0.87, confirming the reliability of the conclusions based on the visual assessment of the dendrogram, since values above 0.7 indicate a good adjustment of the dissimilarity matrix and the graphical representation of the distances (Sokal and Rohlfe, 1962). The accessions of the species *H. densiflora* (accessions 1, 2, 3, and 4) were grouped in the same group (G I), while the accessions of the species *H. psittacorum* were separated in two groups.

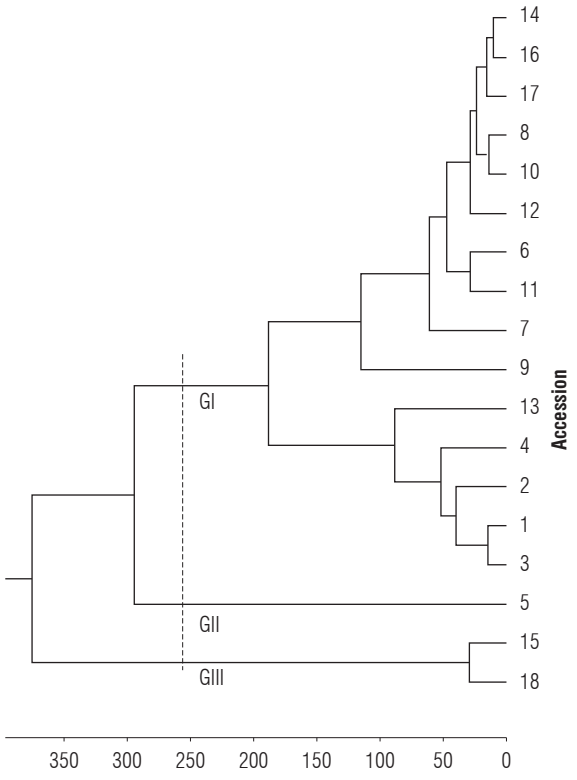

FIGURE 1. Dendrogram of genetic dissimilarity between *Heliconia densiflora* (accessions 1-4) and *H. psittacorum* (accessions 5-18), obtained by the UPGMA method, based on 15 quantitative traits, using Mahalanobis' generalized. Cutting distance 255 (Cophenetic correlation: 0.87; Distortion: 8.3%; Stress: 28.9%).

The highest contributions to the determination of the divergence of the studied accessions (Tab. 4) were related to the traits longevity of the flower stem (SL) with 28.78%, and inflorescence length (IL) with 22.93%, both accounting for 51.71% of the variation among accessions. Stem durability was an important trait for the genetic divergence among accessions, since the accessions 15 and 18 with highest SL formed one group (Group III). On the other hand, the flower stem diameter (FSD) and leaf length (LL) forcing the isolation of accession 5, are traits with no significant contribution to the genetic divergence among accessions (Tab. 4; Fig. 1)

To ensure the success in parental selection for breeding programs, less relevant traits should not be considered in characterization and evaluation studies and the other traits with high discriminatory potential maintained (Cruz *et al.,* 2012). The traits that contributed least to divergence were number of flower inflorescences (NFI) (relative contribution to variation 0.13%) and number of inflorescences per clump (contribution 0.29%) (Tab. 4), which were therefore discarded.

Among the 10 qualitative traits evaluated, six indicated no polymorphism among accessions, e.g.: upright inflorescence position, absence of hairiness on flower stem and leaves, and firmness and flat arrangement of the bracts. Moreover, the accessions differed in terms of flower and inflorescence colors and presence of waxiness on the inflorescence, leaves and stem (Tab. 5).

Tropical flowers differ from other more traditional plants on the market in their diversity of shapes, resistance to transport and durability (Loges *et al.,* 2005), aside from attractive visual aspects, being widely used for floral arrangements, event and party decor, cultivation in gardens, and projects of ornamental garden design. In studies of *Heliconia* sp species focused on cut flowers, Castro *et al.* (2007) reports that the high contrast of bract colors is one of the traits responsible for the appeal of this ornamental plant on the market. In the accessions, eight colors were identified in the inflorescences and two in the flowers (Tab. 5). Thus, the existence of variability for the traits in

TABLE 5. Qualitative traits evaluated in 18 *Heliconia* sp. accessions, collected in municipalities of the state of Mato Grosso, Brazil.

Acessions	Inflorescence				Bracts		Flor	Stem		Leaf
	Growth	Color	Hairiness	Waxiness	Firmness	Arrangement	Color	Hairiness	Waxiness	Waxiness
					H. densiflora					
1	Upright	Orange-red	Absent	Absent	Resistant	Flat	Orange	Absent	Absent	Absent
2	Upright	Orange-red	Absent	Absent	Resistant	Flat	Orange	Absent	Absent	Absent
3	Upright	Orange-red	Absent	Absent	Resistant	Flat	Orange	Absent	Absent	Absent
4	Upright	Orange-red	Absent	Absent	Resistant	Flat	Orange	Absent	Absent	Absent
					H. psittacorum					
5	Upright	Red	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
6	Upright	Red	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
7	Upright	Red	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
8	Upright	Red	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
9	Upright	Light pink	Absent	Present	Resistant	Flat	Orange	Absent	Present	Present
10	Upright	Red	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
11	Upright	Red	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
12	Upright	Yellow	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
13	Upright	Dark red	Absent	Absent	Resistant	Flat	Yellow	Absent	Absent	Absent
14	Upright	Dark orange	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
15	Upright	Dark pink	Absent	Present	Resistant	Flat	Orange	Absent	Present	Present
16	Upright	Dark orange	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
17	Upright	Orange	Absent	Absent	Resistant	Flat	Yellow	Absent	Present	Absent
18	Upright	Dark pink	Absent	Present	Resistant	Flat	Orange	Absent	Present	Present

flower and inflorescence color is fundamental for successful breeding.

Waxiness on inflorescences and leaves was detected in 21.43% of the accessions and 92.85% of the floral stems of *H. psittacorum* accessions (Tab. 5). Waxiness is an undesirable trait, since the visual aspect can be affected by handling (Loges *et al.*, 2005). This trait was not observed in *H. densiflora* accessions (Tab. 5).

Considering only the qualitative traits, two groups were determined using 0.148 as a cut distance. Group II contained the accessions 9, 15 and 18 and Group I the remaining accessions (Fig. 2). The cophenetic correlation was 0.94, also considered high. No polymorphism was detected for accessions of the species *H. densiflora* (accessions 1, 2, 3, and 4). Once again, the accessions of species *H. psittacorum* were classified in two distinct groups, with polymorphism within the species.

The correlations between dissimilarity matrices quantitative, estimated by Mahalanobis (0.74), qualitative Simple Match (0.62) and combined Gower distance (0.84) the whole set of traits, were all significant and high.

In the combined analysis (Gower General Coefficient of Similarity), showing correlation between the matrices of original distances and the cluster matrix. Using 3.6 as cut

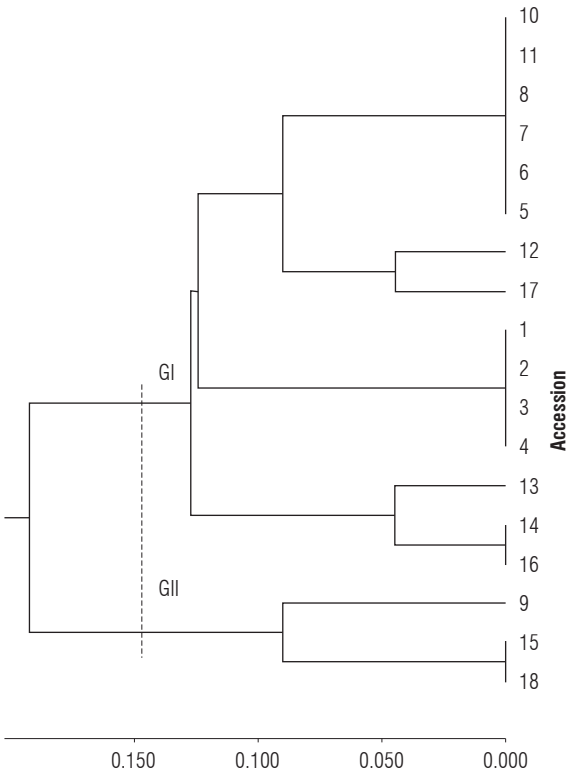

FIGURE 2. Dendrogram of genetic dissimilarity between *Heliconia densiflora* (accessions 1 to 4) and *H. psittacorum* (accessions 5 to 18), based on the UPGMA method, using 11 qualitative traits and the Simple Match. Cutting distance 0.148 (Cophenetic correlation: 0.94. Distortion: 2.7%. Stress: 16.4%).

distance three groups were determined: Group I contained the accessions 5, 6, 7, 8, 10, 11, 12, 13, 14, 16, and 17, Group II accessions 1, 2, 3, and 4, and Group III accessions 9, 15 and 18 (Fig. 3). The *H. psittacorum* accessions were assigned to Group I and III. The *H. densiflora* accessions were clustered in Group II, showing the low variability of these accessions, probably due to the low number of accessions.

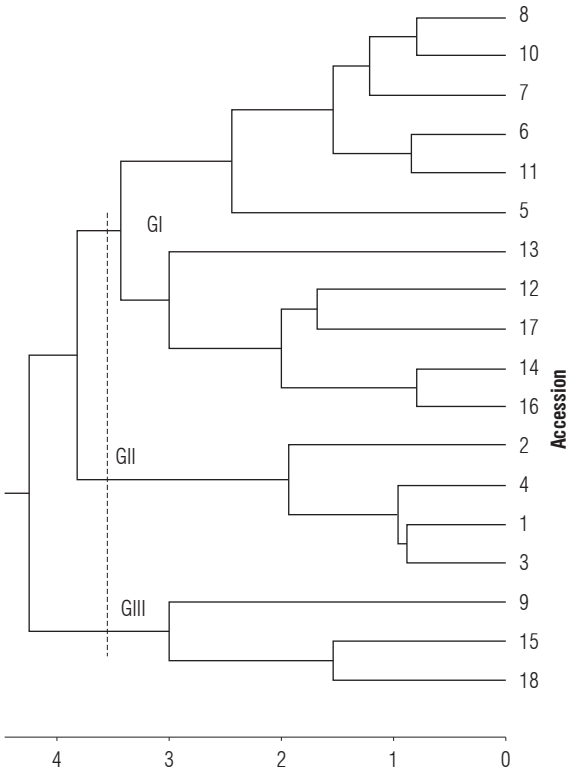

FIGURE 3. Dendrogram of genetic dissimilarity between *Heliconia densiflora* (accessions 1 to 4) and *H. psittacorum* (accessions 5 to 18), obtained by the method UPGMA, based on 15 quantitative and 11 qualitative traits, using the Gower distance. (Cophenetic correlation: 0.84. Distortion: 2.9%. Stress: 17.0%). Cutting distance 3.6.

In Group I, accession 13 stood out with a lower FSL and FSW, along with the absence of waxiness on the inflorescence, stem and leaf. In addition, the inflorescences are dark red and the flowers yellow.

In Group II, the accessions 1, 2 and 4 had low FSL and FSW values and no waxiness on the inflorescence, stem and leaf, as well as highest FSD and orange-red inflorescences and orange flowers.

In Group III, accessions 15 and 18 stood out for good postharvest shelf-life. In breeding programs, parents with high genetic divergence are recommended, to promote the occurrence of superior segregating plants in subsequent generations. However, the choice of individuals with superior plant, flower and fruit traits is also important, and finally, performance analysis for later recommendation of use *per se*. Thus, crosses should be performed between different genotypes with desirable agronomic traits (Cruz *et al.*, 2012).

To this end, we suggest to cross accessions 15 and 18 (Group III) with accessions 1, 2 and 4 of Group II and/or accession 13 of Group I, to breed superior genotypes.

Studies of Gomes *et al.* (2016) pointed out that among *Heliconia*s species, *H. psittacorum* cultivars and hybrids have a number of interesting traits, such as year-round production, terminal and upright inflorescences, varied bract number, and different types of flower colors, as observed in this study.

In this sense, based on the traits evaluated, the studied accessions are of interest for ornamental use, enabling a diversification of the ornamentals market. Traits such as bract length are relevant for playing an important role in the composition of floral arrangements and to raise the interest of consumers, as was expressed by Albuquerque *et al.* (2010).

The diameter and length of the flower stem are also key traits to characterize *Heliconia* sp. accessions. They make the flower stem more resistant to winds, to the transport from the field to the location of cleaning and the selection steps, and extend the postharvest shelf-life.

Conclusions

Morphological characterization of *Heliconia* species allowed the recognition of those descriptors which contribute to the detection of genetic divergence and provided key knowledge of the patterns of variation phenotypic of the target species.

The genetic variability among accessions of germplasm collection of *Heliconia* sp. uncovered in the present study can be explored in the breeding programs.

The characteristics that contributed most to the detection of genetic divergence were floral stem durability and inflorescence length.

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Heterosis for ether extract production and its components in seed of *Cucurbita argyrosperma*

Heterosis para producción de extracto etéreo y sus componentes en semilla de *Cucurbita argyrosperma*

Magda Piedad Valdés[1]*, Sanín Ortiz[1], and Franco Alirio Vallejo[1]

ABSTRACT

Twenty-one genotypes (six parental lines and 15 direct crosses) of butternut squash (*Curcurbita argyrosperma* subsp. *sororia*) were evaluated at the Experimental Center of the Universidad Nacional de Colombia in Candelaria (Valle del Cauca, Colombia) during the second semesters of 2012 (2012B) and the first semester of 2013 (2013A). Heterotic effects (average, variety, and specific) were determined for ether extract production per plant (EEPP) and the following components: percentage ether extract (EE) in seed, seed weight per fruit (SWF), 100-seed weight (100-SW), and number of fruits per plant (NFP). The methodology of Gardner and Eberhart (1966) was used in a randomized complete block experiment design with four replicates (five plants per replicate). Variety heterosis accounted for 48% of the variation of total heterosis for EEPP. Variety heterosis was better expressed in 2013 first semester, with significant differences for EEPP, SWF, and 100-SW; average heterosis was significant for EEPP and EE. In 2012 second semester, heterosis was significant for SWF, indicating genetic divergence between hybrids and parents. Introductions 256 and 132 expressed the greatest effects of variety heterosis for EEPP and superior segregants should be selected from these two parental lines. No significant differences were observed on specific heterosis; however, the best hybrid in terms of EEPP at 2013 first semester came from crosses 256×132 and 140×260.

Key words: heterosis, pumpkins, oilseeds, diallel analysis

RESUMEN

En el Centro Experimental de la Universidad Nacional de Colombia (Candelaria, Valle del Cauca, Colombia), se evaluaron 21 genotipos de *Curcurbita argyrosperma* subsp. *sororia* (6 padres y 15 cruzamientos directos) en semestres 2012B y 2013A, para estimar los efectos heteróticos promedio, varietal y específico para producción de extracto etéreo por planta (EEPP) y sus componentes: extracto etéreo en la semilla (EE), peso de semilla por fruto (PSPF), peso de 100 semillas (PUS) y número de frutos por planta (NFP). Se utilizó la metodología de Gardner y Eberhart (1966) y bloques completos al azar con cuatro repeticiones (cinco plantas por repetición). La heterosis varietal explicó el 48% de la variación de la heterosis total para el carácter EEPP y se expresó mejor en 2013A, con diferencias significativas ($P \leq 0,05$) para EEPP, PSPF y PUS. La heterosis promedia fue significativa para EEPP y EE. En 2012B fue significativa ($P \leq 0,05$) para PSPF, indicando que hay divergencia genética entre híbridos y padres. Las introducciones 256 y 132 expresaron los mayores efectos de heterosis varietal para EEPP. Se sugiere seleccionar segregantes superiores provenientes de estos dos padres. La heterosis específica no presentó diferencias significativas; sin embargo, los mejores híbridos para EEPP en 2013A provenían de los cruzamientos 256×132 y 140×260.

Palabras clave: heterosis, calabaza (Cucurbita), semillas oleaginosas, análisis dialelo.

Introduction

The Cucurbitaceae family is composed of approximately 118 genera and 825 species. One of its most important genus is *Cucurbita*, which is composed of 20 to27 species according to Esquinas and Gulick (1983), 15 species according to Lira (1995), and 12 to 13 species according to Nee (1990).

Five species of the genus *Cucurbita*are were domesticated in the New World and have been grown for centuries (Merrick, 1990; Smartt and Simmonds, 1995; Valdés, 2014): *C. argyrosperma* Huber, *C. ficifolia* Bouché, *C. maxima* Duchesne, *C. pepo* L., and *C. moschata* (Duchesne ex lam) Duchesne ex Poir.

Nee (1990) and Lira (1995) suggested that the species *C. argyrosperma* is constituted by two subspecies: *argyrosperma* and *sororia*. The subspecies *argyrosperma* is formed by four varieties: *argyrosperma*, *callicarpa*, *stenosperma*, and *palmieri*. The first three varieties include all cultivated types, while the fourth corresponds to related wild populations. The subspecies *sororia* L.H. Bailey comprises wild

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populations distributed extensively from Mexico to Nicaragua. Whitaker (1981) and Lira (1995) suggested that the subspecies *sororia* is the wild ancestor of the domesticated species based on both its reproductive compatibility and its morphological similarity. Its scarce diffusion worldwide is probably attributable to its poor pulp quality as compared to *C. moschata* or *C. pepo*. Different parts of the plant (flowers, young stems, tender or mature fruits) are consumed as vegetables (Lira, 1995), and its seeds are consumed whole, roasted, toasted, or ground, being the main ingredient in an array of sauces (Valdés *et al.,* 2010). Due to their high oil (39%) and protein (44%) contents, seeds are the most important product of the fruit (Ortiz *et al.,* 2009; Nawirska*et al.,* 2013; Valdés, 2014).

In Colombia, a native or *creole* butternut squash variety, *C. moschata*, produces, on average, 400 $kgha^{-1}$ of seed, and an average yield of 200 Lha^{-1}ether extract. This variety may be consumed as food or destined to medicinal and agroindustrial uses (Ortiz *et al*., 2009). Its oil is characterized by a high unsaturated fatty acids content (55.28%), in particular linoleic acid (55.11%). Oilseed cake contains approximately 51.11% protein and 4604.66 $kcal\ kg^{-1}$ energy (Ortiz *et al*., 2009).

In Greece, the oil obtained from *C. pepo* is well-known as an alternative source of biodiesel (Schinas *et al*., 2009). The seed of this species has a high oil content and is rich in polyunsaturated fatty acids, such as linoleic (43-56%) and oleic (24-38%), beta and gamma tocopherols (vitamin E), and carotenoids such as luteolin and beta-carotene (López *et al*., 2009).

The seed oil of *C. maxima* has antioxidant properties (Nawirska-Olszańska *et al.,* 2013) and it is well known for its multiple health benefits such as the prevention of prostate growth, reduction of hypertension, mitigation of hypercholesterolemia and arthritis, reduction of gastric levels, and prevention of cancer.

Most of the studies conducted on *Cucurbita* seed have focused on oil content, fatty acid profile and intrinsic seed properties. To date no studies have been reported on the heterosis for ether extract production in seeds of *Cucurbita argyrosperma* subsp. *sororia*.

This study aimed to evaluate the average, variety, and specific heterotic effects related to the character of ether extract production per plant and its components in *C. argyrosperma* subsp. *sororia*.

Materials and methods

Study area

The Fieldwork was carried out at the Experimental Centre of the Universidad Nacional de Colombia (UNAL, as its Spanish acronym), located in the municipality of Candelaria, Valle del Cauca, Colombia (2°6' N and 63°3' W) (Espitia *et al*., 2006). The experimental Centre, located at 972 m above sea level, presents an average annual temperature of 26°C, average annual precipitation of 1100 mm, and 76% relative humidity. Based on rainfall and sunshine rates, the climate is classified as sub-humid (Valdés *et al*., 2010). The macromolecular evaluation was carried out at the Seeds Laboratory of UNAL-Palmira Campus.

Genetic material

Twenty-one genotypes of *C. argyrosperma* subsp. s*ororia*, obtained from diallel crosses between six selected parental lines (introductions 256, 140, 260, and 132 from Central America and 107 and 68 from Mexico) and their corresponding 15 direct F1 crosses, were evaluated (Fig. 1, 2, 3, 4, 5, and 6). A randomized complete block design was used with four replicates and five plants per replicate, and the three central plants of each plot were evaluated. A planting distance of 3 m between and within the furrow was used. The diallel cross was evaluated in two consecutive semesters: 2012B and 2013A.

Evaluated features

- Ether extract production per plant (EEPP): the number of fruits per plant (NFP) multiplied by seed weight per fruit (SWF) and percentage ether extract (EE) in seed, expressed in g.
- Ether extract (EE) in seed: ether extract content (%) in seed.
- Seed weight per fruit (SWF): average weight (g) of three fruits randomly taken from each genotype and conditioned at 12% moisture.
- 100-seed weight (100-SW): weight (g) of 100 seeds randomly taken from three fruits of each genotype and conditioned at 12% moisture.
- Number of fruits per plant (NFP): average number of fruits per plant based on three plants of each genotype.

Heterosis analysis

The fixed model (selected parental lines) (Griffing, 1956) and method 2 (parental lines and direct hybrids) of the

FIGURE 1. Introduction 256 of *Cucurbita argyrosperma* subsp. *sororia*.

FIGURE 2. Introduction 140 of *Cucurbita argyrosperma* subsp. *sororia*.

FIGURE 3. Introduction 132 of *Cucurbita argyrosperma* subsp. *sororia*. A, whole fruit; B, fruit cut open at the equatorial diameter.

FIGURE 4. Introduction 260 of *Cucurbita argyrosperma* subsp. *sororia*. A, whole fruit; B, fruit cut open at the equatorial diameter.

FIGURE 5. Introduction 107 of *Cucurbita argyrosperma* subsp. *sororia*. A, whole fruit; B, fruit cut open at the equatorial diameter.

FIGURE 6. Introduction 68 of *Cucurbita argyrosperma* subsp. *sororia*. A, whole fruit; B, fruit cut open at the equatorial diameter.

methodology proposed by Gardner and Eberhart (1966) was used. The statistical genetic model is the following:

$$Y_{jj'} = \mu_v + ½ (V_j + V_{j'}) + \theta \hat{h}_{jj'}$$

Where:

$Y_{jj'}$: expected average value of a parental variety (j = j') or hybrid (j ≠ j'). If j = j', then θ = 0 and if j ≠ j, then θ = 1.

μ_v: mean of *n* parental varieties.

V_j: variety effect of the j-*th* parental variety.

$\hat{h}_{jj'}$: effect of heterosis that results when variety j is crossed with variety j'.

Total heterosis ($\hat{h}_{jj'}$) break down as follows:

$$\hat{h}_{jj'} = + (h_j + h_{j'}) + S_{jj'}$$

Where:

$\hat{h}$: mean heterosis of all hybrids.

h_j and $h_{j'}$: variety heterosis produced by the j-*th* variety.

$S_{jj'}$: specific heterosis for the cross between varieties j and j'; this is a deviation from the expected mean based on the effects $\hat{h} + h_j$.

The analysis of variance proposed by Gardner and Eberhart (1966) included the main sources of variation: replicates, genotypes (parental lines and hybrids), error, and total. The source of variation "genotypes" was broken down into effects of varieties (V_j) (parental lines), and heterosis ($h_{jj'}$). On the other hand, total heterosis effect was broken

down into average heterosis ($\hat{h}$), variety heterosis (h_j), and specific heterosis ($S_{jj'}$).

- Average heterosis ($\hat{h}$) is the difference between the average of all hybrids and the average of all parental lines.
- Variety heterosis (h_j) is the difference between the heterosis given by parental lines or variety j in its corresponding hybrids and the average heterosis of all the parental lines.
- Specific heterosis ($S_{jj'}$) between parental lines or varieties j and j' measures the deviation between the observed performance of a specific hybrid and its expected performance, based on variety effect (V_j), average heterosis ($\hat{h}$), and variety heterosis (h_j) (Espitia*et al.*, 2006).

Software developed by SAS Institute Inc. (2000), SAS-9.1 (Windows version), and Microsoft Excel 2010 were used to process results.

Results and discussion

Analysis of averages

In both semesters, prevailing environmental conditions affected the performance of parental lines and hybrids. In semester 2012B, evaluated features presented higher averages due to the semester precipitation and temperature and how they influenced the phenotypic expression. In 2013A, the excess precipitation negatively affected the evaluated features.

Tab. 1 shows the parental lines and hybrids averages regarding EEPP and its components on *C. argyrosperma* subsp. *sororia*. The average of both parental lines and hybrids in 2012B was higher than that of 2013A to all the evaluated features, except to the character EE for which parental lines presented a lower average in 2012B.

For the feature EEPP, the average of hybrids was superior to that of parental lines in both semesters, indicating high genetic diversity. However, no hybrid exceeded the best parental line 107, which presented 293.8 g/plant in 2012B and 86.3 g/plant in 2013A. Only hybrid 256×107 yielded a similar value: 216.8 g/plant in 2012B and 125.1 g/plant in 2013A.

For the components of the feature EEPP (EE, SWF, 100-SW, and NFP), the performance of hybrids was similar to that of parental lines in both semesters evaluated, indicating low genetic diversity for these components.

Table 2 presents the mean squares of the analysis of variance for the character EEPP and its components in semesters 2012B and 2013A.

For the character EEPP, the average squares associated with varieties (six parental lines) and heterosis (15 hybrids) for semester 2012B were highly significant, confirming the existence of genetic diversity between parental lines and between hybrids for this feature. There were no significant differences in semester 2013A, possibly because its genetic expression was affected by unfavorable environmental conditions. These data agree with the environmental effects found in other experiments on the agroindustrial traits of *C. moschata* Duch. (Valdes *et al.,* 2010; Valdes *et al.*, 2013).

The average heterosis of the character EEPP and two of its components, EE and 100-SW, was significant in semester 2012B, indicating that genetic variation exists between parental lines to explore heterosis. For the remaining components (SWF, 100-SW, and NFP), average heterosis was non-significant, indicating absence of genetic diversity between parental lines to explore heterosis.

Variety heterosis was highly significant for the character EEPP and two of its components, SWF and 100-SW, only in semester 2012B, confirming the presence of genetic diversity between the parental lines and these features. Variety heterosis was non-significant for components EE and NFP in both semesters, showing little genetic divergence between parental lines.

Specific heterosis was non-significant to the character EEPP and its components in both semesters, indicating that parental lines did not present favorable gene complements in the different hybrids. Similar results were observed on the species *Cucurbita moschata* (Valdés *et al.*, 2014).

Estimating heterosis effects on *C. argyrosperma* subsp. *sororia*

Effect of average heterosis

Table 3 shows the effect of average heterosis on the character EEPP and its components. Average heterosis was best expressed in 2012B, where there were significant differences for EEPP and the component EE. In 2013A, only the component SWF presented significant differences. The significance of these characters indicated that, overall, the parental lines used are suitable to explore the effect of heterosis on hybrids regarding the aforementioned features. Regarding the other characters (100-SW and NFP), the parental lines used are not suitable to tapping heterosis.

TABLE 1. Averages of parental lines and hybrids for the character of ether extract production per plant (EEPP) and different components in *Cucurbita argyrosperma* subsp. *sororia* for two semesters (2012B and 2013A).

Parental line	EEPP		EE		SWF		100-SW		NFP	
	2012B	2013A	2012B	2013A	2012B	2013A	2012B	2013A	2012B	2013A
256	62.6	25.9	40.00	43.21	16.30	11.87	7.62	6.33	9.17	4.83
140	52.4	85.0	37.17	36.84	41.23	30.18	15.86	13.83	3.17	4.00
260	37.5	43.5	30.96	36.12	6.58	8.62	2.55	3.45	19.50	11.00
107	293.8	86.3	36.92	47.05	61.05	21.10	12.17	6.30	13.00	6.25
68	80.8	57.8	36.67	33.58	57.25	39.48	19.77	16.89	3.75	3.13
132	40.1	34.0	34.15	34.60	55.57	54.15	21.91	22.12	2.42	2.13
Average of parental lines	94.5	55.4	35.98	38.57	39.66	27.57	13.31	11.49	8.50	5.22
Hybrids										
256X140	122.9	49.1	40.36	38.64	39.54	29.69	12.55	11.04	7.50	4.42
256X260	108.4	51.6	39.93	36.30	17.27	14.22	5.91	5.73	15.67	9.71
140X260	158.0	61.8	38.35	33.33	32.46	15.83	9.88	5.73	13.00	11.75
256X107	216.8	125.1	40.78	39.95	31.80	28.40	9.49	9.21	17.42	7.67
140X107	189.4	59.1	45.29	40.42	38.38	21.70	13.04	10.10	11.25	6.75
260X107	114.4	32.3	35.44	39.61	16.36	10.97	4.56	4.19	16.75	6.75
256X68	104.1	110.4	41.31	41.30	23.63	29.40	8.79	9.90	10.21	8.83
140X68	85.5	50.1	34.56	37.58	51.12	43.87	15.01	14.13	4.75	2.92
260X68	55.7	64.9	36.94	36.73	19.90	26.40	6.85	7.85	6.42	7.13
107X68	111.1	44.0	39.00	37.22	35.57	27.57	9.25	9.39	7.50	4.42
256X132	199.8	50.4	40.70	36.26	57.76	37.57	17.53	13.05	7.83	3.33
140X132	106.4	54.5	36.07	36.76	67.97	64.93	22.62	20.86	4.50	2.08
260X132	72.3	58.5	38.99	37.35	34.55	25.10	10.20	7.67	5.54	5.38
107X132	119.8	86.1	36.48	40.08	47.90	44.82	15.08	17.46	6.75	4.25
68X132	157.5	95.3	40.93	38.98	60.86	50.30	18.17	15.94	5.50	4.25
Average of hybrids	128.1	66.2	39.01	38.03	38.34	31.38	11.93	10.82	9.37	5.98
$LSD_{(5\%)}$	8.99	9.43	6.41	7.31	19.38	21.28	3.88	4.64	6.26	4.51

TABLE 2. Square means of variance analysis of ether extract production per plant (EEPP) and its components according to the methodology of Gardner and Eberhart (1966) in a diallel cross, evaluated in two semesters (2012B and 2013A) in *Cucurbita argyrosperma* subsp. *sororia*.

Source of variation	G.L.	EEPP		EE		SWF		100-SW		NFP	
		2012B	2013A	2012B	2013A	2012B	2013A	2012B	2013A	2012B	2013A
Replicates	3	22.59 ns	118.25 ns	4.63 ns	37.92 ns	3.17 ns	527.93 ns	9.05 ns	14.795 ns	52.40 ns	24.56 ns
Genotypes	20	168.87**	26.80 ns	39.27*	41.04 ns	1254.23**	912.8**	126.,43**	115.084**	101.78**	31.20**
Varieties (vj)	5	358.57**	15.66 ns	54.67*	104.40**	3876.32**	3091.21**	447.45**	408.570**	329.61**	85.97**
Heterosis (hjj')	15	105.64**	30.51 ns	34.13 ns	19.92 ns	380.20*	186.77 ns	19.42**	17.256 ns	25.83 ns	12.94 ns
Average heterosis	1	193.54*	20.08 ns	157.68**	4.87 ns	30.13 ns	249.62 ns	32.86*	7.700 ns	13.04 ns	9.71 ns
Variety heterosis	5	150.20**	34.15 ns	4.62 ns	33.66 ns	936.16**	136.51 ns	39.72**	18.665 ns	30.81 ns	5.15 ns
Specific heterosis	9	71.12 ns	29.64 ns	36.80 ns	13.96 ns	110.24 ns	207.70 ns	6.65 ns	17.535 ns	24.49 ns	17.63 ns
Error	60	40.42	44.52	20.55	26.75	187.75	226.51	7.55	10.802	19.61	10.20

* Significant effect (Pr>F)<0.05; ** Highly significant effect (Pr>F)<0.01; ns: not significant.

TABLE 3. Effect of average heterosis of the feature ether extract production per plant (EEPP) and its components during two semesters (2012B and 2013A) to the species *Cucurbita argyrosperma* subsp. *sororia*.

Character	Semester	
	2012B	2013A
EEPP	3.36*	1.08
EE	3.03*	-0.53
SWP	-1.33	3.82*
100-SW	-1.38	-0.67
NFP	0.87	0.75

* Significant effect (Pr>F)<0.05

Effect of variety heterosis

Table 4 shows the effects of variety heterosis for all evaluated characters.

Variety effects varied between semesters. However, parental line 256 presented positive effects in both semesters to the character EEPP and its components SWF, 100-SW, and NFP, indicating that it is a stable parental line and can be used in recurrent selection programs to multiply these characters.

Parental line 132 presented positive effects for both semesters to the character EEPP and its components EE and SWF, indicating that this parental line also has important additive genetic effects that can be used in selection programs to multiply these characteristics.

If the objective of a breeding program is to increase the percentage of ether extract on seed and, at the same time, ether extract production per plant, parental lines 256 and 132 should be considered for hybridization and selection programs.

Effect of specific heterosis

Specific heterosis helps identify hybrids with better characteristics than those of their parental lines. The significance of this heterosis indicates presence of dominance effects, probably attributable to favorable gene complementation in the crosses in which they form part.

Table 5 shows the effects of specific heterosis, which varied between semesters. For the character EEPP, the effects of hybrids 256×132 and 140×260 were significant for semester 2012B, indicating that these hybrids were superior to the average of their parental lines and the overall average. In semester 2013A, no significant values were obtained.

Hybrid 140×107 was outstanding in terms of percentage of EE in semester 2012B, presenting positive, significant values. In semester 2013A, although there were hybrids presenting positive values, these were non-significant, suggesting a reduced variability among parental lines and possibly a marked environmental effect even on hybrids.

TABLE 4. Effects of variety heterosis of the character ether extract production per plant and its components during two semesters (2012B and 2013A) for *Cucurbita argyrosperma* subsp. *sororia*.

Parental line	Character		Component							
	EEPP		EE		SWF		100-SW		NFP	
	2012B	2013A	2012B	2013A	2012B	2013A	2012B	2013A	2012B	2013A
256	4.38	2.87	-0.001	-1.75	6.26	3.44	1.50	1.29	2.61	1.22
140	2.64	-2.89	-0.70	0.001	8.66	3.47	2.09	0.78	1.20	0.12
260	-0.45	-0.95	1.16	-0.49	-1.25	-6.63	-0.18	-1.71	-2.87	-0.18
107	-7.19	-1.16	0.02	-2.46	-16.11	-2.63	-1.49	1.66	0.95	-0.52
68	-2.48	0.72	-0.92	2.91	-8.95	-0.80	-3.62	-1.92	-0.75	0.47
132	3.10	1.42	0.44	1.80	11.38	3.16	1.69	-0.10	-1.14	-1.10

TABLE 5. Effects of specific heterosis of the character ether extract production per plant and its components during two semesters (2012B and 2013A) for *Cucurbita argyrosperma* subsp. *sororia*.

Hybrid	Character		Component							
	EEPP		EE		SWF		100-SW		NFP	
	2012B	2013A	2012B	2013A	2012B	2013A	2012B	2013A	2012B	2013A
256×140	-3.85 ns	-1.69 ns	-0.56 ns	0.90 ns	-2.82 ns	-2.06 ns	-1.40 ns	-0.44 ns	-3.35 ns	-2.09 ns
256×260	-1.46 ns	-1.30 ns	0.26 ns	-0.59 ns	2.15 ns	3.35 ns	0.89 ns	1.93 ns	0.72 ns	0.00 ns
256×107	3.32 ns	4.11 ns	-0.72 ns	-0.43 ns	4.31 ns	7.29 ns	0.96 ns	0.62 ns	1.90 ns	0.68 ns
256×68	-2.02 ns	2.19 ns	0.87 ns	2.28 ns	-9.14 ns	-2.73 ns	-1.40 ns	-0.41 ns	1.02 ns	2.42 ns
256×132	4.01 *	-3.31 ns	0.15 ns	-2.16 ns	5.51 ns	-5.86 ns	0.95 ns	-1.70 ns	-0.30 ns	-1.02 ns
140×260	5.75 *	2.52 ns	0.79 ns	-2.13 ns	2.46 ns	-4.23 ns	0.15 ns	-1.30 ns	2.46 ns	3.56 ns
140×107	2.82 ns	0.31 ns	5.90 *	1.47 ns	-3.98 ns	-8.60 ns	-0.20 ns	-1.73 ns	0.14 ns	1.28 ns
140×68	-1.63 ns	-1.04 ns	-3.77 ns	-0.01 ns	3.49 ns	2.56 ns	0.11 ns	0.59 ns	-0.04 ns	-1.99 ns
140×132	-3.09 *	-0.10 ns	-2.36 ns	-0.23 ns	0.85 ns	12.32 ns	1.34 ns	2.88 ns	0.78 ns	-0.76 ns
260×107	-0.84 ns	-2.23 ns	-2.71 ns	1.51 ns	1.23 ns	1.55 ns	0.25 ns	0.04 ns	1.55 ns	-1.92 ns
260×68	-0.78 ns	0.58 ns	-0.14 ns	0.00 ns	-0.50 ns	5.96 ns	0.88 ns	1.98 ns	-2.46 ns	-0.98 ns
260×132	-2.66 ns	0.44 ns	1.80 ns	1.21 ns	-5.34 ns	-6.63 ns	-2.16 ns	-2.64 ns	-2.28 ns	-0.66 ns
107×68	-1.31 ns	-3.45 ns	0.08 ns	-3.00 ns	2.80 ns	-3.10 ns	-0.23 ns	-1.27 ns	-1.95 ns	-0.96 ns
107×132	-3.98 ns	1.25 ns	-2.55 ns	0.45 ns	-4.36 ns	2.85 ns	-0.78 ns	2.35 ns	-1.64 ns	0.93 ns
68×132	5.73 ns	1.73 ns	2.96 ns	0.72 ns	3.34 ns	-2.69 ns	0.65 ns	-0.88 ns	3.43 ns	1.50 ns

It is therefore recommended to conduct studies on the response of parental lines and hybrids in additional contrasting environments.

The effects of specific heterosis to components SWF, 100-SW, and NFP presented positive but non-significant values, indicating that no genetic complementation exists between their parental lines, even though their geographic origin differs.

Conclusions

The group of parental lines used allowed the effect of average heterosis to be explored to the character EEPP and its EE components.

Parental lines 256 and 132 proved best to tap heterotic effects, based on the effect of average and variety heterosis. Furthermore, of all the parental lines evaluated, their stability was the highest to begin a crossbreeding program to select superior-performing segregants to the character EEPP.

The average heterosis of hybrids 256×132 and 140×260 was superior to that of their parental lines and the overall average to the character EEPP in 2012B. Hybrid 140×107 was outstanding regarding the EE component, also during 2012B.

In the case of semester 2013A, although several hybrids presented positive values, these were non-significant.

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A tailor-made crop growth model for the tomato production systems in Colombia

Modelo de crecimiento de cultivo diseñado a medida para los sistemas de cultivo de tomate de Colombia

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ABSTRACT

Potential crop models simulate the plant growth under non-limiting biophysical conditions with no other factor than the climate to which the plants are exposed to. These models may fail to adequately represent the crop performance if they are not adapted to the local conditions. The particularities of Colombian tomato systems (greenhouse and open field) demand the recalibration of existing models to make a more realistic representation of those systems. Therefore, a locally calibrated crop model was proposed considering both production systems. To this purpose, four on-farm calibration experiments were carried out, two under greenhouse conditions with average temperatures of 17.4 and 17.9°C in Santa Sofía (Boyacá) and two under open field conditions in Páramo and San Gil (Santander), with average temperatures of 20.6 and 24.0°C, respectively. The crops were commercially managed according to the local practices. Plant data was collected through destructive measurements carried out on a fortnightly basis, while climate data were collected for the entire crop growth cycle. Independent calibration of the dry matter fractions allocated at the plant organs in function of thermal time resulted in an acceptable model performance. The calibration of the model under commercial conditions gave a better representation of the local systems but at the expense of accuracy since on-farm experiments cannot be controlled as those performed in research facilities.

Key words: crop modeling; cropping system; protected cultivation; thermal time; yield potential

RESUMEN

Los modelos de cultivo potenciales simulan el crecimiento de la planta bajo condiciones biofísicas no limitantes, sin más que el clima al que están expuestas las plantas. Estos modelos pueden no representar adecuadamente el rendimiento del cultivo si no se adaptan a las condiciones locales. Las particularidades de los sistemas colombianos de tomate (invernadero y campo abierto) demandan la recalibración de modelos existentes para hacer una representación más realista de esos sistemas. Por lo tanto, en el presente trabajo se propone un modelo de cultivo calibrado localmente considerando ambos sistemas de producción. Para ello, se realizaron cuatro experimentos de calibración en finca, dos en condiciones de invernadero con temperaturas promedio de 17,4 y 17,9°C en Santa Sofía (Boyacá) y dos en campo abierto en Páramo y San Gil (Santander), con temperaturas promedio de 20,6 y 24,0°C, respectivamente. Los cultivos fueron manejados comercialmente según las prácticas locales. Los datos de las plantas se recolectaron mediante muestreos destructivos realizados cada dos semanas, mientras que los datos climáticos se recolectaron durante todo el ciclo de cultivo. La calibración independiente de las fracciones de materia seca asignadas a los órganos de las plantas en función del tiempo térmico dio como resultado un rendimiento aceptable del modelo. La calibración del modelo en condiciones comerciales dio una mejor representación de los sistemas locales, pero a expensas de la precisión, ya que los experimentos en las fincas no pueden ser controlados como los realizados en instalaciones de investigación.

Palabras clave: modelado de cultivos; sistema de cultivo; cultivo protegido; tiempo térmico; rendimiento potencial

Introduction

During the last 40 years, crop systems simulation has evolved from a neophyte science with inadequate computer power into a robust and increasingly accepted science supported by improved software and computing capabilities (Boote *et al*., 2012). Over the last decade, the most significant demand of cropping system models has aimed to assess climate change impact on agriculture and to evaluate mitigation and adaptation strategies, conducted over different spatial scales and degrees of agricultural systems complexity (Stöckle *et al*., 2014).

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Most crop system models have evolved as elaborations of crop components and soil models focusing on modeling a single point in space over time to explore the of crop responses to soil, management and weather variability (Jones, *et al*., 2016). Regarding the crop modeling component, they simulate phenology and partitioning, and integrate processes of C, N and water balance from planting to maturity, showing the final yield and production as well as the daily values of crop components over the time to maturity (Boote *et al*., 2013). This potential yield is related to an adapted cultivar mainly determined by solar radiation, temperature, carbon dioxide, and genetic traits that lead the length of the growing period, light interception by the crop canopy and its conversion to biomass, and the biomass partition to the harvestable organs (Grassini *et al*., 2015). Under this approach, crop growth is not constrained by factors such as water, nutrients or pests.

While in the developed world the description and testing of single crop models have lost relevance (Stöckle *et al*., 2014), the development of crop growth models remains as a distant study subject to less developed agricultural productive areas. Di Paola *et al.* (2015) showed an overview of the crop growth and yield models through the unbalanced situation in which most of the available models have been applied for temperate regions and some references exhibiting examples from Brazil and Mexico. Moreover, the development of such models has been oriented towards staple crops such as cereals, sugar beet and potato.

Tomato is among the most important horticultural crops worldwide. A variety of tomato growth models have been developed in the past (i.e. Soto *et al*., 2014; Valdés-Gómez *et al*., 2014; Heuvelink, 1999; Scholberg, *et al*., 1997; Jones *et al*., 1991) with different levels of complexity and for different purposes. Tomato crop growth models have been included in decision support systems such as the world-renowned DSSAT (Jones *et al*., 2003) as well as many others (Massa *et al*., 2013; Jizhang *et al*., 2006). Moreover, 3D models of tomato plants have been developed for purposes such as optimizing LED lighting to increase light absorption and crop growth (de Visser *et al*., 2014).

In Colombia, Cooman (2002) evaluated the feasibility of a protected tomato cropping in the high altitude tropics by locally calibrating the second version of the Tomgro model through controlled experiments in the Bogota plateau. He modified the model by reducing the leaf expansion rate at low temperature and incorporating a direct effect of temperature on the distribution of dry matter between vegetative and generative plant organs. This modified version of the Tomgro model was later applied by Bojacá *et al*. (2009) to evaluate the variability of greenhouse tomato yield caused by spatial temperature variations.

However, most of Colombian tomatoes are grown throughout the year in several Andean mountain valleys and hills in warmer climates at altitudes below those of the Bogota plateau. Regarding the production context, tomato is a small-scale business represented by clusters of growers cultivating tomato by one of the two established systems: open field or greenhouse production. Under both systems, growers apply suboptimal practices despite the differences on the demand for resources per unit area (Bojacá *et al*., 2013; 2014).

As process based, crop models closely reflect the behavior of particular crops, the features of Colombian tomato systems demand the development of a locally calibrated growth model. However, this calibration is a highly data-demanding task as well as specific for the available data constraining a broader applicability (Robertson *et al*., 2013). On the other hand, most calibration experiments are carried out under controlled conditions, which in some cases are not representative of those observed under the natural field conditions (Craufurd *et al*., 2013).

Thus, the objective of the present work is to propose a simple crop growth tomato model with the ability to simulate different growth habits (open field or greenhouse) calibrated through on-farm trials, which reproduce the growing patterns and management practices applied by local farmers in Colombia. While on-farm calibration entails a series of experimental challenges, it allows the development of a more realistic model reproducing the production management practices applied by growers in the considered zones.

Materials and methods

Model description

The model structure, as presented in Figure 1, is based on earlier crop growth and transpiration models (Gil *et al*., 2017; Cooman, 2002). The model runs on a daily basis, exception made for the gross photosynthesis and maintenance respiration routines, which run on an hourly basis. All model calculations are done on a per plant basis. At the beginning of the simulation, once the climate (air temperature, relative humidity and global radiation) for the corresponding day is updated, the total dry matter production is simulated followed by its distribution among the above-ground plant organs.

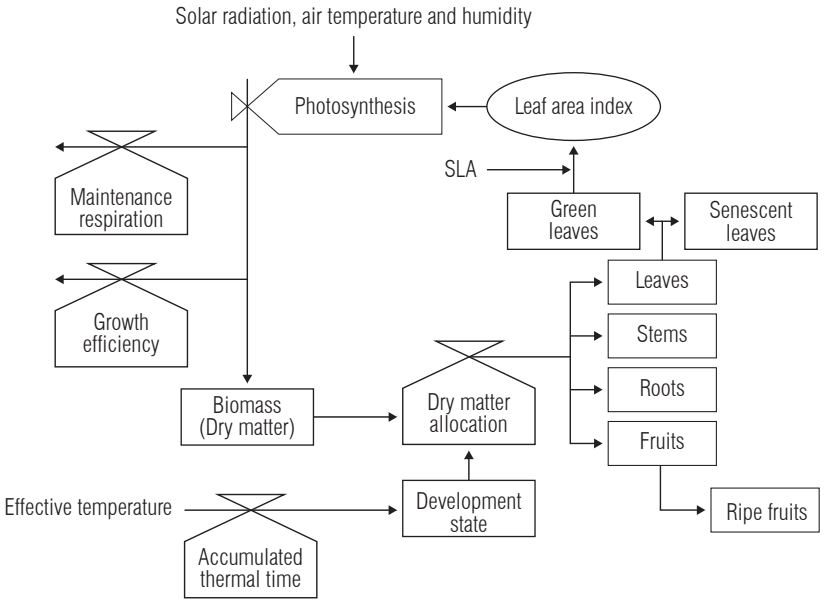

FIGURE 1. Schematics of the proposed tomato crop growth model for the open field and greenhouse production systems.

Dry matter production

The amount of dry matter available for growth is calculated at the end of the day as the difference between gross photosynthesis and total respiration. The daily gross photosynthesis results from the integration of the photosynthetic rates calculated on an hourly basis. The photosynthetic rate depends mainly on the photosynthetic active radiation (*PAR*) absorbed by the canopy, the air temperature and the CO_2 concentration, as modeled by Acock *et al.* (1978). The model considers restrictions on the photosynthetic rate due to extreme temperatures and vapor pressure deficit. These processes were modeled with the following equations:

$$GP_h = \frac{P_{MAX}}{XK} \times ln\left(\frac{(1-XM)+QE\times XK\times PPFD}{(1-XM)+QE\times XK\times PPFD\times e^{-XK\times LAI}}\right) \quad (1)$$

$$PPFD = (SolRad\times 0.47)\times 4.57 \quad (2)$$

$$P_{MAX} = \tau \times CO_2 \times PVPD \times PGRED \quad (3)$$

$$PVPD = e^{(CK\times(VPD-VPDL))} \quad (4)$$

$$PGRED = \begin{cases} 0, & if\ TMP < 9ºC \\ TMP - 9, & if\ 9 \le TMP < 10ºC \\ 1, & if\ 10 \le TMP < 28º \\ -0.083\times TMP + 3.33, & if\ 28 \le TMP < 40º \\ 0, & if\ TMP \ge 40ºC \end{cases} \quad (5)$$

Where GP_h is the hourly gross photosynthesis (g CH_2O h^{-1}), P_{MAX} is the maximum leaf photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), *XK* is the light extinction coefficient, *XM* is the leaf light transmission coefficient, *QE* is the leaf quantum efficiency (µmol CO_2 μmol^{-1} photon), *PPFD* is the photosynthetic photon flux density (µmol m^{-2} s^{-1}), *LAI* is the leaf area index, *SolRad* is the hourly solar radiation (MJ m^{-2}), τ is the CO_2 use efficiency (µmol CO_2 m^{-2} s^{-1} ppm^{-1}), CO_2 is the carbon dioxide concentration in air (ppm), *PVPD* is a function that correct the P_{MAX} for air vapor pressure deficit, *CK* is a factor used to determine the effect of vapor pressure deficit on photosynthesis (kPa^{-1}), *VPD* is the air vapor pressure deficit (kPa), *VPDL* is a factor used to determine the effect of vapor pressure deficit on photosynthesis (kPa), *PGRED* is a function that correct the P_{MAX} for sub optimal temperatures and *TMP* is the hourly mean temperature (ºC).

At the end of each day hourly gross photosynthesis is integrated, and the results is transformed into the amount of carbohydrates synthesized by the plant at the current day, following this formula:

$$GP_d = \sum_{h=1}^{24}(GP_h\times 3600)\times\frac{30\times 10^{-6}}{PLM2} \quad (6)$$

where GP_d is daily gross photosynthesis (g CH_2O d^{-1}), GP_h is hourly gross photosynthesis (g CH_2O h^{-1}) and *PLM2* is the plant density (plants/m^2).

The total respiration is represented by the maintenance respiration and the growth efficiency. Daily maintenance respiration is calculated as a fraction of the accumulated dry matter in stems, active leaves and growing fruits at a reference temperature of 20ºC. Afterwards, the maintenance respiration is corrected for temperature using a Q_{10} value. Next we present the equations that describe this module.

$$M_{RES} = Q_{10}^{0.1(TM\times P-2.0)}\times(\text{RMRL}\times(DMl + DMs) + RMRF \times DMf) \quad (7)$$

where M_{RES} is the maintenance respiration per day (g CH_2O d^{-1}), TMP_{avg} is the daily average temperature (°C), *RMRL* is a respiration coefficient for stem and leaves tissues (g CH_2O g^{-1} DM d^{-1}), *DMl* is the dry matter in leaves (g DM), *DMs* is the dry matter in stems (g DM), *RMRF* is a respiration coefficient for growing fruits (g CH_2O g^{-1} DM d^{-1}) and *DMf* is the dry matter in fruits (g DM).

Based on the above, the daily biomass production per plant is calculated using the following expression:

$$DMp = (GP_d - M_{RES})\times GREF \quad (8)$$

Where *DMp* is the total dry matter produced at day (g DM d^{-1}), GP_d is the daily gross photosynthesis (g CH_2O d^{-1}), M_{RES} is the maintenance respiration per day (g CH_2O d^{-1}) and *GREF* is a growth efficiency coefficient (g DM g^{-1} CH_2O).

Dry matter distribution

We considered the organs in the model as single units (i.e. big leaf model approach), meaning no dry matter distribution occurred among cohorts or sympodial units. The dry matter fabricated each day is allocated to the plant organs through thermal time (*TT*, °Cd) dependent functions. Each function describes the proportion (on a scale from 0 to 1) of the daily dry matter assigned to the considered organ. The base temperature at which plant growth starts was set at 10°C (Valdés-Gómez *et al.*, 2014). A fixed fraction (9%) of the dry matter produced was allocated to the roots. The equations describing dry matter distribution are:

$$DM_l = DMp\times\left(\frac{c_l}{1+a_l\times e^{-b_l\times ATT}} + d_l\right) \quad (9)$$

$$DM_f = DMp\times\left(\frac{c_f}{1+a_f\times e^{-b_f\times ATT}}\right) \quad (10)$$

$$DM_s = DMp - \left(DM_l + DM_f + DM_r\right) \quad (11)$$

Where *DMp* is the total dry matter produced at day (g DM d^{-1}), DM_l is the daily dry matter allocated to leaves (g DM d^{-1}), DM_f is the daily dry matter allocated to fruits (g DM d^{-1}), DM_s is the daily dry matter allocated to stems (g DM d^{-1}), DM_r is the daily dry matter allocated to roots (g DM d^{-1}), *ATT* is accumulated thermal time (°Cd) and a_i, b_i, c_i and d_i are the parameters that must be fitted for each function, and the sub index *i* represents the corresponding plant organ (leaves and fruits).

Once leaf senescence starts, the fraction of dry matter distributed to these leaves was estimated as a function of the ATT. When the harvest begins, we applied the same approach for the ripe fruits. The daily leaf area was calculated as the total dry matter corresponding to the active leafs multiplied by the specific leaf area (SLA). The proportion of senescent leaves was calculated in the following way for open field tomatoes:

$$SEN_l = \begin{cases} 0, & if\ ATT < 677 \\ 1.08\times10^{-3}\times ATT - 0.73, & if\ 677 \le ATT \le 1600 \\ 1, & ATT > 1600 \end{cases} \quad (12)$$

While for tomatoes under greenhouse it was calculated as follows:

$$SEN_l = \frac{c_{sen}}{1+a_{sen}\times e^{-b_{sen}\times ATT}} \quad (13)$$

where SEN_l is the rate of senesces for leaves, *ATT* is accumulated thermal time (°Cd), a_{sen}, b_{sen}, and c_{sen}, are the parameters to be fitted. On the other hand, ripe fruits ready to be harvested were calculated with the following function:

$$F_{hvt} = TDM_f\times\left(\frac{c_{rf}}{1+a_{rf}\times e^{-b_{rf}\times ATT}}\right) \quad (14)$$

where F_{hvt} is the dry matter of ripe fruits (g DM), TDM_f is the accumulated dry matter in fruits throughout the crop cycle (g DM), *ATT* represents the accumulated thermal time (°Cd) and a_{rf}, b_{rf}, and c_{rf}, are the parameters that must be fitted. The leaf area (*LA*) per plant was calculated based on the *DM* allocated to leaves and the *SLA* according to the following expression:

$$LA = TDM_{gl}\times SLA \quad (15)$$

$$DM_{gl} = TDM_l\times(1 - SEN_l) \quad (16)$$

where *LA* is the plant leaf area (m^2 $plant^{-1}$), TDM_{gl} is total dry matter belonging to photosynthetically active leaves (g DM), *SLA* is the specific leaf area (m^2 g^{-1}) estimated as 0.019 and 0.021 for greenhouse and open field tomatoes, respectively, TDM_l is total dry matter allocated in leaves (g DM) and SEN_l is the rate of senesces for leaves. Finally, the *ATT* was calculated as follows:

$$ATT = \sum_{d=1}^{nd} T_{ef} \quad (17)$$

$$T_{ef} = \begin{cases} 0, & if\ TMP_{avg} \le T_b \\ TMP_{avg} - T_b, & if\ TMP_{avg} > T_b \end{cases} \quad (18)$$

where *ATT* is accumulated thermal time (°Cd), *nd* is the number of days of the growing cycle, T_{ef} is the daily effective temperature (°C), TMP_{avg} is the daily average temperature (°C) and T_b is the base temperature (10°C). The values of the parameters included in the previous models equations are shown in Tab. 1; while the values of the fitted parameters values (a_i, b_i, c_i, d_i) for the equations 9, 10, 13 and 14 are shown in the Results section.

TABLE 1. Parameters included in the tomato growth model.

Meaning	Abbreviation	Value	Units
Air carbon dioxide concentration	CO_2	360	ppm
Light extinction coefficient	*XK*	0.58	Dimensionless
Leaf light transmission coefficient	*XM*	0.091	Dimensionless
Leaf quantum efficiency	*QE*	0.0645	μmol CO_2 μmol^{-1} photon
Carbon dioxide use efficiency	τ	0.0693	μmol CO_2 m^{-2} s^{-1} ppm^{-1}
Sensitivity to temperature	Q_{10}	1.4	Dimensionless
Effect of VPD on photosynthesis	*VPDL*	4.0	kPa
Effect of VPD on photosynthesis	*CK*	-0.8	kPa^{-1}
Respiration rate for leaves and stems	*RMRL*	0.015	g CH_2O g^{-1} DM d^{-1}
Respiration rate for fruit	*RMRF*	0.01	g CH_2O g^{-1} DM d^{-1}
Overall conversion efficiency	*GREF*	0.75	g DM g^{-1} CH_2O

Crop model calibration

Field experiments

The experimental work was conducted during 2016 in four commercial tomato production plots, with two of them planted under open field and the other two under greenhouse conditions. On each case, the crops were planted and managed according to the commercial practices regularly applied by growers under each production system. The crops were planted in two of the most representative tomato production areas of Colombia. The Alto Ricaurte province, located in the department of Boyaca, is one of the major greenhouse tomato production areas in Colombia, while the Guanenta province situated in the department of Santander is an important production area for open field vegetables including tomato. Tab. 2 describes the general characteristics of the experiments carried out to calibrate the proposed crop growth model. Next, we present a general description of the management practices applied in the experimental fields for both production systems.

The protected experiments were carried out under plastic naturally ventilated greenhouses with wooden structure. Plants were grown on a single stem of indeterminate length by periodically removing side shoots. Plants were tutored following the high wire system and no fruit pruning was done whatsoever. After harvest began, the leaves located under the harvested truss were removed since these no longer contributed to the plant growth and were more susceptible to be infected by fungal diseases. Nutrients were delivered through a fertigation system along with the irrigation water.

For the open field experiments, determinate growth cultivars grew freely without doing any leaf or fruit pruning, hanging the shoots to an elevated wire. Solid fertilization was done throughout the cropping cycle with amounts and timing defined by each grower. Fertilization for each location was based on soil analysis results, and the nutrients demanded by the plant to achieve potential yields. Under greenhouse, the fertilization of macronutrients was defined based on the following reference extractions: 10, 6.7 and 20 g/plant for nitrogen (as total nitrogen), phosphorus (as P_2O_5) and potassium (as K_2O), respectively. For the open field plots, the reference values were 12.3, 6.9 and 19.3 g/plant of nitrogen (as total nitrogen), phosphorus (as P_2O_5) and potassium (as K_2O), respectively. These values were obtained from the literature (Besford and Maw, 1974; Hernández *et al*., 2009; Atherton and Rudich, 2012) and adjusted based on previous trials conducted. The fertilization was divided into two periods taking into account the plant development stage. The establishment stage was defined from the sowing until the appearance of the first truss while the second one corresponded to the fruits development.

We took into account that the first stage has a shorter duration and that during most of the tomato cycle, the plants alternate between vegetative and generative growth. The fertilization fragmentation was done as follows: 30% of nitrogen and phosphorus, and 20% of potassium during the establishment and the rest during the fruit development stage. In the open field, the fertilizers used were ammonium nitrate, diammonium phosphate and potassium chloride, while under greenhouse the sources were calcium nitrate, monoammonium phosphate, and potassium sulfate. In the open field plots, we applied the fertilizers manually on a fortnightly basis, while under greenhouse we did it through the fertigation system three times a week. Under both systems, pest management was entirely based on chemically synthetized pesticides and with a spraying schedule defined according to the grower's criteria.

Data collection

The model calibration data were collected through a series of destructive measurements carried out for each experimental plot. Starting at transplanting time and on

TABLE 2. General characteristics of the on-farm experiments used to calibrate the tomato crop growth model.

Plot code	Production system	Location	Altitude (m a.s.l)	Planting date	Cycle length (days)	Density (plants/m^2)	Cultivar	Plot area (ha)
GH1	Greenhouse	5º 42' 26.7'' N – 73º 36' 4.1'' W	2346	28/01/2016	131	3.0	Libertador	0.28
GH2	Greenhouse	5º 44' 8.0'' N – 73º 36' 13.1'' W	2347	28/03/2016	113	3.9	Roble F1	0.28
OF1	Open field	6º 25' 15.4'' N – 73º 11' 56.7'' W	1703	27/01/2016	82	1.3	DRD	4.0
OF2	Open field	6º 28' 55.4'' N – 73º 6' 54.7'' W	1140	27/01/2016	97	1.3	Roble F1	1.0

a fortnightly basis, the aerial part of three plants from each experimental plot was removed. Under all conditions, the sampled plants were surrounded by edge plants. Afterwards, the plants were divided into its organs and weighted after being oven dried at 70ºC for at least 72 h. The leaf weight included the weight of the blades and all petioles.

Once fruit harvest and leaf pruning began, the amount of biomass removed from the plant was registered, and a sample was taken to determine its dry matter content. The grower defined the frequency and amount of biomass harvested or removed according to his criteria. the leaf area was determined by taking digital pictures of all the active leaves present at the moment of the destructive measurement. From the digital pictures, the number of pixels representing the leaves was extracted, including a reference object of a known area. To discriminate the image pixels as leaves, a reference object included into a prediction tree algorithm was used. All pictures were taken at the same height through a fixed mount tripod. The corresponding leaf area was estimated through the relation between the number of pixels of the reference object and the number of pixels corresponding to the leaf surface. This image processing step was carried out with the R statistical software (R Core Team, 2015). All the data collected from fruit harvest, leaf pruning and leaf area was later then integrated on a per plant basis.

The length of the data collection calibration was a function of the grower's decision to continue with his crop. Therefore, the number of destructive measurements was variable particularly to each experimental plot. Regularly, greenhouse growers are able to extend the cropping cycle for a longer period than those of the open field system. For the greenhouse plots we were able to carry out ten and nine destructive measurements for *GH1* and *GH2*, respectively, while for the open field plots seven and eight destructive measurements for *OF1* and *OF2* were respectively performed. In all cases, the destructive measurements were carried out until the end of the cropping cycle, ensuring that the complete plant cycle was characterized through these measurements. Tab. 1 includes the duration of the crop cycle for each experiment.

As global radiation, air temperature, relative humidity and wind speed are input variables for the model, Data was collected by placing the required sensors within the experimental plots. The hourly weather was recorded using a Vantage Pro2 Weather System (Davis Instruments, Hayward, CA, USA) for each of the open field experimental plots. For the greenhouse plots, Two copper-constantan thermocouples were installed and linked to a datalogger (Cox-Tracer Junior, Escort DLS, Edison, NJ, USA) to register dry and wet bulb temperatures. Through the psychrometric relationship between these two temperatures the air relative humidity was derived. Thermocouples were placed inside a ventilated white capsule to avoid altered readings due to the sun direct radiation. The global radiation within the greenhouses was measured throughout the measurement period with a pyranometer (Model LI200RX, Campbell Scientific, Inc., Logan, UT, USA) placed at 2.5 m above the ground. A weather station was deployed (Model Vantage Pro2, Davis Instruments, Hayward, CA, USA) outside the greenhouses, registering the external hourly climate.

Dry matter partitioning calibration

The calibration of the proposed model was focused on the dry matter partitioning among organs while it is considered as a key process to define the overall growth and development of the plant. For this purpose, on each tomato system, individual models to the fractions were fitted defining the amount of daily dry matter allocated at the leaves (Equation 9) and fruits (Equation 10) as a function of *TT*. The parameters for each model were estimated through the Nelder-Mead algorithm for a derivative-free optimization (Kelley, 1999) implemented in the dfoptim package (Varadhan, 2016) of the R statistical computing software (R Core Team, 2015). The same procedure was followed for the models that defined the fractions of senescent leaves (Equation 13) and ripe fruits (Equation 14).

A fixed fraction of 9% was allocated to the dry matter of the roots (Gil *et al*., 2017). The fraction of dry matter allocated

to the stems was calculated as the remaining fraction after discounting those allocated to fruits, leaves and roots.

The statistical analysis comparing the observed field data and the simulated values included the following statistical criteria: *Bias* (g DM/plant), root mean square error (*RMSE*, g DM/plant), and model efficiency (*EF*, dimensionless). These goodness-of-fit measures are defined according to the following equations:

$$Bias = \frac{1}{N}\sum_{i=1}^{N} D_i \quad (19)$$

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(D_i)^2} \quad (20)$$

$$EF = \frac{\sum_{i=1}^{N}(Y_i-\hat{Y}_i)^2}{\sum_{i=1}^{N}(Y_i-\bar{Y})^2} \quad (21)$$

Where N is the total number of observations, D_i is the difference between the measured value (Y_i) and the observed one ($\hat{Y}_i$) for the ith observation. *Bias* quantifies the average difference between measured and simulated values, with the best fit indicated when the *Bias* index is closer to zero. The *RMSE* is in the same units of the original variable and is a measure commonly used to check the agreement between measured and simulated results. *EF* is the most widely used distance measure including upper and lower bounds (Wallach, 2006). A model with an *EF* equal to one indicates a perfect fit between observed and predicted values. A full description of these goodness-of-fit measures can be found in Wallach (2006).

Results and discussion

Experimental climate conditions

The climate conditions under which the calibration experiments were carried out are summarized in Tab. 3. The climate conditions for the greenhouse experiments were similar since both greenhouses were located near to each other in the same municipality. However, by being planted in different dates resulted in some climate differences, especially those related to radiation levels. The global radiation level experienced by plants of the *GH1* experiment was higher than the one observed for the *GH2* experiment.

As the open field experiments were located at a lower altitude, these plants grew at higher temperatures with averages above 20°C. The climate of the *OF2* experiment showed the highest temperature and radiation levels as compared to the other three experiments. The lower radiation levels of the other experiments are explained due to the plastic covering in the case of the greenhouse experiments and by the geographical location of the *OF1* experiment. This open field experiment was located on top of a mountain with a permanent cloud cover, observed throughout the data collection period.

TABLE 3. Daily averages of the climate variables registered during the calibration experiments carried out under greenhouse and open field conditions

Plot code	Temperature (°C)	Global radiation (W m^{-2})	Relative humidity (%)
GH1	17.4	132.4	75.5
GH2	17.9	113.3	76.4
OF1	20.6	123.9	82.6
OF2	24.0	220.3	76.9

The temperatures registered in the open field experiments were more suitable for tomato cropping than those of the greenhouse experiments. Despite the use of plastic coverings, the average temperature of the night hours (18:00-5:00) were 15.4 and 14.8°C for the *GH1* and *GH2* experiments, respectively, while for the *OF1* and *OF2* experiments were 18.5 and 21.3°C, respectively. During the day hours (6:00-17:00), the *GH1* experiment showed an average temperature of 19.4°C while the temperature within the *GH2* experiment was warmer with an average of 21°C. Higher daily temperatures were observed for the open field experiments with averages of 22.8 and 26.7°C for *OF1* and *OF2*, respectively.

Dry matter distribution calibration

The dry matter allocation to the plant organs is a process linked to the total dry matter accumulation of the plant. Since the daily amount of assimilates produced by the photosynthesis process is a function of the climate conditions but also of the available leaf area, then the dry matter fraction allocated to the leaves defines the daily dry matter produced by the plant. Therefore, with the calibration of the dry matter distributed to leaves and fruits, we simultaneously calibrated the total dry matter plant accumulation. The fitted parameters of the functions defining the fractions of daily dry matter allocated to leaves (Equation 9), fruits (Equation 10), senescent leaves (Equation 13) and ripe fruits (Equation 14) as a function of *TT* are presented in Tab. 4.

As the dry matter distribution fractions were calibrated as a function of *TT*, next we present the cumulated *TT* of the four experiments. The highest accumulation of *TT* was achieved by the *OF2* experiment with a value of 1,371.5°Cd and followed by GH1 with 972.5°Cd. *GH2* and *OF1* experiments reached similar *TT*s of 888.7 and 885.6°Cd, respectively. Since the dry matter distribution

TABLE 4. Fitted parameters for the dry matter allocation in leaves (DM_l) and fruits (DM_f), leaves senescence rate (SEN_l) and ripening fruit rate (F_{hvt}).

Fraction	Open field				Greenhouse			
	a	***b***	***c***	***d***	***a***	***b***	***c***	***d***
Dm_l	0.044	-0.005	0.547	0.251	0.006	-0.012	0.377	0.301
Dm_f	51.34	0.006	0.521	-	136.23	0.012	0.543	-
SEN_l	-	-	-	-	3,239.37	0.011	0.735	-
F_{hvt}	10,830	0.009	0.668	-	1,000.00	0.009	0.668	-

fractions are calibrated in function of temperature we remove the time effect, allowing a more general application of these temperature-dependent functions. Therefore, the temperatures under which the plants grew are determinant for their development process rather than the cycle length.

The graphical representation of the dry matter distribution functions to the plant organs is depicted in Figure 2. The initial calibration procedure considered unique dry matter distribution functions for both tomato types. However, the results of this calibration procedure and the lower values of the goodness of fit measures indicated that independent calibration procedures should be followed for each tomato production system.

As stated previously, the fraction allocated to the roots was fixed to 0.09, while for the aboveground organs, the calibration was carried out for the leaves and fruits fractions. After adding up all the fractions, the stem fraction was the one needed to reach the total amount of dry matter produced as a function of *TT*. The comparison between production systems, exhibit the differences in the dry matter allocation to the plant organs. Under greenhouse conditions, tomatoes showed a higher decline in the dry matter allocated to the leaves and stems as compared to the situation observed for the open field tomatoes. Even under open field conditions, the plant starts allocating a higher proportion of assimilates to the leaves and then, the stem fraction increases and stabilizes to a value of 0.2.

The observed behavior of the organ fractions is defined by the growing habit of each tomato type and the way each production system is handled by the growers. Under open field conditions, tomato cultivars are mostly related to a determinate growth rate and growers do not apply any shoots pruning. Therefore, these plants have a higher stem fraction as compared to the indeterminate single-stem tomatoes planted under greenhouse conditions.

After a longer vegetative growth stage, the photosynthetically active leaves fraction of the open field plants declines to a minimum at the end of the growing cycle. Most of

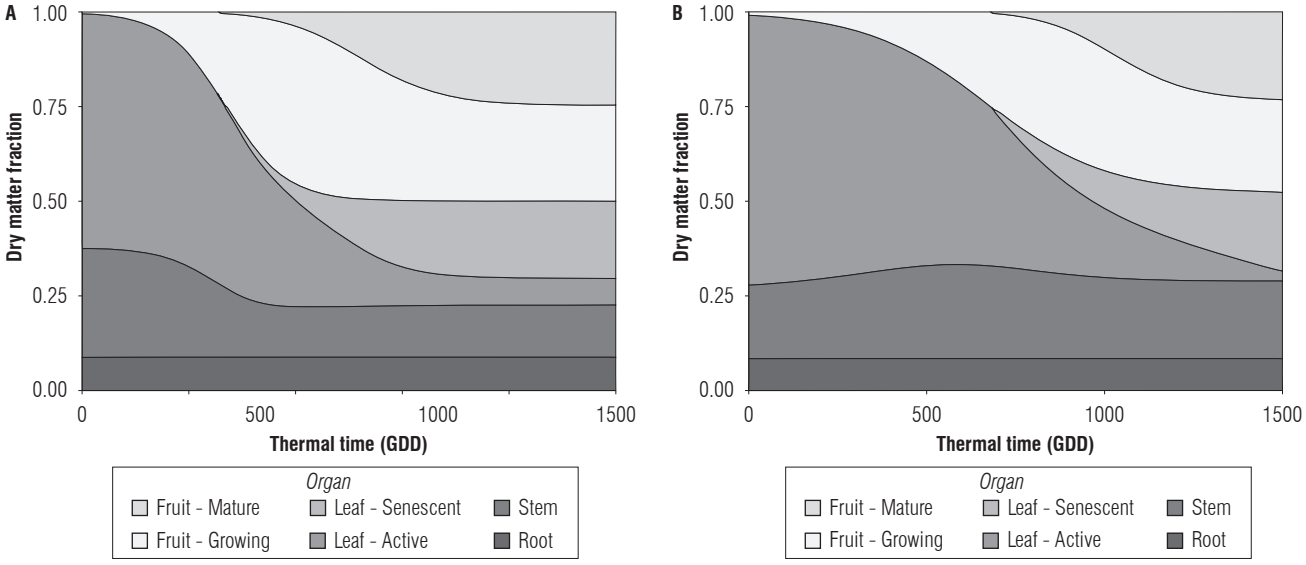

FIGURE 2. (A) Greenhouse and (B) open field tomato dry matter distribution fraction as a function of thermal time for each of the plant organs and its stages.

the remaining leaves hanging on the plant belong to the senescent fraction. On the other hand, a higher and constant fraction of active leaves is observed for the greenhouse plants, a situation that is characteristic of their indeterminate growth habit.

The fraction allocated to the fruits in the open field tomatoes showed a gentle slope as compared to the trend observed for the greenhouse conditions. However, at the end of the growth cycle, the fraction of ripe and growing fruits takes over about half of the dry matter produced by the plant. While the same pattern is observed for greenhouse tomatoes, in this case the fruits fraction is stabilized and remains constant at around the 1000°Cd. Under both production systems, it is important to note the fraction of growing fruits that remain on the plant. As the crop is reaching the end of its production cycle, the amount of harvested fruit should be higher than the one remaining in the plant, especially in the case of open field tomatoes. Nevertheless, under the local conditions growers do not properly balance the vegetative and generative growth of the plant nor apply proper pollination and pruning strategies, leading to this kind of results.

Once the dry matter distribution functions for each tomato type were calibrated, they were incorporated into the model. The observed and simulated total dry matter per plant and its allocation to the plant organs is presented in Figure 3. In most cases, the simulated dry matter properly followed the pattern depicted by the observed field data. The observed data also included not only the average of the sampled plants but also the standard deviation as a dispersion measure. Especially for the open field experiments and in particular for the last destructive measurements, there was an important variation in the data collected in the field.

Table 5 presents the goodness of fit measures selected to establish the crop growth model performance as compared to the observed field data. As the dry matter allocation fractions to the plant organs were estimated independently to each tomato type, we also present the goodness of fit measures per type of production system. According to the results for the whole plant and for each organ, a better model is considered to fit to the open field condition since values were closer to zero than the obtained ones for greenhouse tomatoes (Tab. 5). In most cases, the *Bias* results are positive indicating that the model tends to under-predict especially for the fruit dry matter since the higher *Bias* value was obtained for this organ and for both systems. The under-prediction reported by this index is a common pattern observed in particular for the first measurement dates (Fig. 3). Only the *Bias* for the total dry matter per plant in the open field condition was negative, indicating an overall over-prediction of the model but the *Bias* as such was close to zero (Tab. 5).

According to the results, the highest *RMSE* was obtained for the total dry matter per plant of the open field plants. The *RMSE* for the other plant organs and also for the results of the greenhouse plants yielded comparable *RMSE* values. Looking only at the results for the organs, the simulated dry matter allocated to the fruits gave the lowest fit under both production systems.

For the present case, the crop model reached similar *EF* values when considering the simulated dry matter per plant for both production systems. The lowest degree of agreement was observed for the simulated stem dry matter allocated to the greenhouse plants. For both production systems, the simulated fruit dry matter yielded a better fit than the one simulated for the leaves (Tab. 5).

Previous modeling efforts applied to Colombian greenhouse tomatoes such as the one carried out by Gil *et al.* (2017) whom yielded a *RMSE* of 4.21 g DM/plant for the simulated total plant dry matter. This potential crop growth model was calibrated based on experimental crops planted in the Bogota plateau and carried out under the best management possible practices without any technical constraints.

The calibration of the present model yielded comparable results to those obtained on other tomato models calibrations. For instance, Battista *et al*. (2015) calibrated a

TABLE 5. Goodness of fit measures of the simulated dry matter per plant and per organ by the calibrated tomato crop growth model.

Plant organ	Greenhouse system			Open field system		
	Bias **(g DM/plant)**	***RMSE*** **(g DM/plant)**	***EF***	***Bias*** **(g DM/plant)**	***RMSE*** **(g DM/plant)**	***EF***
Plant	13.77	22.68	0.97	-2.46	61.24	0.91
Stem	0.67	10.66	0.57	0.81	19.89	0.82
Leaf	10.0	14.76	0.86	3.69	25.42	0.80
Fruit	14.72	22.51	0.91	11.8	27.09	0.94

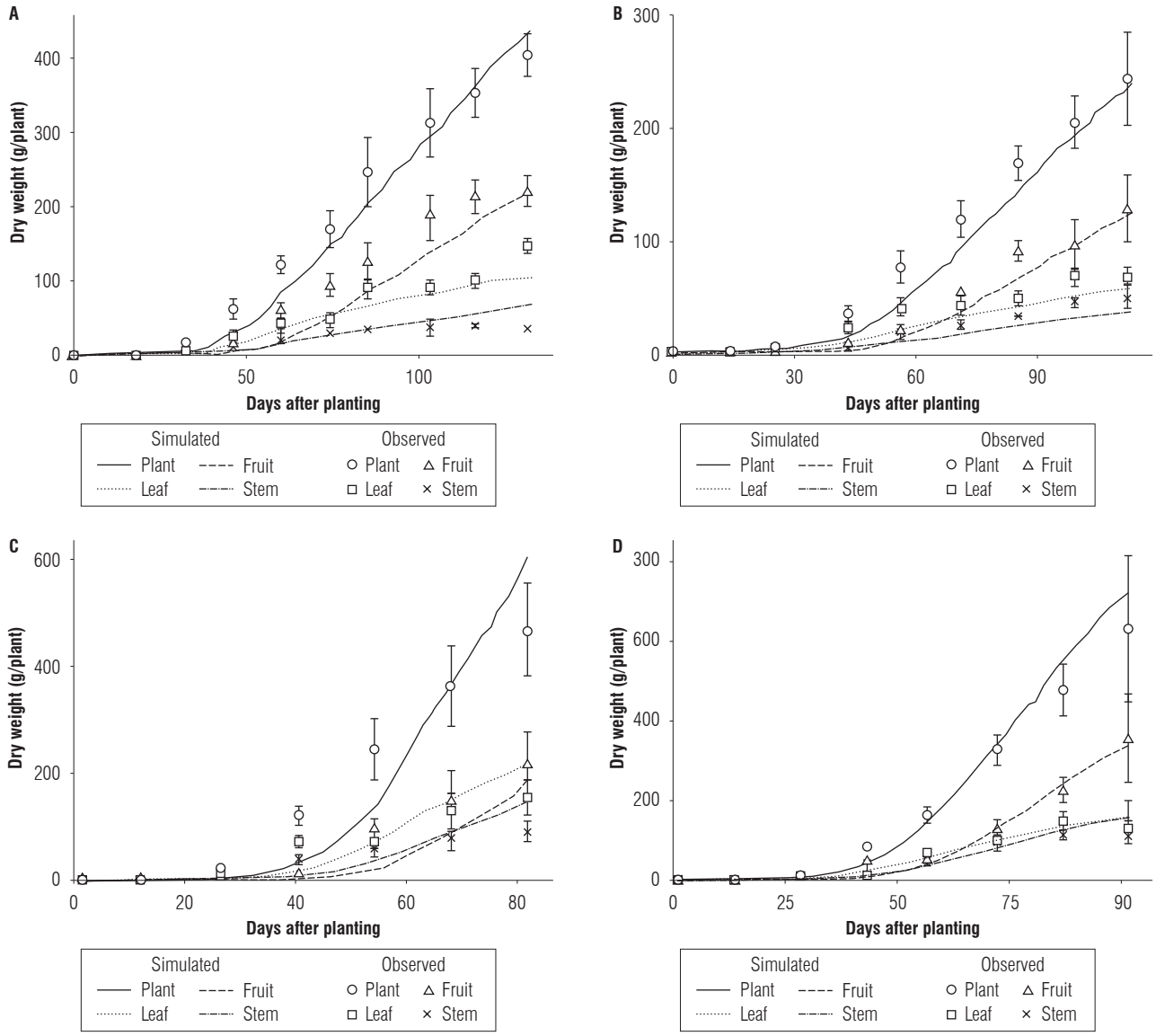

FIGURE 3. Observed and simulated dry matter accumulation and distribution over the plant organs for the calibration experiments carried out under (A, B) greenhouse and (C, D) open field conditions. Vertical bars represent the estimated standard deviation.

modified version of the Tomgro model on tomatoes growing under low-tech Italian greenhouses. The plant dry matter calibration for three cultivars indicated *RMSE* values ranging from 15.4 to 48.5 g/plant and *EF* values between 0.852 and 0.976. The paper of Fan *et al.* (2015) described a knowledge and data-driven modeling approach to simulate the growth of the tomato plant. In this case, the *RMSE* for the plant dry matter simulated with different modeling techniques ranged from 20.95 to 35.73 g/plant. The present study results are comparable to those results (exception made for the plant dry matter estimated for the open field tomatoes).

Under non-limiting growth conditions but with the biophysical constraints imposed by developing the model calibration through on-farm experiments, the proposed tomato growth model yielded acceptable results. It is important to highlight that the on-farm calibration experiments were carried out with the required rigor from the data collection point of view but were developed under the current set of management practices applied by most growers in the included zones. It is well known that on-station research results often do not reflect crop yield when technologies are applied onto surrounding farms (Leeuwis, 2004). Therefore, the model calibration was carried out through

on-farm experimentation while accounting for real world factors due to less consistent crop management.

Yield gap represents the difference between yield achieved by farmers and potential yield (Guilpart *et al.*, 2017). Different yield gaps can be established depending on the reference point used to evaluate the current yield obtained by local growers (Titonell and Giller, 2013). The first gap is obtained by comparing potential yields, with no restrictions other than those imposed by climate conditions, and those currently obtained by local farmers. Potential yields can be calculated based on models calibrated with data obtained from perfectly-controlled conditions. This gap is narrow in areas where production is characterized by high technological levels and where factors such as soil fertility and pests and diseases pressure do not impose major restrictions on the crop development. However, the current gap for both production systems is huge, and therefore impractical to establish improvement strategies, since both systems are characterized by a low technological level, which causes a high susceptibility to biotic (e.g. pest and diseases pressure) and abiotic (e.g. low soil fertility) constraints.

The second gap corresponds to the difference between the attainable yields, which correspond to the maximum yields that could be obtained given technological and environmental restrictions at a certain region and the yields currently obtained by growers. In the present work, the proposed model is calibrated using maximum achievable yield data given the local conditions; therefore, constitutes a useful tool to determine a gap that serves as a reference to design strategies that allow its reduction. Additionally, the model opens the possibility to add modules to study the factors (e.g. fertilization and irrigation strategies) that should be optimized to gradually move towards potential yields.

Another driving factor to explain the model performance is the variability introduced by the genetic factor. Mavromatis *et al.* (2001) stated that successful use of crop models in technology transfer requires coefficients describing new cultivars to be available as soon as the cultivars are marketed. On the other hand, current market trends including specialization have led to genetic differentiation in contemporary tomato varieties (Sim *et al.*, 2011). While the genetic variation is recognized, this factor was overlooked since the purpose of the proposed model is to be as generic as possible. Future improvements on the model performance can be achieved by including the genetic variation since temperature effects on crop yield are also recognized as cultivar-dependent (Vanhoor *et al.*, 2011).

Conclusions

The particularities of cropping systems, such as the case of Colombian tomatoes, demand local calibration of crop growth models. Potential growth models are far from depicting the real behavior of the crop since the conditions under which these models are calibrated are not representative of the local practices. While the tomato cultivars planted in Colombia have all the potential to achieve higher yields, these are restricted by the conditions under which the crop is managed.

The present crop growth model was developed bearing in mind this situation, therefore we calibrated it through on-farm experiments. Although the calibration of a model will never be considered complete or sufficient, the present model sets a baseline for further improvements to get a closer picture of the current tomato production systems. Contrary to our original expectations, differences in the dry matter distribution to the plant organs among greenhouse and open field tomatoes were found, therefore it was necessary to derive independent functions to characterize each tomato type. Despite including these two sets of functions, the crop model is conceived as one entity able to simulate the plant behavior for both types of tomato.

The tomato model proposed in this study is characterized by a fair compromise between representativeness and accuracy. The on-farm calibration experiments entailed a series of challenges and technical issues, commonly tackled in commercial agriculture, reducing the potential yield achievable by the crop. Consequently, by doing the calibration under these settings, the outcome model resembles more closely the reality of the current crop performance. This result comes at the expense of accuracy since higher variability is observed in the field as compared to experiments carried out in dedicated facilities and with all the resources at disposal to achieve the best possible results.

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Combined effect of green-colored covers and shading on the growth of sisal (*Furcraea hexapetala*) plants

Efecto combinado de coberturas de color verde y el sombreado sobre el crecimiento en plantas de fique (*Furcraea hexapetala*)

Fánor Casierra-Posada[1]*, Fernando Portilla-Fuentes[1, 2], and Julián Molano-Díaz[1]

ABSTRACT

Plants have the ability to respond in different ways to the quality of light, its intensity, and the combination of both. In addition, chlorophyll absorbs light in the blue and red bands of the spectrum, but green and infrared light are poorly absorbed or not absorbed, yet they affect plant morphogenesis. An experiment was carried out in Tunja, Colombia, in which the influence of shading on sisal plants (*Furcraea hexapetala*) was evaluated. The plants were placed under green polypropylene filters that induced 55.4, 85.8 and 90.1% light reduction and were compared with open exposure plants in a greenhouse. As a consequence of shading, the values of the following variables were reduced: chlorophyll content index by between 52.2 and 55.0%, dry mass by between 80.8 and 94.0%, water uptake by between 40.9 and 44.9%, water use efficiency by between 23.0 and 53.7% and relative growth rate by between 35.1 and 58.4%, as compared to the control, while the values for the root to shoot ratio, specific leaf area and leaf area ratio were increased by ranges of 24.2 to 73.5%, 107.5 to 132.4% and 116.6 to 174.9%, respectively. The shading with green filters induced a reduction in the red/far red ratio of light. Based on these results, it was possible to infer that the sisal plants presented low plasticity for tolerating the abiotic stress induced by green-filter shading.

Key words: transmittance, red/far red ratio, abiotic stress, crassulacean acid metabolism, light quality, chlorophyll.

RESUMEN

Las plantas tienen la capacidad de responder de diferentes formas a la calidad de la luz, a su intensidad, y a la combinación de ambas. Adicionalmente, las clorofilas absorben la luz en las franjas azul y roja del espectro, pero la luz verde e infrarroja se absorben poco o no se absorben, no obstante, afectan la morfogénesis en plantas. Se desarrolló en Tunja, Colombia, un experimento en el que se evaluó la influencia del sombreado sobre plantas de fique (*Furcraea hexapetala*). Se colocaron las plantas bajo filtros de polipropileno de color verde que indujeron 55,4; 85,8 y 90,1% de reducción de la luz y se compararon con plantas a libre exposición dentro de un invernadero. Como consecuencia del sombreado, en relación con las plantas control, se redujeron los valores de las siguientes variables: el índice de contenido de clorofila entre 52,2 y 55,0%, el contenido de masa seca entre 80,8 y 94,0%, la toma de agua entre 40,9 y 44,9%, la eficiencia en el uso del agua entre 23,0 y 53,7%, la tasa de crecimiento relativo entre 35,1 y 58,4%, mientras que los valores de la relación raíz/parte aérea, el área foliar específica y la relación de área foliar se aumentaron en rangos de 24,2 a 73,5%; 107,5 a 132,4% y 116,6 a 174,9%, respectivamente. El sombreado mediante los filtros de color verde indujo una reducción en la relación rojo/rojo lejano de la luz. Con base en estos resultados se pudo inferir que las plantas de fique presentan poca plasticidad para tolerar el estrés abiótico inducido por el sombreado a través de los filtros de color verde.

Palabras clave: transmitancia, relación rojo/rojo lejano, estrés abiótico, metabolismo ácido de las Crasuláceas, calidad de luz, clorofila.

Introduction

Light wavelengths between 300 and 900 nm can affect the growth and development of plants. However, the quality of light is not the only factor that can influence the growth processes of plants because other properties, such as light intensity and duration as well as climatic factors, are equally involved. The research using a single wavelength range is very useful in identifying the spectrum most suitable for the agricultural application (Casierra-Posada and Peña-Olmos, 2015).

The study of the response of plants to light quality has aroused the interest of researchers in order to improve

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production (Casierra-Posada *et al*., 2015). Among the bands of the visible spectrum, the green and infrared ranges are of particular interest because they are poorly absorbed or not absorbed; nonetheless, they induce important photomorphogenic responses (Wang and Folta, 2013). Although it is presumed that the amount of light being absorbed in the green range is very low and, therefore, not important for photosynthesis, the response spectrum used for photosynthesis shows that green light is in an effective spectrum band for increasing plant photosynthesis (Kim *et al*., 2004). On the other hand, CAM-type species can growth in contrasting environments in terms of light intensity. However, it is still unknown how these species can meet energy and carbon requirements when exposed to shadow conditions (Ceusters *et al*., 2011).

Sisal is a CAM-type plant grown in Colombia to produce natural fibers (Casierra-Posada and Gonzalez, 2009); therefore, because of its physiology, this species is of great interest, especially for the study of its adaptation to contrasting conditions of light intensity and quality. Based on previous approaches, this study was developed to evaluate the effect of shading and filtered sunlight through green polypropylene films on the growth of sisal plants (*Furcraea hexapetala* Jacq.).

Materials and methods

This experiment was carried out under greenhouse conditions in Tunja, Colombia, at the facilities of the Universidad Pedagógica y Tecnológica de Colombia (UPTC) located at an altitude of 2,690 m a.s.l. at 5°33'10.62" N and 73°21'23.97" W. During the study, an average temperature of 19.0°C, 72.6% relative air humidity and 606.0 ± 362.9 µmol m^{-2} s^{-1} illumination were maintained in the greenhouse. The illumination was natural, with a photoperiod of ± 12 h of light / 12 h of darkness, corresponding to tropical conditions.The plants were obtained from(*Furcraea hexapetala* Jacq., plant known as Fique or Cabuya) bulbs, which were placed under hydroponic conditions in 4 L containers filled with the following solution: (mg L^{-1}): Nitrogen, 200; Phosphorus, 100; Potassium, 50; Calcium, 2; Magnesium, 10; Sulfur, 15; Iron, 0.2; Manganese, 1; Copper, 3; Zinc, 6; Boron, 4; Molybdenum, 0.1 and Cobalt, 0.05. The pH of the solution was adjusted to 6.2. The solution in each container was aerated with an aquarium pump to provide oxygen to the solution.

One month after planting, when leaf emergence was observed, the plants were divided into four groups consisting of ten plants each. Three groups of plants were exposed to sunlight filtered through green polypropylene films with a thickness of 15 µm, placed 1 m above the plants and mounted on wooden frames that covered all sides of each group of plants, except underneath. The frame of the first group was covered with a polypropylene filter, the second with two and the last with three. The remaining group of plants was left exposed to the environmental conditions inside the greenhouse without any cover and was used as a control treatment. As a consequence of placing the polypropylene filters on the frames, the illumination in each group of plants was reduced by 55.4, 85.8 and 90.1%, respectively from 606.0 ± 362.9 µmol m^{-2} s^{-1} (Tab. 1).

The trial lasted 256 d counted from the moment of sowing, at the end of which the plants were collected for destructive tests and final measurements. With the information collected at the end of the trial, the absolute growth rate (AGR), the relative growth rate (RGR), the root to shoot ratio (based on dry weight), the leaf area ratio (LAR), the specific leaf area (SLA), and the leaf weight ratio (LWR) were determined according to Hunt (1990). The total dry weight per plant was recorded by drying the plants in an oven at 80°C until reaching a constant dry weight. The dry matter partitioning was taken as the percentage of dry weight of leaves, stem and roots, separately, in each plant, depending on the total dry weight in the plant. Twice a week, the amount of water missing in each container was recorded and taken as the water uptake. The water use efficiency (WUE) was calculated as the dry mass quantity

TABLE 1. Environmental conditions in the sisal (*Furcraea hexapetala* Jacq.) plants exposed to shading with green-colored covers.

Cover color	Polypropylene filters	Illumination (μmol $m^{-2}s^{-1}$)	Red light* (μmol $m^{-2}s^{-1}$)	Far red light** (μmol $m^{-2}s^{-1}$)	Red / far red ratio
Without cover	0	606.0 ± 362.9	43.5 ± 27.4	41.0 ± 25.3	1.1 ± 0.7
Green	1	269.8 ± 234.5	15.0 ± 16.2	16.9 ± 17.3	0.8 ± 0.06
Green	2	85.6 ± 54.7	2.6 ± 1.6	4.2 ± 2.1	0.5 ± 0.1
Green	3	59.6 ± 20.9	1.1 ± 0.3	2.5 ± 0.8	0.4 ± 0.1

The illumination measurements were taken under the polypropylene filters, 10 cm over each plant.

* Red light was measured at 660 nm, 40 nm FWHM (Full width at half maximum) (± 20 nm).

** Far red light was measured at 730 nm, 30 nm FWHM (Full width at half maximum) (± 15 nm).

produced by the whole plant as a function of the amount of water consumed, according to Sinclair *et al*. (1984). The chlorophyll concentration index (CCI) was determined on all leaves of each plant and the average value per plant was taken using a CCM 200 Plus meter (Opti-Sciences, Hudson, USA). The leaf area was determined using a Li-cor 3000A analyzer (LI-COR Biosciences, USA). The illumination and red/far-red ratio were measured with a Field Scout Quantum Meter and Field Scout Red/Far Red Meter (Spectrum, Plainfield IL, USA), respectively. Additionally, the light transmitted through one, two and three polypropylene filters in the wavelength range of 300-880 nm was determined using a Shimadzu UV-1603 spectrophotometer (Tokyo, Japan).

The experiment was arranged in a totally-randomized one-factor design. Each treatment consisted of 10 plants. The measurements were taken individually on each plant. The collected data were subjected to an analysis of variance (P<0.05) and Tukey's significance test using the statistical software IBM-SPSS Statistics version 20.0.0 (IBM Corporation, New York, USA).

Results and discussion

Quality and decrease of the incident light

The transmittance of light through the green filters presented the behaviour shown in Figure 1. The maximum transmittance occurred at 533 nm; however, the transmittance was reduced as more polypropylene filters were used. Therefore, it was shown that the plants were exposed to a spectrum enriched in the green range, but also that the incident radiation on the plants was enriched in light in the far red range, as the plants were grown under the influence of more polypropylene filters, as shown in Tab. 1 and Figure 1. In this respect, light in the green range (510-550 nm) was considered to be an inactive portion of the solar radiation since it was thought to be reflected by the leaf blades and not used as an energy source. However, there has been increasing interest in the study of the effect of this spectrum range on the behaviour and life cycle of plants (Efimova *et al*., 2017) and plant responses to green light exposure, which may be dependent or independent of the action of light receptors in the blue range, involving cryptochrome (CRY) activity. CRYs act, functionally and physically, together with phytochromes (PHYs) in the regulation of physiological processes in plants, without excluding other photoreceptors in the perception of light in the green range nor the interaction of several photoreceptors (Dhingra *et al*., 2006).

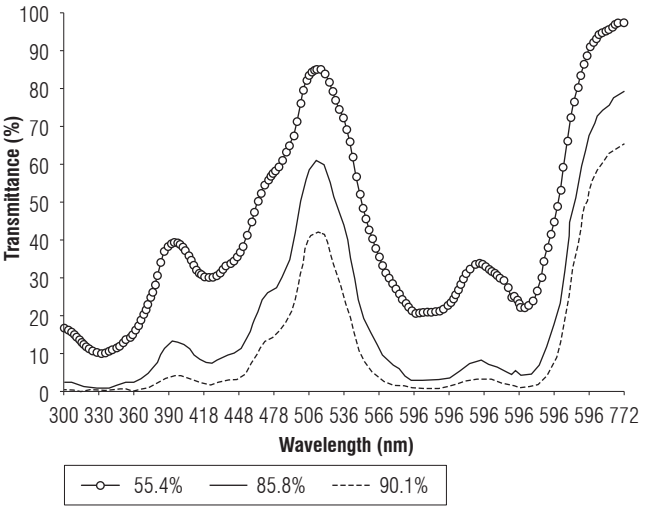

FIGURE 1. Transmittance of light filtered through one, two, and three green polypropylene films, which caused a light decrease of 55.4, 85.8 and 90.1%, respectively.

On the other hand, light in the far red range has a strong effect on plants. For example, the results reported by Turk *et al*. (2003) who found that light in the red, blue, and white ranges suppresses CYP72B1 transcript accumulation in *Arabidopsis*, but increases with the exposure of plants to light in the far red range, given that genetic and physiological effects of hypocotyl response to exogenous blue light and varying intensities of white and monochromatic light revealed that CYP72B1 modulates photomorphogenesis, mainly with exposure to the far red band and, to a lesser extent, with exposure to blue and red light. Therefore, it is possible to infer that the responses of the sisal plants in the present study were the result of the joint action of light in the green and far red bands and the shading.

Kim *et al*. (2004), based on the approaches of several studies, stated that light in the green band is not important for photosynthesis since it has a low absorption coefficient in the absorption ranges of chlorophyll. However, although the amount of light absorbed in the green range is very small, unabsorbed wavelengths are repeatedly reflected from one chloroplast to another in the complex network of photosynthetic cells. With each reflection, a percentage of these wavelengths is absorbed, until finally half or more end up being absorbed by most leaves and used in photosynthesis, since it has been found that the response spectrum used for photosynthesis shows that green light is in an effective spectral band to boost photosynthesis in plants. This process involves some auxiliary pigments and the transfer of energy to the reaction centers. In leaves, the absorption of light by carotenoids shifts from the blue portion of the spectrum towards the green, also used in photosynthesis,

since most carotenoids (both β-carotene and xanthophylls) present in the tilakoids efficiently transfer their excitation energy to the same reaction centers as that of chlorophyll, also contributing to photosynthesis.

Chlorophyll content

The chlorophyll content index (CCI) values were significantly reduced with exposure to shading, with statistically significant differences only between the control and the set of shaded plants with the green filters (Tab. 2). This reduction in the plants exposed to shading was in the range of 52.2-55.0% in relation to the control plants. In this respect, the shading caused alterations in the plant pigment content, given that Casierra-Posada *et al*. (2012b) found that, in *Calendula officinalis* plants, the value of the *a*/*b* chlorophyll ratio was higher in leaves of the shaded plants, while the value of carotene/chlorophyll ratio was higher in plants that grew with full exposure, implying that shading increased the content of chlorophyll, relative to that of the carotenes. Additionally, in *Beta vulgaris* plants, Casierra-Posada *et al*. (2015) found an increase in the value of the chlorophyll a/b ratio and a decrease in the carotene/chlorophyll ratio, in plants placed under a green cover, in relation to plants placed under transparent cover. However, the *a*/*b* chlorophyll ratio showed a similar behaviour in the plants exposed to the green cover as in the red and blue color coverings. For the carotene/chlorophyll ratio, there was no statistical difference between the values found under the green cover and those found under the blue and yellow covers.

Additionally, Materová *et al*. (2017), in *Hordeum vulgare* plants exposed to green monochromatic light, found an increased amount of geranylgeranyled chlorophyll, as compared to plants exposed to white light, as a consequence of an altered activity of the enzyme geranylgeranyl reductase, responsible for the reduction of phytyl chain double bonds in chlorophyll synthesis, indicating that chlorophyll synthesis is severely affected as a result of exposure to green light. Therefore, it can be inferred that the values found for CCI in the present study were due to the combined action of shading and green color of the cover since both have a marked influence on the content of photosynthetic pigments.

Growth variables

We found a significant difference in the absolute growth rate (AGR) values between the control treatment and the group of shaded plants with the green cover, regardless of the level of shading. The AGR was reduced by between 61.3 and 83.0% in the plants placed under the green covers relative to the control plants (Tab. 2). Casierra-Posada *et al*. (2012a) reported an 85.6% decrease in the AGR value in *Fragaria* sp plants exposed to green polypropylene covers, as compared to plants grown under transparent covers. On the other hand, a reduction of 35.1, 44.0 and 58.4%, relative to the control plants, was observed in the relative growth rate (RGR) values in plants exposed to 55.4, 85.8 and 90.1% shading using green covers, respectively, with statistically significant differences between all treatments (Fig. 2). In this regard, Casierra-Posada *et al*. (2014b) found no difference in the RGR value in *Beta vulgaris* plants exposed to green and transparent polypropylene covers, whereas Casierra-Posada *et al*. (2012a) found a 47.3% decrease in the RGR value in *Fragaria* sp plants placed under green covers, as compared to plants developed under transparent covers. For RGR and AGR values, these authors suggested that, when the quality and intensity of light on plants is evaluated, the degree of illumination is more determinant than the spectral composition and the response of plants in terms of growth since acclimatization to the environmental supply depends on the amount of light rather than its quality. Additionally, Casierra-Posada *et al*. (2014b)

TABLE 2. Variables evaluated to determine the growth of the sisal plants (*Furcraea hexapetala* Jacq.) exposed to shading using green polypropylene filters.

Variable	Light reduction (% of the control)			
	0.0 (Control)	55.4	85.8	90.1
Chlorophyll content index	35.6 ± 9.8 a	17.0 ± 6.0 b	16.0 ± 6.5 b	16.3 ± 6.1 b
Water uptake (L)	7.0 ± 1.3 a	4.1 ± 1.0 b	3.8 ± 0.7 b	3.9 ± 0.9 b
Leaf area (cm^2)	164.1 ± 56.2 a	163.5 ± 80.6 a	150.7 ± 74.3 a	125.5 ± 62.7 a
Absolute growth rate ($g\ d^{-1}$)	0.0089 ± 1.7 10^{-3} a	0.0058 ± 1.6 10^{-3} b	0.0050 ± 1.5 10^{-3} b	0.0037 ± 9.0 10^{-4} b
Leaf area ratio ($m^2\ g^{-1}$)	0.0024 ± 6.0 10^{-4} b	0.0052 ± 8.0 10^{-4} a	0.0055 ± 1.4 10^{-3} a	0.0066 ± 2.1 10^{-3} a
Water use efficiency ($g\ L^{-1}$)	1.07 ± 0.4 a	0.82 ± 0.3 ab	0.80 ± 0.40 ab	0.49 ± 0.1 c
Specific leaf area ($m^2\ g^{-1}$)	0.0057 ± 0.001 b	0.0118 ± 0.001 a	0.0120 ± 0.001 a	0.0132 ± 0.003 a
Leaf weight ratio	0.41 ± 0.07 b	0.43 ± 0.09 ab	0.50 ± 0.07 a	0.49 ± 0.07 a

Mean values are shown followed by the standard deviation. For each variable, different letters indicate statistical significance according to Tukey's honest significance test ($P \leq 0.05$).

stated that certain spectral wavelength bands that are not useful for plant metabolism can significantly influence the morphology, composition and adaptive strategy of a plant, optimizing light capture when the light quantity or quality is unfavourable.

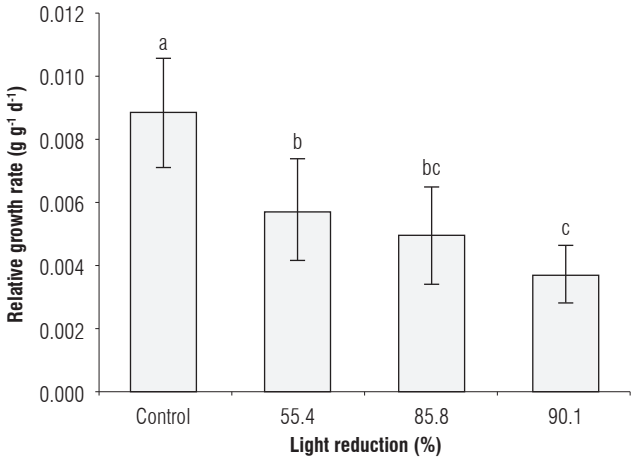

FIGURE 2. Relative growth rate in sisal plants (*Furcraea hexapetala* Jacq.) exposed to shading with green polypropylene filters. The bars in each column indicate the standard deviation. Different letters indicate statistical significance according to Tukey's honest significance test ($P \leq 0.05$).

As for the specific leaf area (SLA) values, an increase in the shaded plants with the green covers was evidenced in relation to the control plants, with significant differences. The group of treatments with plants that were exposed to shade using green covers, without taking into consideration the level of light reduction, showed an increase of 107.5-132.4% in the SLA value as compared to the control plants (Tab. 2). In agreement with these results, Casierra-Posada *et al*. (2012a) reported an increase in the value of this variable of 97.5% in *Fragaria* sp plants maintained under green polypropylene covers, as compared to plants that grew under a transparent cover. On the other hand, Kim *et al*. (2004) found a SLA value of 49.4 m^2 kg^{-1} in *Lactuca sativa* plants exposed to green fluorescent lamps, whereas in plants placed under cool-white fluorescent lamps, the value of this variable was reduced to 33.9 m^2 kg^{-1}. In this regard, these authors mentioned that a larger leaf area per unit of dry leaf mass obtained in plants placed under green light is indicative of greater photosynthetic surface area per unit of leaf tissue inversion, which is associated with the behaviour of plants exposed to shading. Therefore, they inferred that, in the plants exposed to light in the green band, a great amount of resources was assigned with the purpose of obtaining a greater leaf area, whereas the capacity of assimilation of carbon in relation to the leaf area was reduced, as compared to other treatments. In addition, changes in the leaf structure of plants exposed to different spectral environments could also have a significant impact on the chloroplast density in leaves, as well as the amount of light captured by the photosynthetic pigments.

The values found for the leaf area ratio (LAR) showed a statistically significant difference between the control treatment plants and the set of plants that grew exposed to the green filters. In the set of plants that grew under the green filters, regardless of the intensity of light reduction, the LAR value increased between 116.6 and 174.9% in relation to the control plants (Tab. 2). In agreement with this result, Casierra-Posada *et al*. (2012a) reported a 320.6% increase in the LAR value in *Fragaria* sp plants exposed to a green polypropylene cover, as compared to plants exposed to a transparent cover. These authors mentioned this result as an adaptation of the plants to the shade induced by the green cover; therefore, plants exposed to conditions of shading and green light allocate more resources for the formation of a greater assimilation surface to overcome the stress induced by the joint action of the shading and the color of the cover.

The leaf weight ratio (LWR) values increased 4.2, 20.3 and 19.8% in the plants exposed to 55.4, 85.8 and 90.1% shading with green covers, respectively, in relation to the control plants, with statistically significant differences between all treatments (Tab. 2). Casierrra-Posada *et al*., (2012a) found an increase of 113.3% in the value of this variable in *Fragaria* sp plants exposed to a green polypropylene cover in relation to plants placed under a transparent cover. In this regard, these authors mentioned that many plant species show an increase in the leaf weight ratio as an adaptive response to conditions of low light availability. In the present study, the LWR value increased proportionally to the intensity of the shading, as compared to the control plants; therefore, in these plants, the biomass was allocated more to the assimilating surface formation, so the LWR value in plants placed under a green cover indicated the importance of light quality in addition to the amount of light in the parameters related to growth.

On the other hand, it was found that the light in the far red and green bands accentuated the effects induced by the shading, rather than the green light or the far red light separately. In this respect, this effect has been observed both in the morphology and in the gene expression of plants exposed to these factors (Wang and Folta, 2013). In this sense, the effects found in the present study in terms of growth and other evaluated variables were the result of the combined action of shading and light filtering through the

green filters, which, according to Tab. 1, induced a value in the far red/red ratio that was inversely proportional to the reduction of the light, since the combination of the shade, the green light and the infrared light induced morphogenic responses in the plants that helped them to adapt better to the abiotic stress caused by an environment of this nature.

Water uptake and water use efficiency

The water uptake was reduced by 40.9-44.9% in the plants grown under the green covers, in comparison with the control plants, with statistically significant differences between the control plants and the set of plants exposed to the covers (Tab. 2). In relation to this variable, Casierra-Posada *et al*. (2014b) did not find statistical difference in the water uptake of *Beta vulgaris* plants placed under green and transparent polypropylene covers even though the leaf area in these plants was higher under the green cover than under the transparent one. In contrast to the results found in the present study for this result, the leaf area showed no statistical difference in any of the treatments, despite which, the water uptake was higher in the control plants in relation to all the plants under the green covers, suggesting that stomatal activity would be involved in this response, as indicated by Wang *et al*. (2009), who found that *Cucumis sativus* plants exposed to green, yellow and red light had a lower stomatal index than those exposed to white light, suggesting that stomatal density and stomatal index depend on the quality of light and purple and blue light also regulate most of the genes encoding the key enzymes in the Calvin cycle, while the green, yellow and red light regulates them. Additionally, Schoch *et al*. (1980) confirmed that the stomatal index is a function of the radiation received and its variations during the days preceding the differentiation of leaf stomata.

The values recorded for the water use efficiency were by 23.0, 25.3 and 53.7% in the plants exposed to the green covers with 55.4, 85.8 and 90.1% light reduction, respectively, as compared to the control plants, with statistically significant differences (Tab. 2). In contrast to this result, Casierra-Posada *et al*. (2014a) did not find statistical differences in the WUE value in *Capsicum annuum* plants, which grew without cover and those that were under the influence of a green polypropylene cover. Therefore, according to Hatfield *et al*. (2001), who stated that the WUE is a measure of the efficiency of the stomata to maximize the photosynthesis, reducing the loss of water by transpiration. Then it can be inferred that, in the present study, both the shading and the color of the cover induced a very low yield in relation to the WUE in the sisal plants. In this regard, Gil-Marín *et al*. (2006) found that water stress, plastic mulch and partial shading did not affect stomatal resistance or leaf transpiration in CAM *Aloe vera* plants. Stomatal resistance increased exponentially with the increase in air temperature and vapor pressure deficit and decreased with higher relative humidity. Additionally, these authors also indicated that the transpiration rate of the *Aloe* plants was remarkably low. The highest perspiration occurred at night, when the stomata were open, and, during the day, the transpiration was negligible or absent because the stomata were partially closed when the air temperature reached its maximum level. It could be thought that the lack of cover in the control plants of the present study was responsible for the increase in water uptake in the control plants, since the others were under cover, despite the fact that the plants of the present study and those studied by Casierra-Posada *et al*., (2014a) were exposed to similar environmental conditions although the results were very different. In addition, *Fucraea hexapetala* (syn. *Furcraea macrophylla*) is a CAM plant (Crassulacean acid metabolism), according to Casierra-Posada and Gonzalez (2009), and, therefore, has high energy requirements; however, CAM species can thrive in habitats experiencing contrasting light intensities, ranging from shaded habitats to full sun exposure. In addition, it is unknown how CAM species can meet energy and carbon requirements when they are suddenly exposed to shaded conditions in order to ensure survival (Ceusters *et al*., 2011). For this purpose, CAM plants must make use of their plasticity to adapt to the environmental offer of shading, which implies a low luminous intensity and low red/far red ratio. Therefore, based on the drastic reduction in the values of all the variables involved in growth, it can be inferred that the *Furcraea hexapetala* plants presented a low plasticity that allowed them to adapt to and survive the shading conditions evaluated in the present study.

Allometric distribution and production of dry material

The total dry weight per plant was reduced by between 80.8 and 94.0%, as compared to the control plants, in the group of plants exposed to shading with green filters, without taking into consideration the level of light reduction, with significant differences between the control treatment and the shaded group (Fig. 3). In this regard, Casierra-Posada *et al*. (2015) did not find statistical differences in *Beta vulgaris* plants exposed to polypropylene covers that were transparent, green, red and blue. These authors mentioned that, although the blue filter with the highest reduction (62.3%) did not show statistical differences with the response of the plants that were exposed to red, green or transparent color filters, phytochrome is involved in the response of plants to the production of dry mass since the yellow cover induced the greater production of dry mass in the plants.

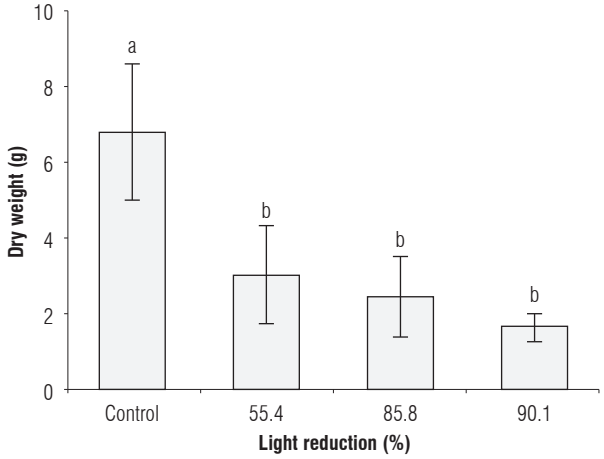

FIGURE 3. Total dry weight of sisal plants (*Furcraea hexapetala* Jacq.) exposed to shading with green polypropylene filters. The bars in each column indicate the standard deviation. Different letters indicate statistical significance according to Tukey's honest significance test ($P \leq 0.05$).

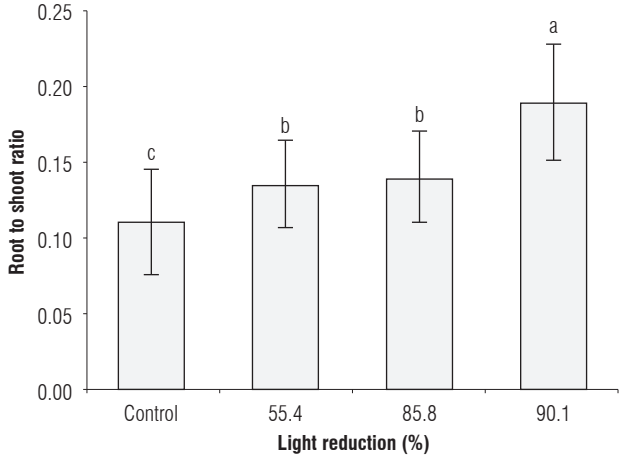

FIGURE 4. Root to shoot ratio in sisal plants (*Furcraea hexapetala* Jacq.) exposed to shading with green polypropylene filters. The bars in each column indicate the standard deviation. Different letters indicate statistical significance according to Tukey's honest significance test ($P \leq 0.05$).

A variable related to the production of dry matter in plants is the electron transport rate (ETR). Casierra-Posada and Ávila-León (2015) found that, in *Calendula officinalis* plants exposed to shading, the ETR value was reduced by 17.3%, as compared to plants placed in full illumination. These authors mentioned that the magnitude of the electron transport rate in low light conditions depends on the incident radiation and the size of the antenna. Therefore, given that, in the present study, the CCI was reduced and that the genus *Furcraea* sp is light demanding because it is a CAM plant, the amount of dry matter per plant was severely compromised as a result of shading.

We found an increase of 24.2, 28.2 and 73.5% in the values of the root to shoot ratio in the plants placed under 55.4, 85.8 and 90.1% light reduction with the green filters, respectively, as compared to the control plants, with statistically significant differences (figure 4). In *Fragaria* sp plants, Casierra-Posada *et al*. (2012a) found that the value of the root to shoot ratio in plants placed under a green polypropylene cover was 86.7% above the value found in plants under a transparent cover, while Casierra-Posada *et al*. (2014b) found no difference in the value of this variable in *Beta vulgaris* plants exposed to green and transparent polypropylene covers. In agreement with the findings of the present study, Casierra-Posada *et al*. (2014b) found that, in *Fragaria* sp plants, a green polypropylene cover presented a greater accumulation of dry mass in the roots, in relation to yellow, blue, red, transparent covers and a control without cover. These authors commented that both the variations of the root to shoot ratio and the changes in the dry mass allocation in the different organs of the plants were the result of the interaction of the quality of the light and the shading provided by the covers because there are anatomical, morphological and physiological parameters that induce greater or lesser plasticity, given variations in the quality of light incident on the plants. In addition, according to Hirose (1987), the optimal partition theory assumes that plants respond to variations in the availability of resources through the preferential partition of resources between different organs in order to optimize the capture of light and maximize the growth rate.

For dry matter partitioning, statistically significant differences were observed in the amount of dry mass assigned to the roots and to the stem, but not to the leaves. In the roots, a statistically significant difference was observed between the control treatment plants and the set of plants placed under the green covers, independently reducing the light through the green covers. In this case, the amount of dry mass assigned to the roots of the plants in the treatment group under shading increased 2.9-6.0%, as compared to the value recorded in the control treatment plants (Fig. 5). The light reduction levels of 55.4, 85.8 and 90.1% reduced the amount of dry mass accumulated in the stems, as compared to the stems of the control treatment plants (Fig. 5), by 4.6, 10.9 and 13.4%, respectively. Although no statistical difference was found in the amount of dry matter assigned to the leaves, an increase of this variable can be observed in Figure 5 in the plants placed under the green covers, in relation to the control plants. The leaf area values for all treatments were in the range of 164.1-125.5 cm^2. In agreement with these findings, Casierra-Posada *et al*. (2014b) found that *Fragaria* sp plants under a green polypropylene cover presented a greater accumulation of dry mass in the roots, in relation to yellow, blue, red, and transparent covers

and a control without cover. These authors commented that variations in the root to shoot ratio and changes in the dry mass allocation in the different organs of plants are the result of the interaction of the quality of the light and the shading provided by covers; therefore, it can be inferred that, in the present study, the color of the cover, the shading and the low far red/red ratio value induced photomorphogenic modifications in the *Furcraea hexapetala* plants with the purpose of adapting to the environmental supply.

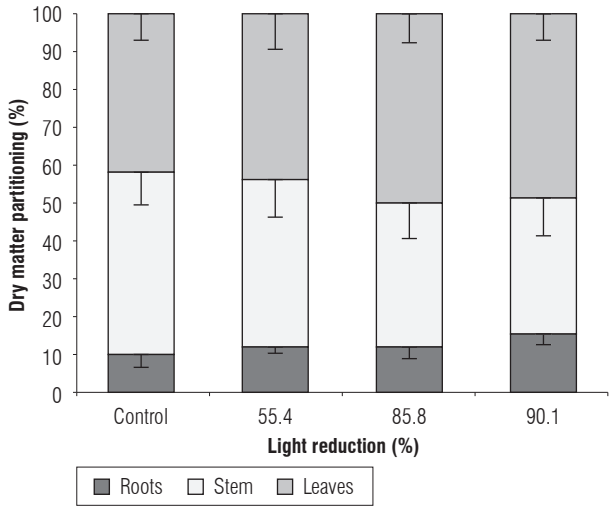

FIGURE 5. Dry matter partitioning in sisal plants (*Furcraea hexapetala* Jacq.) exposed to shading with green polypropylene filters. The bars in each column indicate the standard deviation.

Finally, Kim *et al*. (2004) pointed out that the response of different species of plants or cultivars that grow exposed to different light environments is too complicated to interpret using a quantitative parameter of light quality. Additionally, according to Casierra-Posada and Peña-Olmos (2015), the detection of influences of the adjacent spectrum segments on plants is difficult because the spectrum is continuous, with no apparent margins between adjacent colors. When analyzing the results, it can be inferred that the *Furcraea hexapetala* plants presented little tolerance to the conditions of low illumination and low red/far red ratio induced by the green polypropylene covers; in addition, the difficulty of the studied plants having a CAM-type metabolism was seen, which were not only exposed to shading, but also to the placement of superimposed, green polypropylene layers that reduced the red/far red ratio. This environmental supply constituted a factor of severe abiotic stress for the plants, which did not have enough plasticity to tolerate it and, therefore, the growth variables were severely affected.

Conclusions

With the present results, it can be concluded that sisal plants (*Furcraea hexapetala*) have a low phenotypic plasticity to adapt to the conditions of an environment with diminished illumination and an enriched green color band. This environment induced profound changes in the values of the allometric variables and, therefore, in the architecture of the plant bodies, such as the root to shoot ratio and the leaf area ratio, whose values were severely increased. Similarly, the values of the variables involved in plant growth, such as chlorophyll content index, water use efficiency and relative growth rate, among others, were strongly diminished.

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Fitting of photosynthetic response curves to photosynthetically active radiation in oil palm

Ajuste de las curvas de respuesta fotosintética a la radiación fotosintéticamente activa en palma de aceite

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ABSTRACT

Light saturation curves represent the response of the net photosynthetic rate to the photosynthetically active radiation. These curves were obtained from individual leaves of oil palm genotypes (*Elaeis guineensis, E. oleifera* and the O×G interspecific hybrid) without any type of biotic or abiotic stress, fitting three nonlinear models: the rectangular hyperbolic model (Michaelis-Menten), the non-rectangular hyperbolic model (Prioul and Chartier) and the exponential model (Mitscherlich). The research was conducted at Barrancabermeja (Santander, Colombia) with the aim to compare the adaptations of these models and to identify the most suitable model for the crop. The rectangular hyperbolic model was qualitatively and quantitatively the most appropriate to describe the oil palm response under different conditions, in terms of the coefficient of determination (R^2_a), the mean squared error (MSE) and the standard error (SE); therefore, using this model, the photosynthetic parameters showed higher and more realistic correlation (r) with the measured values. The non-rectangular hyperbolic model was the least appropriate model to estimate the maximum photosynthesis, dark respiration, saturation points, light compensation and photosynthetic efficiency. Thus, the rectangular hyperbolic model is the fastest, simplest and most appropriate option to access the light curve information in oil palms and can be incorporated into the gas exchange and growth models into the whole palm production system.

Key words: Colombia, dark respiration, light compensation point, light saturation point, maximum photosynthesis, photosynthetic efficiency.

RESUMEN

Las curvas de saturación de luz representan la respuesta de la tasa de fotosíntesis neta a la radiación fotosintéticamente activa. Éstas se obtuvieron a partir de hojas individuales de genotipos de palma de aceite (*Elaeis guineensis*, *E. oleífera* y el híbrido interespecífico O×G) sin ningún tipo de estrés biótico o abiótico, y fueron utilizadas para ajustar tres modelos no lineales: el hiperbólico rectangular (Michaelis-Menten), el hiperbólico no rectangular (Prioul y Chartier), y el exponencial (Mitscherlich). La investigación se llevó a cabo en Barrancabermeja (Santander, Colombia), y buscó comparar las adecuaciones de cada modelo e identificar el más preciso para el cultivo. La hipérbola rectangular fue cualitativa y cuantitativamente el modelo más adecuado para describir tal respuesta en todas las condiciones de estudio, en términos de coeficiente de determinación ajustado (R^2_a), cuadrado medio del error (CME) y error estándar (EE); y por ello sus parámetros fotosintéticos mostraron una correlación (r) más alta y realista con los valores medidos. El modelo hiperbólico no rectangular fue el menos adecuado para estimar la fotosíntesis máxima, la respiración oscura, los puntos de saturación y compensación de luz, y la eficiencia fotosintética. Así, el modelo hiperbólico rectangular es la opción más rápida, sencilla y robusta para acceder a la información de las curvas de luz en palma de aceite, que puede ser incorporada en modelos de crecimiento a nivel de planta y sistema productivo.

Palabras clave: Colombia, eficiencia fotosintética, fotosíntesis máxima, punto de compensación lumínico, punto de saturación lumínico, respiración en oscuridad.

Introduction

Photosynthesis is the process used by plants to transform less than 5% of the incident solar radiation into the energy needed to drive carbon dioxide fixation to form the organic matter of plant tissues, and to promote plant growth (Solarte *et al*., 2010). The radiation used in photosynthesis range between 400 to 700 nm of the solar radiation spectrum and is known as photosynthetically active radiation (PAR) (Azcón-Bieto *et al*., 2008). The PAR intercepted and absorbed by the leaves, and the efficiency of the conversion of carbohydrates into chemical energy are key factors to understanding plant growth (Woittiez *et al*., 2017). One way to study these factors is with light saturation curves, which represent the response of the net photosynthetic rate to the PAR (Fig. 1). These curves

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show that there is no photosynthetic assimilation in the dark, and the CO_2 emitted is a result of mitochondrial respiration (dark respiration); as the photon flux increases, the CO_2 uptake increases until it equals the CO_2 release to respiration (Taiz and Zeiger, 2010). At this PAR value, which is called the light compensation point, the net CO_2 exchange of the leaf is zero, and an increase above it results in a proportional increase in the rate of photosynthesis (Azcón-Bieto *et al*., 2008). The initial part is essentially linear, and its slope corresponds to the light-use efficiency by chloroplasts or photosynthetic efficiency (**Φ**). At a high PAR, the photosynthetic response begins to stabilize and reaches its maximum capacity (maximum photosynthesis), and beyond this point, an increase in PAR does not affect the rate of photosynthesis (light saturation point) (Lobo *et al*., 2013).

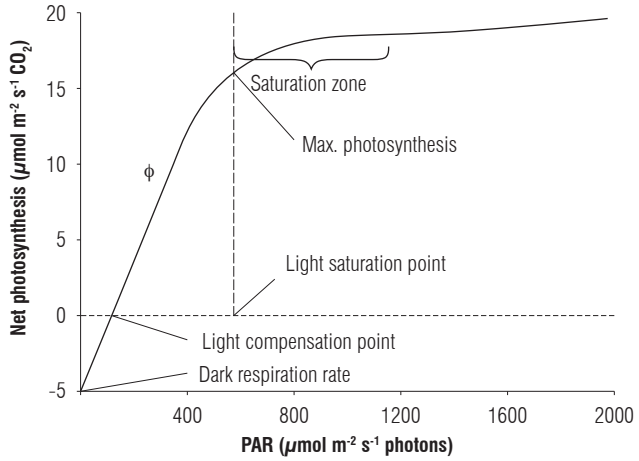

FIGURE 1. Ideal light saturation curve which represents the photosynthetic rate response to the PAR.

To access the information derived from these curves, it is necessary to fit it with nonlinear regression models (Marino *et al*., 2010), because they have simple formulas that can be easily parameterized and interpreted. The effectiveness of the used model depends on the information that is expected to be known and the suitability of the model for the ecophysiology of the species (Gomes *et al*., 2006).

Oil palm is an important crop worldwide due to its oil production for human consumption and industrial uses, especially for the biodiesel industry. So, it is now the most used vegetable oil worldwide, reaching about 32% of the global oils and fats production (Woittiez *et al*., 2017). Oil palm is a perennial crop with a very long growth period and high biomass production which economic cycle may last up to 30 years. In Colombia the crop has an average participation of 9.5% in the production of permanent crops and 5.7 % in national agricultural production, it is the second largest oil palm producer outside Southeast Asia, and the largest producer in South and Central America (Fedepalma, 2016).

Oil palm productive cycle must adjust its photosynthetic and metabolic dynamic based on the soil and climate characteristics of the area where it is grown, aiming to produce the photoassimilates needed to grow and develop reserve structures and fruit bunches (Corley and Tinker, 2015). Thus, the study of the physiological processes that regulate and intervene directly on oil palm production, primarily the increase in photosynthetic capacity (Peláez *et al*., 2010), is a potential tool for the selection of highly productive cultivars (Rivera *et al*., 2013a). Several studies have been conducted on the physiological and morphological characterization of oil palms, particularly in terms of photosynthesis, transpiration and the environmental factors that affect their morpho-physiological processes (Ayala and Gómez, 2000; Corley and Tinker, 2015; Jazayeri *et al*., 2015; Peláez *et al*., 2010; Rivera *et al*., 2012; Rivera *et al*., 2013a; Rivera *et al*., 2013b; Rivera *et al*., 2016; Romero *et al*., 2007; Ruiz and Henson, 2002); however these studies have not parameterized the information provided by the light saturation curves in a simple but robust model for breeding purposes. Thus, the aim of this research study was to identify the most appropriate nonlinear model to fit light saturation curves in oil palm, based on the suitability of the model (goodness of fit criteria), and to provide practical and efficient selection and breeding criteria for the crop.

Materials and methods

Location

The study was carried out at the Experimental field "Palmar de La Vizcaína" (6°59'3.22"N and 73°42'20.93"W), owned by the Oil Palm Research Center (Cenipalma) and located in Barrancabermeja (Santander, Colombia), at an altitude of 125 m a.s.l., with the following climatic conditions:

	Min	Max	Average
Temperature (°C)	16.4	44.3	28.6
Relative humidity (%)	33.0	100.0	81.2
Precipitation (mm year^{-1})	2,843	4,463	3,579

Plant material

A total of 35 plants (n = 35) of three oil palm genotypes, without any type of biotic or abiotic stress, were evaluated: six plants of *Elaeis oleifera* (n = 6), nine plants of *Elaeis guineensis* (n = 9), and twenty plants of interspecific hybrid

(*E. oleifera x E. guineensis*) (n = 20) in both the nursery stage and the adult stage (≥ 6 years after planting).

Plants at the nursery stage received 1 mm d^{-1} of water through a drip irrigation system, while the adult palms (at the definitive site) were not irrigated, because precipitation satisfied their evapotranspiration rate (5 mm d^{-1}). In terms of fertilization, chemical sources were used, in the quantities necessary to reach the levels established as adequate by Rincon *et al*. (2012), and Arias and Beltrán (2010) for nursery and adult palms, respectively.

Experimental conditions

The light saturation curves were obtained using the infrared gas analyzer IRGA - LI-6400 (LI-COR Inc., Lincoln, NE, USA), evaluating the middle section of leaf No. 3 of palms in the nursery stage and the central leaflets of leaf No. 17 in the adult palms under full sunlight conditions (8:30 to 11:30 h). The following conditions were set in the IRGA chamber: 30°C temperature, 400 µmol CO_2 concentration, 2.5 kPa saturation vapor pressure, and 3% of maximum coefficient of variation. Initial experimental condition was a saturating PAR level of 2,000 µmol m^{-2} s^{-1} photons, and then it was reduced every 200 µmol m^{-2} s^{-1} photons to a value of 200 µmol m^{-2} s^{-1} photons (2,000 - 1,800 - 1,600 - 1,400 - 1,200 - 1,000 - 800 - 600 - 400 - 200 µmol m^{-2} s^{-1} photons). At this rate, the spacing between values was reduced to determine the light compensation point (200 - 150 - 50 - 20 - 0 µmol m^{-2} s^{-1} photons).

Statistical analysis

The photosynthesis data based on PAR were fitted to three nonlinear models (Tab. 1), the rectangular hyperbolic model (Michaelis-Menten), the exponential model (Mitscherlich), and the non-rectangular hyperbolic model (Prioul and Chartier), using the statistical package Statistix 9.0® (Analytical Software, USA) and following the methodology proposed by Solarte *et al*. (2010) for the first two models and the Photosyn Assistant® (Dundee Scientific, UK) software for the remaining model. Finally, the photosynthesis data sampled and fitted to the three models were compared using univariate linear regressions with the statistical package of Microsoft Excel 2013. Using the goodness-of-fit criteria, the most appropriate model was selected to predict the photosynthetic parameters: photosynthesis rate as a function of PAR, maximum photosynthesis (A_{max}), light saturation point ($_{saturating}$PAR), dark respiration (D_R), photosynthetic efficiency (Φ) and light compensation point (LCP) in oil palms.

The criteria used were: the adjusted coefficient of determination (R^2_a) which measures the proportion of the total variability explained by the model and further corrected by the sample size (n); the standard error (SE), which explains the variability produced by unknown distorting factors; the mean squared error (MSE), which measures the variance of the prediction error or the difference between the estimated and the actual data; and the correlation (r) or degree of association between the actual data and the data estimated by the model (Montgomery, 1992). Additionally, a significance test (F-test, $P \leq 0.05$) was performed to estimate the overall adequacy of the model.

Results

The sampled values of the photosynthetic response of oil palms to the PAR overlapped, mainly, with the values obtained by fitting the rectangular hyperbolic model (Michaelis-Menten) and the exponential model (Mitscherlich); in contrast, the non-rectangular hyperbolic model (Prioul and Chartier), overestimated the values in all three cases

TABLE 1. Models evaluated to fit the light saturation curves in oil palm. Adapted from: Gomes *et al*. (2006) and Marino *et al*. (2010).

Model	Information provided					
	A_{max}	$_{Saturating}$PAR	D_R	Φ	LCP	θ
Rectangular hyperbolic: Michaelis-Menten $A = \frac{A_{max} * PAR}{(K + PAR)} - R_d$	X	X	X	+	+	
Exponential: Mitscherlich $A = A_{max} * (1 - Exp(-\Phi * (PAR - P_{cl})))$	X		+	X	X	x
Non-rectangular hyperbolic: Prioul and Chartier $A = \left\{ \frac{A_{max} + (\Phi * PAR) - [(A_{max} + (\Phi * PAR))^2 - (4 * \Phi * \theta * A_{max} * PAR]^{0,5}}{(2 * \theta)} \right\} - R_d$	X	+	X	X	+	X

A: photosynthesis based on PAR; A_{max}: maximum photosynthesis; K: light saturation constant = ½ $_{Saturating}$PAR; $_{Saturating}$PAR: light saturation point; D_R: dark respiration; Φ: photosynthetic efficiency; LCP: light compensation point; θ: dimensionless term of convexity. X: information provided directly by the equation; +: estimated information.

of CO_2 assimilation (Fig. 2). Specifically, the rectangular hyperbolic model (which has the shape of a rectangular hyperbola) was quantitatively more accurate to describe the oil palm photosynthetic rate in function of the PAR under the study conditions, and therefore, its predicted photosynthetic parameters showed a higher, significant ($P \leq 0.05$) and more realistic association (correlation) with the measured values (Fig. 3). Hence, this model, displayed four optimal criteria of goodness-of-fit: maximum r, maximum adjusted R^2_a, minimum MSE and minimum SE (Tab. 2).

TABLE 2. Goodness-of-fit criteria of the non-linear models to describe the photosynthesis response of oil palms to the PAR. The optimal values of each criterion are highlighted.

Criteria	Model		
	Rectangular hyperbolic: Michaelis-Menten	Exponential: Mitscherlich	Non-rectangular hyperbolic: Prioul and Chartier
r	0.997**	0.994**	0.905**
R^2_a	0.995	0.988	0.819
MSE	44.69	102.15	1721.24
SE	0.321	0.485	1.989

** Significance F-Test for the model ($P \leq 0.05$). r: coefficient of correlation between the actual and the estimated photosynthesis for the model; R^2_a: coefficient of determination fitted to the model; MSE: mean squared error of the model; SE: standard error of the model.

The rectangular hyperbolic model ($A_{max} * PAR_i / (K + PAR_i) - D_R$), which assumes a reversible photosynthetic response, is simple in its calculation and in the present study allowed to obtain the maximum photosynthesis, saturating PAR and dark respiration directly, and led to the estimation of photosynthetic efficiency and light compensation point. Photosynthetic efficiency was estimated by a linear regression between photosynthesis (A) and 0-300 µmol m^{-2} s^{-1} photons of PAR (ensuring this was significant and with an R^2 greater than 0.90). The light compensation point (LCP), was also estimated using the same linear regression but leveling photosynthesis to zero

$$(A = 0): LCP = - \frac{(\frac{Saturating_{PAR} * D_R)}{2}}{(D_R + A_{max})}$$

Thus, the photosynthetic rates of *E. guineensis*, *E. oleifera* and the O×G hybrid were positive from approximately 20 µmol m^{-2} s^{-1} photons, with lower values at lower PAR intensities. As the PAR increased the photosynthetic rates increased initially linearly (up to 300 µmol m^{-2} s^{-1} photons) and then diverged from the linear response in a small transition stage to a saturation PAR value of approximately 545 µmol m^{-2} s^{-1} photons. Finally the photosynthesis reached a maximum value in a very stable section in which a

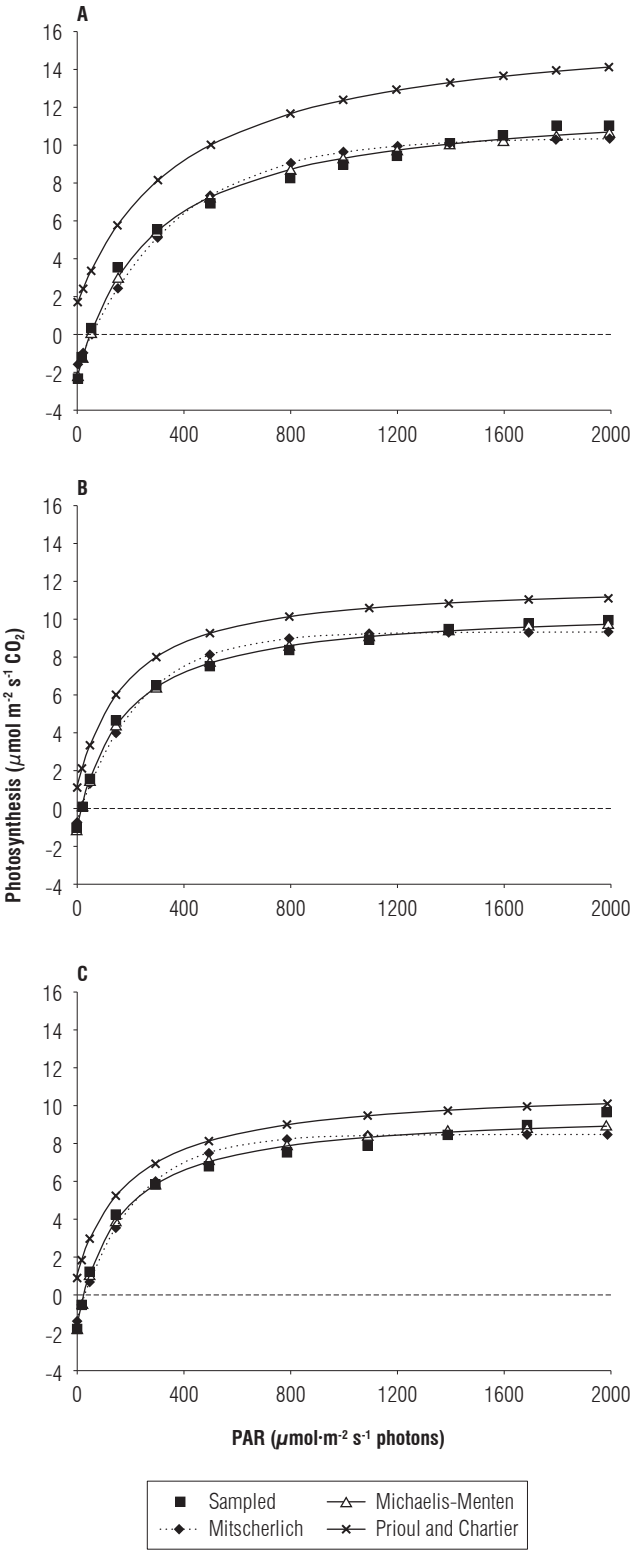

FIGURE 2. An example of light saturation curves fitting in oil palm genotypes with the non-linear models: the rectangular hyperbolic (Michaelis-Menten), the exponential (Mitscherlich) and the non-rectangular hyperbolic (Prioul and Chartier) models: (A) *E. guineensis*, (B) *E. oleifera* and (C) the OxG hybrid.

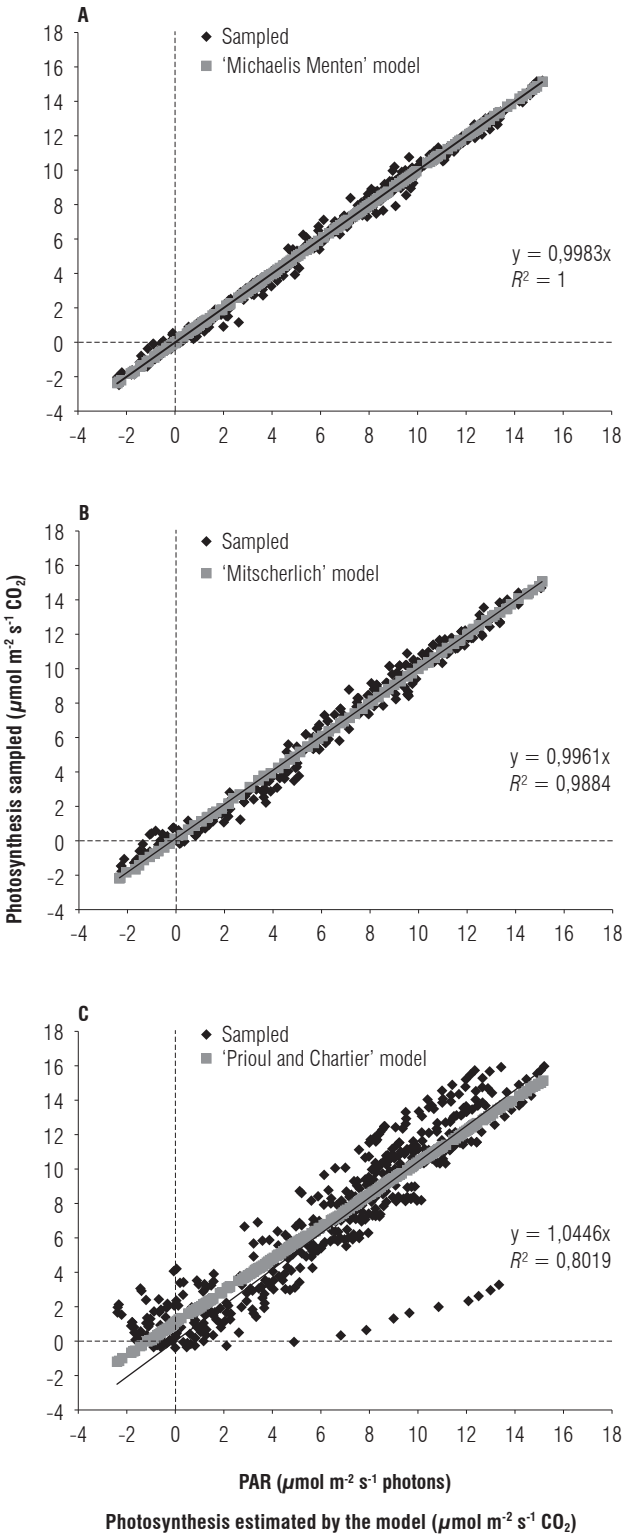

FIGURE 3. Adjusted regression between the actual and the estimated photosynthesis for the non-linear models in oil palms: (A) The rectangular hyperbolic model: Michaelis-Menten; (B) The exponential model: Mitscherlich; (C) The non-rectangular hyperbolic model: Prioul and Chartier.

progressive increase in PAR had no effect on the assimilation rate. Therefore, at approximately 20 μmol m^{-2} s^{-1} photons the net CO_2 exchange of the oil palm leaf was zero and the photosynthetic rate equaled the production of CO_2 by respiratory processes (1.1 μmol m^{-2} $s^{-1}$of CO_2). Moreover, the fraction of photons that reached the leaf tissue and was used to generate the transport of electrons (Φ) was 0.022 mole of CO_2/mole of photons (equivalent to 1 mole of CO_2 for each 45 photons absorbed), and finally, the maximum photosynthesis was 13 μmol m^{-2} s^{-1} CO_2 which became saturated at a PAR of 545 μmol m^{-2} s^{-1} photons (Tab. 3).

Discussion

The non-linear rectangular hyperbolic model (Michaelis-Menten) was selected as a general model to fit the oil palm photosynthetic response curves to PAR, not only because it met the goodness-of-fit criteria commonly used in model fitting, but also because it can be easily stipulated with a common statistical software such as Microsoft Excel® (Lobo *et al*., 2013). This model, which estimates the maximum photosynthesis, the dark respiration, the photosynthetic efficiency and the light compensation and saturation points, can be used in oil palm breeding programs and in eco-physiological performance comparisons of oil palm cultivars. The differences in photosynthetic performance are practical and efficient criteria to predict the yield performance of oil palm cultivars (Peláez *et al*., 2010; Rivera *et al*., 2013a), because the photoassimilates are responsible not only for the dry matter productions required for vegetative growth and plant maintenance but also for the bunch-filling.

Genetic, edaphic, climatic or agricultural management factors can affect through photosynthesis, the production of fresh fruit bunches and plant growth and maintenance (Woittiez *et al*., 2017), and several research studies have shown that there is a relationship between photosynthesis and the production of oil palm; therefore, increased rates of photosynthesis lead to higher productivity. Consequently, conditions that limit the photosynthesis components (water, sunlight, CO_2, nutrients, chlorophyll and leaf area), adversely affect this process, resulting in lower production of bunches, decreased growth and lower resistance to pests and diseases (Cayón, 1999; Romero *et al*., 2007).

Accordingly, it is pertinent to understand the values obtained: the mean photosynthetic parameters estimated using the rectangular hyperbolic model (Michaelis-Menten) correspond to the values reported for C3 plants such as oil palms. The maximum rate of assimilation (12 to 15

TABLE 3. Mean (χ) ± standard error (SE) of the photosynthetic parameters obtained from the light saturation curves fitted to the rectangular hyperbolic model (Michaelis-Menten) in oil palms.

Genotype	A_{max} (μmol m^{-2} s^{-1} CO_2)			Φ (mole of CO_2 / mole of photons)			$s_{aturating}$PAR (μmol m^{-2} s^{-1} photons)			D_R (μmol m^{-2} s^{-1} CO_2)			LCP (μmol m^{-2} s^{-1} photons)		
	χ	±	EE	χ	±	EE	χ	±	EE	χ	±	EE	χ	±	EE
E. guineensis	14.9	±	0.7	0.025	±	0.001	579	±	51	-1.8	±	0.2	37	±	5
E. oleifera	12.0	±	0.9	0.023	±	0.002	393	±	40	-1.3	±	0.1	22	±	3
OxG Hybrid	12.3	±	0.9	0.020	±	0.002	575	±	48	-0.7	±	0.1	16	±	2
General	12.9	±	0.6	0.022	±	0.001	545	±	33	-1.1	±	0.1	22	±	2

A_{max}: maximum photosynthesis; **Φ**: photosynthetic efficiency; $_{saturating}$PAR: light saturation point; D_R: dark respiration; LCP: light compensation point

μmol m^{-2} s^{-1} CO_2) was found within the intervals reported by Corley and Tinker (2015), Ruiz and Henson (2002), Larcher (2003), Romero *et al.* (2007) and Jazayeri *et al.* (2015) for *E. guineensis* (12-16 μmol m^{-2} s^{-1} CO_2), by Peláez *et al.* (2010) and Rivera *et al.* (2013a) for *E. oleifera* (9-17 μmol m^{-2} s^{-1} CO_2), and by Rivera *et al.* (2012) and Rivera *et al.* (2013b) for the O×G hybrids without any type of biotic or abiotic stress (9-18 μmol m^{-2} s^{-1} CO_2). The light compensation (14-40 μmol m^{-2} s^{-1} photons) and saturation (400-600 μmol m^{-2} s^{-1} photons) points were similar to the values obtained in sun plant leaves (LCP: 20-30 and $_{saturating}$PAR: 400-600 μmol m^{-2} s^{-1} photons) and higher than those recorded in shade plant leaves (1-10 and 60-200 μmol m^{-2} s^{-1} photons, respectively), suggesting these parameters vary with the species, the development stage and the orientation of the leaf, as well as with different growth factors, particularly temperature and light (Ögren and Evans, 1993). The photosynthetic efficiency (0.020-0.025 mole of CO_2/mole of photon) was one-half the value reported for C3 plants (0.05 mole of CO_2/mole of photon = 1 mole CO_2 fixed by 20 absorbed photons) under natural conditions, due mainly to a higher photorespiratory rate, but not to a photoinhibition process as the other photosynthetic parameters suggest (Azcón-Bieto *et al.*, 2008). Finally, the mitochondrial respiration under dark conditions (1 μmol m^{-2} s^{-1} CO_2) was two-fold higher than the values reported for C3 plants at 25°C (Byrd *et al.*, 1992). This difference can be attributed to a higher temperature (30°C), and to adequate leaf protein content (leaves did not show chlorosis), since this proportion of the carbon lost by the plant depends on the temperature and the leaf nitrogen content (Machado and Reich, 2006).

Conclusion

The photosynthetic rate of the individual oil palm (*E. guineensis, E. oleifera* and the O×G interspecific hybrid) leaves, showed a curvilinear relationship with the PAR intensity, which increased gradually with the increase of available PAR until a maximum value and then became stable because of inhibition of the photosynthetic apparatus due to a raised leaf temperature and CO_2 limitations. This behavior could be easily accessed and analyzed with the rectangular hyperbolic model (Michaelis-Menten), and could be incorporated into gas exchange or growth models at the plant and ecosystem levels, every time that photosynthesis is not limited by the availability of water, nutrients, light or CO_2. However, it does not imply that the rectangular hyperbolic model is suitable for every plant species, although it is the best model for *Vochysia divergens* (Lobo *et al.*, 2013), *Oryza sativa* (Ye, 2007) and several herbaceous species (Marino *et al.*,2010), because the photosynthetic capacity and the efficiency are represented with dynamic models, whose coefficients must be carefully checked and adjusted to the type of metabolism of the plant (C3, C4 or CAM), the environmental factors (incident radiation, temperature, water, nutrients) and even to the leaf ontogeny (Solarte *et al.*, 2010; Ye, 2007).

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Yield, physicochemical quality, and antioxidant capacity of "beef" and wild tomato fruits (*Solanum lycopersicum* L.) as a function of the electrical conductivity of the nutrient solution

Rendimiento, calidad fisicoquímica y capacidad antioxidante en frutos de tomate bola y silvestre (*Solanum lycopersicum* L.) en función de la conductividad eléctrica de la solución nutritiva

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ABSTRACT

The objective of this study was to evaluate the response of three levels of electrical conductivity (2.0, 2.5 and 3.0 dS m^{-1}) of Steiner's nutrient solution on the yield, physicochemical quality, and antioxidant capacity of fruits from seven tomato genotypes and wild types of tomato (kidney selections). The yield, number of fruits per cluster (NFPC), average fresh fruit weight (AFWF), color, firmness, total soluble solids (TSS), total titratable acidity (TTA), vitamin C (VC), total phenols (TP), lycopene (LY) and antioxidant capacity (AC). The use of 2.5 and 3.0 dS m^{-1} increased the hue angle (49.05°) and TTA (0.35 and 0.36% citric acid). Among genotypes, L-51H and L-76H showed better performance (16.80 and 16.91 kg m^{-2}, respectively), where L-28 stood out for its values of TSS, TTA, VC, TP and AC. Regarding the wild genotypes, the EC modification did not increase the yield; however, the use of 3.0 dS m^{-1} allowed the best results among the wild selections were SS3 (yield, AFWF and LY) and SS5 (NFPC, VC, TP and AC). The modification of the EC did not affect the yield, however, if it affected the physicochemical quality and antioxidant capacity of the analyzed materials.

Key words: total titratable acidity, total soluble solids, ascorbic acid, total phenols, Solanaceae.

RESUMEN

El objetivo de este estudio fue evaluar la respuesta de tres niveles de conductividad eléctrica (CE) (2,0; 2,5 y 3,0 dS m^{-1}) de la solución nutritiva de Steiner, sobre el rendimiento, calidad fisicoquímica y capacidad antioxidante en frutos de tomate bola y silvestre tipo riñón. Se determinó el rendimiento, numero de frutos por racimo (NFPR), peso promedio de fruto fresco (PPFF), color, firmeza, sólidos solubles totales (SST), acidez titulable total (ATT), vitamina C (VC), fenoles totales (FT), licopeno (LI) y capacidad antioxidante (CA). El uso de 2,5 y 3,0 dS m^{-1} incrementaron el ángulo hue (49,05°) y ATT (0,35 y 0,36% de ácido cítrico). Entre genotipos, L-51H y L-76H mostraron mejor rendimiento (16,80 y 16,91 kg m^{-2}, respectivamente), donde L-28 destacó por sus valores de SST, TTA, VC, TP y CA. Con respecto a los genotipos silvestres, la modificación de la CE no incremento el rendimiento; no obstante, el uso de 3,0 dS m^{-1} permitió obtener los mejores resultados. Entre las selecciones silvestres se destacaron SS3 (rendimiento, PPFF y LI) y SS5 (NFPR, VC, FT y CA). La modificación de la CE no modifico el rendimiento, sin embargo, si afectó la calidad fisicoquímica y capacidad antioxidante de los materiales analizados.

Palabras clave: acidez titulable total, sólidos solubles totales, ácido ascórbico, fenoles totales, Solanácea.

Introduction

The fruits of tomato (*Solanum lycopersicum* L.) have a wide versatility as food source whether in fresh or as processed food, tomatoes constitute one of the most widely cultivated and demanded agricultural products worldwide. In Mexico, due to the technological development of protected agriculture and the use of hybrids with high yield potential, between 1980 and 2010 the area under cultivation was reduced by 24%, while production and yield increased by 45% and 90%, respectively (Magaña *et al.,* 2013).

The development of intensive systems of tomato production has led to the import of large volumes of seeds, where the hybrids that are currently cultivated are generated by few transnational companies, so seeds are expensive, not always available, and sometimes inaccessible to small producers. The generation of experimental lines and hybrids as well

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as the search for outstanding wild materials in breeding programs can be a good alternative to generate local materials for both regional and national markets. According to Mendez *et al.* (2011), there are large tomato collections in Central and South America where they are widely cultivated, with indigenous kidney-type varieties almost exclusively to regional use. In some regions of Mexico (Puebla and Oaxaca), native materials known as "kidney" are widely used and are cultivated for local consumption (Estrada *et al*., 2011).

The wild genotypes present acceptable levels of total soluble solids, titratable acidity, vitamin C content (Mendez *et al.,* 2011; Vera *et al.,* 2011), total phenols, antioxidant capacity and lycopene (Kavitha *et al.,* 2014); reasons why they have been used to increase the nutritional quality of fruits (Juárez *et al.,* 2013). In all breeding programs, it is necessary to know the genetic characteristics of the populations, as well as the variations due to environmental effects (Gaspar *et al.,* 2012). It has been detected that some management practices, such as the modification of the nutritional concentration, can positively affect agronomic behavior in tomato including yield and physical characteristics of fruits (Flores *et al.,* 2012), as well as its chemical quality and antioxidant capacity (Krauss *et al.,* 2006; Schnitzler and Krauss, 2010). Therefore, the objective of this study was to evaluate the response of three levels of electrical conductivity (2.0, 2.5 and 3.0 dS m^{-1}) of the Steiner nutrient solution on the yield, physicochemical quality and antioxidant capacity in "beef" tomato and wild type kidney fruits.

Materials and methods

Location of the experiment and plant material

The experiment was carried out from April to September 2016, under medium-tech "full vent" greenhouse conditions, with a 600-gauge polyethylene cover with 70% light transmission, and protected front, side and upper ventilation, protected anti-fouling mesh, located at the Autonomous University of Chapingo, Mexico (19°29' N and 98°53' W; 2,240 m a.s.l.); with an annual average air temperature of 15.9 °C. Eleven tomato materials were used: "beef" type (1 commercial 'Susan' type hybrid (control) and 6 experimental lines (L-51H, L-52, L-43H, L-28, L-76H, and H13-33) as well as 4 selections of wild genotypes ("kidney") (SS1, SS3, SS4, and SS5) (*S. lycopersicum* L.).

Crop management

Sowing was done in expanded polystyrene trays with 200 wells, using peat moss as a substrate. At 30 d, the seedlings were transplanted into black polyethylene bags filled with volcanic rock "tezontle" of 10-20 mm in diameter (13 kg). The plants were led to a single stem with a density of 3.7 m^{-2} plants. The supply of essential elements for the growth and development was performed according to the parameters established by the Steiner solution and complemented with micronutriments at 100, 125 and 150% of its concentration, representing an electrical conductivity of 2.0, 2.5 and 3.0 dS m^{-1}, respectively (Tab. 1) (Steiner, 1984); which was applied by a drip irrigation system with an applied volume of 0.30-2.5 L/plant following each phenological stage.

Harvesting was carried to the fifth cluster, at which point the plant was exposed above the third leaf after the cluster. In order to carry out the corresponding analyzes, the fruits harvested were those located between the second and fourth cluster at the sixth maturity stage, being a stage when the fruit possess 90% red coloration (Choi *et al*., 1995).

Experimental design. The experimental design was completely randomized with six replicates, the experimental unit consisted of one fruit and the variables evaluated were: yield, number of fruits per cluster, average fruit weight, color, firmness, total soluble solids, titratable acidity, vitamin C, total phenols, lycopene, and antioxidant capacity.

Parameter evaluated

Yield. The weight of the fruits (experimental unit) harvested using an OHAUS® portable digital scale was obtained

TABLE 1. Concentration of macroelements and microelements of the nutrient solutions.

Concentration (%)	Anions (meq L^{-1})				Cations (meq L^{-1})				EC (dS m^{-1})
	NO_3	H_2PO_4	SO_4	Total	K^+	Ca^{2+}	Mg^{2+}	Total	
	60	5	35	100	35	45	20	100	
100	12.0	1.00	7.00	20	7.00	9.00	4.00	20	2.0
125	15.0	1.25	8.75	25	8.75	11.25	5.00	25	2.5
150	18.0	1.50	10.50	30	10.50	13.50	6.00	30	3.0

*EC: Electric conductivity (dS m^{-1}).

with approximation to 0.01 g. The data obtained are reported in kg m^{-2}.

Number of fruits per cluster (NFPC). It was obtained by dividing the total of fruits harvested to the total of clusters per experimental unit.

Average fresh weight of fruit (AFWF). The yield value was divided to the total numbers of harvested fruits, the result was expressed in grams (g).

Color. It was determined directly on the epidermis of the fruit with X-Rite® SP62 colorimeter, values L, a, and b were taken in the equatorial region of each fruit. With these values, the tone angle (hue) and the color purity (chromaticity) were calculated applying the formulas: hue=$\tan^{-1}$ (b/a), chromaticity =$(a^2+b^2)^{½}$ and the luminosity L obtained directly with the colorimeter, which correspond to the color space L * a * b (Voss, 1992).

Firmness. The measurement was performed at the equatorial zone of the fruit by means of a Chatillón® AMETEK penetrometer, with a cone-shaped strut. The force applied until the penetration of the strut was expressed in Newtons (N).

Total soluble solids (TSS). The total soluble solids (°Brix) were counted with a PAL-1® portable digital refractometer (ATAGO, USA) using a 0-53° scale. The measurement was carried out by placing a fruit juice drop in the screen of the refractometer to further assessing the result.

Total titratable acidity (TTA). It was determined according to the methodology proposed by the AOAC (AOAC, 1990), with 20 g of pulp neutralized with 0.1 N NaOH, using 1% phenolphthalein as indicator. Results were reported as % citric acid.

Vitamin C (VC). It was estimated according to the method of Tillman (AOAC, 1990), known as DFI-2, 6 dichlorophenol-indophenol, for this 5 g of finely chopped fruit was homogenized with 50 mL of a 5% oxalic acid solution. The titration process was carried out with a 10 mL juice aliquot. The concentration was expressed in mg ascorbic acid 100 g^{-1} by a standard curve of ascorbic acid.

Total phenols (TP). The quantification of the total phenols was carried out by the method of Folin and Ciocalteu described by Waterman and Mole (1994), with the following modifications: 300 µL of ethanolic extract (1.0 g of pulp in 5 mL of ethanol, homogenized with 24 h of rest) to which 8.0 mL of distilled water, 0.5 mL of the Folin and Ciocalteu reagent, respectively, were added and shaken; finally 1.5 mL of a 20% Na_2CO_3 solution was added to each sample and resuspended, allowing it to stand for 2 h under dark conditions. The absorbance reading was taken at 760 nm using a UV-VIS® model digital spectrophotometer (PerkinElmer®, USA). The results were expressed in mg 100 g^{-1} of fresh weight (FW) according to a standard curve of tannic acid.

Lycopene (LY). The determination was realized using the modified method of Sadler *et al.* (1990). 20 g of pulp were homogenized with distilled water, the obtained mixture was placed in a jar wrapped in aluminum foil and dried at 38°C. 0.1 g of the paste was placed in aluminum foil test tubes, 30 mL of a 2: 1: 1 hexane / ethanol / acetone mixture was added and stirred for 10 min. Subsequently, 18 mL of distilled water were added and the mixture was stirred for 5 min. until the aqueous and organic phase separated. The volume of organic phase at which the absorbance value was taken at 470 nm (PerkinElmer UV-VIS®, USA) was measured with separation flasks. Quantification was performed using the formula of Inbaraj and Chen (2008) and the results were expressed in mg 100 g^{-1} FW.

Antioxidant capacity (AC). It was carried out according to the method ABTS (2,2'azinobis (3-ethylbenzothiazolin-6-sulfonic acid) modified by Ozgen *et al.* (2006) $ABTS^{\bullet+}$ was formed after the reaction of ABTS (7 mM) with potassium persulfate (2.45 mM, final concentration) incubated at room temperature and in dark conditions for 24 h. After the $ABTS^{\bullet+}$ radical was formed it was diluted with PBS (sodium acetate buffer solution) (pH 4.5) until an absorbance value of 0.7 ± 0.1 at 734 nm (maximum absorption length) was obtained. For the test, 3.9 mL of the $ABTS^{\bullet+}$ solution and 100 µL of extract from the sample and allowed to stand for 2 h where the absorbance reading was performed at 734 nm. The results are expressed in TEAC (Antioxidant Activity Equivalent to Trolox).

Statistical analysis. An analysis of variance (ANOVA) and Tukey's mean comparison ($P \leq 0.05$) were performed, using the statistical analysis program Statistical Analysis System (SAS), ver. 9.1.

Results and discussion

Effect of the nutrient solution Electrical conductivity: "beef" type genotypes. The variation in electrical conductivity (EC) of the nutrient solution did not affect the yield (13.83 to 14.74 kg m^{-2}), NFPC (3.99 to 4.43), and AFWF (200.15 to 214.95 g) (Tab. 2). Which concurs to Valenzuela

TABLE 2. Effect of the nutrient solution conductivity on the components of yield, physicochemical quality and antioxidant capacity in tomato "beef" fruits.

EC (dS m^{-1})	TSS (ºBrix)	TTA (% c. acid)	VC (mg 100 g^{-1} asc. acid)	TP (mg 100 g^{-1})	LY (mg 100 g^{-1})	AC (mm TEAC g^{-1})
2.0	3.67 a	0.34 b	3.95 a	2.67 a	16.09 a	33.24 a
2.5	3.72 a	0.35 ba	3.59 a	2.56 a	15.71 a	32.83 a
3.0	3.74 a	0.36 a	3.88 a	2.70 a	17.22 a	34.22 a
MSDH	0.271	0.018	0.856	0.206	3.457	3.274

EC (dS m^{-1})	Y (kg m^{-2})	NFPC	AFWF (g)	L	C	H (ºhue)	F (N)
2.0	14.67 a[§]	4.09 a	214.95 a	43.12 a	40.86 a	47.69 b	1.62 a
2.5	14.74 a	4.43 a	200.15 a	43.78 a	43.35 a	49.05 a	1.61 a
3.0	13.83 a	3.99 a	205.88 a	43.51 a	42.11 a	47.75 b	1.71 a
MSDH	1.609	0.573	18.725	1.133	3.553	1.268	0.196

EC: electrical conductivity. Y: yield, NFPC: number of fruits per cluster, AFWF: average fresh weight of fruit, L: luminosity, C: chromaticity, H: hue angle, F: firmness, TSS: total soluble solids, TTA: total titratable acidity, VC: vitamin C, TP: total phenols, LY: lycopene, AC: antioxidant capacity. MSDH: Minimum Significant Difference Honest. [§]Means with equal letter within the same column are statistically equal according to Tukey`s test ($P \leq 0.05$).

et al. (2014) who reported yield values between 14.49 and 14.99 kg m^{-2} using lower concentrations (50 to 100%) of Steiner's solution (1.0 to 2.0 dS m^{-1}).

The variation of EC did not affect the behavior of two components of fruit color: luminosity (43.12 to 43.78) and chromaticity (40.86 to 43.35), as well as firmness (1.61 to 1.71 N); however, the color hue (hue angle) had an increase from 47.69 to 49.05º (Tab. 2). Similar fruit color results were reported by Cruz and Sandoval-Villa (2012) with values of brilliance (39.57 to 42.75), chromaticity (21.86 and 22.43) and hue angle (58.0 to 62.95°) for tomato fruits grown with conductivity electrical from 50, 75, and 100%. The change in the hue values may be associated with a decrease in the red coloration of the epidermis, from a bright red coloration (41.3º) to a red orange color (48.0º) (Batu, 2004).

"Beef" tomatoes fruit cultivated with a EC of 2.0 to 3.0 dS m^{-1} did not modify the TSS content (3.67 to 3.74 °Brix), however, it was significant in relation to the variation of the organic acids concentration after presenting fluctuations of 0.34 to 0.36% of citric acid (Tab. 2). In this sense, after evaluating five levels of electrical conductivity of nutrient solution (1-5 dS m^{-1}) Brasiliano *et al*. (2006) found a linear increase in the total titratable acidity values of 9.4%. On the other hand, Schnitzler and Krauss (2010) indicated even a higher increase of citric acid contents in tomato fruits (10.7, 52.2, and 78.3%) when using EC of 3.0 to 6.5, 10 and 13.5 dS m^{-1}. These results, according to Wakeel (2013), may be related to the presence of K^+, which directly affects the cation-anion charge balance mechanism that occurs when this nutrient element is transported without the presence of a companion anion in the cytoplasm.

As shown in Tab. 2, EC modification of nutrient solution did not allow significant differences in VC content (3.59 to 3.95 mg ascorbic acid 100 g^{-1}), TP (2.56 to 2.70 mg 100 g^{-1}), as well as LY (16.98 to 18.70 mg 100 g^{-1}) and AC (32.83 to 34.22 mm TEAC g^{-1}). However, Krauss *et al*. (2006); Schnitzler and Krauss (2010) reported a significant increase in vitamin C content (8.1, 10.0, and 11.1 mg ascorbic acid 100 g^{-1}), lycopene (57.5, 112.5 and 135%), total phenols (28.5 to 48.1 mg 100 g^{-1}), and antioxidant capacity (26.1 to 38.8 mm TEAC g^{-1}) after apply a higher electrical conductivity in the nutrient solution (6.5, 10, and 13.5 dS m^{-1}).

Fruit quality: "beef" type genotypes. When comparing genotypes, with exception of L-43H (10.11 kg m^{-2}), all experimental lines showed a similar yield value as commercial 'Susan' (12.90 to 16.91 kg m^{-2}) (Tab. 3). These results contrasted with those reported in twenty-four "beef" type hybrids (6.73 to 11.80 kg m^{-2}) (Martinez *et al*., 2005). Also, our findings agreed with the yield data (10.79 to 15.23 kg m^{-2}) presented by Pérez *et al*. (2012) in four commercial "beef" type hybrids.

All analyzed materials had a NFPC that fluctuated from 2.90 to 4.53, where the experimental line H13-33 (7.65) (Tab. 3) stands out, which main characteristic was a "beef" type with small fruit. Magaña *et al*. (2013) and Pérez *et al*. (2012) reported a number of fruits per similar cluster (2.71 to 3.94 and 2.93 to 5.26) for seven and four commercial fruit "beef" hybrids. In contrast, Martinez *et al*. (2005) reported the highest number of fruits in six bunches per plant in twenty-four "beef" type hybrids (19.0 to 56.3).

The experimental line L-76H presented higher AFWF (282.75 g) than the one observed in commercial hybrid

'Susan' (230.5 g), but with a similar trend to the lines L-51H and L-52 (266.36 and 252.97 g, respectively) (Tab. 3). Similar data are reported by Magaña *et al*. (2013) and Martinez *et al*. (2005) from 104.62 to 151.63 g and 37.1 to 116.3 g respectively, in seven and twenty-four commercial hybrids of beef tomato. Nevertheless, Pérez *et al*. (2012) and Grijalva *et al*. (2011) indicate a lower AFWF (146.5 to 215.4 g and 152.5 to 211.3 g), in four and ten commercial "beef" type hybrids.

The fruits from experimental lines L-51H, L-52, and 'Susan' hybrid had significantly higher values of luminosity (44.5 to 45.49) than the rest of the genotypes (41.41 to 42.64) (Tab. 3). The data obtained in this study are similar to those reported by Hernández *et al*. (2007) in five commercial varieties cultivated in Spain (44.2 and 44.6). On the other hand, Gaspar *et al*. (2012) obtained lower luminosity values in eight advanced lines (33.2 and 37.6). All experimental lines analyzed showed higher brightness values than the optimal levels (38.0 to 40.0) described by Preczenhak *et al*. (2014) for this species, which might indicate that all materials presented fruits with desirable characteristics for this quality character.

All experimental lines evaluated in this study as well as commercial 'Susan' control showed statistically similar chromaticity values (37.70 to 45.98) (Tab. 3). These results agree with those obtained by Gaspar *et al*. (2012) and Kacjan *et al*. (2011) (44.0 to 53.5 and 39.22 to 43.35, respectively) on eight advanced lines and eleven tomato cultivars; though, those are chromaticity levels higher than the described by Hernández *et al*. (2007) (30.8 to 34.3) in five commercial cultivars of tomato grown in Spain.

Comparing between genotypes (Tab. 3), it was observed that the experimental lines of "beef" tomato H13-33, L-52, L-51H and L43H presented a fruit tonality statistically similar to the control with values that fluctuated between 48.10 and 50.55º. These results are within the range (44.9 and 53.2º) indicated by Kacjan *et al*. (2011) in eleven cultivars managed in different climatic conditions. Likewise, the data obtained surpassed the 35 and 40º mencionated by Cantwell *et al*. (2006) in tomato fruits with red tonality, showing values close to the color red-orange (48.0º).

When evaluating fruit firmness (Tab. 3), it was found that all the experimental lines (except L-76H) and the commercial 'Susan' showed firmness levels (1.50 to 1.93 N) higher than 1.46 N indicated at least by Batu (2004) on tomato fruits intended for fresh consumption. In the same way, they coincide with that reported by Hernández *et al*. (2013) (1.3 to 2.4 N) in mature fruits (90% red) of seven commercial tomato hybrids.

TABLE 3. Yield, physicochemical quality, and antioxidant capacity components of "beef" type tomato fruits.

Genotype	Y (kg m^{-2})	NFPC	AFWF (g)	L	C	H (ºhue)	F (N)
'Susan'	15.44 ba[§]	3.65 cb	230.51 b	44.90 a	44.40 ba	48.10 a	1.69 bac
L-51H	16.80 a	3.43 cb	266.36 ba	45.04 a	41.21 ba	49.66 a	1.88 ba
L-52	13.64 b	2.90 c	252.97 ba	45.49 a	40.67 ba	49.78 a	1.93 a
L-43H	10.11 c	4.53 b	120.14 d	42.41 b	45.98 a	48.94 a	1.78 bac
L-28	12.90 c	3.76 cb	188.35 c	42.64 b	45.20 a	45.62 b	1.51 bdc
L-76H	16.91 a	3.28 c	282.75 a	42.41 b	37.70 b	44.51 b	1.25 d
H13-33	15.12 ba	7.65 a	107.85 d	41.41 b	39.59 ba	50.55 a	1.50 dc
MSDH	3.134	1.117	36.466	2.197	6.890	2.460	0.381

Genotype	TSS (°Brix)	TTA (% c. acid)	VC (mg 100 g^{-1} asc. acid)	TP (mg 100 g^{-1})	LY (mg 100 g^{-1})	AC (mm TEAC g^{-1})
'Susan'	3.07 c	0.35 ba	4.74 ba	2.71 ba	19.65 a	36.84 a
L-51H	3.76 ba	0.33 b	3.09 bdc	2.40 b	15.89 ba	30.48 bc
L-52	3.51 bc	0.35 ba	3.01 dc	2.38 b	19.01 a	32.72 ba
L-43H	4.24 a	0.29 c	4.66 bac	2.99 a	18.74 ba	36.30 ba
L-28	4.21 a	0.38 a	5.19 a	3.09 a	13.28 ba	37.23 a
L-76H	3.13 c	0.35 ba	3.37 bdc	2.41 b	15.77 ba	26.30 c
H13-33	4.06 a	0.38 a	2.61 d	2.51 b	12.04 b	34.14 ba
MSDH	0.525	0.035	1.660	0.399	6.704	6.349

Y: yield, NFPC: number of fruits per cluster, AFWF: average fresh weight of fruit, L: luminosity, C: chromaticity, H: hue angle, F: firmness, TSS: total soluble solids, TTA: total titratable acidity, VC: vitamin C, TP: total phenols, LY: lycopene, AC: antioxidant capacity. MSDH: Minimum Significant Difference Honest. [§]Means with equal letter within the same column are statistically equal according to Tukey`s test ($P \leq 0.05$).

Experimental lines L-43H, L-28, H13-33 and L-51H presented statistically higher TSS contents (3.76 to 4.24 °Brix) to the commercial 'Susan' (3.07 °Brix) commercial indicator (Tab. 3). The results obtained were approximate to those of Gaspar *et al*. (2012), Pérez *et al*. (2012) and Chattopadhyay *et al*. (2013) in eight tomato lines, four commercial cultivars and thirty-one hybrids produced in India (3.9 to 5.2, 4.6 to 5.1 and 3.82 to 5.1 ºBrix, respectively).

When comparing TTA between genotypes (Tab. 3), all experimental lines, except L-43H, showed levels (0.33 to 0.38%) statistically similar to commercial "Susan" hybrid (0.35%). The results found are within the ranges reported by Gaspar *et al*. (2012) and Chattopadhyay *et al*. (2013) (0.24 to 0.39 and 0.27 to 0.52% of citric acid, respectively) in fruits of eight lines and 31 hybrids of tomato.

It was possible to detect that the highest levels of VC (4.66 to 5.19 mg ascorbic acid 100 g^{-1}) were statistically higher than the lines H13-33 and L-52, with the experimental lines L-28, L-43H and the hybrid 'Susan' (2.61 and 3.01 mg ascorbic acid 100 g^{-1}, respectively) (Tab. 3). High concentrations of vitamin C were reported by Gaspar *et al*. (2012) and Chattopadhyay *et al*. (2013) (9.7 to 16.0 mg, and 14.63 to 40.50 mg ascorbic acid 100 g^{-1}, respectively). The variation between the results obtained with respect to those reported in the literature could be related to the freezing (-30 ºC) and thawing to which the fruits were subjected during their analysis, since according to Barankevicz *et al*. (2015) freezing tomato fruits at -18 °C reduces to 67.18% of ascorbic acid content, which is associated to the enzymatic and non-enzymatic oxidation of this acid in the presence of oxygen.

Regarding the TP content (Tab. 3), all the experimental lines and 'Susan' hybrid showed statistically similar levels (2.38 to 3.09 mg 100 g^{-1}). Independently, Hernández *et al*. (2007) and Bhandari *et al*. (2016) reported higher concentrations in commercial tomato cultivars (19.7 to 21.1 and 13.28 to 23.65 mg GAE 100 g^{-1}, respectively).

All experimental lines (except H13-33) and commercial 'Susan' control showed statistically similar LY contents (13.28 to 19.65 mg 100 g^{-1}). This is consistent with those reported in eight advanced tomato lines by Gaspar *et al*. (2012) (9.6 to 16.8 mg 100 g^{-1} FW). Nevertheless, Hernández *et al*. (2007) and Chattopadhyay *et al*. (2013) indicated a lower concentration of lycopene (1.89 to 2.56 and 1.25 to 4.91 mg 100 g^{-1}, respectively).

In relation to AC (Tab. 3), the experimental lines L-28, L-52, H13-33 and L-43H were found to have levels (32.72 to 37.23 mm TEAC g^{-1}) statistically superiors to the commercial control 'Susan' (36.84 mm TEAC g^{-1}). The results of this experiment surpassed those reported by Kavitha *et al*. (2014) (5.5 to 11.1 mm TEAC g^{-1}) in commercial hybrids and tomato varieties.

Effect of EC of nutrient solution: wild type tomato. Yield and NFPC were not affected by EC variation in nutrient solution from 2.0 to 3.0 dS m^{-1} (Tab. 5) whose values ranged from 6.95 to 8.81 kg m^{-2} and from 11.63 to 12.29 (Tab. 4); but, AFWF showed a significant decrease (40.69 to 31.81 g). In this sense, Flores *et al*. (2012) obtained a lowest yield (3.17 to 3.27 kg m^{-2}) after evaluating values of electrical conductivity from 2.0 to 3.0 dS m^{-1}. This study coincides with Bertoldi *et al*. (2008) who reported that increasing the conductivity of nutrient solution from 3.0 to 9.0 dS m^{-1} does not generate variations in the NFPC (19.96 to 17.14); nevertheless, the AFWF (7.8 to 6.0 g) is significantly reduced.

TABLE 4. Effect of nutrient solution conductivity on yield components, physicochemical quality and antioxidant capacity of fruit in wild tomato.

EC (dS m^{-1})	Y (kg m^{-2})	NFPC	AFWF (g)	L	C	H (ºhue)	F (N)
2.0	8.81 a[§]	11.63 a	40.69 a	46.37 a	36.72 a	39.22 b	0.74 a
2.5	7.52 a	12.29 a	33.77 b	44.05 b	38.97 a	44.86 a	0.85 a
3.0	6.95 a	12.29 a	31.81 b	44.09 b	38.56 a	41.01 b	0.84 a
DMSH	1.964	3.134	3.918	1.653	2.901	3.108	0.247

EC (dS m^{-1})	TSS (ºBrix)	TTA (% c. acid)	VC (mg 100 g^{-1} asc. acid)	TP (mg 100 g^{-1})	LY (mg 100 g^{-1})	AC (mm TEAC g^{-1})
2.0	4.40 b	0.51 b	6.61 b	3.39 b	22.00 b	52.09 b
2.5	5.16 ba	0.57 a	8.98 ba	3.26 b	19.07 b	50.67 b
3.0	5.59 a	0.56 a	9.38 a	3.90 a	28.42 a	60.37 a
MSDH	0.888	0.036	2.462	0.351	4.281	8.241

EC: electrical conductivity. Y: yield, NFPC: number of fruits per cluster, AFWF: average fresh weight of fruit, L: luminosity, C: chromaticity, H: hue angle, F: firmness, TSS: total soluble solids, TTA: total titratable acidity, VC: vitamin C, TP: total phenols, LY: lycopene, AC: antioxidant capacity. MSDH: Minimum Significant Difference Honest. [§]Means with equal letter within the same column are statistically equal according to Tukey's test ($P \leq 0.05$).

As for the physical variables (Tab. 5), the increase in EC levels of the nutrient solution did not affect the color intensity (chromaticity) (36.72 to 38.97) and fruit firmness (0.74 to 0.85 N). Similar chromaticity values are reported by Cruz and Sandoval-Villa (2012) on "Charleston" tomato fruits (21.86 and 22.43) grown with concentrations of Steiner's solution of 0, 50, 75 and 100%. Flores *et al*. (2012) reported firmness values that surpass what was found in this study (2.45 to 2.59 N) in 10 native genotypes and two commercial hybrids cultivated with three levels of Steiner's solution (1.0, 2.0, or 3.0 dS m^{-1}).

The change on EC values from 2.0 to 2.5 dS m^{-1} caused a significant decrease in fruit brightness of the four wild tomato genotypes evaluated (46.37 to 44.05) (Tab. 5). Non-significant variations on fruit luminosity (31.87 to 32.34) was presented from ten native genotypes of tomato cultivate with three levels of Steiner's solution (1.0, 2.0 and 3.0 dS m^{-1}) (Flores *et al*., 2012). On the other hand, Borghesi *et al*. (2011) found that increasing the conductivity of the nutrient solution from 3.5 to 5.5 dS m^{-1} decrease fruit brightness values by 11.2%.

The wild genotypes fruit tonalities were affected when changing CE values from 2.0 to 2.5 dS m^{-1} (39.22 to 44.86º) (Tab. 5). In contrast, Flores *et al*. (2012) studied ten native genotypes of tomato cultivated with three conductivity levels (1.0, 2.0 and 3.0 dS m^{-1}) and found no significant variation on the hue angle values (33.40 to 34.90°). In this sense, it is important to note that the color of the tomato fruit goes from bright red (41.3º) to orange red (48.0º) (Cantwell *et al.,* 2006); so that the increments found in the hue angle in the present study, reflect a decrease in red coloration.

The variation of the EC from 2.0 to 3.0 dS m^{-1} generated a positive effect on the concentration of the TSS from 4.40 to 5.59 ºBrix, as well as of the TTA (0.51 to 0.57% of citric acid) (Tab. 5). In contrast, Preczenhak *et al*. (2014) found a TSS content maximum of 7.3 °Brix after characterizing sixty-four genotypes of mini tomatoes. Wu and Kubota (2008) reported increases from 5.3 to 6.1 °Brix on tomato fruits by raising the conductivity of the nutrient solution from 2.3 to 4.5 dS m^{-1}. Likewise, Cruz and Sandoval-Villa (2012) found significant increases in percentage of citric acid from 0.348 to 0.383 after increasing concentration of Steiner's solution from 50 to 100%. The behavior observed in data of TSS and TTA according to Ruiz *et al*. (2014) may be related to a decrease in the accumulation of water inside the fruit without a significant change in the sugar concentration.

In this work, the EC modification allowed to detect increases in the VC content from 6.61 to 9.38 mg 100 g^{-1} ascorbic acid. This result differs from the reported by Juárez *et al*. (2013) who evaluating the effect of three levels of the Steiner's solution of EC (1.0, 1.5 and 2.0 dS m^{-1}) did not find variation on the concentration of VC. On the other hand, Krauss *et al*. (2006) indicated an increase in the ascorbic acid concentration up to 35%, when studying the electrical conductivity of 3, 6.5, and 10 dS m^{-1}. The EC of 3.0 dS m^{-1} allowed to increase the TP content (3.90 mg 100 g^{-1}) (Tab. 5). In this sense, Krauss *et al*. (2006) detected significant increases (28.5 to 48.1 mg 100 g^{-1}) on solutions with higher conductivities (6.5 to 13.5 dS m^{-1}), which according to Bhandari *et al*. (2016) is associated to the activation of certain defense mechanisms against the conditions of stress caused by the presence of salts. Non-significant variations on phenols content of tomato fruits was reported by Kubota *et al*. (2012) when evaluating electrical conductivity ranges from 2.4 to 4.8 dS m^{-1}.

Similarly, EC modification favored significantly the LY content in fruits of wild tomato genotypes (22.00 to 28.42 mg ascorbic acid 100 g^{-1}) (Tab. 5), concurring to Krauss *et al*. (2006) whom stablished that the biosynthesis pathway of carotenes (lycopene and β-carotene) is very sensitive to stress caused by environmental factors (light and temperature) and those related to the soil (water deficit and salinity), where the presence of a concentration of salts in the nutrient solution of irrigation may be linked to this behavior (Borghesi *et al.,* 2011). In contrast, Urrieta *et al*. (2012) reported non-significant variations in LY content after evaluating the conductivity levels (1.0 and 2.0 dS m^{-1}). Independently, Juárez *et al*. (2013) reported significant increases of lycopene in cherry-type tomato fruits (42.0 to 49.4 mg 100 g^{-1}) by increasing the conductivity of Steiner's solution from 1.0 to 2.0 dS m^{-1}. These results show that LY content in genotypes of small tomato, such as wild and cherry type, they are more sensitive to variations in the concentration of the nutrient solution, perhaps due to their lower proportion of water compared to fruits of tomato "saladette" and "beef" type. The AC of fruits increased significantly when modifying EC from 2.0 to 3.0 dS m^{-1} with values from 39.44 to 42.93 mm TEAC g^{-1} (Tab. 5). The same behavior was observed by De-Pascale *et al*. (2003) in tomato fruits cultivated in hydroponics using a nutrient solution with a conductivity range between 0.5 and 8.5 dS m^{-1}.

Fruit quality: wild type tomato. When comparing genotypes (Tab. 5), wild selection SS5 presented the statistically highest yield (11.25 kg m^{-2}) relative to SS1 and SS4, which showed the lowest values (5.23 and 5.92 kg m^{-2}, respectively).

These yields were higher than those reported by Vázquez *et al*. (2010) and Ramos *et al*. (2009) (0.46 to 1.66 and 0.53 to 1.53 kg per plant) in wild type kidney fruit. The wild selection SS3 presented the NFPC regarding to SS1, SS4 and SS5 which presented values that fluctuated from 10.51 to 11.04 (Tab. 5). This agronomic characteristic contrasts to those reported by Vázquez *et al*. (2010) and Carrillo *et al*. (2013) (4.2 to 7.2 and 1.86 to 7.33, respectively) in eleven and fifteen collections of kidney-type wild tomatoes. On the other hand, Ramos *et al*. (2009) reported similar of NFPC (60 to 72) in two kidney-like wild genotypes of Oaxaca, Mexico. The same authors also indicate that the great variability in the number of fruits per cluster produced among genotypes of wild tomato is a characteristic directly related to the degree of domestication.

When comparing genotypes (Tab. 5), the wild selection SS5 stands out for its higher AFWF (56.20 g), this result is congruent to the fruit weight ranges indicated by authors such as Vázquez *et al*. (2010) and Carrillo *et al*. (2013) (17.3 to 58.8 and 36.5 to 116.9 g per fruit, respectively); but, AFWF results were lower than those reported by Estrada *et al*. (2011) in four wild type kidney materials with 42.63 and 91.61 g. Among the wild selections, fruits harvested from SS4 wild selection showed the highest color luminosity (43.78 to 44.50) (Tab. 5). These values were lower than those reported for twenty-six genotypes (thirteen wild type kidney and thirteen native and wild) by Vera *et al*. (2011) and Méndez *et al*. (2011) on fruits with brightness ranges from 35.5 to 40.6 and 36.5 to 40.7, respectively). All the wild selections presented fruits that exceed the ideal levels (38.0 and 40.0) described for this species by Preczenhak *et al*. (2014). The chromaticity values of the fruits did not present differences between genotypes (Tab. 6) (37.05 to 39.15). Nevertheless, what was observed for this color component was similar to that reported by Vera *et al*. (2011). As can be seen in Tab. 3, the fruits from the SS3 wild selection showed the highest color tone (45.16°) (Tab. 5) which are within the levels of 35 to 40° of hue angle reported by Cantwell *et al*. (2006) for fruits with red epidermis suitable for commercialization.

The value of firmness found on the wild materials harvested fruits can be considered not adequate (0.73 to 0.92 N), because they did not exceed the minimum firmness level suggested by Batu (2004) for commercial use (1.40 N). This could be considered one of the reasons to consider at the time of marketing these genotypes, and according to Vázquez *et al*. (2010) after 8 d of storage the fruits of these wild genotypes lose consistency being very sensitive to mechanical damage, which makes difficult their postharvest management.

On the other hand, the fruits of the wild selections SS1 and SS3 presented the highest accumulation of TSS (5.35 and 5.86 °Brix) (Tab. 5). This contrasts with the values reported by Vera *et al*. (2011) on 13 samples of kidney type tomato from Mexico (3.4 to 5.2 °Brix). A similar behavior was described by Méndez *et al*. (2011) among 13 native and wild samples from Mexico (4.38 to 8.01 °Brix).

Among genotypes, wild selection SS4 presented a significantly higher percentage of citric acid (0.63%) than the rest of wild materials (0.51 to 0.52%) (Tab. 5). regarding these

TABLE 5. Yield, physicochemical quality and antioxidant capacity components of wild tomato fruits.

Genotype	Y(kg m^{-2})	NFPC	AFWF (g)	L	C	H (°hue)	F (N)
SS1	5.23 c[§]	11.04 b	25.63 b	44.50 b	37.05 a	41.94 b	0.73 a
SS3	8.65 b	15.96 a	29.60 b	43.78 b	38.93 a	45.16 a	0.92 a
SS4	5.92 c	10.51 b	30.26 b	46.90 a	37.21 a	38.89 b	0.81 a
SS5	11.25 a	10.77 b	56.20 a	44.17 b	39.15 a	40.79 b	0.78 a
MSDH	2.507	4.000	5.001	2.104	3.692	3.956	0.315

Genotype	TSS (°Brix)	TTA (% c. acid)	VC (mg 100 g^{-1} asc. acid)	TP (mg 100 g^{-1})	LY (mg 100 g^{-1})	AC (mm TEAC g^{-1})
SS1	5.35 a	0.51b	9.79 b	3.58 b	17.11 c	54.36 ba
SS3	5.86 a	0.5 b	13.23 a	4.10 a	21.92 bc	64.21 a
SS4	4.17 b	0.6 a	6.13 c	3.30 cb	24.81 ba	50.23 b
SS5	4.83 ba	0.5 b	4.13 c	3.08 c	28.81 a	48.70 b
MSDH	1.131	0.046	3.134	0.447	5.449	10.490

Y: yield, NFPC: number of fruits per cluster, AFWF: average fresh weight fruit, L: luminosity, C: chromaticity, H: hue angle, F: firmness, TSS: total soluble solids, TTA: total titratable acidity, VC: vitamin C, TP: total phenols, LY: lycopene, AC: antioxidant capacity. MSDH: Minimum Significant Difference Honest. [§]Means with equal letter within the same column are statistically equal according to Tukey's test ($P \leq 0.05$).

results, Brasiliano *et al.* (2006) indicate a low acidity level when the plants are cultivated in low salinity conditions, but it increase linearly with increasing concentration of salts in nutrient solution. Our results coincide with those reported by Méndez *et al.* (2011) and Vera *et al.* (2011) with values of 0.30 to 0.72 and 0.26 to 0.61%, respectively.

The wild selection SS3 presented the statistically higher content of VC (13.2 mg ascorbic acid 100 g^{-1}), which exceeded the rest of wild materials whose values fluctuated between 2.61 and 5.19 mg ascorbic acid 100 g^{-1}) (Tab. 5). Similar values are reported by Vera *et al.* (2011) (5.5 to 11.6 mg 100 g^{-1}); nevertheless, Méndez *et al.* (2011) found a higher concentration (12.4 to 22.9 mg 100 g^{-1}).

As can be seen in Tab. 5, the TP concentration was higher on the wild selection SS3 (4.10 mg 100 g^{-1}), but was lowest than that reported by Kavitha *et al.* (2014) for four species of wild tomato (53.8 to 96.4 mg GAE 100 g^{-1}). Likewise, LY contents (Tab. 5), wild selections SS4 and SS5 had the highest concentrations (24.81 and 28.81 mg 100 g^{-1}), which exceed those indicated by Méndez *et al.* (2011) on native tomato (12.4 to 22.9 mg 100 g^{-1}) and Vera *et al.* (2011) (10.0 to 16.0 mg 100 g^{-1}) for wild "kidney" type tomato.

The SS3 genotype showed the highest AC value (64.21 mm TEAC g^{-1}) to those observed in SS4 and SS5 genotypes (50.23 and 48.70 mm TEAC g^{-1}, respectively), likewise it was similar to SS1 (54.36 mm TEAC g^{-1}). This result contrasts with that described by Kavitha *et al.* (2014) (30.7 to 48.7 mmol TE·kg^{-1}) in four species of wild tomato.

Conclusions

The EC levels studied in this work did not affect the yield but the physicochemical characteristics and antioxidant capacity of the fruits of "beef" tomato and wild type kidney. So its implementation in the different production systems, can be an alternative of agronomic management that enable obtaining fruits with characteristics of nutraceutical qualities and very attractive for its fresh consumption. Among the kidney genotypes, SS3 and SS5 stood out for their high content of bioactive compounds, which could be very useful as a selection material of genetic breeding.

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Toxicity evaluation of two insecticides on *Tetragonisca angustula* and *Scaptotrigona xanthotricha* (Hymenoptera: Apidae)

Evaluación de la toxicidad de dos insecticidas sobre *Tetragonisca angustula* y *Scaptotrigona xanthotricha* (Hymenoptera: Apidae)

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ABSTRACT

Stingless bees (Hymenoptera: Apidae, Meliponini) have crucial roles in the ecosystem, offering pollination service and contributing to genetic diversity of species, and also providing honey and wax to humankind. *Tetragonisca angustula* and *Scaptotrigona xanthotricha* are species that have been used since ancient times for beekeeping. Currently these and other species have been exposed to agronomic practices, among which the use of synthetic pesticides used for crop protection stands out. The aim of this study was to evaluate the toxicity of the insecticides thiamethoxam and fipronil, which are used in agriculture in several countries in the tropical and subtropical belt in order to establish the risk that these products represent to the survival of these two species. The oral and topical LD_{50} was obtained by Probit analysis. Comparisons with the LD_{50s} of other stingless bees and *Apis mellifera* were realized. Although further studies are required to calculate the real risk of the two compounds, the results showed an evident susceptibility of both species. We concluded that it is essential to use tools and practices that reduce the risk, and perform toxicological evaluations of new and existing pesticides on stingless bees.

Key words: pollination, stingless bees, Meliponini, pesticide risk, toxicology.

RESUMEN

Las abejas sin aguijón (Hymenoptera: Apidae, Meloponini) hacen parte fundamental del ecosistema, ofreciendo el servicio de polinización y diversificación genética de las especies, además de proporcionar miel y cera para los seres humanos. *Tetragonisca angustula* y *Scaptotrigona xanthotricha* son especies que han sido usadas en la meliponicultura por tiempos ancestrales. En la actualidad ellas, junto con otras especies, han estado expuestas a las prácticas agronómicas entre las cuales se destaca la protección de cultivos. El objetivo de este trabajo fue evaluar la toxicidad de tiametoxam y fipronil, insecticidas usados en la agricultura de varios países de la franja tropical y subtropical, a fin de establecer el riesgo que pueden representar para la supervivencia de estas dos especies. Se obtuvo por medio del análisis Probit las DL_{50} para la exposición oral y tópica de los dos insecticidas. Se realizaron comparaciones con la DL_{50} de otras especies de abejas sin aguijón y *Apis mellifera*. Aunque se requieren más estudios para calcular el riesgo real de los dos compuestos, se evidenció la susceptibilidad de las dos especies y el riesgo para su supervivencia. Se concluyó que es fundamental el uso de herramientas y prácticas que disminuyan el riesgo que corren estas especies, además de la realización de evaluaciones toxicológicas sobre las abejas sin aguijón a la hora de aprobar el uso de nuevos y existentes pesticidas.

Palabras clave: polinización, abejas sin aguijón, Meliponini, riesgo de pesticidas, toxicología.

Introduction

Many American indigenous bees pollinate native and domesticated plant species, in some cases even, more effective and efficiently than the imported bee *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae). According to Klein *et al*. (2007) from 57 crops accounting the 95% of the world production, 42% are visited by a native bee.

Among stingless bees those of the tribe Meliponini stand out, they do not sting because the sting is atrophied. They are distributed throughout the tropical and subtropical region of America, Africa, Asia and Oceania (Michener, 2002; Nogueira-Neto, 1997). In Latin America the exploitation of honey and wax from meliponines or the so called meliponiculture, a term created by Nogueira-Neto. (1997), has been reported before the arrival of Columbus (McGregor,

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1976). In Colombia, few documents about this culture exist, however the use of 25 species has been recorded (Nates-Parra and Rosso-Londoño, 2013). In Brazil, until the introduction of the domestic bee in 1839, they were the only producers of honey (Witter and Tirelli, 2014), while in Colombia they were of great importance to the development of native communities, long before the conquest. The native tribes Chibchas, Muiscas and Tayronas, among others, consumed their honey to sweeten the "chicha" and used its wax in gold melting for jewelry production, using the lost wax technique (Falchetti and Nates-Parra, 2002, Nates-Parra, 1996).

Meliponines are social insects that offer a great service by effectively pollinating native and cultivated plants (Witter and Tirelli, 2014). Bees of the genus *Scaptotrigona* and *Tetragonisca* can be used to pollinate field and greenhouse crops, due to their docility, absence of functional sting and adaptation to artificial domains (McGregor, 1976; Nates-Parra, 2005).

Tetragonisca angustula Illiger, 1806, according to Nogueira-Neto (1997) is one of the bees with very good characteristics to "meliponiculture", both to honey production and pollination. Workers can cover a distance of 500 m, are generalist and polylectic, which means they can visit flowers of various plant species and are rustic (Nates-Parra, 2005; Venturieri, 2008). In Colombia, the species has been recorded from 0 to 2,000 m a.s.l. in most of the national territory (Nates-Parra and Rosso-Londoño, 2013).

Scaptotrigona xanthotricha Moure, 1950 called in Brazil "mandaguari-amarillo", covers a distance of approximately 750 m (Nogueira-Neto, 1997); its behavior is more aggressive than *T. angustula*. They do not possess a functional sting, but defend their hive by tangling in operator´s hair and clinging with legs and mandibles to the cloth and sensitive skin areas like eyelids and lips, without causing any physical damage. They show great potential as a producer species of special honey with antimicrobial activity (Borsato *et al*., 2013); distributed in several states in Brazil (Camargo and Pedro, 2008) and registered in Colombia in the department of Santander (Nates-Parra, 2001).

Although there is a considerable number of articles over the toxicology and the effect of insecticides on meliponines, as those published by Costa *et al*. (2015), Jacob *et al*. (2013), Lourenço *et al*. (2005), Moraes *et al*. (2000), Soares *et al*. (2015), Tomé *et al*. (2015), Valdovinos-Nuñez *et al*. (2009), among others, there is few information on the toxicology of the two species mentioned above and even less about neonicotinoids and phenylpyrazoles based insecticides, such as thiamethozam and fipronil.

Thiamethoxam belongs to the neonicotinoids, agonistic molecules of the nicotinic acetylcholine receptor, that mimic the action of the neurotransmitter acetylcholine, blocking receptors and disrupting the transmission impulse between nerve cells (Devine *et al*., 2008, Group IRAC International MoA Working, 2015); it is systemic and acts by ingestion and contact, as well as its metabolite clothtianidin. Both compounds are persistent in soil and water and are highly toxic to *Apis mellifera* (Lewis *et al.,* 2016). Nowadays, Collapse Colony Disorder (CCD) is one of the most important problems that threaten *A. mellifera* and, consequently, agriculture in North America and Europe. CCD is defined as: "the loss of bee colonies without the presence of dead adult bees; only the queen is found, sometimes honey and immature bees" (USDA ARS, 2008). As other insecticides belonging to this group, the use of thiamethoxam is one of the possible causes of this disorder, both directly and indirectly, by increasing the susceptibility of bees to pathogens such as *Nosema* that affects the digestive tract of bees (CCD Steering Committee, 2012, Henry *et al*., 2012). Due to these results, several countries of the European Union have banned and / or limited its use in several crops (Agriculture forêt, 2013).

Fipronil belonging to phenylpyrazoles, it is also a neurotoxic antagonist of the chloride channel regulated by γ-aminobutyric acid (GABA); due to its mode of action interferes with the chloride channels in the nerve membrane, disrupting ion transfer and impulse transmission between nerve cells (Devine *et al*., 2008, Group IRAC International MoA Working, 2015). It is systemic and can act by ingestion and contact; persistent in soil as well as its most important metabolites, product of its transformation, fipronil amide, sulfone and sulphide, of which the toxicity to bees has been only studied for fipronil sulfone, product, orally, highly toxic (Lewis *et al*., 2016). It is reported as a toxic compound for bees and also as a possible, partial, CCD responsible (Reuber, 2015); in addition, causes a greater susceptibility of bees to *Nosema* (Vidau *et al*., 2011).

In Colombia there are two products registered with thiamethoxam as active ingredient and two in mixture with lambda-cyhalothrin; for fipronil more than 50 products with the active ingredient are registered alone and in mixture. Its use is permitted in rice, maize, potato, pasture, tomato, bean, citrus, onion, soybean, green beans, chrysanthemums, rose, avocado and cotton (ICA Instituto Colombiano Agropecuario, 2016).

In order to contribute to the knowledge of the toxicology of these two products commonly used in agriculture and to formulate strategies to increase productivity, toxicity and the effect of thiamethoxam and fipronil on *T. angustula* and *S. xanthotricha* was evaluated, estimating the LD_{50} applied both topically and orally.

Materials and methods

The research was carried out at the phytosanitary laboratory of the Agronomy Faculty Eliceu Maciel, Federal University of Pelotas, Capão do Leão Brazil, where artificial hives of *Scaptotrigona xanthotricha* and *Tetragonisca angustula* (Hymenoptera: Apidae) are available.

To perform the tests, the methodologies proposed in the Guide 213 Honeybees, Acute oral Toxicity Test and Guide 214 Acute Contact Toxicity Test of the Organization for Co-operation and Economic Development (OECD/OCDE, 1998); OCSPP 850.3020 : Honeybee Acute Contact Toxicity Test and OCSPP 850.3030: Honey Bee Toxicity of Residues on Foliage, The United States Environmental Protection Agency (EPA, 2012a, 2012b), the Guideline for Evaluating Side Effects of Plant Protection Products on Honeybees from The European and Mediterranean Plant Protection Organization (OEPP/EPPO, 2010) and the Standard Methods for Toxicology Research in *A. mellifera* of the International Bee Research Association (Medrzycki *et al*., 2013) were considered.

Collection of bees: The collection was made with an aspirator, lifting the top of the hives. Initially, 180 bees per test, ten bees for three replicates and six treatments were collected, then deposited in a plastic container with a lid and a feeder with a 50% sucrose solution (EPA, 2012a, 2012b; Medrzycki *et al*., 2013, OECD/OCDE, 1998; OEPP/EPPO, 2010). The bees were kept for 24 h in a dark room at 26°C and 70% humidity (EPA, 2012b; OECD/OCDE, 1998; OEPP/EPPO, 2010).

Specimen conditioning prior to application: The importance of food availability at the collection event has been observed. Maintaining these meliponines for more than 30 minutes without food causes a high natural mortality in the vials. For *A. mellifera* it is not recommended to have an individual alone for more than 1 h (OEPP/EPPO, 2010); since the meliponines are also social insects, this recommendation was considered.

The individuals were removed from the darkroom and all dead bees or those that did not exhibit normal and active behavior were discarded. The normal ones were anesthetized with CO_2 for 20 s or until sleeping.

Preliminary tests were performed to evaluate the response of individuals to the anesthesia procedure, to determine the volume of solution to be applied to the individual for topical application, and to know the effect of solvents for both topical application and oral exposure.

After the application of the chemical the individuals were transferred to an aluminum box with two holes and metal mesh where they had a 50% sucrose based feeder; all 18 experimental units corresponding to each test were taken back to the dark room.

Insecticide Solutions: The dilutions were prepared according to the commercial formulated products guidelines: Actara 250 WG containing 25% m/v of thiamethoxam and Standak® containing 25% m/v of fipronil. As base solution the maximum recommended dose for field applications (MDRC) of the insecticides was taken, prepared with water

TABLE 1. Doses used for the determination of the topical LD_{50} and oral LC_{50}

Species	Insecticide	Administration	Dosage used (A.I. ng μL^{-1})							
			1	2	3	4	5	6	7	8
Tetragonisca angustula	thiamethoxam	Oral	750.00	46.88	1.46	0.18	0.02			
		Topical	750.00	46.88	11.72	2.93	1.46	0.73	0.09	0.01
	fipronil	Oral	750.00	46.88	5.86	0.18	0.09	0.01		
		Topical	750.00	46.88	5.86	0.73	0.18	0.09		
Scaptoatrigaona xanthotricha	thiamethoxam	Oral	750.00	23.40	11.72	1.46	0.37	0.09	0.01	
		Topical	750.00	11.72	1.46	0.09	0.01			
	fipronil	Oral	750.00	46.88	5.86	2.93	1.46	0.09	0.05	0.01
		Topical	750.00	46.88	5.86	1.46	0.73	0.09		

and 1% detergent due to the insolubility of both commercial products in acetone. Serial dilutions were prepared from the base solution until the required doses for topical application and oral exposure were reached (Medrzycki *et al.,* 2013).

The doses used (Tab. 1) varied according to the behavior of each species. To determine the definitive doses, first serial dilutions were made, however, not all were applied to the sample. A selection of five dilutions was made, based on assumptions of the mortality that they could cause, and after the evaluation, along with the analysis of the data, the tests were adjusted, adding or not adding other doses. Thus, the number of doses of each test was variable.

Determination of LD_{50} by means of topical application: Topical application was performed using the Burkard® precision microapplicator. Each dose was applied to an experimental group of 30 bees, 10 bees from each hive, using three replicates (EPA, 2012b), plus an experimental control group without application, only was applied the aqueous solution with detergent and hold under the same conditions (Medrzycki *et al*., 2013; OECD/OCDE, 1998).

Each bee received 1 µL of the solution corresponding to each treatment on the pronotum (Medrzycki *et al*., 2013, OECD/OCDE, 1998, OEPP/EPPO, 2010); then the individuals were placed in a Petri dish to allow evaporation of the compound and subsequently deposited per replicate in an aluminum box and taken to the dark room where mortality and poisoning symptoms were observed at 4, 24 and 48 h (Medrzycki *et al*., 2013; OECD/OCDE, 1998).

Determination of oral LC_{50}: Subjects were anesthetized to be placed in the aluminum boxes, where they were fed 10µL/bee of a 50% sucrose solution contaminated with the doses proposed for the test for 4 h (Medrzycki *et al.,* 2013; OECD/OCDE, 1998; OEPP/EPPO, 2010); afterwards the contaminated food was removed and a tube with clean food solution was placed.

The number of boxes or experimental units was initially 18 for each test; but with increasing doses in some of them this number varied; however, all boxes were maintained under the conditions described above and observations were made with the same periodicity as in the previous evaluation. No repetition in time was performed.

Statistical Analysis: Due to the nature of the data and the objective of this work to determine the LD_{50} applied both topically and orally with their respective 95% confidence intervals and Chi square values, the Probit analysis was performed using EXCEL software, XLSTAT 2015 statistical package and GraphPad Prism software.

Results and discussion

Oral and topical lethal dose: Oral LC_{50} is a measurement that allows to obtain the lethal concentration of a pesticide in a food solution. However, its effect depends on the amount ingested by the target organism; LC_{50} is then converted into LD_{50}, and represents the estimated amount of pesticide that causes the mortality of the organism. The oral exposure diet to reach the LC_{50} to thiamethoxam in *T. angustula* of 6.664 ng μL^{-1} diet, and to *S. xanthotricha* of 0.1848 ng μL^{-1} diet was estimated. With fipronil the following data were obtained: *T. angustula*: 0.1864 ng μL^{-1} diet and *S. xanthotricha*: 0.8162 ng μL^{1} diet.

The oral LD50 was calculated after considering the concentration of the pesticide in the solution with which the bees were fed, it was also necessary to estimate the average consumption of the specimens during the 4 h of exposure, in order to know the amount of pesticide they consumed and subsequently calculate the LD50 in ng/bee; for *S. xanthotricha* it was 2 µL and for *T. angustula* 0.8 µL; results are presented, with the respective statistics, in Tables 2 and 3.

Slope interpretation of the curve Dose-Response: Figures 1 and 2 show the model used to determine of the topical and oral LD_{50} of the two insecticides. The different doses corresponding to the estimated percentages of mortality can be identified in the graphs. In addition, the risk represented by the insecticides to the bees can be interfered from the data in Tables 2 and 3. The slopes resulting from the oral fipronil tests in *T. angustula* and fipronil topical in *S. xanthotricha* were superior to 2, indicating a high sensitivity of both species to small modifications in the used dose; therefore, the existing range between LD_{50} and LD_{90} in these two cases is less than in the other tests.

In the oral thiamethoxam test for *T. angustula*, the lowest slope inclination was obtained, which explains the great difference between the LD_{50}andLD_{90}; second is the thiamethoxam topical test on *S. xanthotricha* where the same behavior is observed between the two lethal doses. This low slope inclination agrees with the higher doses of the LD_{50} calculated in this study and make the different reaction of the two species to thiamethoxam evident. According to Zhu *et al.* (2015) the risk is influenced by the products concentration at field application and the slope of the dose response curve, a high inclination of a slope indicates a high sensitivity to the pesticide; small dose increase causes high mortality.

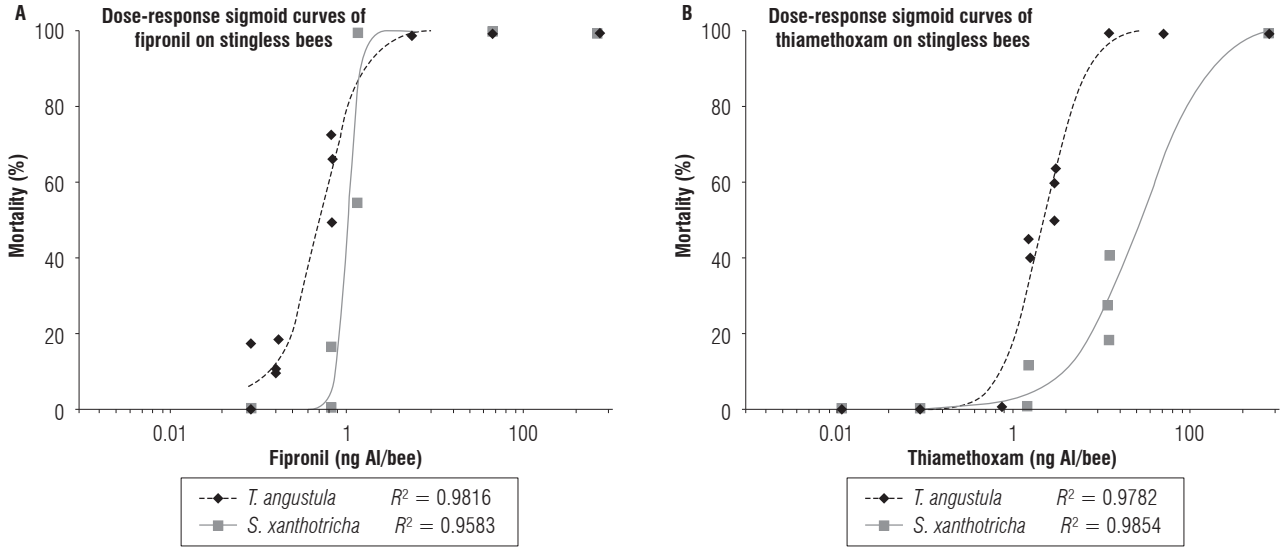

FIGURE 1. Dose curve response evidencing the lethal effects of a dilution series based on the respective MDRC of fipronil (A) and thiamethoxam (B) (1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, 1/4096); topical application on *T. angustula* and *S. xanthotricha* (mortality was measured 48 h after treatment).

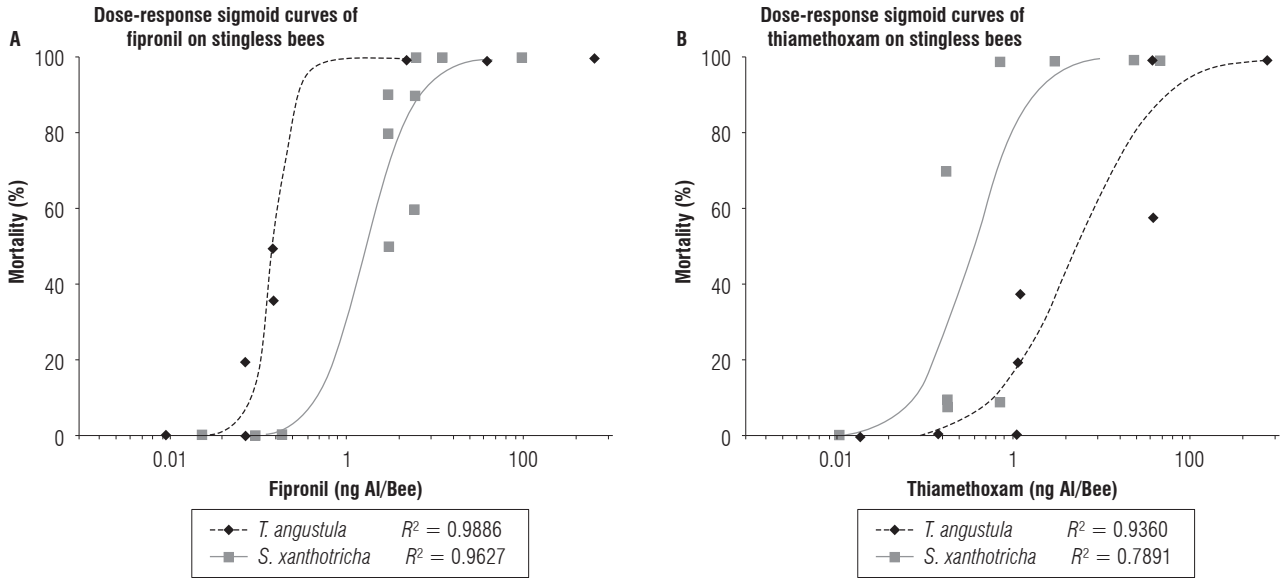

FIGURE 2. Dose curve response evidencing the lethal effects of a dilution series based on the respective MDRC of fipronil (A) and thiamethoxam (B) (1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, 1/4096), oral exposure of *T. angustula* and *S. xanthotricha. (*Mortality was measured 48 h after treatment).

TABLE 2. Effect of thiamethoxam and fipronil o≠n the stingless bee *Tetragonisca angustula*.

Insecticide	Exposure	Slope±SE	LD_{50} (95% LC[1])	LD_{90} (95% LC)	Fraction MRFD[2]	Pr>Chi
Thiamethoxam	Oral $_{(ng/bee)}$	0.91±0.30	5.331 (1.41-20.16)	49.68 (24.36-148.66)	1/140	<0.0001
	Topical $_{(ng/bee)}$	1.857±0.27	1.86 (1.79-2.51)	5.097 (3.77-8.94)	1/403	<0.0001
Fipronil	Oral $_{(ng/bee)}$	3.536±0.93	0.15 (0.14-0.17)	0.272 (0.2-0.75)	1/5000	<0.0001
	Topical $_{(ng/bee)}$	1.932±0.61	0.56 (0.42-0.76)	1.783 (1.11-4.05)	1/1339	<0.0001

[1] Confidencial level; [2] maximum recommended field dose.

TABLE 3. Effect of thiamethoxam and fipronil on the stingless bee *Scaptotrigona xanthotricha.*

Insecticide	Exposure	Slope±SE	LD_{50} (95% LC[1])	LD_{90} (95% LC)	Fraction MRFD[2]	Pr>Chi
Thiamethoxam	Oral $_{(ng/bee)}$	1.34±0.65	0.37 (0.148-0.921)	1.235 (0.084-2.46)	1/2032	<0.0001
	Topical $_{(ng/bee)}$	1.11±0.53	27.78 (9.54-80.88)	126.7 (52.55-919.32)	1/27	<0.0001
Fipronil	Oral $_{(ng/bee)}$	1.564±0.60	1.632 (0.84-3.17)	5.89 (4.37-9.62)	1/459	<0.0001
	Topical $_{(ng/bee)}$	6.56±2.302	1.124 (0.89-1.418)	1.415 (1.3-1.53)	1/667	<0.0001

[1] Confidencial level; [2] maximum recommended field dose.

It is of great importance to know the sensitivity and the reaction of species to insecticides, based on the slope of the dose response curve. This slope describes the doses that may or may not cause a sub-lethal effect can be inferred and it also implies a contribution when planning the doses for toxicological studies of sub-lethal effects.

Routes of exposure to pesticides: There are multiple routes of exposure to pesticides that affect bees, being direct contact with a pesticide solution and with the powder from treated seeds or the application of pesticides in granular form to the soil the most common ones. Oral exposure to residues; or products translocated by the plant that are present in the pollen or nectar of planted or associated plants within or at the crops border and nearby crops; all the exposed aerial applications; the application of granules to the soil; the seed treatment or the absorption of residues from previous grown crops (EFSA European Food Safety Authority, 2013c; Godfray *et al.*,2014) are considered highly hazardous .In addition, it is assumed, that its response also depends on intrinsic factors, such as ecology, biology, physiology, morphology and extrinsic factors such as climate and flora.

No research was found on the consumption of free environmental water by stingless bees; however, a topical or oral exposure to insecticides can be potentially considered either by the guttation drops from some crops treated with systemics, the consumption of contaminated water from puddles or ditches or droplets present on plant surfaces or on soil as a remain product of the irrigation, as mentioned by Girolami *et al.* (2009) for *A. mellifera*. These studies should be carried out for stingless bees. It is of special importance to consider the pesticide or its metabolites risks and how to measure their effects, matter slightly studied so far, once these routes of exposure have been verified.

If bees use guttation drops to support the hive, it is important to study the content of thiamethoxam and its metabolites in plants such as *Fragaria* x *ananassa* (Strawberry), where the product is used by means of foliar application and in regular irrigation water. Strawberry flowers are those normally visited by *T. angustula* (Imperatriz-Fonseca *et al.,* 2015). Many Gramineae such as *Zea mays* although they do not require bee-assisted pollination, could provide guttation droplets to bees after through secretions by maize and pollen. This solution has been found to contain thiamethoxam residues when treated seeds are planted or granules are applied to the soil (Godfray *et al*., 2014; EFSA European Food Safety Authority, 2013a, 2013b; Simon *et al*., 2013).

Intoxication symptoms: After topical application and oral exposure, acute poisoning symptoms were observed with the highest doses of both insecticides. Intoxication with fipronil of *S. xanthotricha*, initially induced a high excitation condition, represented by wing movement, vibration and circular movements at the same site. Later, **s**lowness in movement, tremor and a displacement difficulty was observed, which also occurred in another stingless bee, *M. scutellaris* when exposed orally and topically to the same compound (Lourenço *et al*., 2012); *T. angustula* symptoms were less evident due to their slower movement dynamic, but it was also observed that individuals struggled with difficulty and trembled to death. After less than 4 h after the application event the individuals were dead showing a full spread of wings and legs. With thiamethoxam poisoning, individuals of both species started to move rapidly throughout the box; minutes later they slowed down and were paralyzed, agreeing with the symptoms observed for *S. postica* when exposed to imidacloprid (Soares *et al.,* 2015); in some cases the bees died clinging to the mesh of the experiment boxes (Fig. 3). The dead individuals showed a swollen abdomen (Fig. 4) and legs and wings contracted.

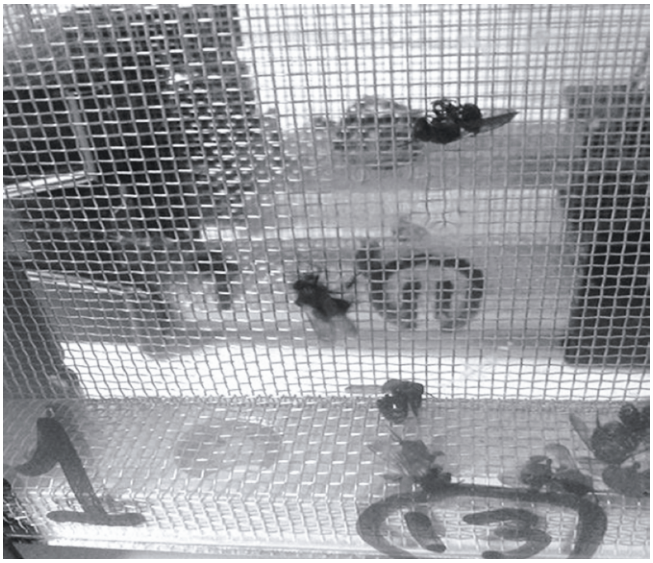

FIGURE 3. *Scaptotrigona xanthotricha* dead and firmly entangled on the mesh of the cage after topical exposure to thiamethoxam.

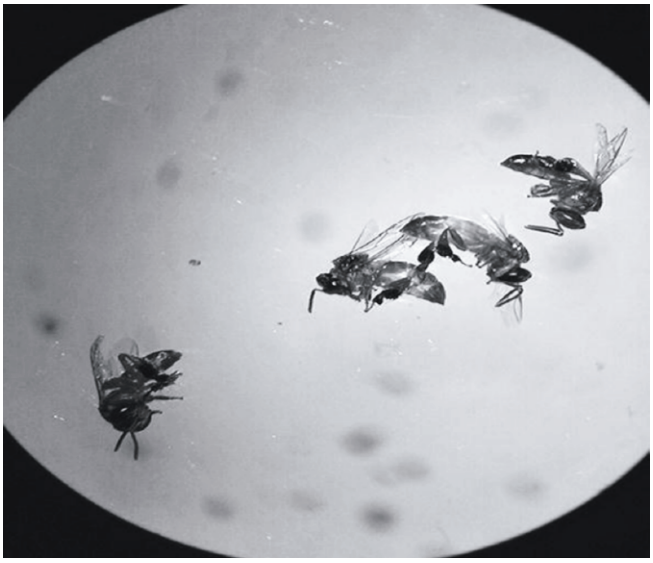

FIGURE 4. *Tetragonisca angustula* dead with swollen abdomen after oral contamination with thiamethoxam.

Toxicological comparisons: Fipronil has been evaluated on several species of stingless bees, as well as on domestic bee *A. mellifera*. Jacob *et al.* (2013) reported a topical LD_{50}: 0.54 ng/bee and oral LC_{50}: 0.24 ng μL^{-1} diet to *S. postica*. On the other hand, Lourenço *et al.* (2012) recorded a topical LD_{50} of 0.41 ng/bee and an oral LC_{50} of 0,011 ng μL^{-1} diet on *Melipona scutellaris*. Comparing the results of this study with those obtained by the mentioned authors, it is observed that both species are more susceptible than *S. xanthotricha* with both topical exposure and oral administration, whereas *S. postica* is slightly more tolerant to the insecticide than *T. angustula* provided in oral form, but in turn more tolerant than *M. scutellaris*. When *A. mellifera* was exposed topically to Fipronil, Vidau *et al.* (2011) obtained an LD_{50} of 417 ng/bee, which shows that both species are more susceptible than the domestic bee.In relation to thiamethoxam, Valdovinos-Nuñez *et al.* (2009) obtained a topical LD_{50} of 4 ng/bee to *Nanotrigona perilampoides*, which reflects a susceptibility, approximately 6 times greater than *S. xanthotricha*, but a higher tolerance to the product than *T. angustula*. Even so, the topical LD_{50} reported by Zhu *et al.* (2015) on *A. mellifera* was equivalent to 40 ng/bee, twice the obtained value to *S. xanthotricha* and more than 30 times greater than the LD_{50} for *T. angustula*.

Finally, the research carried out by Tomé *et al.* (2015) showed that Imidacloprid is lethal in doses used in the field for the *S. xanthotricha*, as has also been observed in the present study, where thiamethoxam and fipronil were lethal to all individuals of the two species, when exposing the bees to the doses used during field application.

The EFSA European Food Safety Authority (2013c) states that both bumblebees and solitary bees, because of their own biology, ecology and physiology, are more susceptible to pesticides than *A. mellifera*. Based on the results of this study, the stingless bees could be included in this concept amplifying EFSA´s statement.

Comparing the susceptibility of both bees to insecticides, as well as their capacity to adapt to agroecosystems, allows to recognize their potential as pollinators that could replace *Apis mellifera*. Jaffe *et al.* (2015) found that *Trigona snipes* is able to colonize degraded environments and may persist in highly altered landscapes, being an example of a "rescue pollinator".

Susceptibility and risk: In general, a greater susceptibility of both species to fipronil was detected. *S. xanthotricha* also presents a high susceptibility to thiamethoxam in oral exposure. Furthermore, fipronil is toxic to the 50 and 90% of the sample in doses lower than 2 ng/bee. Representing a high toxicity after applying the classification criteria proposed by Atkins *et al.* (1981), which indicates that insecticides with LD_{50} less than 2 µg/bee (2,000 ng/bee) are highly toxic to this group of insects.

In the case of thiamethoxam, *S. xanthotricha* showed a higher tolerance to topical exposure than *T. angustula*, although there is a high toxicity to oral exposure. This result is considered concerning, due to the high systemicity of the product, which, being soluble in water, is rapidly translocated to all parts of the plant and represents a risk

due to in foliar application, soil or seed treatment. Based on this mode of action, the European Union banned its use in many crops, considered attractive to bees or in those in which the bees could be exposed to the compound (The European Commission, 2013).

The maximum recommended dose for field applications represents the dosage suggested by the formulator of the commercial product. In both cases the dose from which the dilutions were made was 3 g L^{-1}. Therefore, direct contact in the field during spraying or exposure to fresh or dry residues could be of great detriment to the survival of the hive of both species.

The evaluation of the potential risk of a pesticide requires not only the calculation of the LD_{50} but also the evaluation of larval toxicity, the effects on their behavior, the effect on the hypopharyngeal gland as well as the cumulative effect of the pesticides (EFSA European Food Safety Authority, 2013c). So far, no research on this topic for Meloponini has been carried out.

This basic research demonstrated the susceptibility of the *T. angustula* and *S. xanthotricha* to the action of thiamethoxam and fipronil when administered orally or topically. A greater susceptibility of these two species of native bees than that of *A. mellifera* to these pesticides was confirmed. There are several reasons that could explain this greater susceptibility, such as an adaptation and selection processes of *Apis* being over time exposed to insecticides, size and the presence or absence of structures that could protect from direct contact with the compound, foraging habits associated with weather and the moment an application is made, among other causes.

There is very little information and studies on stingless bees and without them it is impossible to establish with any certainty the actual risk represented by pesticides for these bees. It is of great importance to carry out studies on native stingless bees, taking into account their role as pollinators in the diversity of fauna and flora. At present for registration and use of pesticides only the LD_{50} on *A. mellifera* is considered, without knowing fully the real risk even on this species. The consumption of pollen and nectar has to be quantified, as well as the use of guttation water, to evaluate all routes of exposure and to fully identify the risk that for the meliponines can represent pesticides, used under field conditions.

In front of CCD and the continuing degradation of ecosystems, meliponines can play a crucial role regarding global food production and food security, up to now, no reports of CCD affecting meliponine hives have been reported, probably due to differential characteristics of *A. mellifera* and stingless bees. Eventually they could recover the role they had as pollinators before the introduction of the *A. mellifera* in the tropical and subtropical region.

The great Meloponini biodiversity richness of Latin America is, among many others, a justification to increase efforts in native bee species conservation and reducing the impact of agriculture practices on ecosystems.

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Effect of deficit irrigation on yield and quality of pear (*Pyrus communis* cv. Triumph of Vienna)

Efecto del riego deficitario en la producción y calidad de la pera (*Pyrus communis* L.) variedad Triunfo de Viena

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ABSTRACT

Crop demands for irrigation require different technologies to optimize the use of water. Regulated Deficit Irrigation (RDI) is a strategy that enables a significant reduction of water application without affecting the crop yield and quality, with the advantage of being a tool for control of vegetative growth. The present study was conducted in Sesquilé, Cundinamarca (Colombia) between 2015 and 2016. The objective was to evaluate the quality and development of pear crop (*Pyrus communis* L. cv. Triumph of Vienna) on field conditions, using three treatments of 100%, and 25% of water requirement (ETc) and no irrigation, applied at the rapid fruit growth stage. The mid day stem water potential, plant water relations, pressure-volume curve, fruit yield and quality were evaluated. There were no significant differences in the yield and quality of the fruits among the different irrigation treatments. The trees had the mechanisms of osmotic adjustment, which allowed water stressed trees to cope with irrigation restrictions during the rapid fruit growth stage without affecting the yield.

Key words: water savings, osmotic adjustment, water consumption, water relations, deciduous.

RESUMEN

La demanda de riego en los cultivos requiere diferentes tecnologías para optimizar el uso del agua. El Riego deficitario controlado (RDC), es una estrategia que permite una reducción significativa en la aplicación de agua sin afectar el rendimiento y la calidad del cultivo, con la ventaja de ser una herramienta para controlar el crecimiento vegetativo. El presente estudio se realizó en Sesquilé, Cundinamarca (Colombia) entre 2015 y 2016 con el objetivo de evaluar la calidad y el desarrollo del cultivo de pera (*Pyrus communis* L. cv. Triumph of Vienna) en condiciones de campo, utilizando tres tratamientos: 100%, 25% de requerimiento de agua (ETc) y sin riego, aplicados en la etapa de crecimiento rápido del fruto. Se evaluó el potencial hídrico del tallo del mediodía, las relaciones hídricas de la planta, la curva presión-volumen y el rendimiento y la calidad del fruto. No se presentaron diferencias significativas en el rendimiento y la calidad de las frutas entre los diferentes tratamientos de riego. Los árboles mostraron mecanismos de ajuste osmótico, lo que permitió a aquellos con estrés hídrico hacer frente a las restricciones de riego durante la etapa de crecimiento rápido del fruto sin afectar el rendimiento.

Palabras clave: ahorro de agua, ajuste osmótico, consumo de agua, relaciones hídricas, caducifolio.

Introduction

Worldwide, it is estimated that the total extraction of fresh surface water and groundwater for agricultural purposes is 69%, including irrigation, livestock and aquaculture (FAO, 2014). Around 50% of this water consumption corresponds to crop evapotranspiration (Kohli, *et al*., 2010) including fruit trees which are highly dependent on irrigation (Naor, 2006).

In Colombia, Boyacá is considered the most important area in production of deciduous fruit crops (pear, peach, plum and apple) About 5.382 ha were grown in 2010, which 31% relates to pear production. These crops continue their expansion, thus, it demands technological advances in the improvement of plant material, sanitary conditions, and irrigation techniques (Miranda *et al*., 2013).

The growers are conditioned by the available water that is scarce in some areas (De la Rosa *et al*., 2015), which makes it necessary to employ strategies to improve efficiency and optimize its use (Cui *et al*., 2009). Those strategies are based on the detection of plant response to water deficit (De Swaef*et al*., 2008) by means of indicators of plant and soil water status (water potential, sap flow, and trunk diameter

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variation) to determine the best irrigation scheduling (Rallo *et al*., 2017).

Regulated deficit irrigation (RDI) has been widely used in deciduous and other fruit tree crops, such as citrus and grapevine (McCarthy, 2005) and consists of reducing water supply only in periods when fruit growth is less sensitive. The trees have shown various mechanisms of adaptation to water deficit, which usually imply osmotic adjustment, changes in tissue elastic properties, stress evasion by stomatal closure, and change in leaf area, amongst others (Torrecillas *et al*., 2001).

Several plant indicators have been considered as a tool for irrigation scheduling; one of the most used is the midday stem water potential (Ψ_t) (Intrigliolo and Castel, 2006). Based on this indicator, it is possible to know the plant water condition and its response to RDI. Techniques such as the Pressure-Volume (PV) curve are useful to determine if there is an osmotic adjustment due to water restriction (Mellisho*et al*., 2011).

The aim of this study was to evaluate the response of pear trees to water restriction and the influence of RDI during the rapid fruit growth stage on yield and quality of fruits in order to develop strategies to optimize the use of water on deciduous crops.

Materials and Methods

Location

The experiment was conducted from October2015 to April 2016, in an open field pear orchard (*Pyrus communis* L. cv. Triumph of Vienna) at the plot known as "Finca San Benito", located in Sesquilé, Cundinamarca (5°02' N and 73°47' W, elevation 2,595 m a.s.l.). Grafted pear trees of the cv. "Triumph of Vienna" with a well-developed root system were planted in 1998, in a spacing pattern of 4×4 m. All of them had similar management of pest and disease control, edaphic fertilization was applied twice a year and fertigation through the drip irrigation system every 15 d. The total amount applied was 60, 44, 100 k of N, and, respectively, using N32 liquid nitrogen (32%, N); 83% phosphoric acid (53%,); potassium nitrate (46% N, 13%). Additional applications of phosphorus, potassium and boron were performed.

The soil was identified as Histosol, with loamy and clay texture. The volumetric moisture at field capacity and at permanent wilting point was 26.9% and 15.3%, respectively. The content of organic matter, potassium and phosphorus were 5.06%, 78.2 and 23.9, respectively and pH was 4.6 (Molina *et al*., 2015).

Climate and Irrigation

The crop irrigation needs (ETc) were calculated according to Penman-Monteith method using a maximum crop coefficient (Kc = 0.8) at full canopy growth (Allen *et al*., 2006). The irrigation was applied through a drip system with two lines per row of trees and six 8 drippers per plant. Three treatments were applied from 60 to 140 d after full bloom (DAFB), corresponding to the rapid fruit growth stage (December 26, 2015 to March 9, 2016): (T1) Control in which the plants were irrigated at 100% of Etc, (T2) irrigation at 25% of T1 and (T3) no irrigation. During the rest of the season all plants were irrigated at 100% ETc. Weather conditions (temperature, relative humidity, precipitation, wind speed, solar radiation) were measured using an automatic weather station WS-GP1 (AT delta-T Devices Ltda., Cambridge, UK) located on the plot. The volume of irrigation water applied was measured with 12 mm volumetric counters Controlagua® (F.F. Soluciones S.A, Colombia). The accumulated volume of irrigation water applied during the treatments (60 to 140 DAFB) was 1460, 394, and 0 L per tree respectively in T1, T2, and T3.

Plant water relations

The midday stem water potential (Ψ_t) was measured every 8 d on two leaves of two trees in each replicate (16 leaves per treatment) selected from the inner part of the canopy, enclosed in an aluminum covered plastic bag during 90 min before the measurement with a pressure chamber (Model 600 Pressure Chamber Instrument, PMS Instrument Company, Oregon, USA) (Naor *et al*., 1995).

At mid-water restriction season (February 19, 2016), PV curves were performed according to the free transpiration technique (Tyree and Hammel, 1972) in order to determine the osmotic potential at the turgor loss point(Ψ_{oppt}), modulus of elasticity (ε), apoplastic relative water content (RWC_a) and relative water content at the turgor loss point (%RWC_{ppt}) (Mellisho*et al*., 2011). To obtain the curve, 20 leaves were cut per replicate and the petioles were immersed in distilled water for 24 h. Once saturated, the fresh weight of each leaf was measured with a precision electronic balance 0.1 mg (Precisa XT2202A, Dietikon, Switzerland) and then the leaf water potential (Ψ) was determined using the Scholander pressure chamber, finally, leave fresh weight was measured again. The procedure was performed successively at regular intervals as the leaves were dehydrated under ambient conditions until the minimum weight loss was found (Corcuera, 2003), then leaves were oven dried at 60° C for 72 h to determine the dry weight.

To find the osmotic potential at full turgor (Ψ_{100}) four saturated leaves were taken from each replicate and wrapped in aluminum foil and immediately frozen in liquid nitrogen in order to halt their metabolic activity (Abril, 2015). 72 h after, the tissue was macerated and centrifuged for 10 min at 10,000 rpm in order to extract the cellular fluids and determine Ψ_{100} using an Osmometer (Vapro®, Wescor, USA). The osmotic adjustment (ΔO) was estimated as the difference between Ψ_{100} of the plants of T2 Ψ_{100} and T3 and of the T1 Control treatment (Ruiz *et al*., 2000).

Yield and quality

All the fruits at physiological maturity were harvested from all the trees. Two fruits were randomly picked by treatment and replicate (8 fruits per treatment), to which they were determined: The higher (a) and minor (d) equatorial diameter and length (L) using a manual calibrator; fresh weight of each fruit using a precision balance 0.1 mg (Precisa XT2202A, Dietikon, Switzerland); the volume from the displacement of distilled water in a graduated 1,000 mL container; the color of the pulp and the peel using a colorimeter Chromameter CR-400 (Konica Minolta®, Japan); titratable total acids (TTA) using Titroline® 6000 (SI Analytics, Japan) automatic titrator with 0.1 N sodium hydroxide (NaOH); total soluble solids (TSS) with manual optical refractometer (Kikuchi Precision Optics, Tokyo, Japan); maturity index (IM) as the relationship: IM = , (Rodríguez *et al*., 2010) and firmness using 2 mm test with CT3 TextureAnalyzer (Brookfield Engineering Labs., USA) in the equatorial direction and at the poles of each fruit.

Statistical analysis

The experiment was carried out in a randomized block design with four replicates (12 plots with 12 to 20 trees each). Measurements were taken on two adjacent trees per replicate, considering the edge effect. Data were analyzed with ANOVA, and means were compared using Duncan's test at $P \leq 0.05$ using the software InfoStat 2016 from InfoStat, FCA, Universidad Nacional de Córdoba, Argentina (Di Rienzo *et al*., 2016).

Results and Discussion

Climate and irrigation

During the study, the weather variables were measured. The minimum temperature, in January 2016 was 2.9°, and the maximum was 27.8°C, the mean was 14°C. The mean relative humidity was 81%, the highest was in April (98%) and the lowest was in January (60%). Mean ETo was 3.1 mm d^{-1}, a 14% lower than the maximum ETo recorded in the year (3.6 mm d^{-1}). The average vapor pressure deficit (VPD) was in the range of 0.62 (in January) to 0.03 (in April). The total precipitation during the restriction period was 218 mm (Fig. 1).

Plant water relations

The Ψ_t was influenced by precipitation. From 80 to 140 DAFB a significant difference was observed between T3 and other treatments according to Duncan's test ($P \leq 0.05$), indicating that Ψ_t was a good indicator of the irrigation regimes applied. The Ψ_t had the same values for all three treatments at 150 and 170 DAFB due to rainfall. During the treatments, there was an increase of Ψ_t represented by more negative values in T3 (mean -1.17 MPa) than T2 (mean -0.9 MPa) and T1 (mean -0.9 MPa). T3 had a maximum of -1.78 MPa at 126 DAFB while there were no significant differences between T1 and T2, which means that the amount of

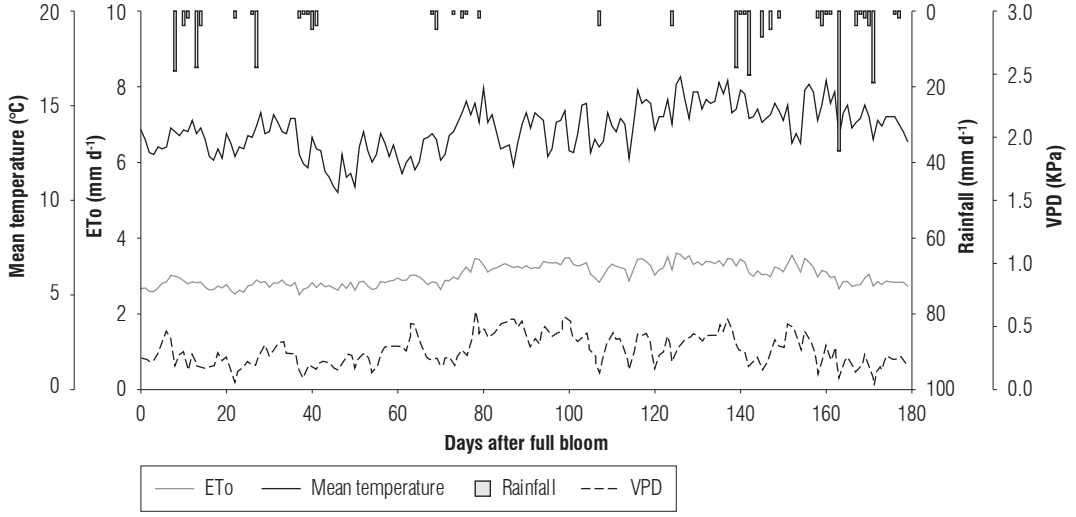

FIGURE 1. Climatic parameters during the crop cycle. Mean temperature, potential evapotranspiration (ETo), Rainfall and vapor pressure deficit (VPD).

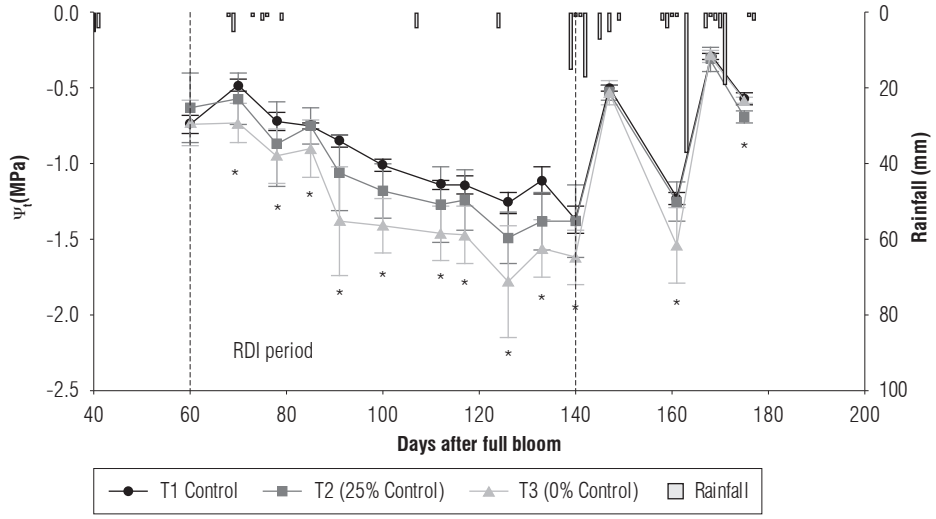

FIGURE 2. Variation of the midday stem water potential (Ψ_t) in T1, T2 and T3 obtained as the mean of 4 replicates per treatment and rainfall during the crop cycle. Bars indicate standard error and asterisks indicate significant difference between treatments.

water applied in T2 allowed to maintain water conditions of the plant similar to the Control (Fig. 2). Similar results in the same crop were reported by Abril (2015), with Ψ_t between -0.3 MPa and -1.22 MPa.

In other studies in pear, Caspari *et al*. (1994) reported more negative values of in trees variety 'Hosui' under water restriction (-2.0 MPa to -1.7 MPa), while Morandi *et al*. (2014) found values of Ψ_t between -0.6 MPa and -1.25 MPa for the 'AbbeFettel' variety, very similar to that reported by Marsal *et al*. (2002) (-0.5 MPa to -1.5 MPa) in pear 'Blanquilla' and Naor (2001) (-1.2 MPa and -3 MPa) in pear variety 'Spadona' under RDI treatments.

The Ψ_t was slightly higher in comparison to other species such as apricot (-1.08 MPa to -1.87 MPa) (Pérez *et al*., 2014) and jujuba (-1 MPa and -4 MPa) (Cruz *et al*., 2012). On the other hand, Ψ_t had a behavior similar to that reported by Samperio *et al*. (2015) in 'Red Beaut' plum (between -0.59 MPa and -0.70 MPa) and Podestá *et al*. (2010) who found a maximum Ψ_t of -1.53 MPa in cherry trees under water deficit.

At 119 DAFB (59 d of water restriction, the day on which the PV curve was performed) there was a significant difference that showed osmotic adjustment in Ψ_{100} between T3 and T1 (-3.1and -2.53 MPa, respectively). The greatest difference respect to Control was observed in the treatment T3 (0.57 MPa) that coincides with that reported in the apricot tree by Ruiz*et al*. (2000). The decrease in the osmotic potential can be attributed to the active solute accumulation on the leaf tissues which has been considered as a mechanism of osmotic adjustment in mature peach plants and depends on the species, variety, severity of water restriction, leaf maturity and the time in which the restriction was applied (Marsal and Girona, 1997; Cruz *et al*., 2012).

The other parameters derived from the PV curve did not present a significant difference according to the Duncan's test ($P \leq 0.05$) due to the treatments, similar to the results reported by Mellisho *et al*., 2011 in peaches under water restriction where were no differences respect the Control in the turgor loss point (Ψ_{ppt}, WRC_{ppt}), or in de modulus of elasticity (ε), however they found osmotic adjustment of 0.18 MPa, that is lower than the values founded on this study.

It is important to note that these plants were under RDI treatments in previous years, which may have been preconditioned or affected (Ruiz*et al*., 2000) causing their water status to be maintained. It is also observed that the increase of in Ψ_{100} T3 could contribute to maintain the turgor in the plant as observed in the values of Ψ_{oppt} (-5.9 MPa) and ε (11.5 MPa) slightly higher for T3.

Yield and quality

The harvested fruits were classified into three size categories according to diameter: category I larger than 65 mm, category II between 65 and 50 mm and category III less than 50 mm. Fruit yield per tree (kg/tree) was higher for T1 (23.59 kg) without significant difference with T2 and T3 according to the Duncan's test ($P \leq 0.05$). The mean weight of the fruits was homogeneous between treatments with 160g, 170 g and 170 g for I, II and III respectively, it was similar to data found by Molina (2014) (140 g) and slightly lower than the reported by Abril, (2015) (200 g) in the same orchard. The number of fruits per tree varied between treatments

TABLE 1. Parameters of the pressure-volume curve for T1, T2 and T3 obtained as the mean of 4 repetitions per treatment and its standard error (S.E). Equal letters between columns indicate that there was no significant difference between treatments according to Duncan's test ($P \leq 0.05$).

	T3	S.E	T2	S.E	T1	S.E
Osmotic potential at full turgor (Ψ_{100})(MPa)	-3.1 b	0.08	-2.86 ab	0.11	-2.53 a	0.22
Apoplastic relative water content (RWC_a)(%)	89.51 a	1.54	90.54 a	3.14	90.46 a	2.03
Osmotic potential al turgor loss point (MPa) (Ψ_{oppt})	-5.9 a	2.93	-5.3 a	0.49	-5.02 a	0.60
Modulus of elasticity (ε) (MPa)	11.5 a	0.78	10.74 a	0.594	9.17 a	1.982
Relative water content at turgor loss point (%) (RWC_{ppt})	94.85 a	3.64	95.35 a	1.82	95.16 a	1.10
Osmotic adjustment (MPa) (Δ0)	0.57		0.33			

TABLE 2. Fruit yield for T1 (1460 L/tree), T2 (394 L/tree) and T3 obtained as the mean of 4 replicates per treatment and their standard error (SE). Equal letters between columns indicate that it was not significantly different between treatments according to the Duncan's test ($P \leq 0.05$).

		T1	S.E.	T2	S.E.	T3	S.E.
Yield (kg/tree)	I	14 a	3.28	10.7 a	1.43	10.7 a	1.43
	II	9.35 a	2.39	6.5 a	1.32	8.18 a	1.1
	III	0.28 a	0.11	0.63 a	0.19	0.63 a	0.21
	Total	23.59 a		17.86 a		19.5 a	
Fruit mean weight (g)	I	200 a	0	230 a	0.03	230 a	0.03
	II	180 a	0.03	180 a	0.03	200 a	0
	III	100 a	0	100 a	0	100 a	0
	Media	160 a		170 a		170 a	
Fruits/tree	I	61 a	14	46 a	7	45 a	6
	II	49 a	9.03	40 a	8.26	51 a	5.96
	III	3 a	1.11	6 a	1.66	6 a	2.12
	Total	113 a		92 a		102 a	

without significant differences according to Duncan's test ($P \leq 0.05$). More fruits were obtained per tree in T1 (113 fruits) than in T2 (92 fruits) and T3 (102 fruits). The highest number of fruits corresponded to categories I and II in all treatments, with no significant differences, meaning that the majority of the fruit corresponded to fruits larger than 50 mm coinciding with Pérez *et al.* (2014); De la Rosa *et al.* (2015), who did not find significant differences in peach yield under moderate RDI treatments.

Although there was no significant difference in yield, there is a slight variation of T1 considering that the water status of the tree (Ψ_t) did not show significant differences between T1 and T2. The results suggest that the T2 represents water saving up to 75% during the rapid fruit growth stage (obtaining similar yield results).

As the plant water relations did not present significant differences between treatments, there was no difference in the quality parameters: volume, density, sphericity, color index (CI) of pulp and peel, firmness and TSS according to Duncan's test ($P \leq 0.05$) (Tab. 3) similar to that found by De la Rosa *et al.* (2015) in nectarines, indicating that the fruits in the three treatments present similar quality characteristics.

The sphericity of the fruits was 0.96 which is very close to one (1) indicating an almost spherical shape typical of the Triumph of Vienna pears what may be a desired attribute of quality for consumers. The titratable total acidity (TTA) and maturity index (MI) were the only parameters with significant difference according to the Duncan's test ($P \leq 0.05$) between Control and T2 and T3. Because the MI is derived from the relation between TSS and TTA, its variation is due to the reduction of malic acid quantity for T1. Parra *et al.* (1998) reported mean TSS values similar to those found (12.67 °Brix) indicating that as the fruit grows and develops in the plant, °Brix increases, while the acid content decreases. The MI had values higher than those reported by Arenas (2012) for the same species under normal irrigation conditions and lower than those obtained by Abril (2015) (41, 48 y 46 °Brix gL^{-1} for T1, T2

TABLE 3. Fruit quality parameters for T1, T2 and T3 obtained as the mean of 4 replicates per treatment and their standard error (SE). Equal letters between columns indicate that it was not significantly different between treatments according to the Duncan's test ($P \leq 0.05$).

	T1	SE	T2	SE	T3	SE
Volume (cm^3)	186 a	13.212	178.38 a	8.831	173.38 a	10.72
Density ($g\ cm^{-3}$)	1.07 a	0.037	1.03 a	0.038	1.15 a	0.075
Sphericity	0.96 a	0.008	0.96 a	0.006	0.96 a	0.008
CI of peel	-1.78 a	0.902	-1.05 a	0.698	-1.54 a	0.618
CI of pulp	-1.78 a	0.902	-1.05 a	0.698	-0.53 a	0.205
Poles firmness (N)	11.51 a	1.020	11.9 a	1.295	12.26 a	0.609
Ecuador firmness (N)	15.19 a	1.138	15.71 a	0.748	13.99 a	1.001
TSS (°Brix)	14.21 a	0.315	14.38 a	0.514	14.91 a	0.488
Titratable total acids (% malic acid)	0.33 a	0.021	0.43 b	0.037	0.48 b	0.024
Maturity index ($°Brix\ gL^{-1}$)	44.1 a	2.744	34 b	3.157	30.76 b	2.079

y T3 respectively) and Molina (2014) (61, 65 y 61 $°Brix\ gL^{-1}$ for T1, T2 y T3 respectively).

Conclusions

Pear trees were resistance to the water deficit as they performed osmotic adjustment, but osmotic potential was similar in all treatments. The application of RDI did not affect the yield or quality of the fruits in relation to the well irrigated trees, indicating that a similar production can be achieved with savings of water up to 100% during the rapid fruit growth stage.

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Isolation and evaluation of the antagonist activity of lactic acid bacteria in raw cow milk

Aislamiento y evaluación de la actividad antagonista de bacterias ácido lácticas en leche cruda de vaca

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ABSTRACT

Lactic acid bacteria (LAB) are considered as a good alternative to reduce the risk of food borne diseases in food industry. In addition to the improvement effects on the organoleptic characteristics of fermented foods from the LAB metabolites, they can inhibit the growth of microorganisms responsible of the food spoilage. This work is an advance on the biodiversity exploration of natural additives in food. Isolation, identification and screening of potential antimicrobial activity of LAB were the aims on this work. Species of *Lactobacillus (Lb. casei, Lb. brevis, Lb. paracasei,* and *Lb. plantarum*) and *Pediococcus acidilactici* were identified and their antagonism against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 7644 was demonstrated.

Key words: antagonistic bacteria, raw milk, *Lactobacillus*, isolation, pathogen inhibition.

RESUMEN

Las bacterias ácido lácticas (BAL) son una alternativa en la industria de alimentos para la reducción del riesgo que representan las enfermedades transmitidas por algunos alimentos. Los metabolitos típicos de las BAL, además de sus reconocidos efectos sobre las características organolépticas de los alimentos fermentados, pueden inhibir el crecimiento de microorganismos responsables del deterioro e incluso la aparición de patógenos. Este trabajo es un avance en la exploración de la biodiversidad y en la búsqueda de aditivos naturales con aplicación en la industria de alimentos. Los principales logros de este trabajo fueron el aislamiento, identificación y evaluación de la actividad antimicrobiana de BAL. Se identificaron especies de *Lactobacillus (Lb. casei, Lb. brevis, Lb. paracasei* y *Lb. plantarum*) y *Pediococcus acidilactici* que presentaron antagonismo frente a *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 y *Listeria monocytogenes* ATCC 7644.

Palabras clave: bacterias antagónicas, leche cruda, *Lactobacillus*, aislamiento, inhibición de patógenos.

Introduction

Foodborne diseases are the most serious and expensive issues in food industry. According to the USDA (United States Department of Agriculture) annually in USA, 48 million people suffer foodborne illnesses and 3000 are reported as deadly cases (FDA and Administration, 2013). New meals, manufacturing processes, and the growing demand for minimally processed products (ready-to-eat) increase the possibility of microbiological contamination. Alternative food preservation technology such as bio-preservation is a reliable option to extent the shelf-life and to enhance the hygienic quality, minimizing the impact on the food nutritional and organoleptic properties (García *et al*., 2010). Bio-preservation uses the antimicrobial potential of non-pathogen microorganisms or their metabolites to inhibit the growth of pathogens or spoilage related microorganisms (Nath *et al*., 2014; Settanni and Corsetti, 2008; Smid and Lacroix, 2013).

Different strains of microorganisms with potential use as bio-preservative agents have been reported (Ghanbari *et al*., 2013; Henning *et al*., 2015; Hwanhlem *et al*., 2014). Dairy products are one group of foods commonly used to obtain strains with antagonistic features. The most important microorganisms with antagonistic characteristics and potential use in food industry are lactic acid bacteria (LAB). They have been traditionally associated to food and are considered safe (García *et al*., 2010). LAB are Gram positive bacteria, non sporulating, anaerobic facultative, catalase and coagulase negative, tolerant to acidic conditions and with low content of guanine and cytosine in their DNA. LAB belong to the group of Firmicutes, Lactobacillales order and the most representative genera are *Aerococcus,*

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Alloiococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Symbiobacterium, Tetragenococcus, Vagococcus, and *Weissella* and in phylum Actinobacteria with genera *Atopobium* and *Bifidobacterium* (Giraffa, 2012). Many LAB are considered as probiotics or live microorganisms, that consumed in adequate amounts, confer a health benefit on the host (Fijan, 2014).

LAB are successful habitat competitors due to their ability as competitive exclusion (Settanni and Corsetti, 2008) which consists on releasing antimicrobial substances that are able to affect the development of other microorganisms (Smid and Lacroix, 2013). The main product of LAB metabolism is lactic acid but they can produce other organic acids and compounds as hidrogenus peroxide (H_2O_2), carbon dioxide (CO_2), diacetyl (2,3-butanediona), reuterin and bacteriocins (Ammor *et al.*, 2006; Khan *et al.*, 2010; Nath *et al.*, 2014). Organic acids, especially lactic acid, are metabolites produced as a result of sugar metabolism. They are released to the environment reducing its pH, inhibiting the development of some populations of undesirable microorganisms (Okano *et al.*, 2010). Hydrogen peroxide (H_2O_2) has an oxidizing effect over sulfhydryl groups of membrane proteins and over lipids, also damaging the cell wall of some other microorganisms (Finnegan *et al.*, 2010). Additionally, H_2O_2 reacts with O_2, forming CO_2 reducing free O_2 and creating an anaerobic environment that can reduce the development of anaerobic populations (Šušković *et al.*, 2010). Diacetyl is associated to a characteristic smell of some dairy products, but it also has antagonistic activity when interacts with the cell membrane of some bacteria altering some metabolic ways (Lanciotti *et al.*, 2003). Bacteriocins are antimicrobial peptides produced by Gram positive and negative bacteria. In general, those peptides have low molecular weight and are heat stables, they do not affect the producer cells due immunity mechanisms (Cotter *et al.*, 2005; Karumathil *et al.*, 2016).

In this work, were isolated and identified LAB with antimicrobial activity against *Escherichia coli, Staphylococcus aureus,* and *Listeria monocytogenes,* three of the most important foodborne pathogens. These results are very important for the possibility of their application on biopreservation of foods.

Experimental procedures

Isolation and identification

Lactic acid bacteria were isolated from raw milk obtained from the Veterinarian Medicine Faculty of the Universidad Nacional de Colombia. Briefly, milk was incubated at 37°C during 48 h in order to allow fermentation and to obtain bacteria strains that survive on acid milk. 11 ml of fermented milk were added to 99 ml of sterile peptone water (0.1%) and tenfold dilutions until 10^{-7} were made. 1 ml of dilutions 10^{-5}, 10^{-6} and 10^{-7} were poured in petri dishes and covering with MRS agar (Man, Rogosa and Sharpe) Oxoid, petri dishes were incubated at 37°C during 48 h. In order to obtain more diversity in the isolation, colonies with different morphologies were plated onto the surface of MRS agar. After incubation at 37 °C during 24 h, Gram stain was performed. Isolates were stored at -20°C on cryovials (CRIOBANK®).

In order to know some features of the isolates some biochemistry tests were performed. Gas production test in Durham tubes on MRS broth; growth in MRS broth at different temperatures (10, 30, 37 and 45°C) and tolerance to NaCl 6.5% tests were performed. Growth was measure as optic density (O.D) on spectrophotometer (Genesys, USA) at 600 nm. Catalase and oxidase were also tested (Muñoz *et al.*, 2012). Isolates were partially identified on the basis of their biochemical features according to fermentation ability by using API 50CHL (Api System S.A., Bio-Merieux, France) (Todorov *et al.*, 2013).

To obtain the molecular identification, isolates were activated in 5 mL of MRS broth (Oxoid, UK) and incubated at 37°C during 48 h. After incubation the DNA was extracted using the kit PureLink™ Genomic DNA Mini Kit (Invitrogen) following manufacture instructions. Extraction was verified by electrophoresis in agarose gel 1.4% (w/v) at 70 Volts, 400mA during 30 minutes and the DNA was stained with SybrSafe. Concentration was measured by spectrophotometry using Nanodrop (Thermo Scientific, UK). Genera and specie were determined by amplification and sequencing of 16S ribosomal subunit by PCR technique using a Veriti® (Life Technologies, UK) thermo cycler. Conditions of PCR were: 94°C for 5 min, 30 times 94°C for 30 s, 55°C for 30 s, 72°C for 1.5 min and 72°C for 7 min. Reaction mixture contained 38.2 µL of water, 5 µL of buffer 1X, 1.5 µL $MgCL_2$ 1.5 Mm, 0.4 µL DNTs 0.2 Mm, 1.25 of primers (27F: 5' AGAGTTTGATCMTGGCTCAG 3', 1492R: 5' TACGGYTACCTTGTTACGACTT 3'), 0.4 µL Taq and 2 µL of DNA (Doi *et al.*, 2013). Amplification products were separated by electrophoresis in 1.2% agarose gel. Amplified samples were sent to the Instituto de Genética (SSiGMol) in the Universidad Nacional de Colombia (Bogota). Samples were purified and sequenced in bidirectional way. The sequences were compared to those deposited in GenBank, using the BLAST algorithm

(http://www.ncbi.nlm.nih.gov/BLAST) and phylogenetic tree was constructed using the MEGA 7.0.14 software.

Antimicrobial activity screening

Screening to find isolates with antagonistic features was performed using the spot-on-lawn method described by Schillinger and Lucke (1989) with some modifications. Briefly, overnight cultures of the isolates were spotted onto the surface of agar plates with MRS (1.2% agar) and incubated for 24 h at 30°C to allow colonies to develop and produce their metabolites. Approximately 5 x 10^7CFU/mL of the indicator strains important in food industry (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Listeria monocytogenes* ATCC 7644) were inoculated into 100 ml of soft TSA (Trypticase soy) agar (containing 0.7% agar), and poured over the plate in which the isolated LAB were grown. After incubation at 37°C during 24 h, diameter of inhibition zones was measured from the edge of the zone with a caliper, and expressed in mm.

Results

64 lactic acid bacteria were isolated from raw cow milk. 23.1% of all isolates were Gram positive and 76.9% of them presented rod shape, minor proportion presented sphere shape. Rods were large and short, some of them thinner than others, they were arranged on pairs or short chains. Most of cocci were arranged on tetrads and some of them on pairs. 24 isolates were chosen for further analysis according to their antimicrobial activity potential.

The isolates growth at 6.5% of NaCl and at temperatures of 10, 30, 37 and 45°C (Fig. 1) showed that the optimal temperature for all isolates was 30°C followed by 37°C, some of them grew at 10°C and at 45°C was the least suitable temperature for growth. 46.13% of isolates were tolerant to 6.5% of NaCl. Those tests allow knowing the technological potential of isolates in food industry applications. Gas production was negative for all cases.

The isolates that presented rod morphology (19 of them) were tested with API 50CHL (Api System S.A., Bio-Merieux, France) designed as *Lactobacillus* sp. according with the manufacturer, in order to determine phenotypic characteristics showed in biochemistry profiles (fermentative profiles). Tab. 1 shows the percentage of isolates that were able to use each sugar on the API CHL 50 panels. All of the isolates fermented N-acetyl glucosamine, glucose, ribose and fructose and none of them was able to use D-fucose, L-fucose, D-arabitol, erythritol, D-arabinose, L-xylose, adonitol, methyl-D-xiropyranose or glycerol. Galactose and gluconate were used almost for all isolates, except for the identified at molecular level as *Pediococcus*, this result can be explained because some strains of this genera do not use the sugar sources mentioned (Vos *et al.*, 2009). Arbutinine, aesculine, maltose and salicin were consumed by 89.5% of the isolates. Sugars consumed for one strain were rhamnose, metyl-D-manopiyanoside, starch, glycogen o xylitol. Partial identification according to biochemical profiles gave as a result that isolates belong to genera *Lactobacillus* spp. with species *Lb. brevis* 16%, *Lb. plantarum* 21%and *Lb. paracasei spp. paracasei* 63%.

Molecular identification

DNA extraction was performed to 19 isolates. Molecular weight marker of 1000 pb was used in order to know size of the product amplified. This size was approximated to 1400 pb according to observed bands. Chromax software was used to observe, interpreter, and depurate sequences obtained from the Genetics Institute (Universidad Nacional de Colombia, Bogota). Results were analyzed and compared to data in GenBank using BLAST (Basic Local Alignment Search Tool), phylogenetic tree was made using Mega 7.0.14 program (Fig. 2).

TAB 1. Percentage of positive isolates of each carbon source on API CHL 50.

Sugar	%	Sugar	%	Sugar	%	Sugar	%	Sugar	%
Glycerol	0	Rhamnose	4,5	Mannose	86	Xilitol	4,5	Sorbose	27
Erythritol	0	Dulcitol	9,1	Cellobiose	73	Gentiobiose	59	Salicine	91
D-arabinose	0	Inositol	41	Maltose	91	Turanosa	68	Glycogen	4.5
L-arabinose	27	Mannitol	68	Lactose	77	Lixosa	23	Esculin	91
Ribose	100	Sorbitol	68	Melobiose	36	Tagatose	64	Starch	4.5
D-xylose	55	Methyl-D-manopiranoside	4,5	Sucrose	64	D-fucose	0	Arbutine	91
L-xylose	0	Methyl-D-glucopiranoside	41	Trehalose	82	L-fucose	0	Rafinose	9.1
Adonitol	0	N-acetylglucosamine	100	Inulin	27	D-arabitol	0	Amigdaline	64
Metil-D-xiropiranosa	0	Glucose	100	Melezitose	73	5-ketogluconate potassium	64	2-cetogluconato potassium	59
Galactose	95	Fructose	100	L-arabitol	14	Gluconate	95		

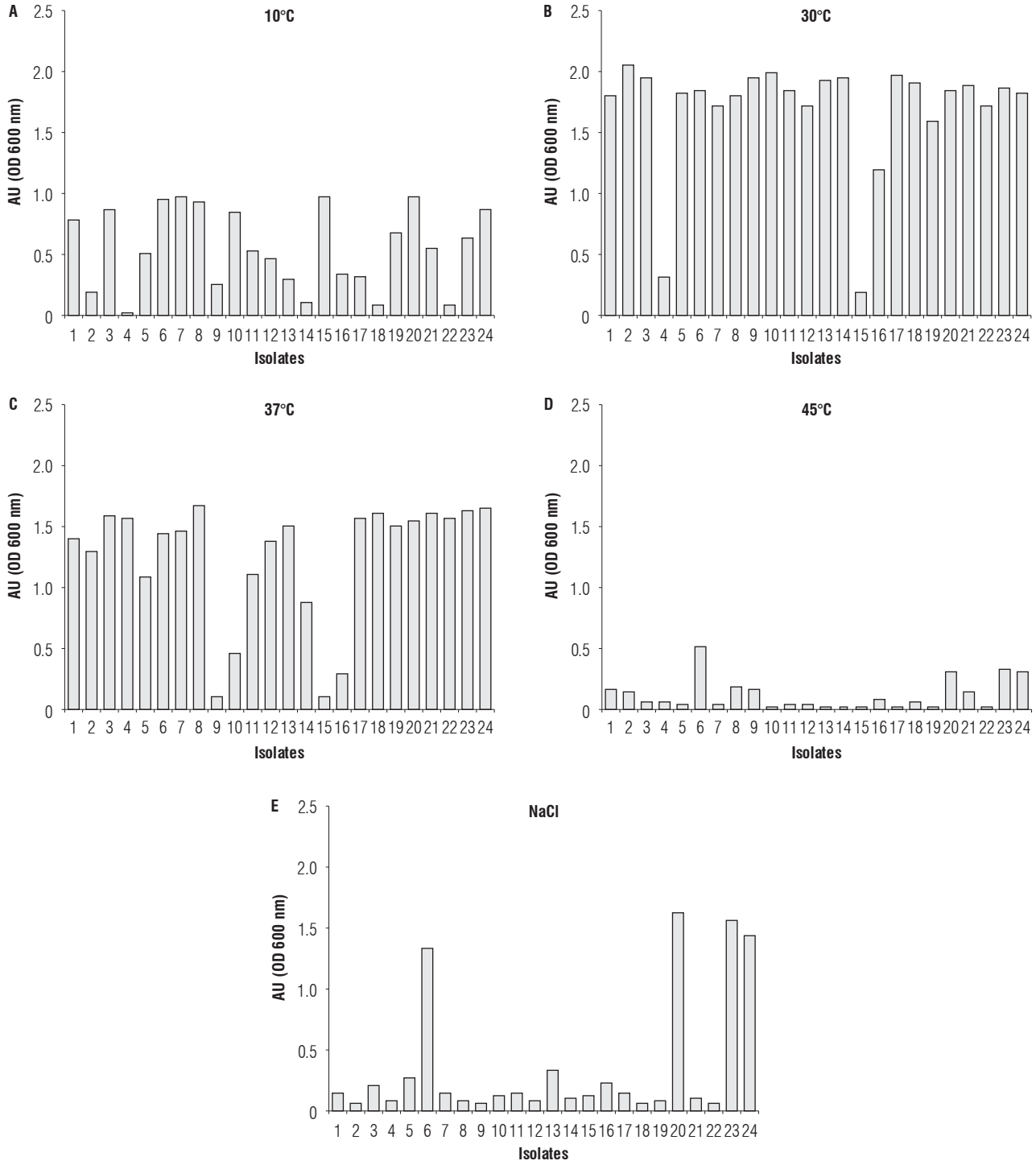

FIGURE 1. Growth of isolated LAB at different conditions of incubation,(A) 10°C, (B) 30°C, (C) 37°C, (D) 45°C, and (E) with 6.5% of NaCl. Numbers represent the isolated LAB; AU = absorbance units.

13 of the isolates were congregated on *Lb. casei/paracasei* cluster with a similarity percentage of 66% (isolates AL3, AL5, AL7, AL8, AL9, AL10, AL11, AL12, AL13, AL14, AL15, AL16 and AL18). Six isolates remaining were associated with 100% of similarity with *Lb. plantarum* (isolates AL1, AL2, AL4 and AL6), and *Lb. brevis* with 99% of similarity (isolates AL17 and AL19). According to the bioprospection concept about the obtaining of bioactive products from nature, in this case for applications in the food industry, the results showed the possibility to considerate the raw milk as an important source of antagonistic *Lactobacillus* strains with potential application on biopreservation. The

above depends mainly of the natural behavior of bacteria due that the strains express different survivor strategies, as the release of antagonist substances as response to population density (Cornforth and Foster, 2013).

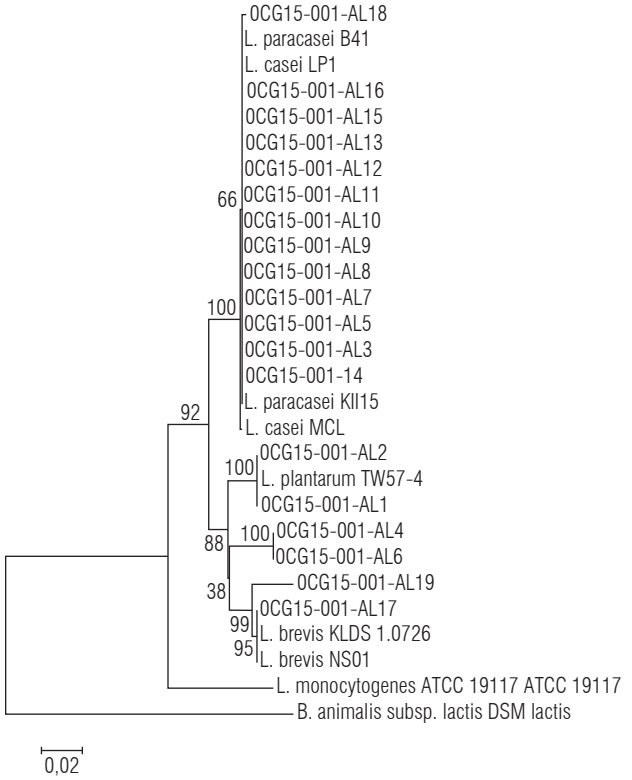

FIGURE 2. Phylogenetic tree of the isolates.

Antimicrobial activity evaluation

Fig. 3 shows the growth of the isolated BAL on MRS agar (1.2%) after incubation for 24 h at 30°C and the inhibition zones obtained after a second incubation with *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 7644.

Tab. 2 shows inhibition zones in millimeters against indicator strains.

Discussion

Biochemistry tests made by using API® CHL50 (BioMerieux, France) showed metabolic characteristics of the isolates, this information allowed to estimate sugar fermentation profile allowing growth media optimization. According to the results obtained on apiweb™, 11 strains corresponded to *Lb. paracasei*, 9 of them were identified at molecular level as *Lb. casei*, one of them as *Lb. casei/paracasei*, and one as *Lb. paracasei*. The close related species were *Lb. casei, Lb. paracasei,* and *Lb. casei/paracasei* and they were all identified by the apiweb™ as *Lb. paracasei*. On the other hand, those profiles are not enough to identified microorganisms, so it is necessary to use molecular techniques to obtain more robust results. Tab. 3 shows that names obtained by API CHL 50® to isolates AL2, AL4 and AL8 did not correspond to the identification obtained by sequencing and contrasted with databases.

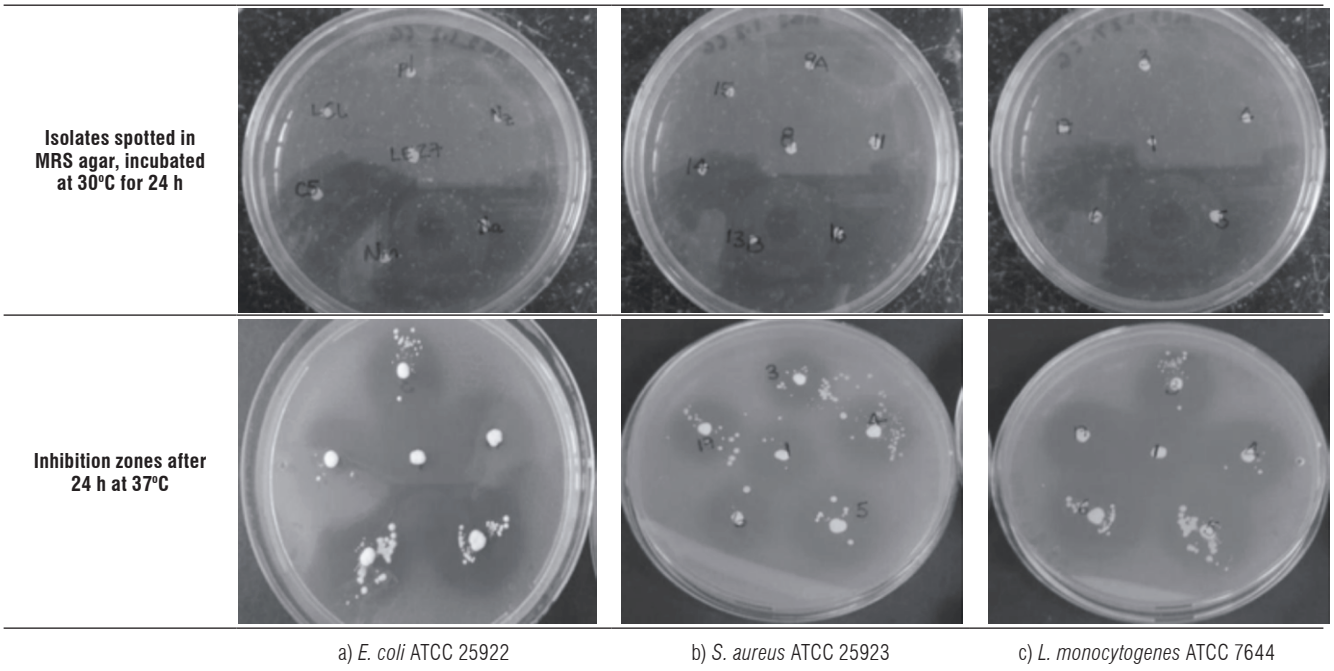

FIGURE 3. Antimicrobial activity screening. Upper part shows the growing of isolated LAB, the lower shows inhibition zones against *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 7644 performed by the isolated strains.

TABLE 2. Inhibition zones obtained on the screening.

	Inhibition zones (mm)					
	S. aureus		*E. coli*		*Listeria*	
	Diameter	SD	Diameter	SD	Diameter	SD
AL1	20	± 0.3	17	± 0.3	23	± 0.0
AL2	23	± 0.0	21	± 0.0	19	± 0.3
AL3	28	± 0.7	25	± 0.3	16	± 0.3
AL4	21	± 0.7	17	± 1.4	14	± 0.7
AL5	20	± 0.7	22	± 0.3	20	± 0.3
AL6	25	± 0.3	19	± 0.3	17	± 0.7
AL7	19	± 0.3	23	± 0.3	18	± 0.7
AL8	19	± 0.7	18	± 0.3	15	± 0.0
AL9	23	± 0.3	19	± 0.3	18	± 0.3
AL10	22	± 0.0	21	± 0.3	21	± 0.3
AL11	22	± 0.0	18	± 0.3	21	± 0.7
AL12	19	± 0.7	18	± 0.3	15	± 0.7
AL 13	13	± 0.7	17	± 0.3	10	± 0.7
AL15	10	± 0.7	12	± 0.3	11	± 0.7
AL17	23	± 0.0	22	± 0.7	20	± 0.7
AL18	17	± 0.7	15	± 1.0	10	± 0.3
AL20	25	± 0.3	24	± 0.3	21	± 0.3
AL21	25	± 0.0	19	± 0.3	24	± 0.3
AL23	23	± 0.3	25	± 0.3	18	± 0.3
AL24	23	± 0.7	19	± 0.3	13	± 0.3

Two out of the 19 strains identified at molecular level corresponded to *Pediococcus* sp. The rest were verified as belonging to *Lactobacillus* sp. (Tab. 3). Five different species of lactobacillus were identified as *Lb. casei*, *Lb. brevis*, *Lb. paracasei*, *Lb. casei/paracasei*, *Lb. plantarum*. One species of *Pediococcus* was identified as *P. acidilactici*. Other researches focused on isolated of native LAB from different foods have shown that dairy products are an important source of this kind of microorganisms (dos Santos *et al.*, 2014). For instance, 319 strains were isolated from different products made with raw buffalo milk on Gansu province (China), authors amplified and sequenced DNA from those isolates and compared results with GenBank database finding genera as *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus* and *Weissella.* Major proportion of isolated corresponds to *Lb. casei* and *Lb. helveticus* (Bao *et al.*, 2012). Sixty different isolates from five Spanish cheeses made without starter microorganisms were identified as *Lactococcus lactis* (Alegría *et al.*, 2010). Davati *et al.* (2015) isolated 64 LAB from camel milk and using Amplified Ribosomal DNA Restriction Analysis (ARDRA) found 12 different profiles. In the same study, identification by amplification and sequencing of 16S region of DNAr were performed and *P. pentosaceus*, *E. faecium* cepa Y-2, *E. faecium* cepa JZ1-1, *E. faecium* cepa E6, *E. durans*, *E. lactis*, *Lc. mesenteroides*, *Lb. casei* and *W. cibaria* were found.

TABLE 3. Comparison of culture and molecular identification of isolated LAB.

	API CHL 50 identification	Molecular identification	ID (%)
AL1	*Lactobacillus plantarum*	*Lactobacillus plantarum*	100
AL2	*Lactobacillus brevis*	*Lactobacillus plantarum*	100
AL3	*Lactobacillus paracasei/ casei*	*Lactobacillus paracasei*	100
AL4	*Lactobacillus brevis*	*Pediococcus acidilactici*	99.8
AL5	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	100
AL6	Did not performed	*Pediococcus acidilactici*	99.6
AL7	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	99.9
AL8	*Lactobacillus plantarum*	*Lactobacillus casei*	100
AL9	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	99.1
AL10	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	100
AL11	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	99.9
AL12	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	99.9
AL13	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	100
AL14	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	100
AL15	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	99.8
AL16	*Lactobacillus paracasei/ casei*	*Lactobacillus casei*	99.9
AL17	*Lactobacillus brevis*	*Lactobacillus brevis*	99.8
AL18	*Lactobacillus paracasei/ casei*	*Lactobacillus casei/paracasei*	99
AL19	*Lactobacillus brevis*	*Lactobacillus brevis*	98.2
AL22	*Lactobacillus paracasei/ casei*	Unrealized	

Other authors obtained and characterized morphologically isolates from paddy rice silage. API CHL50 tests to make the partial identification were performed. Also, analyses of 16S rRNA and recA sequences were made to obtain genera *Lactobacillus, Lactococcus, Pediococcus, Enterococcus,* and *Leuconostoc. P. pentosaceus* was the more abundant microorganism, and the low was *Lb. casei* (Ni *et al*., 2015).

Species most commonly isolated from raw milk belong to genera *Enterococcus* (*E. faecalis* and *E. faecium*), *Lactobacillus* (*Lb. delbrueckii subsp. Lactis, Lb. helveticus, Lb. hilgardii. Lb. fermentum, Lb. gasseri* and *Lb. rhamnosus*), *Lactococcus* (*Lc. lactis subsp. lactis* and *subsp. Cremoris*), *Leuconostoc* (*Ln. mesenteroides* subsp. *mesenteroides*), *Pediococcus* and *Streptococcus* (*St. Uberis* and *St. thermophilus*) (Neviani *et al*., 2013). Also, *Lactobacillus* sp. and *Streptococcus* sp. genera has been reported from raw cow milk (Elgadi *et al*., 2008). Other authors identified *Enterococcus* spp. and some members of *Lactococcus* spp., also they reported a less amount of *Lactobacillus* strains in contrast with the present work (Franciosi *et al*., 2009). Other authors isolated 7 species of *Lb. plantarum* from donkey milk. Results obtained on RAPD-PCR spectrum showed that isolates were replicates of the same strain, for this reason they evaluated one single strain named *Lb. plantarum* LP08AD. Bacteriocin LP08AD was identified and characterized at biochemistry and molecular level, also antimicrobial activity was measure against *L. monocytogenes*, *E. faecium*, *Lb. curvatus, Lb. fermentum* and *P. acidilactici* (Murua *et al*., 2013).

All strains presented antagonistic activity against *L. monocytogenes*, *E. coli* and *S. aureus* showing a potential application on research related with production and recovery of antimicrobial metabolites with broad spectrum against foodborne pathogens. Similar results have been reported with isolated strains belonging to genera *Pediococcus, Enterococcus, Leuconostoc* and *Lactobacillus* showed antimicrobial activity against *S. aureus*, *Bacillus cereus* and *E. coli* (Davati *et al*., 2015). In other study, 17 of the 60 of *Lc. lactis* isolated presented antimicrobial activity against Gram positive bacteria from the *Lc. lactis* cluster (different subspecies), *Lb. sakei* CECT 906T, *Lb. plantarum* LL 441, *L. innocua* 86/26 and *S. aureus* CECT 86T (Alegría *et al*., 2010).

Bioprospecting as a tool in the search for natural substances with potential applications in food is booming worldwide due to the need of developing food products with natural features that allow to replace the use of synthetic additives. This work showed the importance of raw milk as source of LAB with antagonistic against pathogen bacteria features and showed the needed to focus the research in the evaluation of the antimicrobial activity of the native LAB to characterize metabolites responsible of this activity with application in biopreservation of food.

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Contribution of local peasant innovations to the re-configuration of endogenous rural development

Contribución de las innovaciones locales del campesinado en la reconfiguración del desarrollo rural endógeno

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ABSTRACT

In Colombia, Rural Development Institutional programs have made of local agents imperceptible since their trajectories have not been considered in the search for solutions to the problems in their territories. The present work introduces an innovative system to the production of the leaves of the plantain variety 'Cachaco' (*Musa* ABB S). This improvement, which was developed independently by indigenous communities of southern Tolima (Colombia), has been studied in detail throughout the technical, economic and social aspects of the productive and commercial processes of some plantain leaves producing farms. Based on their relation with their natural and socio-cultural environments, these communities have prompted their own process of re-peasantization. This productive innovation improves the life quality of the families involved, ensures their permanence in the territory and favors its cultural appropriation.

Key words: productive innovation, re-peasantization, Cachaco plantain, peasant agriculture, indigenous agriculture.

RESUMEN

Los programas institucionales de Desarrollo Rural en Colombia invisibilizaron a los actores locales, pues no han sido consideradas sus trayectorias para la búsqueda de soluciones a los problemas en sus territorios. Se analiza la innovación productiva de la hoja de plátano 'Cachaco' (*Musa* ABB S) desarrollada autónomamente por comunidades indígenas del sur del Tolima. Fincas productoras de hoja fueron estudiadas profundizando aspectos técnicos, económicos y sociales del proceso productivo y comercial. Las comunidades generaron una experiencia propia de re-campesinización a partir de la relación con su entorno natural y social. La innovación productiva mejora la calidad de vida de las familias, asegura la permanencia y favorece la apropiación cultural del territorio.

Palabras clave: innovación productiva, re-campesinización, plátano Cachaco, agricultura campesina, agricultura indígena.

Introduction

Colombian farmers have faced multiple adversities over a long history of violence that has been recently aggravated by the capitalist globalization policy imposed since the 1990s. The latter has promoted inequality and has accentuated rural poverty since it tends to favor small groups of rural entrepreneurs and marginalize the vast majority of smallholders (UNDP, 2011). The risk implied in these inequities is increased by excessive land concentration, forced displacement and problems associated to climate change, together with the rise of illicit crops and the lack of a strong and inclusive rural institutional framework (Garay, 2013). This has resulted in processes of de-peasantization (i.e., abandonment of the farming activity: Van der Ploeg, 2008), a social and economic phenomenon related to how the peasant population is excluded from their rural territories under the pressure of the economic policies that increasingly empower the business sector (Hocsman, 2015).

Faced with the risk of de-peasantization, rural societies resort to various adaptive resistance strategies related to both work modality and the intrinsic management of the productive units (Bendini and Steimbreger, 2010). In turn, this generates new configurations of the productive systems and their relation with the market.

When these resistance and adaptation strategies are used by a increasing number of farmers, a phenomenon known as re-peasantization takes place. The concept arose from rural sociology studies intended to explain the movement of farmers toward autonomous production models operating in closer association with the logic of peasant production. This has been done to re-structuring productive activities,

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reducing costs, optimizing ecological cycles and strengthening cooperation, together with the development of social and technological production innovations and short circuit marketing (Van der Ploeg, 2008).

What is common to many re-peasantization experiences is that they not only implement production modes based on proper traditional knowledge, but also seek for environmentally sustainable production and endogenous local development (Jenkins, 2000; Ray 1999). This particular farming knowledge constitutes an unexplored source of information that is in danger of being lost over the next generations (Knapp and Fernández-Giménez, 2009).

Traditional knowledge is considered as that acquired by experience and transmitted and adapted from generation to generation (Toledo and Barrera-Bassols, 2008). It is evident not only on adaptive production strategies, but also on skills, procedures and forms of organization and the relation with nature. In managing their productive systems, farmers organize and allocate natural, labor and economic resources under a particular prioritization logic that preserves their life conditions (Vélez, 2015). In the case of traditional farmers, subsistence is determined mostly by its relation to the environment (co-production) than by its linkage to markets (Toledo and Barrera-Bassols, 2008). In this prioritization, local knowledge is particularly relevant.

As result of its dynamic nature, traditional knowledge is enriched over time, resulting in innovations that contribute to increased production (Läpple *et al.,* 2015). Innovations are considered as those human adaptations aimed to generate favorable socio-economic conditions for a given social group, or to solve particular needs (Rodima-Taylor *et al.,* 2012). In the agricultural sector, innovators are farmers who have introduced changes in products or organizational processes, which are innovative to local or regional agriculture (Läpple *et al.,*2015; Mileone, 2005). In the social field, innovation affairs refer to organizational strategies, ideas or arrangements relevant to the strengthening of local organizations and civic institutions, with the further goal to overcoming inequality and exclusion (Rodima-Taylor, 2012). Innovation itself is a mechanism on which society is constantly adapting to changes, which are determined, in turn, by people's social and cultural values (Chhetri *et al.,* 2012).

The focus of the current study was the characterization and recognition of local adaptation and innovation strategies implemented by the 'Cachaco' plantain *(Musa* ABB S) leaves producers from the indigenous peasant communities of southern Tolima (Colombia), as a result of their local knowledge system. Local agents innovation, which has remained hidden to formal experts, is made visible in terms of its viability and social, economic and ecological relevance (Mileone, 2009; van der Ploeg, 2008). Since local innovation is presented as an expression of re-peasantization, its contribution and relevance to the design of local development strategies is analyzed.

Methodology

The study was carried out in the region of *Los Totarcos*, located in the upper basin of the Guaguarco River, which marks the geographical boundary between the municipalities of Coyaima and Natagaima (south of the department of Tolima, Colombia).The region corresponds to a Tropical Dry Forest life zone, with altitudes ranging from 450 to 700 m a.s.l. and average temperature of 28°C. In this territory, there are six *Pijao* native indigenous reservations (*Totarco Niple, Totarco Tamarindo, Totarco Piedras, Totarco Dinde Tradicional,Totarco Dinde Independiente* and *Zanja Honda*). The study analyzed 10 'Cachaco' leaves producing farms.

The study followed the approaches of agent-centered sociological analysis (Long, 2007) and social network analysis (Lugo-Morin, 2011). The former proposes to uncover local agents' knowledge about their natural, social and agricultural practices, assuming that they have the skills and capabilities to transform the environment (Gerritsen *et al*., 2009; Rivas and Quintero, 2014). It proposes a deeper understanding of local knowledge systems, thus addressing the inherent problems of agricultural research and extension processes and how to use local knowledge and technological change. On the other hand, the social networks approach allows the understanding of complex social phenomena originated from the interactions of observable local production systems with individuals, communities and institutions (Lugo-Morin, 2011). In addition, such dynamic not only facilitates the recognition of the processes of negotiation, cooperation and subordination that takes place within the formal relations held by local agents, but also reveals conflicts in a context of social interactions.

Fieldwork included an initial exploratory survey (Fig. 1) that allowed recording the antecedents of the studied productive innovation and to select farms dedicated to this activity along with the basin representative farming's of the region in leaf production. Used as an important research tool, participative observation allows the researcher to immerse into the farms in order to conduct in-depth interviews regarding the family livelihood.

The activities related to the production process were detailed and the relevant agents identified. Finally, an analysis of the 'Cachaco' leaves production network was performed in order to identify its configuration, allong with the basic characteristics of its interactions and their support among the most relevant agents.

The categories of analysis included technical aspects and gradients of plantain leves cultivation (production areas, inputs requirements, management and yields); economic analysis (costs, incomes and profitability); participation degree of the peasant family (addressing both basic and extended family networks and their participation on the productive activities); environmental analysis (crop structure) and social network analysis.

To identify the participation degree of the institutional agents on the cropping activity, interviews were conducted with the local leaders such as indigenous governors, local extension service technicians, institutional representatives and market intermediaries. Social network analysis was performed in UCINET 6.542 (Borgatti *et al.,* 2002). The agents involved in the analysis of the networks included 10 farming systems; the social agents (10 main families and their respective extended relatives and their indigenous territories); the institutional agents (*"Banco Agrario"* and the extension, training and sanitary control services); the commercial agents (supply and labor providers, leaves wholesalers and production partners). The interactions between all these agents were represented through a graph in a "Net draw" software package.

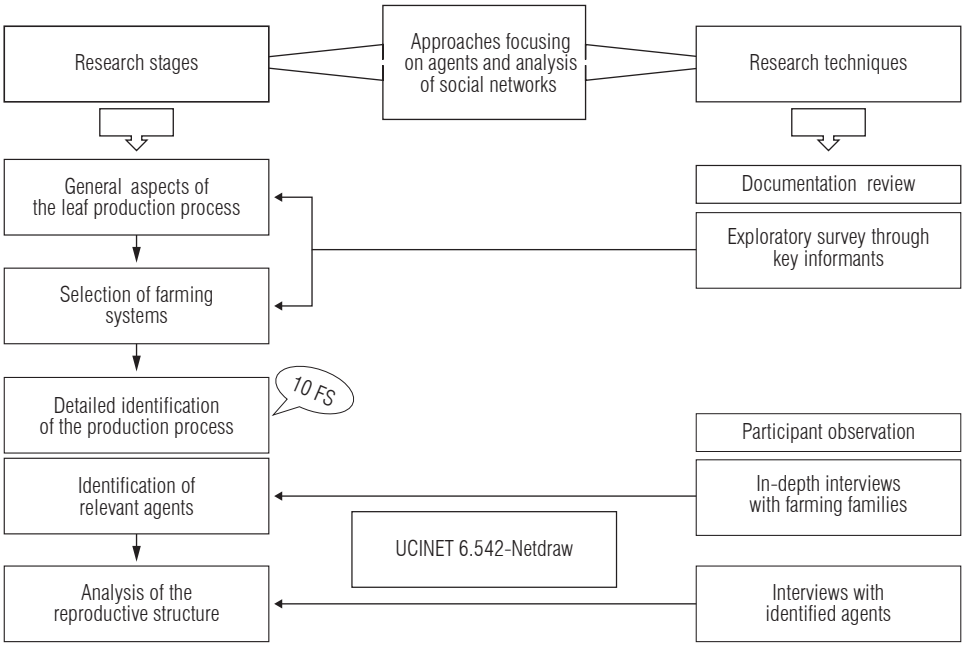

FIGURE 1. Methodological outline of the study.

TABLE 1. Characteristics of the 'Cachaco' leaves productive farms in the region of *Los Totarcos*.

No.	Name of the farm	Area of the farm (ha)	Area under leaves cultivation (ha)	Locality	Land tenure
1	San Nicolás	4.75	1.00	*Totarco Piedras*	Assigned
2	Chaparracena	1.25	1.00	*Coya Mana Grande*	Assigned
3	Los Balsos	6.00	1.00	*TotarcoTamarindo*	Private
4	El Caleño	4.00	0.75	*Coya Mana Grande*	Private
5	El Guásimo	3.50	3.00	*Totarco Tamarindo*	Rented and assigned
6	Jazmín	1.25	1.00	*Totarco Tamarindo*	Awarded
7	Agua Blanca	2.00	2.00	*Totarco Tamarindo*	Rented
8	El Paraíso	3.70	2.16	*Totarco Dinde*	Assigned
9	El Palmar	10.00	5.50	*Totarco Tamarindo*	Private
10	El Salto de Totarco	15.50	5.50	*Totarco Tamarindo*	Private
	Average	5.20	2.29		

Results and discussion

Characteristics of the farms and innovation under study

Considering that the Family Farm Unit (FFU) (administrative unit officially determined in the country as the area to generate the equivalent of two legal minimum salaries per department) size for Coyaima is 12 ha (Tab. 1), the studied plantain leaves productive farms can be considered to be small farmsteads, since only one of them exceeds this limit. Fifty percent of the families work in farms assigned by an indigenous *reservation*; 40% corresponds to owner farming families; and 10% work in rented lands. Only one farm combines the first and the last modalities.

These types of land tenure in the region show how the indigenous families participate in a reservation and how important is to have access to land and, therefore, labor means. In fact, almost all the families of the five reservations at the region have assigned a piece of land, which they use to plantain leaves production.

The distribution of the cultivated areas at the ten farms shows that the area grown with 'Cachaco' leaves, which averages 2.29 ha per farm, constitutes the main crop. The planted area oscillates between 0.75 ha and 5.50 ha.

Although the 'Cachaco' plantain crops were initially used to produce fruit, leaf production and trading started in the second half of the 1980s, when some local producers bought the crop leaves to trade them in Bogota. Since then, the producers processed younger leaves, which were slightly roasted in timber wood fire (a process known under the local Spanish term "*soasar*") and then folded and packed to satisfy the demand of the *tamal* packing industry (*tamal is* a traditional Colombian food). As the demand for plantain leaves increased, the farmers replaced their sugar cane plantations and established plantain leaves crops. Over time, they have progressively introduced technological changes to their farms regarding production, processing, packaging and marketing processes.

A key factor to this innovation was the "*tamal tolimense*" industry of Bogota, promoted by supermarkets and small rural entrepreneurs of the department of Tolima, who opened markets for this typical food in the capital of the city. This exemplifies how innovations generate novelties to the markets, which, in turn, strengthen such innovations (Läpple *et al.,* 2015). The production of *tamales* has become a large industry of great economic impact for supermarkets and family small businesses, thus becoming one of the largest packed typical food industries of the country (Portafolio, 2012).

As a cultivar, 'Cachaco' plantain crops are known for their resistance to drought, which confers strong fitness to the climatic conditions of the tropical dry forest ecosystems of southern Tolima. The 'Cachaco' crop can maintain elevated leaves production for around 8 to 10 years. Nevertheless, it is frequent to find crops with 20 to 40 years of productive life. Production begins 8 months after planting the *colino* (local name given to the vegetative plantlet). To the third year onwards, leaves production allows cutting 6 to 8 leaves per site every 3 weeks, for a rough total number of leaves rolls ranging from 1800 to 2400 y^{-1} ha^{-1} (12.6 to 16.8 tons of leaf per year, with an estimated weight of 7 kg / roll). At the studied farms, yield averaged 5.32 t $ha^{-1}year^{-1}$ (Tab. 2), mainly due to planting density, labor dynamics and management modality.

At the 10 studied farms, an average benefit / cost ratio of 1.44 was calculated after considering monetary and non-monetary costs. This indicator was considered as positive in all cases (1.21 to 1.7). In the ordinary cost structure of the leaf production, the highest expense was represented by labor (94.38% of total costs), family labor accounting (78.53% of total costs) and hired labor force (15.85%).

TABLE 2. Leaf yields for the studied farms in the region of *Los Totarcos*.

No.	Name of the farm	Productivity yield (t ha^{-1})	Performance (B / C ratio)
1	San Nicolás	8.73	1.48
2	Chaparracena	7.28	1.29
3	Los Balsos	10.92	1.70
4	El Caleño	4.03	1.52
5	El Guásimo	1.45	1.13
6	Jazmín	6.91	1.37
7	Agua Blanca	6.37	1.50
8	El Paraíso	1.20	1.56
9	El Palmar	4.00	1.64
10	El Salto de Totarco	2.30	1.21
	Average	5.32	1.44

A local innovation in the cultivation of 'Cachaco' plantain as a re-peasantization experience

The migration of a growing number of cassava and panela producers to 'Cachaco' plantain leaves cropping in the region of *Los Totarcos* caused a series of technological, social and economic changes in this territory. A significant increase of more than 600% in the cultivated areas took place along a 10-year period where the crop production was intensified, involving approximately 960 families in the studied region (Tab. 3). Thus, 'Cachaco' leaves production has become an important strategy for farming families

to remain in the territory. As proposed by Bendini and Steimbreger (2010), new economic activities are likely to counterbalance de-peasantization processes and contribute to those of re-peasantization.

TABLE 3. Trajectory of the production of 'Cachaco' plantain leaves in the region of *Los Totarcos*.

Year	Area planted in 'Cachaco' plantain (ha)	Increase (%)
1994	400	-
1998	1,700	325.0%
2014	2,430	42.5%

Prepared by the authors based on data from the local Agricultural Census of the municipality of Coyaima (Tolima-Colombia). Municipality of Coyaima, 2015.

The emergence of the innovation analyzed in this study combines what Knapp and Fernández-Giménez (2009) denominate main categories of knowledge, named active knowledge applied to management decisions; knowledge that results from local dwelling; and integrative knowledge linking ecological, economic and social aspects.

Farmers who used to produce cassava starch and *panela* sugar cane in the upper basin of the Guaguarco river have migrated to the plantain leaves production as a new productive activity, which they have structured through their interaction with the local environment. As a result of their permanent adaptation to change, they were able to take advantage of the opportunity emerged with the plantain leaves production, which, based on their profound knowledge of their natural environment, triggered a new way of appropriating to the local markets (Toledo and Barrera-Bassols, 2008). This innovative way to interact with the environment (van der Ploeg, 1992; van der Ploeg, 2008), has given rise to both a regional product with specific cultural identity and a proper form of social development. This productive system emerges as a local innovation, made possible by the availability of genetic resources, on a specific ecosystem, occupied by a particular cultural group (anthropic biome) and driven by a specialized market demand (Figure 2).

From an ecological standpoint, the positive effects of this productive activity arise from the crop structure, the length of its lifecycle and its agronomic management. As an herbaceous plant with a semi-permanent cycle, 'Cachaco' plantain has the additional capability of storing moisture in its tissues, thus resisting drought conditions and preventing over-tillage of the soil, which certainly preserves its characteristics. Additionally, and due to the plant architecture, there is a low risk of soil erosion; the plat provides an adequate coverage to heavy rain effects.

In turn, the widespread association with forest species like *Iguá (Pseudosamanea guachapele), Caracolí (Anacardium excelsum)* and *Palma Real (Attalea butyracea)* increase the environmental benefits such as the enhancing of microclimate conditions, pests and diseases protection by biological controllers, symbiotic relationships (nitrogen fixation (e.g., *Iguá*) and the permanence of other beneficial

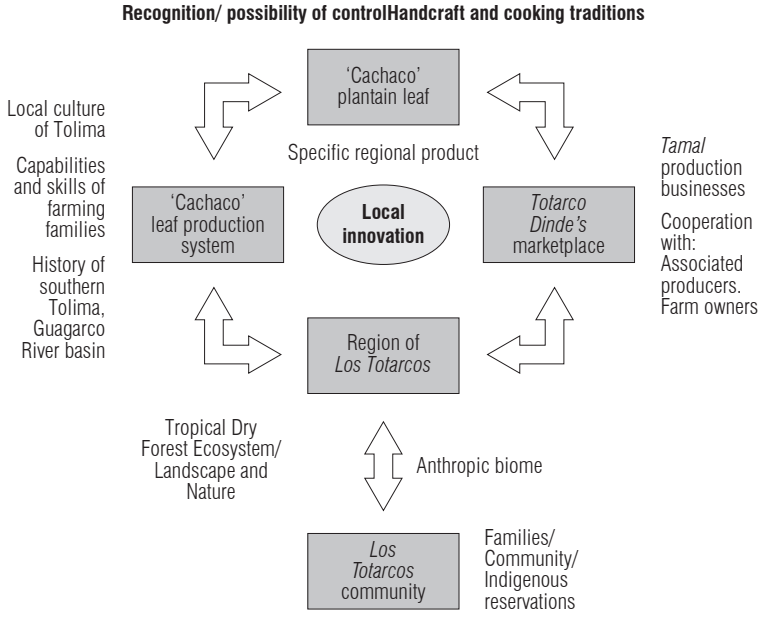

FIGURE 2. Integral appropriation of the territory for the production of 'Cachaco' plantain leaves in the region of *Los Totarcos.* Modified by the authors from Gerritsen *et al.,* 2009.

organisms), and the conservation of associated wild species, among others.

Productivity can be significantly increased through local innovations. An example presented by Läpple *et al*. (2015) emphasizes how innovations can result after applying single or multiple changes to lead the introduction of new components into the productive processes in the farm. Three elements of technological innovation can be mentioned as most relevant in the agricultural system of 'Cachaco' plantain: genetic material, weed management and packing mode.

From a genetic standpoint, the importance of the 'Cachaco' plantain material lies on its unique fitness to the climatic and soil conditions of the area, which were detected by local farmers through observation and interaction with their resources (genetic material, soil, climate and information). The use of herbicides to weed control was a technological adaptation intended to optimize some labor force to the harvest and postharvest activities. The packing system amendment, which went from a leaf stacking to a leaf rolling , due to the difficulties brought by the former system to both leaf and *tamal* producers, since the heavy weight of the packages used to complete handling and transportation. In addition, including the leaf vein on the final product was inconvenient to the farmers, since it is an important source of organic matter for the crop.

As far as the market circuits are concerned, plantain leaf producers travel variable distances from the farms to the local marketplace, using beasts of burden along brida lpaths, which turns the product transport into a very heavy labor. The distance between *Totarco Dinde's* market place, where the totality of the 'Cachaco' leaves are sold to wholesalers, and *Paloquemao* market place in Bogota, where it is distributed, does not exceed 220 km. The distances to alternative market places like those found in the cities of Ibagué and Neiva are located to 138 and 111 kilometers, respectively. Thus, the geographic location of *Los Totarcos* represents a great opportunity to the projection of the plantain leaf market to other places of the country.

According to the network analysis graph (Figure 3), the family units represents the main social agent sustaining the crop. The structure of the families shows that, in average, they have 7.2 members, out of which 3.2 are adults, which shows an elevated percentage of children. From all family members, 91.7% live in the farms; 87.5% of the adults are fully involved on the leaf production, while 33.7% of the children are just partially involved.

These figures not only which are the family terms to remain in the region, but also their dynamic articulation to leaf production through its various activities, namely cropping, harvest and postharvest, which enable the integration of all the family members.

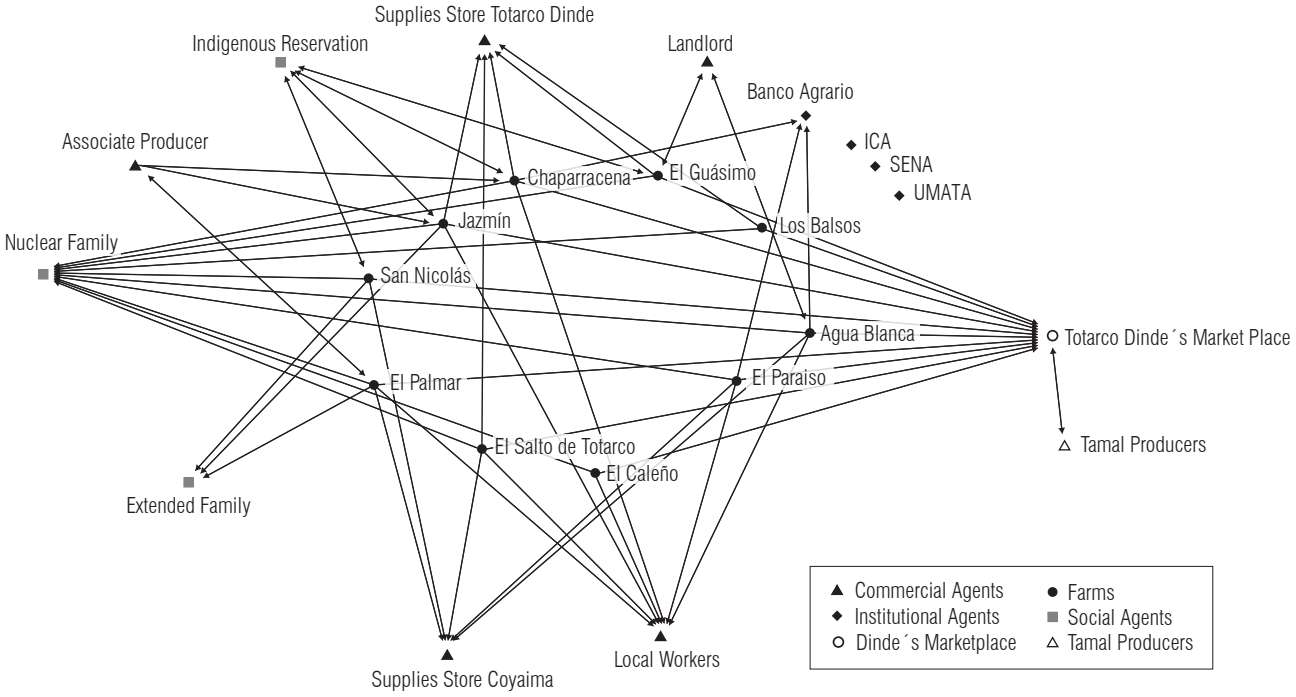

FIGURE 3. Graph illustrating the agents linked to the production of 'Cachaco' leaf in the region of *Los Totarcos*.

In the present case, the farmer´s adaptability has allowed the emergence of a productive innovation featured by technological and social specificities. In other words, the way this innovation transforms and uses the landscape in its favor has allowed not only the generation of a genuine productive activity that improves the local economy, but certain social conditions that generate well-being to the community. There is a close relationship between agricultural innovation and social capital. According to Van Rijn *et al*. (2012), the adoption degrees of productive innovations are related directly to strong and stable social structures. In the region of *Los Totarcos* these structures, which correspond to indigenous families, communities and indigenous reservations, have generated the particular form of production that is reported in the present work.

The social aspect of the 'Cachaco' plantain leaves innovation focuses on the nuclear farming family, since all its members are able to participate in the production, harvest, post-harvest, transport and packaging activities. In this way, each family member acquires specific practical skills within the whole process.

Children and youngsters are related to the cropping activity. From the age of 15 to 17, they independently assume the harvest and post harvest activities, receiving the same payment as an adult worker. Just as well, they can receive from their parents a piece of land to grow their own plot of 'Cachaco' and benefit from it autonomously. In this way, leaf production facilitates not only the articulation and strengthening of the family network, but also the retention of strong labor force in the territory.

The farm systems also show strong relations of dependence with the *Totarco Dinde* marketplace, where all the leaf production is sold. The relations held by the farmers with credit (the BancoAgrario), training (SENA), rural extension (UMATA), and sanitary control (ICA) institutions were analyzed. At present, only the bank is intervening in this local innovation, since they encourage small-scale productive projects for which they offer credits with special interests and repayment plans. No other institution offers any kind of service in the socio-technical network, as these innovations are kept hidden by the expert knowledge system, which are centrally affected by formal bureaucracy.

Van der Ploeg *et al.* (2012) suggest that rural development can be analyzed from market dynamics. In the case of the plantain leaf market, the most important commercial agents are the providers of supplies and services, along to the buyers and associate producers. The buyers, who are either leaves wholesalers or tamal producers, purchase the product in the regional marketplace of *Totarco Dinde*, which is specialized in 'Cachaco' leaves and is considered to be the biggest one regarding this specific product. Although other regions of Tolima such as *El Guamo, Saldaña* and *Purificación* also produce and trade plantain leaves, the largest number of farmers is in the region of *Los Totarcos* (Coyaima). It was precisely in the locality of Totarco Dinde where the homonymous marketplace was established in 1997.

As politically autonomous entities, the indigenous reservations do not interfere on the regulation of the plantain leaf production processes. Nonetheless, they are fundamentally important to the governance of the territory, since they can affect leaf production, environmental protection and sanitary management regulations.

Although several studies suggest that productive innovations necessarily involve extension services and technical assistance (Knapp and Fernández-Giménez, 2009; Chhetri *et al.,* 2012), the case of 'Cachaco' plantain leaves demonstrates innovation emerged from the community itself. Similar cases of innovation arising from local knowledge systems have been demonstrated on shrimp production in Bangladesh (Chowdhury and Khairun, 2014), traditional horticulture in the Argentine Patagonia (Eyssartier *et al*., 2013) and cereals in Tunisia (Dolinska and Aquino, 2016).

The importance of this local innovation in the configuration of Rural Development programs aimed to territorial sustainability

The modernization paradigm that currently dominates Rural Development theory and practice is currently being challenged by new paradigms (Van der Ploeg *et al.,* 2000; Wilson, 2009; Morgan, 2010). The Territorial Development approach (Schejtman and Berdegué, 2003), which corresponds to the formal logic of the institutional megaprojects, follows only the economic interest of integration into globalized markets. As such, it reproduces the logic of mass consumption goods, without integrating any biophysical or socio-cultural aspects (Ramírez-Miranda, 2014; Schneider, 2006).

Such complexity highlights the fact that rural development is not adding new things to old situations. Territorial rural development should not be conceived only as a result of the laws of political economics, the logic of commercialization, and patterns of capital accumulation. Instead, it is the result of complex interactions among diverse circumstances in

particular territories. The rural territorial development must therefore be an ethical and political project strengthened by the action and involvement of the affected social agents and the organizations of society in general. As such, it should be featured not only by multi-level, multi-agent and multifaceted approaches inscribed on the traditional modes of integrating nature and society, but also by a thorough revaluation of agricultural practices and a diversity of other rural activities (Fajardo, 2012; Schneider, 2006; Van der Ploeg *et al*., 2000).

In this ideological context, which stands for a postmodern rural development approach, the concept of re-peasantization (with its many expressions) has been catalogued by Van der Ploeg (2008) as one of the three main trends of contemporary agriculture world-wide. Re-peasantization means giving a new role to farmers and their families in the structuring of rural development territorial initiatives. It implies that farmers are valued for their experiences, knowledge and ideas about local rural problems, such as productivity improvement, conservation of the natural resource base, linkage to markets, and many other aspects of rural life. Re-peasantization, which closely resonates with approaches such as multi-functionality, is achieved through diversification strategies aimed at developing a closer co-production relationship with nature (Rentig *et al.,* 2009; Van der Ploeg *et al*., 2000).

The local innovation of 'Cachaco' production represents a process of re-peasantization that has allowed the farmers to reconfigure their production means, generating new market alternatives with important socio-environmental effects to their ancestral territories. This experience leads us to reflect on the need to flexibilize the expert-designed system of technical and scientific knowledge, and consider the deviations and unexpected results obtained by the communities. This implies valuing the local innovations implicit in traditional agricultural systems, thus entering dialogue with conventional agrarian sciences and improving both traditional and modern agroecosystems (Van der Ploeg, 2008). The traditional knowledge incorporation into our current understanding of the agricultural technology would allow the design of rural development programs that are capable of both transcending the technology transfer model and implementing social knowledge management systems based on local interaction networks. Such processes of revaluation and inclusion will not only allow a better understanding of the reality of national agriculture, but also strengthen a continuous process of traditional knowledge transfer through the agricultural extension services of rural development programs.

Conclusions

The production of 'Cachaco' plantain leaves is important in the region of *Los Totarcos* in terms of how farming families are related to territory appropriation and the production process itself, thus resulting in an important economic base for the region. This activity, which constitutes a particularly productive, economic, social and environmental innovation generated from the farmers rationality, was made possible by the development of creative work processes emerged in the relationship established by the farmers with their natural and socio-cultural environment.

As a local productive innovation, 'Cachaco' leaves have enabled communities in the region to strengthen their social fabric, providing the new generations with an alternative to remain in the territory. The re-peasantization experience has improved life quality, created a cultural territory with genuine characteristics, and contributed to strengthening endogenous rural development processes and, therefore, to regional sustainability.

The institutional recognition of this regional production process and its inclusion in Territorial Rural Development programs is particularly promising. In effect it is likely to constitute an alternative not only to improve life conditions of hundreds of indigenous and peasant families living in this territory, but also for creating better governance opportunities for them, thus strengthening the identity of an indigenous group that is in risk of disappearing.

New horizons to advance in this research include the study and recognition of the rationality of traditional farmers and their own knowledge, to adapt the agriculture models to their particular conditions; the role of re-peasantization in the structuring of territorial initiatives of rural development and the importance of implementing local knowledge management systems based on networks of social interaction. These aspects will be relevant for inclusion in participatory rural extension programs.

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Evaluation of four degree-day estimation methods in eight Colombian coffee-growing areas

Evaluación de cuatro métodos para estimar grados-día en ocho zonas cafeteras colombianas

Carlos Andrés Unigarro[1]*, Leidy Natalia Bermúdez[2], Rubén Darío Medina[3], Álvaro Jaramillo[3], and Claudia Patricia Flórez[3]

ABSTRACT

Methods to estimate the accumulation of degree-days based on maximum and minimum temperaturesare are commonly used to determine relationships or to adjust phenological models based on the "physiological time". Degree-days are obtained indirectly by these methods, this information is not generally available on hourly or shorter time scales due to the type of equipment used to record data or a data loss in historical time series. To compare the performance of such methods, degree-days were estimated with four indirect methods in eight Colombian locations during 1 year. Each indirect method was evaluated in comparison to the numerical integration method by the trapezoidal rule (reference method) using temperatures recorded every 5 min. Based on the percent bias error, the methods proposed by Arnold, Ometto and Snyder tend to overestimate thermal time, whereas the Villa-Nova method underestimates this time, but with a lower performance as regards to the previous ones.

Key words: thermal time, temperature, numerical integration, linear regression, bias.

RESUMEN

Los métodos que estiman la acumulación de los grados-día basados en datos de temperatura máxima y mínima diaria son comúnmente usados para determinar relaciones o hacer ajustes en modelos fenológicos basados en "tiempo fisiológico". La obtención de los grados-día con estos métodos se hace de manera indirecta, dado a que en general no se dispone de información de temperaturas a escala horaria e incuso menor, debido al tipo de equipo utilizado para tomar registros o por la pérdida de datos en series históricas. Con el objetivo de determinar el desempeño de estos métodos, se estimaron los grados-día con cuatro métodos indirectos en ocho localidades colombianas durante 1 año. Cada uno de los métodos se evaluó con respecto al método de integración numérica por regla del trapecio (método de referencia) usando las temperaturas registradas cada 5 min. El desempeño de los métodos se evaluó a partir de un modelo de regresión lineal y sus respectivos errores. Los métodos de Arnold, Ometto y Snyder, según el porcentaje de sesgo, tienden a sobrestimar el tiempo térmico, mientras el método de Villa-Nova lo subestima, pero con un menor desempeño respecto a los anteriores.

Palabras clave: tiempo térmico, temperatura, integración numérica, regresión lineal, sesgo.

Introduction

Temperature affects several physiological processes in plants. Generally, this influence is due to a thermal action on enzymatic activity, with temperature inducing changes in the conformation of enzymes and consequently on their functionality (Sharpe and DeMichele, 1977; Johnson and Thornley, 1985; Higley *et al*., 1986; Bonhomme, 2000). The phenological response of plants to temperature is observed as changes in developmental rates (the occurrence of certain phenological events per time unit) (Raworth, 1994). Jaramillo and Guzmán (1984) found that a significant correlation exists among the number of days elapsed from planting to first harvest, the average air temperature (°C) and the thermal units.

Heat accumulation is referred to as "heat units", "thermal time", "cumulative growing degree days" or "physiological time" and reflects the concept of a quantifiable relationship between temperature and the rate of crop development (Gordon and Bootsma, 1993; McMaster and Wilhelm, 1997; Bonhomme, 2000; Snyder *et al*., 2013). This relationship has been successfully used in agricultural science to predict and quantify the time between the plant phenological stages (Gilmore and Rogers, 1958; Cross and Zuber, 1972; McMaster, 1993; Cardina *et al*., 2007).

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In the study of phenology and crop development, the concept "physiological time" can be expressed in quantifiable terms, with this time measured in growing degree-days (GDD, °C-days) (McMaster and Wilhelm, 1997; Rodríguez *et al.*, 2012) and representing the thermal units recorded between the minimum (Tb) and maximum (TB) threshold temperatures at the hours of a day (Snyder *et al.*, 2013). Mathematically, the daily GDD are obtained by the integration of growing degree-hours (GDH, °C-hours) for each hour of the day divided by the number of hours in a day (24 h) (Roltsch *et al.*, 1999; Cesaraccio *et al.*, 2001; Souza *et al.*, 2011; Rodríguez *et al.*, 2012; Snyder *et al.*, 2013).

However, hourly data are not always available, for example,when historical records are used or automatic weather stations are scarce. In such cases, the GDD are estimated using mathematical methods that use only daily maximum (TM) and minimum (Tm) temperatures (Snyder *et al.*, 1999). Among the methods most frequently used for calculating degree-days are the rectangle (Arnold, 1959), triangle (Lindsey and Newman, 1956) and sine wave (Baskerville and Emin, 1969) methods, including their variants (Villa-Nova *et al.*, 1972; Ometto, 1981; Snyder, 1985).

According to Roltsch (1999), when the TM exceeds the TB, these methods can use two approximations of accumulated thermal units: (1) the vertical cutoff, which assumes that there has been no accumulation of thermal units and (2) the horizontal cutoff, which assumes that the thermal units continue to accumulate until the Tb is reached. The threshold temperatures are the values under which (Tb) or above which (TB) the development rate is zero.

However, the threshold temperature is only a statistical value, which may be distant from the "physiological temperature" for which the development rate is close to zero, essentially because its value may vary depending on the method used for its calculation, on the growth stage or on the physiological process analyzed (Wang, 1960; Durand *et al.*, 1982; Bonhomme, 2000; Litschmann *et al.*, 2008).

In crops such as Brussels sprouts, cabbage, parsley, legumes, fodder, corn, soybeans and tomatoes, the Tb may oscillate between 0 and 10°C (Gordon and Bootsma, 1993). For *Coffea arabica* L. in Brazil, Lima and Silva (2008) estimated the Tb and TB at 12.9 and 32.4°C, respectively, from transplanting to the first flowering event, whereas Pezzopane *et al.* (2008) obtained a Tb value of 10.2°C from flowering to harvesting based on several harvest cycles using the "Mundo Novo" variety. For most tropical plants, including coffee, the Tb and TB values have been defined, respectively, as 10°C (Pedro-Junior *et al.*, 1977; Jaramillo and Guzmán, 1984) and 32°C (Jaramillo and Guzmán, 1984; Hatfield andPrueger, 2015).

Roltsch *et al.* (1999), Souza *et al.* (2011), Rodríguez *et al.* (2012) and Kean (2013) have found variation in performance when comparing different methods to estimate GDD based on maximum and minimum daily temperatures and when applying those methods to different growing regions. These disparities become relevant, for example, should the estimated accumulation of degree-days be greater than the "real" accumulation; this example would imply that a shorter chronological time than estimated would be necessary to reach a certain phenological stage, or *vice versa* (Bryant *et al.*, 1998), which can affect the predictions of phenological models based on "physiological time" (Kean, 2013).

Given the importance of the degree-day calculation in phenological models, the objective of the present research was to evaluate the performance of four horizontal cutoff methods in estimating degree-days within eight Colombian coffee-producing areas, using thermal thresholds associated with tropical plants.

Materials and methods

Study area and meteorological data

Eight localities with contrasting environmental conditions were selected, espatial distribution and economic importance for Colombian coffee crop was considered. Each locality was equipped with an automatic, RAWS-F, Fire Weather, Campbell Scientific® Remote Automated Weather Station from the Coffee Meteorological Network, a part of the Colombian National Federation of Coffee Producers (Federación Nacional de Cafeteros de Colombia) (Tab. 1, Fig. 2). The air temperature was recorded 24 h a day between February 1, 2014, and January 31, 2015, with five-minute intervals between measurements. The differences in the number of daily logs for the meteorological stations, due to the days with missing data are shown in Table 1. The temperature sensors were previously calibrated, by means of the homologation in parallel between conventional and authomatic stations, with amaximum error of ± 0.5 °C.

Degree-day calculation

For all the developed methods, the Tb and TB values were considered as 10 and 32°C, respectively, following the proposal by Pedro-Junior *et al.* (1977), Jaramillo and Guzmán, (1984), Camargo and Pereira (1994) and Hatfield and Prueger, (2015) for coffee cultivation.

TABLE 1. Geographic location of the weather stations used in the study; average annual historical values for precipitation (**P**), maximum temperature (T_{max}), temperature (T_{med}), minimum temperature (T_{min}) and mean relative humidity (**RH**); and the number of days used in calculating the degree-days for each locality (**ND**).

Weather station	Department	Elevation (m)	Latitude	Longitude	P (mm)	T_{max} (°C)	T_{med} (°C)	T_{min} (°C)	RH (%)	ND
El Sauce	Nariño	1609	01°37´	77°07´	1853	25.7	19.9	16.1	77.5	292
Jorge Villamíl	Huila	1420	02°20´	75°31´	1337	24.5	19.8	16.2	76.6	365
Julio Fernández	Valle del Cauca	1381	03°49´	76°32´	1094	25.7	19.9	16.4	78.3	337
Paraguaicito	Quindío	1203	04°24´	75°44´	2179	28.1	21.7	17	78.5	365
Naranjal	Caldas	1381	04°58´	75°39´	2795	26.8	20.9	16.6	76.9	337
Bertha	Boyacá	1677	05°53´	73°34´	1999	26.1	18.8	13.2	77.3	337
El Rosario	Antioquia	1635	05°58´	75°42´	2645	24.9	20.1	16.2	75.3	281
Pueblo Bello	Cesar	1134	10°25´	73°34´	2043	27.2	21.0	15.7	81.1	337

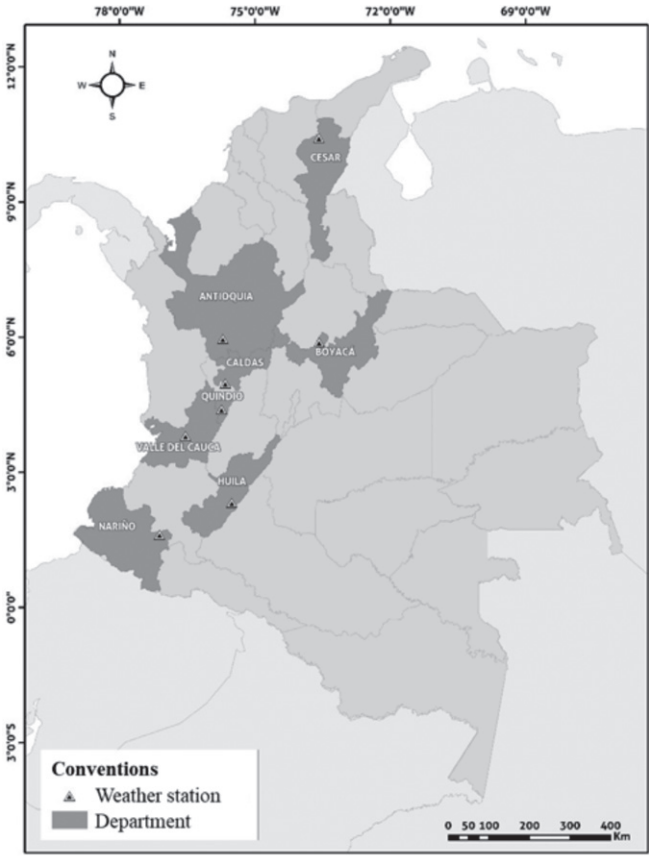

FIGURE 1. Location in Colombia of the weather stations of the Coffee Meteorological Network used in the study.

Numerical integration by trapezoidal rule (reference method)

The integration method was used as the reference method against which the indirect methods were evaluated due to its greater estimation precision and its comparatively frequent data collection at relatively short intervals (Cesaraccio *et al.*, 2001; Souza *et al.*, 2011; Rodríguez *et al.*, 2012).

The estimate of GDD using the reference method (GDD_r) was calculated as follows:

$$GDD_r = GDD_{TT} - (GDD_{TB} + GDD_{Tb}) \quad (1)$$

Where GDD_r = degree-days of the reference method (°C-days), GDD_{TT} = total degree-days (°C-days), GDD_{TB} = degree-days above TB (°C-days), and GDD_{Tb} = degree-days below Tb (°C-days).

To obtain the values that compose expression (1), we used expression (2), in which the areas of individual trapezoids constructed from the temperature records were calculated to apply the "trapezoid rule". These areas were integrated into an area under the curve (AUC), which was divided by the total number of seconds per day (86400), thus obtaining the daily thermal units, representing the total degree-days per day (GDD_{TT}) in this case. To calculate the GDD_{TB} and GDD_{Tb}, the AUC was integrated, assuming that the temperatures (the m_i values in expression 2) were, respectively, higher than or equal to the TB and below or equal to the Tb at corresponding times (Fig. 2).

$$TU = \frac{\sum_{i=2}^{n} 0{,}5 * (m_i + m_{i-1}) * (t_i - t_{i-1})}{86400} \quad (2)$$

Where **TU** = thermal units, $\mathbf{m}_i$ = temperature in the i^{th} measurement, and $\mathbf{t}_i$ = i^{th} time in the daily logs.

Indirect methods evaluated

The daily "cumulative growing degree days" or "physiological time" in GDD was estimated using the daily maximum and minimum temperature and four indirect methodologies formerly described in the literature (Arnold, 1959; Villa-Nova *et al.*, 1972; Ometto, 1981; Snyder, 1985) (Tab. 2), some of them previously applied to coffee production (Lima and Silva, 2008).

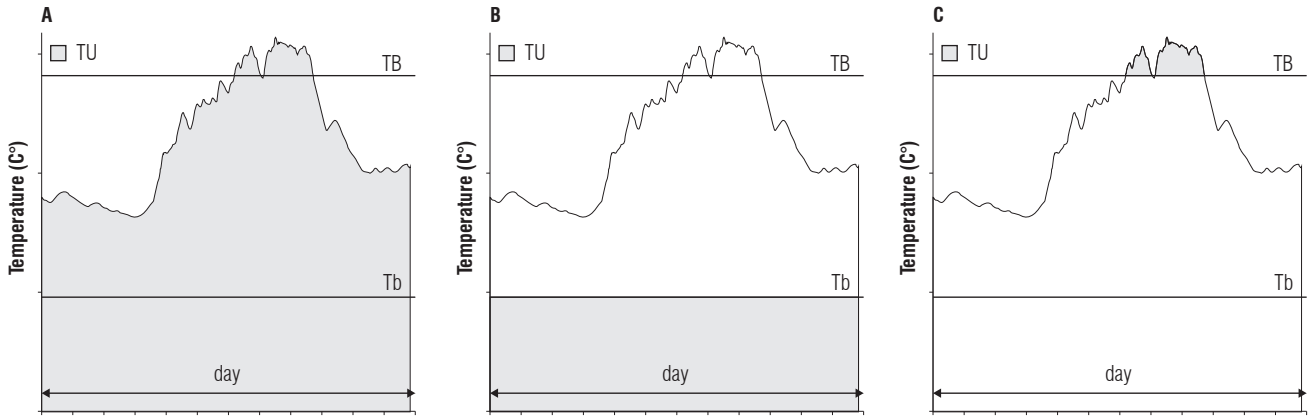

FIGURE 2. Description of thermal units (**TU**) integrated in the **AUC** for $\mathbf{GDD_{TT}}$ (a), $\mathbf{GDD_{Tb}}$ (b) and $\mathbf{GDD_{TB}}$ (c).

TABLE 2. Indirect methods of degree-day estimation used in the analysis.

Method	Use context	Equation	
Arnold (1959)	General	$GDD = \left(\frac{TM + Tm}{2}\right) - Tb$	(3)
Villa-Nova *et al.* (1972)	Tb ≥ Tm	$GDD = \frac{(TM - Tb)^2}{2(TM - Tm)}$	(4)
	Tb < Tm	$GDD = \frac{(Tm - Tb) + (TM - Tm)}{2}$	(5)
	Tb ≥ TM	$GDD = 0$	(6)
Ometto (1981)	TB > TM > Tm > Tb	$GDD = \left(\frac{TM - Tm}{2}\right) + (Tm - Tb)$	(7)
	TB > TM > Tb > Tm	$GDD = \frac{(TM - Tb)^2}{2(TM - Tm)}$ (8)	(8)
	TB > Tb > TM > Tm	$GDD = 0$	(9)
	TM > TB > Tm > Tb	$GDD = \frac{\left(2(TM - Tm)(Tm - Tb)\right) + (TM - Tm)^2 - (TM - TB)^2}{2(TM - Tm)}$	(10)
	TM > TB > Tb > Tm	$GDD = \frac{1}{2}\left(\frac{(TM - Tb)^2 - (TM - TB)^2}{(TM - Tm)}\right)$	(11)
Snyder (1985)		$M = \left(\frac{TM + Tm}{2}\right)$	(12)
		$W = \left(\frac{TM - Tm}{2}\right)$	(13)
		$\theta = arcsin\left(\frac{Tb - M}{W}\right)$	(14)
		$\varphi = arcsin\left(\frac{TB - M}{W}\right)$	(15)
	Tb < Tm	$GDD = \left(\frac{TM + Tm}{2}\right) - Tb$	(16)
	Tb > Tm	$GDD = \frac{\left[(M - Tb)\left(\frac{\pi}{2} - \theta\right) + (W \cos\theta)\right]}{\pi}$	(17)

Continue

TABLE 2. *Continuation*

Method	Use context	Equation	
Snyder (1985)	Tm > Tb; TM > TB	$GDD_1 = \left(\frac{TM + Tm}{2}\right) - Tb$	(18)
		$GDD_2 = \frac{\left[(M - TB)\left(\frac{\pi}{2} - \varphi\right) + (W \cos\varphi)\right]}{\pi}$	(19)
		$GDD = GDD_1 - GDD_2$	(20)
	Tb > Tm; TM > TB	$GDD_1 = \frac{\left[(M - Tb)\left(\frac{\pi}{2} - \theta\right) + (W \cos\theta)\right]}{\pi}$	(21)
		$GDD_2 = \frac{\left[(M - TB)\left(\frac{\pi}{2} - \varphi\right) + (W \cos\varphi)\right]}{\pi}$	(22)
		$GDD = GDD_1 - GDD_2$	(23)

Where: TM = maximum daily temperature (ºC), Tm = minimum daily temperature (ºC), Tb = minimum threshold temperature (ºC), TB = maximum threshold temperature (ºC), GDD = growing degree-days according to the indirect method (°C-days), GDD_1 = degree-days above Tb (°C-days), and GDD_2 = degree-days above TB (°C-days).

Statistical analysis

A linear regression model of the observed values was adjusted to each locality and indirect method, fitting the reference method data (dependent variable, Y) as a function of the data obtained with the indirect method (independent variable, X). The significance of the regression coefficients was evaluated using a t-test with α=5%. To identifying the best adjustment, we also evaluated whether the regression slope coefficient (β_1) differed statistically from one to determine whether the indirect method overestimated or underestimated the GDD. Additionally, the coefficient of determination (R^2) was calculated.

The bias of the degree-days accumulated by each conventional method was estimated as the percent bias (PB) of the residual (expressed as percentage), as shown by expression (24).

$$PB = \left(\frac{\sum_{i=1}^{n}(GDD - GDD_r)}{\sum_{i=1}^{n} GDD_r}\right) * 100 \quad (24)$$

Where: GDD_r = degree-days of the reference method (°C-days); GDD = growing degree-days according to the indirect method (°C-days).

The statistical analyses were performed using SAS software version 9.4 (SAS Institute, 2012).

Results and discussion

The results of adjusting the regression model with the data from the reference method expressed as a function of the data obtained from each of the indirect methods are presented in Table 3 for each weather station. The regression coefficients (β_1) were significantly different from zero for all the methods by the t-test at the 5% probability level, indicating that the values obtained with each of the indirect methods help explain the results observed with the reference method. The proportion of the data variability observed with the reference method that was explained by the indirect method varied from 32 to 82%, according to the coefficients of determination (Tab. 3).

The regression coefficient was equal to one (it means, a 1:1 ratio existed between the indirect method and the reference method) for the indirect method of Villa-Nova *et al.* (1972) and for the localities of Naranjal (β_1 = 0.960°C-days) and El Rosario (β_1 = 1.053°C-days) (t-test, α = 5%). The coefficient was significantly different from 1 for all the other localities.

Regarding goodness-of-fit, R^2 for the methods proposed by Arnold (1959), Ometto (1981) and Snyder (1985) exceeded 0.73 for the El Rosario, El Sauce, Jorge Villamíl, Naranjal and Paraguaicito localities and varied between 0.59 and 0.68 for Julio Fernández and Pueblo Bello. For every locality assessed, the R^2 obtained for the method of Villa-Nova *et al.* (1972) was lower compared to all the other methods. The lowest adjustment values for R^2 were recorded for a locality in Bertha (between 0.32 and 0.44).

The PB showed that when the degree-day errors accumulated, for the methods of Arnold (1959), Ometto (1981) and Snyder (1985) overestimated the GDD relative to the reference method by between 8.3 and 17.2% at all eight

TABLE 3. Regression coefficient (β_1), standard error (STE), coefficient of determination (R^2) and percent bias (PB) obtained in the relation between accumulated degree-days in each indirect method and the accumulated degree-days estimated by numeric integration during the evaluated period in eight coffee-growing areas of Colombia.

Station and degree-days accumulated (reference method)	Indirect method	Regression coefficient $\beta_1 \pm$ SE			R^2	Accumulated (indirect method) (°C-day)	PB (%)
Bertha (ΣGDDr= 3148°C-día)	Arnold(1959)	0.566 ± 0.035	a	b	0.44	3685.4	17.08
	Ometto(1981)	0.569 ± 0.035	a	b	0.44	3686.3	17.11
	Snyder(1985)	0.572 ± 0.035	a	b	0.44	3687.8	17.16
	Villa *et al.* (1972)	0.529 ± 0.042	a	b	0.32	3085.1	-1.99
El Rosario (ΣGDDr= 2907°C-día)	Arnold(1959)	0.908 ± 0.028	a	b	0.79	3264.5	12.31
	Ometto(1981)	0.908 ± 0.028	a	b	0.79	3264.5	12.31
	Snyder(1985)	0.908 ± 0.028	a	b	0.79	3264.5	12.31
	Villa *et al.* (1972)	1.053 ± 0.038	a		0.73	2334.6	-19.68
El Sauce (ΣGDDr= 3004°C-día)	Arnold(1959)	0.798 ± 0.027	a	b	0.75	3490.3	16.20
	Ometto(1981)	0.798 ± 0.027	a	b	0.75	3490.3	16.20
	Snyder(1985)	0.798 ± 0.027	a	b	0.75	3490.3	16.20
	Villa *et al.* (1972)	0.864 ± 0.037	a	b	0.66	2541.0	-15.41
Jorge Villamil (ΣGDDr= 3711°C-día)	Arnold(1959)	0.810 ± 0.020	a	b	0.82	4161.0	12.12
	Ometto(1981)	0.810 ± 0.020	a	b	0.82	4161.0	12.12
	Snyder(1985)	0.810 ± 0.020	a	b	0.82	4161.0	12.12
	Villa *et al.* (1972)	0.826 ± 0.027	a	b	0.73	2946.4	-20.61
Julio Fernández (ΣGDDr= 3650°C-día)	Arnold(1959)	0.677 ± 0.026	a	b	0.68	4183.0	14.61
	Ometto(1981)	0.677 ± 0.026	a	b	0.68	4183.0	14.60
	Snyder(1985)	0.678 ± 0.026	a	b	0.68	4182.9	14.60
	Villa *et al.* (1972)	0.728 ± 0.038	a	b	0.52	3021.3	-17.22
Naranjal (ΣGDDr= 3828°C-día)	Arnold(1959)	0.926 ± 0.023	a	b	0.82	4354.8	13.76
	Ometto(1981)	0.926 ± 0.023	a	b	0.82	4354.8	13.76
	Snyder(1985)	0.926 ± 0.023	a	b	0.82	4354.8	13.76
	Villa *et al.* (1972)	0.960 ± 0.029	a		0.76	3196.8	-16.49
Paraguaicito (ΣGDDr= 4465°C-día)	Arnold(1959)	0.869 ± 0.026	a	b	0.76	5085.7	13.90
	Ometto(1981)	0.870 ± 0.026	a	b	0.76	5085.5	13.90
	Snyder(1985)	0.871 ± 0.026	a	b	0.76	5085.1	13.89
	Villa *et al.* (1972)	0.832 ± 0.027	a	b	0.72	3747.8	-16.06
Pueblo Bello (ΣGDDr= 3871°C-día)	Arnold(1959)	0.780 ± 0.036	a	b	0.59	4193.0	8.32
	Ometto(1981)	0.780 ± 0.036	a	b	0.59	4193.0	8.32
	Snyder(1985)	0.780 ± 0.036	a	b	0.59	4193.0	8.32
	Villa *et al.* (1972)	0.836 ± 0.062	a	b	0.35	3178.8	-17.88

SE = standard error; a = t-test with alpha 5% for β_1 values significantly different from zero; b = t-test with alpha 5% for β_1 values significantly different from one.

localities. Under the thermal thresholds evaluated, these three methods were also consistently observed to show no significant differences. The method described by Villa-Nova *et al.* (1972), with negative PB values, was the only one to underestimate the reference method results, ranging between 2.0 and 20.6%.

In the present study, the methods of Arnold (1959), Ometto (1981) and Snyder (1985) showed no descriptive differences by locality regarding the regression coefficient, R^2 or PB values (Tab. 3). Souza *et al.* (2011) also reported that these three methods produce similar results, which is due to the similar geometric forms usedby these methods for estimating the degree-days based on Tb and TB.

According to the above, these methods did not present differences, because most of the minimum temperatures were higher than the Tb in the daily records. However, in

a very few cases, the maximum temperatures exceeded the TB. This occurrence causes the methods of Ometto (1981) and Snyder (1985) to consider expressions based only on the Tb, a situation bearing similarity to the arising results with the method of Arnold (1959), which only includes Tb in its mathematical expression. According to Higley *et al.* (1986), even though omitting a maximum development threshold could decrease the precision of the degree-day estimation, the introduced error is low as long as the daily maximum temperatures are generally lower than the maximum threshold temperature for development (TB).

The evaluation criteria did indicate different performance among the localities for themethods of Arnold (1959), Ometto (1981) and Snyder (1985). For example, higher coefficients of determination (R^2) were observed for the localities of El Rosario, El Sauce, Jorge Villamíl, Naranjal and Paraguaicito compared to those on Bertha, Julio Fernández and Pueblo Bello (Tab. 3), this implies a better fit for the first. Carlson and Hancock (1991) and Roltsch *et al*. (1999) highlight the importance of R^2 as an evaluation criterion to determine the degree of fit between methods.

The PB also varied among the localities, although the variation was inconsistent with that described for the adjustment. Comparatively high PB values were obtained for Bertha and El Sauce, where as those for El Rosario, Jorge Villamíland Pueblo Bello were low (Tab. 3). The greater PB for Bertha could be attributed to the lower temperatures recorded in this area (Tab. 1). By contrast, the greater PB for El Sauce could be mostly due to variation in cloudiness and wind speed, which affect the daily temperature fluctuation. Roltsch *et al*. (1999) and Worner (1988) found that when degree-days are estimated by indirect methods, a greater error is found for cold areas or climates in which fast thermal changes occur. Both error and bias are important parameters to consider because the prediction of phenological events is linked to the accumulation of physiological time (Rodríguez *et al.*, 2012).

The accumulated error evaluated by the PB did not exceed 17.1% compared to Arnold (1959), Ometto (1981) or Snyder (1985) methods in any locality (Tab. 3). This error could be considered acceptable for the prediction as regards to the PB found by Rodríguez *et al*. (2012) for Colombia (between 7.13 and 30.57%). The different errors involved may cancel each other to some extent, but the greatest source of error, which is thermal accumulation, results from inaccurate temperature estimation (Pruess, 1983). Those methods with positive PB values overestimated the degree-days relative to the ones calculated by the reference method, which was also reported by Souza *et al.* (2011).

An overestimation can be convenient, considering that an underestimation of accumulated degree-days can lead to erroneous conclusions regarding a phenological event in poikilothermic organisms by inaccurately predicting a later event than actually occurs (Bryant *et al*., 1998). Plant development may be further affected by not only nutritional status and water stress but also photoperiod in long-day plants and, to a lesser extent, in short-day plants (Bonhomme, 2000). Therefore, any deleterious effects of these factors should be reduced for cash crops, with all required substrates present in amounts adequate to ensure growth (Higley *et al*., 1986).

The method proposed by Villa-Nova *et al.* (1972) was the only one to present regression coefficients statistically equal to 1 and to underestimate the reference method, contrasting with the results reported by Souza *et al.* (2011).

Conclusion

Considering the PB, the methods proposed by Arnold, Ometto and Snyder tend to overestimate thermal time, whereas the method proposed by Villa-Nova underestimates thermal time, but with a lower performance regarding to the previous ones. However, the performance of each method can vary between zones due to the agri-environmental conditions typical of each locality. Arnold's method can be taken into account when daily temperatures do not exceed the maximum or minimum threshold considered, as in the present study. The use of the different methods depends on the available information and the objective of the thermal time estimation.

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