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Caracterización por colorimetría de accesiones de plátano del Sistema de Bancos de Germoplasma en Colombia

Colorimetry characterization for banana accessions of the Germplasm Bank System in Colombia

Ayda Lilia Enriquez Valencia[1], Alvaro Caicedo Arana[1], Luis Eduardo Ordoñez Santos[2], Eberto Rodriguez Henao[1].*

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Resumen

El color es uno de los atributos sensoriales que mejor define la calidad de un alimento, no obstante las investigaciones sobre el color superficial de pulpa de frutos de musáceas en Colombia son escasas. Esta información es de gran utilidad para identificar materiales promisorios destinados para consumo fresco y procesamiento agroindustrial. En este estudio se caracterizó el color superficial de la pulpa de 25 accesiones de plátano *Musa* sp. (Musaceae) del Banco de Germoplasma de Musáceas del Sistema de Bancos de Germoplasma de la Nación para la Alimentación y la Agricultura (SBGNAA) existente en la Corporación Colombiana de Investigación Agropecuaria (Agrosavia) en Palmira, Colombia. Las coordenadas CIE_{L*a*b*} fueron determinadas por triplicado utilizando un colorímetro, entre ellas se calcularon parámetros de cromaticidad (C*), tonalidad (hº) y diferencia de color (ΔE). Las accesiones de plátano presentaron rangos de L*(17.31-79.30), a*(8.73-30.64). y b*(15.24-95.49), C*(17.77-98.22), hº(38.24-81.00) y ΔE*(147.07-6851.24). Las pulpas de plátano fueron clasificadas colorimétricamente entre amarillos tenues a amarillos intensos. La variación de las coordenadas colorimétricas a*, hº, y ΔE se ajustaron a una cinética de orden cero; y L*, b* y C* describieron una cinética de primer orden. Las accesiones Benedetta, Cachaco espermo, Cachaco sin bellota, Dominico mocho, Dominico mutante, GAEP-2, Hartón tigre y Maia Maoli Risaralda, fueron clasificadas como materiales promisorios para consumo en fresco o procesos agroindustriales, por los bajos valores de la constante de velocidad cinética (k) registrados en el presente estudio.

Palabras clave: Color superficial, *Musa balbisiana*, pardeamiento, polifenol oxidasa, recursos genéticos, Colombia.

Abstract

Color is one of the sensory attributes that best define the quality of a food, however, to date there are no scientific investigations that record the study of superficial color of the fruit pulp of Musaceae, which provides useful information to identify promising materials destined for fresh consumption and agroindustrial processing. Surface color of plantain, *Musa* sp. (Musaceae) pulp from the Musaceae Germplasm Bank of the Corporación Colombiana de Investigación Agropecuaria (Agrosavia) was characterized. The CIE_{L*a*b*} coordinates of the pulp of 25 plantain accessions were determined in triplicate using a colorimeter. From the coordinates of the surface color, parameters of chromaticity (C*), hue (hº), and color difference (ΔE) were calculated. Plantain accessions presented a range of L* (17.31-79.30), a* (8.73-30.64), and b* (15.24-95.49), C* (17.77) -98.22), hº 38.24-81.00) and ΔE* (147.07-6851.24). The plantain pulps can be classified colorimetrically among mild to intense yellows. Variation of the colorimetric coordinates a*, hº, and ΔE were adjusted to zero-order kinetics, and L*, b* and C* described a first-order kinetics. Accessions Benedetta, Cachaco espermo, Cachaco sin bellotae, Dominico mocho, Dominico mutant, GAEP-2, Hartón tigre and Maia Maoli Risaralda, are reported as promising materials for fresh consumption or agroindustrial processes, due to the low values of the constant speed kinetics (k) recorded in the present study.

Keywords: browning, genetic resources, *Musa balbisiana*, polifenol oxidase, superficial color, Colombia.

Introducción

Los plátanos *Musa* sp. (Musaceae) son considerados como la cuarta fuente de energía de mayor importancia, después del maíz, arroz y trigo, con una producción mundial de 37.87 millones de toneladas. Después de Uganda, Colombia es el segundo productor de musáceas en el mundo con 3.30 millones de toneladas (FAO, 2014). Los programas de mejoramiento genético en musáceas se han orientado principalmente al desarrollo de variedades resistentes a plagas y enfermedades; no obstante, algunas de ellas son rechazadas por los consumidores debido a la presencia de atributos no deseables en sus características organolépticas (Dzomeku, Osei, Ankomah, Akyeampong y Darkey, 2006; Arvanitoyannis y Mavromatis, 2009).

En la calidad de los alimentos, la apariencia es el atributo visual más importante, ya que relaciona el tamaño, la forma y el color (Werner, Machado, Poncio y Ferreira, 2009). El color es uno de los factores que mejor define la apariencia de un alimento. En plátanos, cuando la pulpa es cortada, los altos niveles de pardeamiento enzimático son una de las principales causas de pérdida de calidad poscosecha (Martínez, Pagán, Garza e Ibarz, 2010). El pardeamiento enzimático es causado por la acción de la Polifenoloxidasa (PPOs) sobre los fenoles contenidos en el fruto, causando su oxidación y polimerización (García, Giraldo, Hurtado y Mendivil, 2006; Quinde, Ullrich y Baik, 2004).

El color superficial de un fruto o alimento puede ser identificado a partir de varios sistemas, entre ellos el más utilizado es CIE_{L*a*b*} (Commission Internationale de l'Eclairage) que define las coordenadas L*, a* y b*, donde L* es el eje vertical y representa la medida de luminosidad de un color, variando desde cero para color negro hasta 100 para color blanco; a* es uno de los dos ejes horizontales que define la desviación del punto acromático correspondiente a la claridad, hacia el rojo si es mayor y hacia el verde si es menor que cero; y b* es el otro eje horizontal perpendicular al eje a* y define la desviación hacia el amarillo si es mayor y hacia el azul si es menos que cero. Con los valores obtenidos de L*, a* y b* es posible calcular el índice de saturación del color o croma (C*), el ángulo de tonalidad (h°) y el cambio de color total ΔE* (Hernandez, Barrera, Martínez y Fernández, 2009).

Estudios sobre el color superficial en frutos en almacenamiento de *Musa acuminata* Colla (tipo Cavendish) y *Musa paradisiaca* L., demuestran los aumentos en los valores de a* y b*, lo que corresponde a la maduración (Salvador, Sanz y Fiszman, 2007). Werner et al. (2009) evaluaron el cambio de color del banano durante el almacenamiento a partir de escala visual e imágenes digitales, encontrando relación entre ambos métodos. Ji et al. (2013) presentaron una nueva metodología de imagen digital que podría ser utilizada para estimar con mayor precisión los estados de maduración de musáceas. Sin embargo, a la fecha en Colombia no existen investigaciones científicas sobre el color superficial en la pulpa de plátano, información que es de gran utilidad para identificar materiales promisorios. El objetivo de este estudio fue caracterizar el color superficial de la pulpa del fruto de 25 accesiones de plátano del banco de Germoplasma de musáceas de la Corporación Colombiana de Investigación Agropecuaria (Agrosavia), en el Centro de Investigación (C.I.) Palmira, Colombia.

Materiales y métodos

Material vegetal

Se utilizaron frutos de 25 accesiones de plátanos del Banco de Germoplasma de Musáceas del -SBGNAA (Sistema de Bancos de Germoplasma de la Nación para la Alimentación y la Agricultura) establecido en Agrosavia en el Centro de Investigación de Palmira, Valle del Cauca, Colombia, ubicado a 1000 m.s.n.m., 24 °C y 1200 mm de precipitación, promedio anual. El manejo agronómico se realizó con base en protocolos ya establecidos (Belalcázar, 1991), los cuales fueron programados anual, mensual y semanalmente. Los racimos fueron identificaron mediante el cinteo de plantas recién paridas (primera semana de aparición de la bellota). Esto permitió tener un inventario de las posibles plantas que se incluyeron en el estudio. Se observaron los frutos hasta que las aristas de estos se redujeron y el color verde de los dedos de la primera mano del racimo presentó una tonalidad amarilla, momento en el que se cosechó, según la guía técnica INIBAP (Dadzie y Orchard, 1997).

Evaluación fisicoquímica

Todas las evaluaciones fisicoquímicas se realizaron de acuerdo con los protocolos descritos por Dadzie y Orchard (1997), para el efecto se pesaron 30 g de pulpa del dedo central de la segunda mano que se licuaron durante 2 min en un mezclador con 90 ml de agua destilada antes de ser pasados por un filtro. El pH se determinó utilizando un potenciómetro modelo 744 meter metrohm. La acidez se midió por titulación con reacción acido/base y los resultados se expresaron en gramos de ácido málico/100 g. pulpa. El contenido de materia seca (MS%) se obtuvo mediante la diferencia de peso fresco y seco.

Determinación del color superficial

Después de la cosecha de los racimos se seleccionó un fruto central de la cuarta mano, la pulpa fue homogenizada en trazos de un milímetro de espesor y colocada en cajas Petri para realizar la lectura de color en condiciones ambientales. Para la calificación del color se utilizó un colorímetro portátil marca CR410 de Konica Minolta, un ángulo de observador de 10°, iluminante D65, la calibración del equipo se realizó con la placa de valores de referencia X = 79.01, Y = 83.96, Z = 86.76. A partir de los espectros de reflexión se obtuvieron las coordenadas de color de $CIE_{L^*a^*b^*}$, las cuales fueron registradas, para cada muestra por triplicado en diferentes tiempos (0, 15, 30, 60 y 120 minutos, respectivamente) después de la preparación de la muestra. Los parámetros de color calculados fueron croma (C*), ángulo de tonalidad (h°) y cambio de color (ΔE*), según las ecuaciones 1, 2 y 3, respectivamente (Ramírez y Rodríguez, 2012). Los datos registrados en el colorímetro fueron analizados utilizando Microsoft Excel® (versión 2010) y expresados como promedio aritmético.

$$C^* = \sqrt{a^2 + b^2} \quad \textbf{Ec. 1}$$

$$h^* = arctg\left(\frac{b}{a}\right) \quad \textbf{Ec. 2}$$

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad \textbf{Ec. 3}$$

Determinación de parámetros cinéticos

Para la aplicación de la cinética de cambio de color existen varias ecuaciones que permiten determinar dichos cambios de materiales en alimentos como una función en el tiempo (Bal, Kar, Satya y Naik, 2011). La tasa de cambio de un factor de calidad C puede ser representado por la ecuación 4.

$$\frac{dC}{dt} = -kC^n \quad \textbf{Ec. 4}$$

Donde, k es la constante de velocidad cinética, C es el valor del color superficial de interés en el tiempo t, y n es el orden de la reacción. Para la mayoría de los alimentos, las relaciones de dependencia del tiempo son descritas por modelos cinéticos de orden cero (Ecuación 5) y de primer orden (Ecuación 6) (Bal et al., 2011), mediante la integración de la ecuación 4.

$$C = C_0 \pm kt \quad \textbf{Ec. 5}$$

$$C = C_0\, exp(\pm kt) \quad \textbf{Ec. 6}$$

donde, C_0 es el valor inicial de color y C es el valor de color en un tiempo específico. En las ecuaciones, (±) indica la formación y la degradación de cualquier parámetro de calidad (Bal et al., 2011). El orden de la reacción para los parámetros de color superficial de la pulpa de plátano se determinó mediante el ajuste de los datos experimentales a las Ecuaciones 5 y 6, utilizando análisis de regresión lineal. En cada caso, se seleccionó el mejor ajuste y se determinó la constante de velocidad cinética de la reacción.

Análisis estadístico

Los tratamientos fueron registrados por triplicado y presentados en valores medios y desviación estándar. El análisis de color $CIE_{L^*a^*b^*}$ en las muestras se realizó mediante regresión lineal usando Microsoft® Excel® para Office 365 MSO versión 1908. Para determinar las muestras que presentaban el menor nivel de pardeamiento se hicieron análisis de regresión y se determinó el coeficiente de correlación (R^2).

Resultados

Las propiedades fisicoquímicas en las pulpas de plátano evaluadas presentaron un rango de pH entre 4.97 y 6.33, una acidez titulable entre 0.03 y 0.11 g de ácido málico/100g pulpa y un contenido de materia seca entre 2.63 y 45.03%. En los Cuadros 1 y 2 se incluyen los atributos de color superficial en la pulpa de las diferentes accesiones en función del tiempo de evaluación y el parámetro cinético k estimado para las cinéticas de orden 0 y 1. Los valores iníciales de las coordenadas colorimétricas en 25 accesiones analizadas registraron rangos: L*(17.31-79.30), a*(8.73-30.64), y b*(15.24-95.49), C*(17.77-98.22), h°(38.24-81.00) y ΔE*(147.07-6851.24) (Cuadro 1). Durante la evaluación de la pulpa, las coordenadas de color L*, a*, b*, C*, y h* se redujeron mientras que ΔE se incrementó (Cuadro 2).

La modelación de los cambios de color en las muestras analizadas se realizó aplicando las cinéticas de orden 0 y 1, tal como se puede observar en el Cuadro 2. La variación de las coordenadas colorimétricas a*, h°, y ΔE se ajustaron a una cinética de orden cero (0), donde los parámetros cinéticos de k en las variables de color: a* fue 0.0002-0.08 min^{-1} R^2 = 0.00001-0.89; h*, k = 0.04-0.30 min^{-1} R^2 = 0.45-0.99; y ΔE, k = 18.46-53.39 min^{-1} R^2 = 0.67-0.98 (Cuadro2). En el caso de las coordenadas L*, b* y C* los cambios de color se describen con una cinética de primer orden donde los valores de k oscilaron para L*, k = 0.0033-0.01 min^{-1}, R^2 = 0.73-0,99; b*, k = 0.003-0.01 min^{-1}, R^2 = 0.51-0.99 y; C*, k = 0.0021-0.011 min^{-1}, R^2 = 0.54-0.98 (Cuadro 2).

Cuadro 1. Parámetros de color superficial de accesiones de plátano en el Sistema de Bancos de Germoplasma en Colombia. Agrosavia, C.I. Palmira.

Accesión	Tiempo (minutos)	L*	a*	b*	C*	h*	ΔE
¾ naine	0	65.0±1.3	28.7±0.5	84.9±1.3	89.6±1.2	71.4±0.5	
	120	32.3±0.8	26.0±3.4	40.0±1.3	47.8±1.5	57.1±4.0	6452.8±278.0
Benedetta	0	71.3±0.7	16.3±0.3	83.3±1.6	84.9±1.6	78.9±0.2	
	120	42.5±3.4	15.9±0.5	52.6±1.8	54.9±1.6	73.2±1.0	5305.2±374.2
Cachaco espermo	0	76.6±1.1	13.4±0.6	84.3±1.2	85.4±1.1	81.0±0.5	
	120	41.2±1.4	18.1±0.8	45.8±2.7	49.2±2.2	68.4±1.9	6522.7±53.1
Cachaco sin bellota	0	74.3±3.6	16.9±0.8	79.9±1.5	81.7±1.3	78.0±0.7	
	120	46.3±1.1	22.5±0.3	57.0±2.0	61.2±1.9	68.4±0.8	4626.6±750.7
Dominico enano	0	53.9±0.5	22.0±0.3	34.8±0.3	41.2±0.2	57.8±0.6	
	120	28.2±1.6	19.9±0.7	17.3±1.1	26.4±1.2	41.0±0.9	2298.4±102.9
Dominico Hartón Támesis	0	59.2±0.9	25.6±0.4	82.6±1.4	86.5±1.4	72.8±0.1	
	120	26.0±1.0	23.0±0.5	32.4±2.0	39.7±1.9	54.6±1.1	6436.1±206.3
Dominico maqueño	0	59.9±8.8	18.4±1.1	65.2±3.1	67.7±3.3	74.3±0.2	
	120	26.1±0.6	23.2±0.2	30.4±0.1	38.3±0.1	52.6±0.3	4532.6±523.6
Dominico mocho	0	57.9±0.4	21.0±0.9	36.7±0.2	42.2±0.6	60.2±0.9	
	120	38.4±1.7	17.1±0.3	22.1±0.4	28.0±0.5	52.2±0.3	2078.8±88.9
Dominico mutante	0	73.9±0.1	10.3±0.3	62.2±1.3	63.1±1.3	80.6±0.1	
	120	44.0±1.3	20.3±0.1	39.2±0.4	44.2±0.4	62.6±0.3	4234.3±162.1
FHIA 110	0	62.8±0.9	16.1±0.2	60.4±2.1	62.5±2.0	75.1±0.4	
	120	21.6±3.1	24.5±0.5	19.5±3.5	31.4±2.6	38.2±4.3	4782.2±436.8
Figue famile	0	57.7±1.6	17.5±0.3	53.9±0.7	56.7±0.7	72.0±0.2	
	120	25.1±0.9	24.5±0.5	24.9±1.2	34.9±1.2	45.4±1.0	3554.2±165.2
GAEP-2	0	58.7±3.6	16.3±0.3	70.1±0.5	71.9±0.6	76.9±0.2	
	120	35.9±7.6	16.8±3.3	25.1±1.6	30.3±3.2	56.4±3.5	4825.0±357.4
Hartón tigre	0	65.4±0.9	22.4±0.2	89.7±0.6	92.5±0.5	76.0±0.2	
	120	44.2±3.2	25.0±0.4	61.3±4.4	66.2±4.1	67.8±1.4	4871.0±531.9
Hondureño enano	0	71.0±6.1	23.6±2.9	78.5±3.7	81.9±4.4	73.3±1.3	
	120	30.9±3.5	24.2±0.4	32.6±1.6	40.7±1.1	53.4±1.8	6574.6±372.8
Kelongmekintu	0	47.2±1.9	19.6±0.6	62.4±2.5	65.4±2.5	72.5±0.2	
	120	17.3±1.5	8.7±1.5	15.2±4.4	17.8±3.2	58.9±10.4	4147.3±207.5
Maiamaoli Risaralda	0	79.3±0.3	23.0±0.1	95.5±0.8	98.2±0.7	76.5±0.1	
	120	47.4±1.1	27.3±0.4	59.9±3.0	65.9±2.8	65.2±0.9	6851.2±237.9
Mbindi	0	54.1±2.3	15.1±0.8	56.6±0.6	58.7±0.5	74.5±0.8	
	120	30.0±2.1	24.9±0.1	35.7±1.0	43.5±0.8	55.1±0.8	2823.6±84.5
Mbourou kou-1	0	54.1±1.2	22.4±1.3	74.2±0.7	77.5±0.4	73.2±1.1	
	120	26.9±0.5	24.1±0.2	34.6±0.6	42.2±0.4	55.2±0.6	4834.1±176.8
Neypoovan	0	62.2±2.5	13.1±0.4	43.5±1.6	45.4±1.4	73.2±0.9	
	120	27.3±4.7	12.4±0.5	17.4±1.9	21.4±1.8	54.6±1.9	3499.4±194.0
Pompo o comino	0	63.2±3.9	21.7±0.9	66.2±1.2	69.6±1.4	71.8±0.5	
	120	18.3±0.4	23.5±0.7	20.9±0.5	31.5±0.9	41.7±0.2	5395.1±353.3
Hibrido saba2	0	58.0±1.4	16.0±0.8	61.9±0.6	64.0±0.7	75.5±0.5	
	120	33.7±0.6	26.2±0.3	41.0±0.2	48.6±0.1	57.5±0.2	3133.5±118.0
Pompo o comino Risaralda	0	63.1±1.5	26.1±0.2	76.9±0.6	81.3±0.5	71.0±0.1	
	120	35.3±5.8	22.3±0.2	40.7±6.3	46.6±5.6	60.9±3.5	5026.0±725.9
Hartón Santander	0	71.3±0.4	12.0±0.2	69.4±0.1	70.6±0.2	79.5±0.4	
	120	19.7±2.1	19.1±0.2	19.1±2.2	27.1±1.7	44.9±3.3	6464.8±144.5
BLANCO SARDY	0	54.2±2.2	27.0±0.4	73.1±2.2	78.2±2.2	69.1±0.4	
	120	29.2±0.9	30.1±0.1	38.4±1.2	48.8±1.0	51.9±0.8	4401.3±484.5
Hartón Macho	0	61.6±0.4	21.7±0.7	81.6±1.7	84.4±1.7	75.1±0.3	
	120	36.9±0.1	25.2±0.3	47.6±0.6	53.9±0.7	62.0±0.1	5023.2±263.2

Cuadro 2. Parámetros cinéticos estimados para accesiones de plátano en el Sistema de Bancos de Germoplasma en Colombia. Agrosavia, C.I. Palmira.

Nombre	Parámetro	Cinética Orden Cero			Cinética Orden Uno		
		a*	h°	ΔE	L*	b*	C*
¾ naine	k	0.03	0.1	48.2	0.006	0.006	0.005
	R^2	0.31	0.9	0.9	0.97	0.98	0.98
Benedetta	K	0.01	0	36.2	0.004	0.004	0.003
	R^2	0.21	0.5	0.69	0.73	0.84	0.86
Cachaco espermo	K	0.03	0.1	52.6	0.005	0.005	0.005
	R^2	0.57	1	0.94	0.98	0.95	0.95
Cachaco sin bellota	K	0.04	0.1	33.8	0.004	0.003	0.002
	R^2	0.87	0.9	0.79	0.85	0.79	0.77
Dominico enano	K	0.02	0.1	18.5	0.005	0.006	0.004
	R^2	0.55	0.9	0.9	0.96	0.96	0.96
Dominico Hartón Támesis	K	0.03	0.2	53	0.007	0.008	0.007
	R^2	0.52	1	0.96	0.99	0.98	0.98
Dominico maqueño	K	0.03	0.2	35.6	0.007	0.006	0.005
	R^2	0.29	1	0.85	0.94	0.98	0.98
Dominico mocho	K	0.03	0.1	18.5	0.004	0.005	0.004
	R^2	0.6	0.9	0.96	0.95	0.98	0.97
Dominico mutante	K	0.08	0.2	33.2	0.004	0.004	0.003
	R^2	0.89	1	0.94	0.97	0.97	0.95
FHIA-110	K	0.06	0.3	35.2	0.009	0.009	0.005
	R^2	0.6	0.9	0.75	0.93	0.92	0.86
Figue famile	K	0.05	0.2	28.2	0.007	0.006	0.004
	R^2	0.79	1	0.9	0.99	0.99	0.97
GAEP-2	K	0.0002	0.2	40.2	0.004	0.009	0.007
	R^2	1.00E-05	0.9	0.93	0.74	0.98	0.97
Hartón tigre	K	0.02	0.1	41.5	0.003	0.003	0.003
	R^2	0,71	1	0,98	0,94	0,94	0,94
Hondureño enano	K	0.0003	0.16	47.69	0.0068	0.0069	0.006
	R^2	0.0001	0.94	0.79	0.9	0.93	0.91
Kelongmekintu	K	0.08	0.13	31.39	0.009	0.01	0.011
	R^2	0.72	0.45	0.76	0.89	0.93	0.96
MaiaMaoli Risaralda	K	0.03	0.1	53.39	0.0041	0.004	0.0034
	R^2	0.75	0.52	0.69	0.88	0.51	0.54
Mbindi	K	0.07	0.16	22.1	0.0048	0.0038	0.0024
	R^2	0.82	0.97	0.91	0.95	0.99	0.98
Mbourou kou-1	K	0.01	0.14	35.54	0.0053	0.006	0.005
	R^2	0.2	0.97	0.86	0.95	0.97	0.97
Neypoovan	K	0.01	0.14	26.43	0.007	0.0075	0.0062
	R^2	0.13	0.88	0.79	0.86	0.96	0.96
Pompo o comino	K	0.002	0.25	40.26	0.01	0.0098	0.007
	R^2	0.002	0.99	0.8	0.94	0.99	0.98
Hibrido saba-2	K	0.08	0.15	24.32	0.004	0.0033	0.0021
	R^2	0.89	0.96	0.91	0.97	0.97	0.95
Pompo o comino Risaralda	K	0.03	0.08	36.94	0.005	0.0051	0.0044
	R^2	0.57	0.75	0.67	0.75	0.74	0.73
Hartón Santander	K	0.03	0.28	47.58	0.01	0.0106	0.008
	R^2	0.22	0.92	0.76	0.96	0.95	0.94
BLANCO SARDY	K	0.02	0.14	34.57	0.005	0.0053	0.0039
	R^2	0.33	0.97	0.85	0.96	0.97	0.96
Hartón Macho	K	0.03	0.11	43.35	0.004	0.0047	0.004
	R^2	0.75	0.97	0.98	0.97	0.96	0.96

Discusión

Las diferencias en las variables fisicoquímicas en las muestras evaluadas son debidas, principalmente, al efecto del cultivar. Los valores de pH, acidez titulable y materia seca concuerdan con los hallazgos de Barrera, Arrazola y Cayón (2010) quienes evaluaron en plátano hartón (*Musa* AAB Simmonds) cinco estados de maduración y encontraron valores de pH entre 5 y 6.3) y acidez titulable entre 0.1 y 0.41 g de ácido málico/100g pulpa, siendo similares a los del presente estudio. De acuerdo con los valores medios de luminosidad (L*), saturación (C*), y tono (hº), las muestras evaluadas se pueden clasificar colorimétricamente en la zona de los amarillos tenues a amarillos intensos, la variación de color que se observa en los materiales estudiados probablemente es debida a las diferencias en el contenido de carotenoides, compuestos fenólicos y el nivel de actividad de la enzima polifenoloxidasa presentes en los materiales.

Bal et al. (2011) encontraron resultados similares a los hallados en este estudio utilizando modelos cinéticos de orden cero (0) en los cambios de las coordenadas colorimétricas a*, hº y ΔE y, orden uno (1) para los atributos de color L*, b* y C* durante la deshidratación de *Abelmoschus esculentus* (L.) Moench (citado como *H. esculentus*) (Malvaceae) y *Bambusa vulgaris* Schrad. ex J.C. Wendl. (Poaceae). Con base en estudios realizados por Chong, Cheng y Noor (2011) quienes afirman que la enzima polifenoloxidasa actúa sobre los compuestos fenólicos, los cuales al hidrolizarse a orto-difenol se oxidan a o-quinonas y orto-quinonas, y éstas, a su vez, se polimerizan a compuestos pardos como la melanina, es posible asumir que en este estudio el cambio de color superficial de pulpa, se debe principalmente a la acción de la enzima polifenoloxidasa. Otra reacción responsable de la modificación del color superficial en la pulpa de plátano es consecuencia de los procesos de isomeración y oxidación de los carotenoides, reacciones químicas que degradan estos pigmentos (Rodríguez, 2001). Estas reacciones pueden modificar las propiedades ópticas en las muestras de estudio, al reducir el nivel de la refracción de la luz, desencadenando una mayor absorción del espectro visible , aumentando así las zonas pardas en la pulpa de plátano. Las accesiones Benedetta, Cachaco espermo, Cachaco sin bellota, Dominico mocho, Dominico mutante, GAEP-2, Hartón tigre, MaiaMaoli Risaralda, Mbindi, Hibrido saba-2, Pompo-comino Risaralda, Blanco Sardy y Hartón Macho, presentaron menor cambio de color en el tiempo de evaluación con respecto a la constante de velocidad cinética k, en la coordenada colorimétrica L*(0.003–0.005 min^{-1}) (Cuadro 2), por tanto se consideran materiales promisorios para consumo en fresco o procesos agroindustriales, por los bajos valores de pardeamiento en pulpa. No obstante, en futuros estudios se sugiere cuantificar la concentración de compuestos fenólicos, carotenos y determinar el nivel de actividad de la enzima PPOs.

Conclusiones

La variación de las coordenadas colorimétricas a*, hº, y ΔE se ajustaron a una cinética de orden cero (0), y L*, b* y C* se pueden describir con una cinética de orden uno (1). El análisis cinético de las pulpas de plátano evaluadas evidencia que las accesiones Benedetta, Cachaco espermo, Cachaco sin bellota, Dominico mocho, Dominico mutante, GAEP-2, Hartón tigre, Maia Maoli Risaralda, Mbindi, Hibrido saba-2, Pompo-comino Risaralda, Blanco Sardy y Hartón Macho son materiales promisorios para consumo en fresco o procesamiento agroindustrial, ya que presentaron los menores valores de pardeamiento en pulpa.

Se sugiere continuar con la investigación validando estos resultados con la determinación de la actividad enzimática de la polifenoloxidasa presente en las accesiones de plátano evaluadas en el presente estudio.

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Electrophoretic behavior of ewe milk proteins from local breeds Rembi and Ouled-Djellal of the Algerian central steppe

Comportamiento electroforético de las proteínas de la leche de oveja de las razas locales Rembi y Ouled-Djellal de la estepa central Argelina

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Abstract

In order to characterize the production of sheep milk in Algeria (North of Africa) and to detect molecular markers related to the constitution of the protein phase of this milk, we proposed to analyze the electrophoretic behaviors of caseins and serum proteins under various migration conditions (native, urea and SDS-PAGE), from milk collected during the first three months of the year, from two breeds Ouled-Djellal and Rembi, living in the central area steppe. The profiles obtained show a great similarity and homogeneity between the different samples of the milk of the two breeds of ewes studied as to the number and intensity of the revealed migration bands. Some of the latter are nevertheless distinguished from cow's milk by different levels of migration and intensity, which require partial sequencing to be able to identify them with certainty.

Key words: Algerian steppe; electrophoresis; ewe's breed; ewe's milk; proteins.

Resumen

Para caracterizar la producción e identificar marcadores moleculares relacionados con la constitución de la fase proteica de la leche producida por ovejas de razas Ouled-Djellal y Rembi en la zona central de la estepa de Argelia (Norte de Africa) se analizaron los comportamientos electroforéticos de las caseínas y proteínas séricas en diversas condiciones de migración (nativa, urea y SDS -PAGE). Las muestras de leche fueron recolectadas durante los primeros tres meses del año. Los perfiles obtenidos muestran una alta similitud y homogeneidad entre las diferentes muestras de ambas razas en relación con número e intensidad de las bandas de migración reveladas. No obstante algunos de ellos se distinguen de la leche de vaca por diferentes niveles de migración e intensidad, que requieren de una secuencia parcial para identificarlos con certeza.

Palabras clave: Estepa argelina; electroforesis; raza de oveja; leche de oveja; proteínas

Introduction

The Algerian steppe covers about 20 million hectares and contains an estimated 28.7 million head of sheep (ONS, 2018). This herd is composed mainly of local breeds (Benyoucef, Madani and Abbas, 2000). Ouled-Djellal and Rembi comprise the main ones (Boucif et al., 2007) and represent 63 and 21% of the total sheep population, respectively. Although this livestock is well adapted to the harsh conditions of the environment, milk production remains low, and is used primarily for the breastfeeding of lambs, then consumed as it is or transformed into Djeben (traditional cheese) or Smen (traditional butter). This milk, well appreciated by local populations, has been characterized both microbiologically and physicochemically (Yabrir et al., 2012). This composition varies due to several factors (Yabrir, Hakem (Ex. Akam) and Mati, 2013a), among which breed remains one of the most studied factors. The effect of the breed has been studied on the lipid profile (Yabrir et al., 2016), on the mineral composition (Yabrir et al., 2014) and on the different nitrogen fractions (Yabrir et al., 2013b) of ewe's raw milk collected in Algerian steppic environment. This work aims to carry out an extension of these studies by focusing on the characterization of the major proteins of milks derived from the two Ouled Djellal and Rembi breeds and to look for specific molecular markers for each of these breeds.

Materials and methods

Sampling

The samples of raw sheep milk analyzed were collected from two dairy breeds, Ouled-Djellal and Rembi, located in the region of Djelfa (300 km south of the capital Algiers). For each breed, three individual milk samples were taken for three months (January, February and March) and three times a month.

Milk skimming

The milk was heated and stirred gently for 10 minutes in a water bath at 30-35 °C to allow the rise of the fat surface, then it was skimmed by centrifugation at 3500 x g for 20 min at 4 °C then filtered through glass wool. The operation was repeated 2 to 3 times.

Isolation of caseins and serum proteins

From the skimmed milk, the caseins were separated from the serum proteins by precipitation at their isoelectric point (pH 4.6) by adding dropwise 4N hydrochloric acid, followed by centrifugation at 4000 x g for 20 min at 25 °C. The pellet containing the caseins was recovered in a minimal volume of distilled water. This operation was repeated 3 times, in the same way as the supernatant containing the serum proteins, in order to eliminate any trace of contamination after adjusting the pH to 7 by addition of 1N sodium hydroxide and acidification and centrifugation in the same forms. The two major groups of proteins obtained (caseins and whey proteins) were dialyzed (cut-off membrane equal to 10,000 Dalton) for 48 hours at 4 °C. against distilled water, renewed twice a day.

Electrophoretic behavior

The electrophoretic migration of the proteins obtained (total caseins and whey proteins) was carried out under native conditions (native PAGE), or in the presence of dissociating and/or denaturing agents (urea-PAGE or SDS-PAGE).

By applying protocols developed on bovine milk (Laemmli and Favre, 1973; Darling and Butcher, 1975), the electrophoretic migration of ewe's milk proteins has been monitored and the methods were each time optimized by modifying certain electrophoretic parameters (gel porosity, migration time, amperage, voltage, coloring conditions/discoloration) to have resolving and discriminating profiles.

The electrophoresis was conducted on a system of vertical mini-tanks 10x10 and 10x8cm (Hoefer SE 200) in constant voltage and amperage. After migration, the proteins were fixed with 12% tricholoracetic acid, stained with Coomassie blue and decolorized using water/methanol/acetic acid mixture.

Native-PAGE

This electrophoresis was performed according to the method of Hillier (1976) with a polyacrylamide gel (T =12%) in 0.75M Tris-HCl buffer, pH 8.9. Samples (2 mg mL^{-1}) were solubilized in 75mM Tris-HCl buffer, pH8.9, containing 10% (v/v) glycerol, and 0.01% (w/v) bromophenol blue.

SDS-PAGE

In this conditions, the method described by Laemmli and Favre (1973) was used with a stacking gel (T = 4%, C = 2.7%) in Tris-HCl buffer, pH 6.8 and a separating gel (T = 17%, C = 2.7%) in Tris-HCl buffer, pH8.8.

In order to calibrate the gel, a pre-colored kit of known molecular weight (MW) proteins was used. It was composed by the 7 following entities: α2-Macroglobulin (180,000 Da); β-Galactosidase (116,000 Da); Lactoferrin (90,000 Da); Pyruvate kinase (58,000 Da); Fumarase (48,500 Da); Lactic dehydrogenase (36,500 Da); Triose-Phosphate

Isomerase (26,600 Da). After proteins marker migration a standard curve Log (MW) versus migration distance was traced than the MW of the unknown proteins were calculated by resolving generated equation $y = 6.6015x + 33.438$ (y: Log (MW) ; x: migration distance).

Urea-PAGE

In native conditions, caseins, because of their micellar structure, were difficult to separate. In this case, dissociating agents such as urea and an S-S bridge reducing agent (β-mercaptoethanol) were used.

The method used was that described by Shalabi and Fox (1987) with a stacking gel (T = 4.8%, C = 2.7%) containing 5.7 mol/l urea and a separating gel T = 13%; C = 4.15%) containing the same concentration of urea. The gel buffers were identical to those of the SDS-PAGE and the migrating buffer was similar to the native-PAGE buffer.

Result and discussion

Electrophoretic behavior of serum proteins

In native PAGE, the mobility of protein fractions depends both on their charge and their PM. According to Lin et al. (2010), this method was well-adapted for the separation of serum proteins.

An examination of the electrophoretic diagrams (Figure 1) allowed to drawing two remarks. The first was that the separation profiles obtained show a great deal of similarity and homogeneity between the different samples of the raw sheep milk analyzed. The second showed that some bands were characterized by migration levels similar to those of cow's milk proteins. The latter migrate into five distinct bands that can be characterized by their electrophoretic mobility as being: Ig, PP, BSA, α-La, and β-Lg, based on the bibliographic data of Egito et al. (2001).

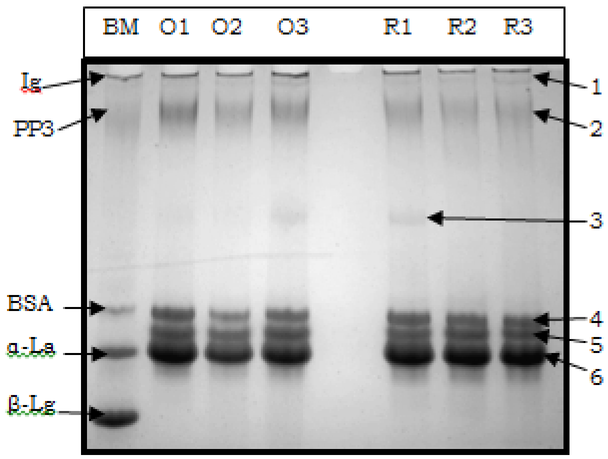

Figure 1. Electrophoregram of whey proteins of ovine milk in native PAGE.

BM: Bovine milk; O: *Ouled-Djellal* breed; R: *Rembi* breed; (O or R): 1, 2, 3: respectively for the months of January, February and March. 1 to 6: protein bands detected in the different samples.

The electrophoretic profile obtained for the raw sheep milk analyzed shows the existence of six bands (1 to 6) of which three well focused (4, 5 and 6). Among these, bands 4 and 6 appeared with an identical migration levels to those of BSA and α-La, respectively. While band 5 was between these two proteins. Furthemore, as reported by Pesic et al. (2011b), whey proteins from ovine milk followed the increasing order of electrophoretic mobility: SA, α-La and β-Lg and the migration level of the last two proteins was lower than that found in bovine milk. This leaded us to hypothesize that the bands 4, 5 and 6 might correspond respectively to SA, α-La and β-Lg. However, confirmation requires the isolation of each fraction and the sequencing of their N-terminal part.

Although ovine β-Lg showed two major variants β-LgA and β-LgB (Amigo et al., 1992; Mayer, 2005; Pesic et al., 2011a and b), these appear only in the form of a single intense band whose electrophoretic mobility was similar to that of the bovine α-La. This observation is in agreement with the results obtained by the latter authors. The highlighting of these two bands may be possible using other techniques such as isoelectrofocusing (Amigo et al., 1992; Moatsou et al., 2005), capillary electrophoresis (Recio et al., 1997), chromatofocusing (Fernandez-Espla, Lopez-Galvez, and Ramos, 1993). With the highest electrophoretic mobility compared to all other proteins, bovine β-Lg was targeted on PAGE-native to detect a possible adulteration of ovine with bovine milk (Pesic et al., 2011a).

Electrophoretic behavior of caseins

Due to their micellar structures and interactions of their groups, electrophoretic separations of caseins require the utilization of agents dissociating hydrogen bonds (such as urea) and S-S bridge reducers (such as 2-mercaptoethanol). In bovine milk, four well separated casein bands were distinguished in the profiles of urea-PAGE. Starting from the deposition and based on their electrophoretic mobility (Pesic et al., 2011a), these bands corresponded to γ-CN, β-CN, αS_2-CN and αS_1-CN.

Regardless of breed and sampling period, sheep caseins migrate in five bands of high intensity divided into two distinct zones: zone 1 with two bands and zone 2 with 3 bands (Figure 2). The first zone was located at the boundaries of γ-CN and β-CN bovines while the second zone was characterized by a lower electrophoretic mobility than αS bovine caseins. This same behavior was observed by Dall'Olio, Davoli and Russo (1990) and Moatsou et al. (2004). Pesic et al. (2011a) report that κ-CN and β-CN were characterized by the same electrophoretic mobility in the milk of the three species (sheep, goat and cattle) and the ovine αS-CN showed a lower migration with greater bands intensity than caprine and bovine milks. The migration of sheep caseins investigated by Dall'Olio et al. (1990) in the decreasing order of mobility was: κ-CN, β-CN+κ-CN, αS_1-CN and αS_2-CN. Whereas for Trujillo, Casals and Guamis (2000), the order was of type κ-CN, αs_2-CN, αs_1-CN and β-CN. While, Recio et al. (1997) and Clement, Agboola and Bencini (2006), were referred to the following migration order of ovine caseins: αS_2-CN, αS_1-CN, κ-CN and β-CN.

According to the electrophoretic mobility in urea-PAGE, Dall'Olio et al. (1990) and Moatsou et al. (2004) notes that sheep caseins αS and β were migrated in two distinct groups: β-CNs (with two bands called β_1 and β_2 caseins) and αS-CNs (with three bands named αS_1, αS_2 and αS_3-CN according to their electrophoretic mobility). Mayer (2005) notes that bovine αS1-CN can be used as a marker to identify the presence of cow milk in sheep or goat milk.

Behavior of serum proteins and caseins in SDS-PAGE

Under this denaturing and dissociating conditions with sodium dodecyl sulfate as detergent, the ovine whey proteins of our samples were homogeneous for the six visualized bands (indicated from 1 to 6) (Figure 3). Three bands (2, 6 and 7) were intense with a migration levels similar to those observed for bovine SA, β-Lg and α-La. Bands 1 and 3 were characterized by the lower electrophoretic mobility which can corresponded to sheep lactoferrin and/or immunoglobulins belonging to two different classes based on calculated and theoretical PM and referring to bibliographic data. Bands 4 and 5 corresponded to caseins (low casein contamination)

The profiles obtained for serum proteins in SDS-PAGE were consistent with those obtained by Pelmus et al. (2012) by examining the protein polymorphism of sheep milk from the local Romanian breed. These authors also note that sheep proteins showed a low electrophoretic mobility, both for serum proteins and for caseins, compared with bovine proteins.

For caseins, four bands were observed in the electropherogram obtained for the different samples of raw sheep milk collected in the region of Djelfa. These bands, noted 1 to 4 according to their increasing electrophoretic mobility, were well focused but with varying intensities (Figure 4). Vairo Cavali et al. (2008) report that these bands corresponded respectively to αS2-CN, αS1-CN, β-CN and κ-CN. Nevertheless, the presence of κ-CN was difficult to identify (Calavia & Burgos, 1998).

Molecular weights of isolated proteins

The measured molecular weights (Table 1 and 2) of ovine proteins of milk samples collected from breeds Ouled-Djellal and Rembi corresponded globally to those reported by different authors. but not corresponded to those reported by Ameur Ameur et al. (2016). Similarly, any major differences in the MW of proteins between the samples of these two breeds were detected. However, the slight variations detected might be due to genetic differences in the dairy females considered.

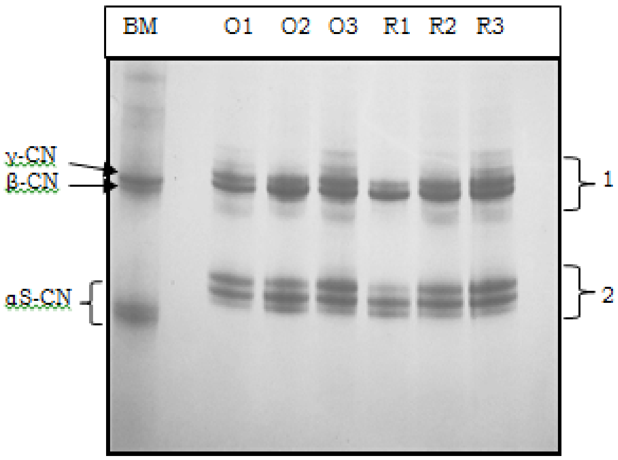

Figure 2. Electrophoregram of caseins of ovine milk in PAGE-urée.

BM: Bovine milk; O: *Ouled-Djellal* breed; R: *Rembi* breed; (O or R):1, 2, 3: respectively for the months of January, February and March. 1 and 2: distinct areas of protein found in different samples.

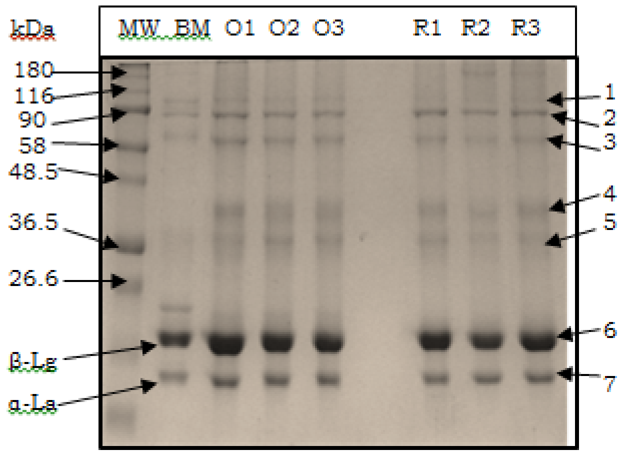

Figure 3. Electrophoregram of serum proteins of ovine milk in PAGE-SDS.

MW: Molecular weight (proteins marker); BM: Bovine milk; O: *Ouled-Djellal* breed; R: *Rembi* breed; (O or R):1, 2, 3: respectively for the months of January, February and March. 1 to 7: protein bands detected in the different samples

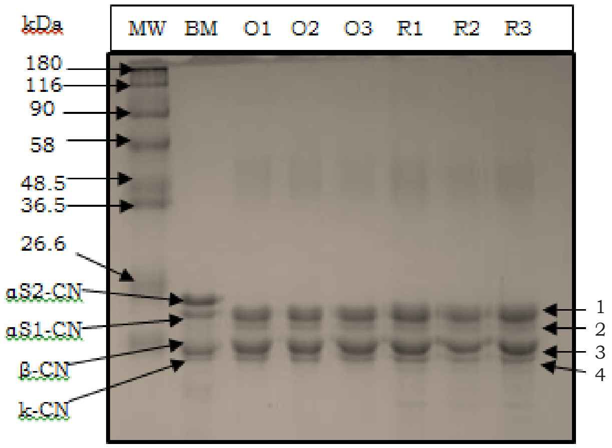

Figure 4. Electrophoregram of caseins of ovine milk in PAGE-SDS.
MW: Molecular weight (proteins marker); BM: Bovine milk;
O: *Ouled-Djellal* breed; R: *Rembi* breed;(O or R):1, 2, 3: respectively for the months of January, February and March. 1 to 4: protein bands detected in the different samples

Table 1. Molecular weight (Da) of whey proteins in ovine milk compared to those of bovine milk.

Protein*	Ovine milk				Bovine milk
	Trujillo et al., 2000	Hernandez - Ledesma et al., 2011	Fernandez-Espla et al., 1993	Present study	Farrell et al., 2004
Ig	/	/	/	67350	150000-1000000
SA	66322	/	/	66000	66399
PP	/	/	/	50483	/
β-lg	18148-18170	18300	16100	17782	18277-18363
α-La	14152	14000	/	14125	14178

*Ig: immunoglobulin, SA: serum-albumin, PP: proteose-peptone, lg: lactoglobulin, La: lactalbumin.

Table 2. Molecular weights (Da) of ewe milk casein's compared with those of bovine milk.

Protein	Ovine milk		Bovine milk
	Trujillo et al., 2000	Present study	Farrell et al., 2004
αs_1-CN	23411	23442	23615-23542
αs_2-CN	25616	25118	25226
β-CN	23750	23273	23983-24092
κ-CN	19373	19498	19006-19037

CN : casein.

Conclusion

The aim of this work was to situate the level of similarities and singularities of the protein fraction of milk from dairy breeds Rembi and Ouled-Djellal of the central steppe of Algeria. The analysis of the caseins and whey proteins under several conditions showed a pattern with great similarity and homogeneity between the different milk samples, regardless of the period during which the milk was collected. The electrophoretic profiles obtained revealed the presence of homologues to major bovine proteins with some differences in electrophoretic mobility, linked to the presence of structural features (composition and arrangement of amino acids, nature and size of prosthetic groups) of these proteins.

Further investigations had been explored as a follow-up to this preliminary study to highlight the nature of these proteins and the post-translational modifications they contain.

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Effects of vitamin pre-sowing treatment on sweet maize seedlings irrigated with saline water

Efecto del tratamiento presiembra con vitaminas en el desarrollo de plántulas de maíz dulce regadas con agua salina

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Abstract

Salinity stress represents an obstacle for the production of plants of commercial interest, including sweet maize. Among the techniques used to suppress the effects caused by excess salts in the soil is the exogenous application of vitamins. Thus, the objective of this study was to evaluate the effect of the pre-sowing treatment of sweet maize seeds in solutions of thiamine, niacin and these two vitamins combined on the development of the plants irrigated with saline water. The treatments were composed by the 24 h pre-sowing treatment with water, thiamine solution (thiamine 100 mg L^{-1}), niacin solution (niacin 100 mg L^{-1}) and a combined solution with these two vitamins (thiamine 50 mg L^{-1} + niacin 50 mg L^{-1}) irrigated with saline water. A control (pre-sowing treatment with water and irrigation with non-saline water) was also used. It was observed that salinity stress affects the sweet maize initial development. Application of isolate thiamine or niacin and its combined application improves the relative contents of chlorophyll 'a' and total, height of shoot, leaf number, diameter of stem and shoot, root and total dry matter, reducing dry matter losses by 8.89%, 25.46% and 39.60%, respectively. Soaking seeds for 24 h in thiamine and niacin solutions improve the initial growth of sweet maize plants under salt stress and a combined vitamin solution (thiamine 50 mg L^{-1} + niacin 50 mg L^{-1}) effectively reduces the salt stress negative effect on the initial development of these plants.

Keywords: abiotic stress, defense mechanism, vitamin B1, vitamin B3, *Zea mays* L. *saccharata* Strut.

Resumen

El estrés por salinidad es un limitante para la producción de plantas de interés comercial, incluido el maíz (*Zea mays*) dulce. Entre las técnicas utilizadas para suprimir los efectos causados por el exceso de sales en el suelo se encuentra la aplicación exógena de vitaminas. Teniendo en cuenta esta condición, el objetivo de este estudio fue evaluar el efecto del tratamiento previo a la siembra de semillas de maíz dulce con tiamina, niacina y ambas vitaminas combinadas, en el desarrollo de plantas sumergidas en soluciones de éstas durante 24 h antes de la siembra. Las concentraciones utilizadas consistieron en 100 mg L^{-1} de las vitaminas solas y 50 mg L^{-1} de cada una cuando fueron aplicadas en mezcla, más un tratamiento testigo con agua no salina. La salinidad afectó el desarrollo inicial de las plantas; por el contrario, el tratamiento con los antibióticos solos o en mezcla, mejoró el contenido relativo y total clorofila 'a', la altura de brotes, el número de hojas, el diámetro del tallo, el desarrollo radicular y la producción de materia seca total, siendo las pérdidas de esta última de 8.89%, 25.46% y 39.60% menores en relación con el testigo, cuando se aplicaron, respectivamente, tiamina, niacina y la mezcla de ambos antibióticos.

Palabras clave: estrés abiótico, mecanismo de defensa, vitamina B1, vitamina B3, *Zea mays* L. *saccharata* Strut.

Introduction

The sweet maize (*Zea mays* L. *saccharata* Strut.) is considered a horticultural species with potential for in natura and industrial use through the manufacture of canned products (Oliveira-Jr. et al., 2006). The United States of America is the world's largest producer of sweet maize. By 2015, the country had cultivated an area equivalent to 242,090 acres, where the average yield was 8.09 tons (USDA, 2017). In Brazil, this species also presents good profitability indexes and can be used to increase the income of small and medium producers (Jesus et al., 2016). Despite the growing demand, however, the cultivation of sweet maize requires further information in regions with abiotic stress occurrences.

Salinity stress represents an obstacle to the production of plants of commercial interest due to the susceptibility of these species (Medeiros et al., 2012). It is estimated that global losses in agricultural productivity are caused by high levels of salinity in the soil, which is a problem that affects about 20% of the world's arable land (Taiz et al., 2017). Among the causes for salinity increase, mainly anthropic actions such as mistaken fertilization practices jointly with poorly conducted irrigation and drainage systems that increase the concentration of soluble salts in the soil solution (Na^+ and K^+) up to toxic levels (Eloi et al., 2011, Eschemback et al., 2015, Taiz et al., 2017) can be mentioned.

Regarding to the maize, it was observed that they are genotypes with different levels of resistance to salt stress (Kaya et al., 2015). The occurrence of this stress results in deleterious effects throughout the crop cycle, which reduces carbon fixation, accumulation of nutritional and energy reserves, damaging photosynthetic and enzymatic reactions, causing productivity losses (Hassanein et al., 2009, Farooq et al., 2015, Cunha et al., 2016). Thus, despite advances achieved through genetic improvement, alternatives that will help increase resistance to abiotic stresses, such as the use of vitamins, should be sought.

The application of exogenous vitamins has been studied as a stress-relieving technique, caused by biotic and abiotic factors (Ahn et al., 2005; Hassanein et al., 2009; Boubakri et al., 2012; Abddalah et al. 2016). Its use can also improve physiological conditions through increases in energy (Barakat 2003; El-Bassiony 2005) and nutritional reserves, essential to the correct development of plant organs (Taiz et al., 2017). In addition, vitamins can interact with phytohormones, helping to maintain the activities of cell division and stretching (Oertli, 1987). The objective of this study was to evaluate the effect of the pre-sowing treatment of sweet maize seeds in solutions of thiamine, niacin and these two vitamins combined on the development of the plants irrigated with saline water.

Material and methods

Plant material and growth condition

This research was undertook in a protected environment at the experimental area of the Agronomy School, Federal University of Goiás, Brazil (16° 35' 55.2" S, 49° 16' 39.1" W) in March 2017. Both, the protected environment as well as the sides of the structure were covered with plastic.

According to the classification of Köppen-Geiger (Cardoso et al., 2014), the region presents an Aw climate, characterized by tropical climate with rainy season from October to April and a period with monthly precipitations below 100 mm between May and September. Average monthly temperatures range from 20.8 °C in June and July to 25.3 °C in October (Cardoso et al., 2014). During the experiment, climate data were collected from a digital datalogger (AK172, Akso, São Leopoldo, RS, Brazil) inside the protect environment. The registered mean values for maximum, minimum and mean temperature and air humidity were 34.6 °C, 20.6 °C, 27.6 °C and 70.2%, respectively.

Seeds of sweet maize (cv. BRS Vivi) were soaked in four solutions as pre-treatment (Distilled water; 100 mg L^{-1} thiamine solution; 100 mg L^{-1} niacin solution; 50 mg L^{-1} thiamine + 50 mg L^{-1} niacin solution) for 24 h in a room at 25±0.5°C. Hereafter, two seeds were sown in plastic pots (4.4 cm diameter, 20 cm height) with commercial organic substrate (pine bark, vermiculite, peat and coconut fiber).

The pots containing the seeds were distributed in a completely randomized scheme, with five repetitions for the following treatments: T1 – Control (seed soaking and irrigations made with water); T2 –Stress control (seed soaking in water and irrigations made with NaCl 100mM solution); T3 -NaCl + thiamine (seed soaking in thiamine solution and irrigations made with NaCl 100mM solution); –T4 NaCl + niacin (seed soaking in niacin solution and irrigations made with NaCl 100mM solution); T5 –NaCl + thiamine + niacin (seed soaking in thiamine + niacin solution and irrigations made with NaCl 100mM solution).

To avoid drought stress, irrigations were carried out every day with 50 ml of the respective solution per pot. Ten days after plant emergence a seedling was removed and 50 ml of a nutrient solution (N - 10%; P - 9%; K - 28%; Ca - 18%; Mg - 3.3%; S - 4.3%; Fe EDDHA - 6%; B - 0.06%; Cu - 0.01%; Mo -0.0746%; Mn - 0.05%; Zn -0.02 %) was applied.

Relative chlorophyll content

Hand-held chlorophyll meter (CFL1030; Falker, Porto Alegre, RS, Brazil) was used to record the Falker chlorophyll index (FCI) readings from the topmost fully expanded leaves on each plant at the 21 days after sowing. FCI values were measured two times along the flag leaf blade and, then, the readings were averaged to have a single value for a plant.

Biometric parameters

After 21 days of sowing, plants were harvested. Shoot height and root length were measured with a ruler. Leaf number was counted and the stem diameter was measured with a digital caliper (Metrotools, São Paulo, SP, Brazil). Having dried the plants in an oven at 65 °C for 72 h, the shoot, the root and the total dry weight were measured in a digital scale (ML 600, Marte, São Paulo, SP, Brazil).

Dickson quality index

The Dickson quality index (Dickson et al., 1960) was calculated using the equation 1:

$$DQI = \frac{TDM}{(\frac{SL}{SD}) + (\frac{SDM}{RDM})} \quad \textbf{Eq 1}$$

where, *DQI* is the Dickson quality index, *TDM* is the total dry matter, *SL* is the shoot length (cm), *SD* is the stem diameter (mm), *SDM* is the shoot dry matter (g) and *RDM* is the root dry matter (g).

Statistical analysis

Analysis of variance (ANOVA) was applied to all data and the parameters values were expressed as the mean ± standard deviation (SD) with a minimum of five replications. The difference significance between the treatments was obtained with the Tukey test at P < 0.01and P < 0.05 using the statistical software SISVAR 5.6 (Ferreira, 2014).

Results

Analyses of variance indicated no significant difference for chlorophyll 'b' content and Dickson quality index (Table 1). Significant difference was found for the chlorophyll 'a' and total content, height of shoot, leaf number, diameter of stem and shoot, root and total dry matter.

Compared to the control treatment, salinity stress did not reduce the relative contents of chlorophyll 'a', 'b' and total. Nevertheless, the application of Niacin increased the relative contents of chlorophyll 'a' and total, compared to the controls with or without application of saline solution, even when the seedlings were submitted to the salinity stress. The applications of Thiamine and Niacin + Thiamine were not statistically different from the treatment where the Niacin solution was applied by soaking seeds (Figure 1).

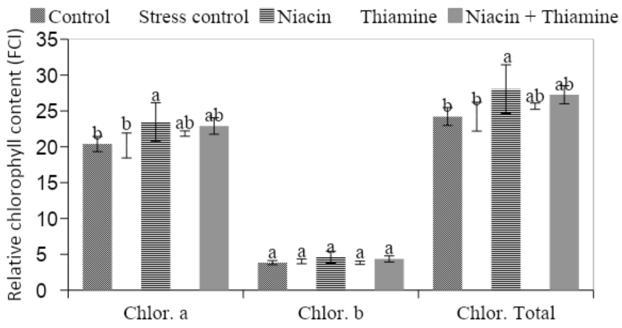

Figure 1. Relative chlorophyll 'a', 'b' and total contents on seedlings of sweet maize submitted to salt stress and seed soaking pre-treatment in different vitamins solutions. Agronomy School, Federal University of Goiás, Brazil.

Table 1. Analysis of variance for sweet maize plants initial growth components under salt stress conditions and seed soaking in different vitamins solution. Agronomy School, Federal University of Goiás, Brazil.

		Mean square				
Source of variation	**DF**	**Chlor. 'a'**	**Chlor. 'b'**	**Chlor. Total**	**Height of shoot**	**Length of root**
Replications	4	10.66	0.58	15.25	1.73	15.69
Treatments	4	4.51**	0.16 ^{ns}	5.93*	0.31**	11.96*
Error	16	2.10	0.24	3.25	0.20	5.84
CV (%)	—	6.67	11.85	6.96	7.47	10.62

			Mean square				
Source of variation	**DF**	**Leaf number**	**Stem diameter**	**DM shoot**	**DM root**	**Total DM**	**Dickson quality index**
Replications	4	1.06	0.39	0.00155	0.00108	0.00436	0.00064
Treatments	4	0.06**	0.03*	0.00004**	0.00019*	0.00034**	0.0001 ^{ns}
Error	16	0.11	0.11	0.00026	0.00028	0.00086	0.0002
CV (%)	—	9.42	10.82	2.08^{1}	2.16^{1}	3.53^{1}	1.90^{1}

P<0.01 very significant (**); P<0.05 significant (*); P>0.05 no significant (^{ns}); data transformed by square root of X + 0.5 (^{1}); Degrees of freedom (DF); Coefficient of variation (CV), DM = dry matter.

It was also verified that the leaf number was significantly expanded when seeds were soaked in solutions containing niacin, increasing the values in 33.33% when compared with the stress control treatment. Despite of the fact that most of the characteristics related to the development and accumulation of dry matter were strongly affected by the vitamins combination, any statistical difference from the isolate thiamine and niacin applications was presented. In general, it also observed that for most of the variables analyzed there was no statistically significant difference between the treatments composed by the application of the vitamins and the control treatment without the application of salt stress (Table 2).

Compared to the stress control treatment, it was possible to observe that the height of shoot, stem diameter, dry matter of shoot and total dry matter were increased in 15.09%, 25.38%, 100.00% and 76.92%, respectively, when the seeds were previously soaked in solution containing the combination of niacin and thiamine. For the remaining characteristics it was observed an increase of the values when the seeds were soaked in vitamins solutions without statistical difference to the stress control treatment, however (Table 2).

Compared to the control treatment, salinity effect was strongly suppressed by the presence of both vitamins. It was observed that the use of combined vitamins solutions as seed soaking pre-treatment decreases losses in 39.60% for the total seedling dry matter. In addition, isolate vitamins also provide reduction in the total seedling dry matter losses, 8.89% and 25.46% for thiamine and niacin, respectively (Figure 2).

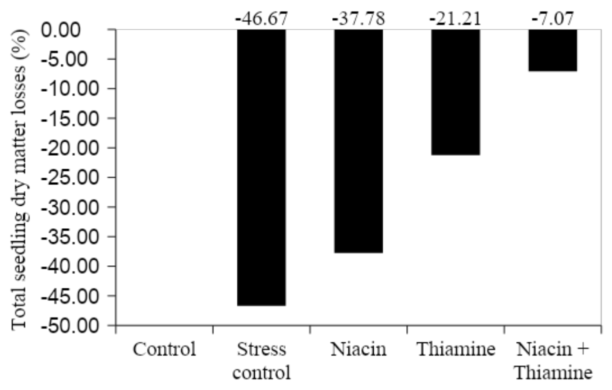

Figure 2. Total dry matter losses for seedling of sweet maize submitted to salinity stress and seed soaking in different vitamins solutions. Agronomy School, Federal University of Goiás, Brazil.

Discussion

The obtained results are strictly related to the salinity stress that can cause a significant damage in the maize plant tissues by promoting a low osmotic potential of the soil solution. This affects the water absorption and, consequently, the cell development (Oliveira et al., 2009). Salinity stress can also promote leaf injuries, decreasing growth and essential nutrient absorption (Munns, 2005).

The sweet maize cultivar which was used in the present study can be denominated as glicofitic once the Na^+ concentration (100 mM) becomes toxic and results in the photosynthesis and other biosynthetic processes inhibition by ions accumulating in leaves (Taiz et al., 2017). Thus, the stress could have induced reduction in relative contents of chlorophyll 'a', 'b', carotenoids, total pigments (Hassanein et al., 2009; Kaya et al.,

Table 2. Mean values of sweet maize plants initial growth components under salt stress conditions and seed soaking in different vitamins solution. Agronomy School, Federal University of Goiás, Brazil.

Treatments	Height of shoot (cm)	Length of Root (cm)	Leaf number	Stem Diameter (mm)
Control	6.85±0.30a	25.43±4.90a	4.00±0.00a	3.20±0.23ab
Stress Control (NaCl)	5.30±1.30b	20.70±1.30b	3.00±0.00b	2.64±0.39b
NaCl + Thiamine	5.86±0.55b	21.86±0.55ab	3.20±0.45b	2.80±0.21ab
NaCl + Niacin	5.60±2.00ab	22.45±2.00ab	4.00±0.00a	3.05±0.28ab
NaCl + Thiamine + Niacin	6.10±2.31a	23.30±2.31ab	3.40±0.55ab	3.31±0.36a
Treatments	**Dry matter of shoot (g)**	**Dry matter of root (g)**	**Total dry matter (g)**	**Dickson quality index**
Control	0.12±0.02a*	0.13±0.02a	0.25±0.03a	0.082±0.01a
Stress Control (NaCl)	0.06±0.02b	0.07±0.02b	0.13±0.04b	0.046±0.02a
NaCl + Thiamine	0.08±0.01ab	0.08±0.02ab	0.15±0.03ab	0.050±0.02a
NaCl + Niacin	0.09±0.03ab	0.11±0.03ab	0.20±0.05ab	0.074±0.02a
NaCl + Thiamine + Niacin	0.12±0.03a	0.11±0.04ab	0.23±0.07a	0.080±0.03a

*Means followed by the same letters in a column do not differ significantly by the Tukey test ($p < 0.05$).

2015), accumulation in soluble sugar and level of starch (Farooq et al., 2015) and an increase in reactive oxygen species (Azevedo-Neto et al., 2004; Kaya et al., 2015; Farooq et al., 2015).

The positive results, when the treatments composed with vitamin where compared to the stress control, are related to the action of thiamine as a regulating factor of carbon metabolism and protein synthesis (Kaya et al., 2015), since this vitamin acts as a coenzyme in several metabolic pathways (Goyer, 2010) and may exert a protective effect against oxidative stress caused by abiotic elements (Kaya et al., 2015).

For maize seeds submitted to salinity stress, optimizing effects obtained by application of thiamine up to 150,00 mg L^{-1} were observed. Germination and germination time of 50,00% of the seeds, depending on the cultivar, were increased with the application of thiamine by immersion of the seeds for 24 hours (Kaya et al., 2015).

Other studies showed that the thiamine exogenous application increased vegetative, flowering, biochemical and physiological characteristics of marigold plants (*Calendula officinalis* L.) when 50 and 100 mg L^{-1} thiamine solutions (Soltani et al., 2014) were used. For *Foeniculum vulgare* var. *azoricum* it was observed that the foliar application of 25, 50 and 100 mg L^{-1} thiamine solutions promoted a significant increase of plant growth and yield (Hendawy and El-Din, 2010).

The exogenous application of niacin induced the production of carbohydrates and the accumulation of energy reserves (Barakat 2003; El-Bassiony 2005) improving the absorption and the storage of nutrients in the plant tissues, as it was observed for wheat plants under salinity stress (El-Bassiouny et al., 2014). These reserves can be used for the maintenance of plant tissues during developmental periods or when the plant is under stress, providing energy and collaborating for the continuity of plant development (Taiz et al., 2017).

For maize cultivated under salinity stress, exogenous application of nicotinamide as grain soaking alleviated the stress effects on growth parameters which were accompanied by marked increases in IAA, GA, photosynthetic pigments and carbohydrate contents, and decreases in ABA and amylases activity as compared to those of the reference controls. Also, significant reduction in the activities of SOD, peroxidases and lipid peroxidation, as well as significant increase in catalase activity and reduced glutathione content were observed (Hassanein et al., 2009).

Studying the development of quinoa (*Chenopodium quinoa* Willd.) cultivated under sandy soils conditions, Abdallah et al. (2016) verified significant increases in leaf number, shoot length, fresh and dry shoot weight, fresh and dry root weight, comparing control treatment and leaf application of a nicotinamide solution (100 g L^{-1}). Likewise, for wheat cultivated under newly reclaimed sandy soil, an increase in all morphological criteria (plant height, leaves number, fresh and dry weights of shoots), metabolism (photosynthetic pigment, total soluble sugar, total carbohydrates, total amino acids and proline), mineral contents (N, P, K, Ca and Mg) and yield (grain, straw and biology) for two cultivars fertilized with the recommended or half recommended doses of NPK (El-Bassiouny et al., 2014) was observed.

The combined thiamine and niacin used as a seed soaking pre-treatment wasn't previously reported. The significant result can be related to the action of those two vitamins, creating an antioxidant protection to the photosystems cells, promoting the production of carbohydrates, accumulation of energy reserves and storage of nutrients, promoting plant development and decreasing the salinity stress effect.

Conclusion

Pre-sowing treatment with the vitamin solutions effectively reduces the negative effect of salt stress on the initial development of sweet maize.

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Effect of *Bacillus subtilis* and *Bacillus amyloliquefaciens* culture on the growth and yield of off-season potato (*Solanum tuberosum* L.)

Efecto de la aplicación de *Bacillus subtilis* y *Bacillus amyloliquefaciens* en el crecimiento y rendimiento de patata (*Solanum tuberosum* L.) cultivada fuera de temporada

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Abstract

A study was carried out in order to investigate the effect of microbial fertilizer application on off-season potato (*Solanum tuberosum* L. cv.Universia) under field conditions in early spring and late autumn in 2016. The experiment included Control (C, no application), Standard Fertilizer Application (SFA), Microbial Fertilizer Application (MFA) and SFA+MFA treatments. An equal mixture of three strains of *Bacillus subtilis* VKPM B-10641(DSM 24613), *Bacillus amyloliquefaciens* VKPM B-10642 (DSM 24614) and *Bacillus* amyloliquefaciens B-10643 (DSM 24615) was applied at $1x10^9$cfu/ml to tubers before planting and to plants at flowering stage in MFA and SFA+MFA plots. Treatments affected the plant growth parameters. SFA plots produced the highest tuber yields in both seasons. MFA plots had earlier emergence time (40 days), higher number of stems per plant (3), higher number of leaves per plant (37.2), thicker stem diameter (10.15 mm), higher above ground biomass yields (5.42 t/ha), higher single tuber weight (104.21 g) and higher tuber yields (22.06 t/ha) compared with control plots (42 days, 2.2, 31.2, 9.15 mm, 4.40 t/ha, 90.88 g and 20.14 t/ha respectively). Tuber yield in MFA plots (26.56 t/ha) was equal to SFA plots (26.81 t/ha) in warmer autumn planting. Combination of SFA and MFA produced lower tuber yields (21.72 t/ha) than SFA treatment (26.81 t/ha). In conclusion, *Bacillus subtilis* and *Bacillus amyloliquefaciens* gave comparable tuber yields to chemical fertilizer application in warmer autumn plantings, but lower tuber yields in colder spring conditions warranting further experiments with cold tolerant psychrophilic bacterial strains for off-season potato production.

Key words: Microbial fertilizer,potato, tuber yield

Resumen

Se estudió el efecto de la aplicación fuera de temporada de fertilizantes microbianos en patata (*Solanum tuberosum* L. cv. Universia) en condiciones de campo al comienzo de primavera y finales de otoño de 2016. El experimento incluyó tratamientos de control (C, sin aplicación), aplicación de fertilizante estándar (SFA), aplicación de fertilizante microbiano (MFA) y la combinación de SFA + MFA. Se aplicó la misma mezcla de tres cepas de *Bacillus subtilis* VKPM B-10641 (DSM 24613), *B. amyloliquefaciens* VKPM B-10642 (DSM 24614) y *B. amyloliquefaciens* B-10643 (DSM 24615) en concentración$1x10^9$ ufc/ml a tubérculos antes de plantar y plantas en floración en las parcelas MFA y SFA + MFA. Las parcelas MFA presentaron un tiempo de emergencia más temprano (40 días), un mayor número de tallos (3.0) y de hojas (37.2) por planta, un mayor diámetro de tallo (10.15 mm), una mayor biomasa aérea (5.42 t/ha), un mayor peso de tubérculo (104.21 g) y una mayor producción (22.06 t/ha) en comparación con las parcelas control (42 días, 2.2, 31.2, 9.15 mm, 4.40 t/ha, 90.88 g y 20.14 t/ha, respectivamente). El rendimiento en las parcelas MFA (26.56 t/ha) fue similar al de las parcelas SFA (26.81 t/ha) en el experimento de otoño. La combinación de SFA y MFA produjo rendimientos más bajos (21.72 t/ha) que el tratamiento con SFA (26.81 t / Ha). En conclusión, *Bacillus subtilis* y *Bacillus amyloliquefaciens* dieron rendimientos comparables al fertilizante químico en otoño, estación más cálida, pero no en condiciones de primavera (más frías).

Palabras clave: fertilizante microbiano, patata, rendimiento de tubérculos.

Introduction

Potato (*Solanumtuberosum* L.) is one of the most commonly cultivated crop plants in the world. As a food source it includes a good amino acid balance, vitamins C, B1, B3 and B6, folate and minerals such as potassium, phosphorus, calcium, magnesium and micronutrients iron and zinc. Potatoes are globally grown on 19.4 million hectares and its production exceeds 368 million tons (FAO, 2015). It is grown in all continents excepting Antarctica (Rowe and Powelson, 2002). Despite being a cool temperate climate crop plant, it adapted also to the areas with subtropical climate characteristics. Turkey has a suitable climate for potato production with an acreage of 130.200ha and a production exceeding 4.2 million tons (Turkstat, 2016). Most of the production is carried out as main crop in April-November period. In some regions it is grown as second crop after cereals (İlisulu, 1986; Samancı et al., 1998; Arıoğlu and Çalışkan, 1999; Arıoğlu et al., 2002). Mediterranean climatic conditions (Mauromicale et al., 2003; Günel et al., 2002) allow off season production of potatoes during winter in coastal belt of Mediterranean and Aegean Regions (Arıoğlu and Çalışkan, 1999; Arıoğlu, et al., 2002).

Off-season potato cultivation allows for earlier product introduction to the market and attracts premium prices and higher income from unit area. Off season crop cultivation has the advantage of low input sustainable production. However, under the effect of low air and soil temperatures, emergence takes longer and plants may suffer from cold stress limiting yield and quality (Çalışkan et al., 2002; Foti, 1999). Use of early maturing and cold tolerant cultivars (Arıoğlu and Çalışkan, 1999; Beukema and Van der Zaag, 1990; Mauromicale et al., 2003) and application of special cultivation techniques (Turgut, 1988; Logan and Turnbull, 2002; Samancı, et al., 1998) may increase the success of off season production.

There is well established information and technology for the production and fertilization of main potato production. But, off season potato production requires a new approach for fertilization program because of cold soil conditions prevailing during plant growth. The use of bio-fertilizers for off season potato production may offer environment friendly, cheap and sustainable alternativeplant nutrition. An important part of soil microflora consists of aerobes and facultative anaerobe and gram positive *Bacillus* sp.(Berkeley and Logan, 1997; Berkeley et al., 1984). *Bacillus subtilis* grows and survives under adverse mesophilic temperature conditions (below an optimal temperature of 25-35 ^{O}C) owing to the formation of stress-resistant endospores and genetic adaptation (Bandow et al., 2002). *Bacillus* based bio-fertilizers promoted plant growth hormone synthesis (Chabot et al.,1996; Amer andUtkheda, 2000) and released soil organic matter decomposing amyloclastic, celluloselytic, proteoclastic enzymes, enriching the plant tissue N and P concentrations and consequently increasing biomass production (Toro et al., 1997). Bacillius based microbial agents may play important role under sub-optimal air and soil temperatures prevailing in the production of off season potatoes in the field conditions.

This study investigated the possibility of using microbial fertilisers for off season potato (*Solanumtuberosum* L.) production under cold conditions of late autumn and early spring season plantings in comparison and in combination with mineral fertilizers under field conditions.

Materials and methods

An experiment was set up in order to investigate the effect of microbial fertilizer application on plant development and tuber yield of off season potato cv. Universiain the late autumn and early spring sowings in the field conditions. There were four treatments as follows: Control (C, no microbial and commercial fertilizer application), Standard Fertilizer Application (SFA), Microbial Fertilizer Application (MFA, a commercial mixture of *B. subtilis* and *B. amyloliquefaciens)* and SFA+MFA. The experiment was set up in a Randomised Blocks Design with four replicates on the experimental farm of Akdeniz University, Faculty of Agriculture in Antalya in the southwest of Turkey in the growth seasons of early spring and late autumn in 2016. Tubers of 40-50 days old stored under cool conditions (+4^{0}C) were planted by hand on 09 January 2016 in spring and on 15 August 2016 in autumn in the plots of two rows with 30 cm intra and 70 cm inter row spacing in 10-18 cm soil depth. Each plot had 20 plants in an area of 4.2 M^{2} (1.4 M x 3 M) giving 47619.04 plants/ha. Standard Fertilizer Application (SFA) plots were given commercial fertilizers as 50 kg/ha P_2O_5 and 50 kg/ha N_2 prior to sowing and 50 kg/ha N_2+50 kg/ha K_2O at first hoeing (Samancı, et al., 1998). In MFA and SFA+MFA plots, tubers were sprayed thoroughly and saturated with *B. subtilis* and *B. amyloliquefaciens* culture at the rate of 1 ml/ 10 L water immediately before planting. Plants in MFA and SFA+MFA plots were further sprayed with the bacterial culture of 1 ml/100 L water / ha at the beginning of flowering. Bacterial culture used included three strains of *B. subtilis*VKPM B-10641(DSM 24613),*B. amyloliquefaciens* VKPM B-10642 (DSM 24614) and *B. amyloliquefaciens* B-10643 (DSM 24615) in equal parts at a rate of 1x10^{9}cfu/ml in 10 ml commercially available sealed glass vials. Control and SFA plots were planted first by hand in order to prevent unintentional contamination. Potato cv.Universia used was early type cultivar grown commercially in the region (Anonymous, 2017). Experimental soil analysis on the samples taken at sowing from 20 cm soil depth on the experimental site showed it was clay loam with relatively low organic matter content (Table 1). Soil temperature at 20 cm soil depth at the experimental site was daily measured (Figure 1). Climatic data for the duration of experiment were obtained from Meteorological Observation and Record Station of Antalya Directorate of Turkish State Meteorological Service 3 km away from the experimental site (Table 2). Soil from experimental site was analyzed (Anonymous, 1985) in Laben Agricultural Analysis Laboratory (Antalya).

Plants were irrigated in drip irrigation and weeding was done by hand as required. Plant growth were observed and measurements of seedling emergence time, number of stems per plant, plant height, number of leaves per plantand stem diameterwere made on selected 5 plants at full flowering stage on each plot. Leaf chlorophyll content were measured also on 5 plants with a SPAD meter (SPAD 502 Chlorophyll Meter, Spectrum Technologies). Harvest was made on 29 April 2016 and 12December2016 for spring and autumn sowings respectively. Data were collected on above ground biomass yield, tuber number per plant, single tuber weight and tuber yield at harvest on each plot (Samancı, et al., 1998). Data were subjected to analysis of variance using statistical package of SPSS and means were separated by LSD test.

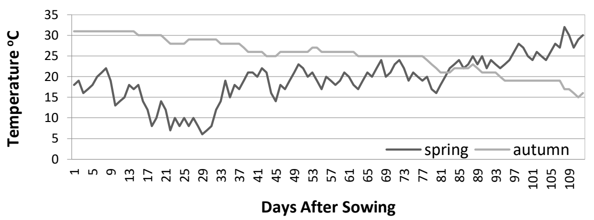

Figure 1. The soil temperature at 20 cm depth at the experimental site during the plant growth period.

Table 1. Chemical and physical properties of the soil sample from 20 cm depth on the experimental site.

Analysis Parameters	Analysis Results	Remarks
pH	7,9	Light Alkali
Lime Content (%)	42,3	Limy
Salt (%)	0,012	Without salt
Saturation (%)	50	Clayic Loam
Organic Matter (%)	1,19	Poor OM content
Total N (%)	0,100	Medium
Available P (kg/ha)	3,88	Poor
Available K (kg/ha)	70,3	Adequate
Extractable Ca (kg/ha)	1940,8	High
Extractable Mg (kg/ha)	88,5	Adequate
Plant Available Fe (ppm)	3,92	Adequate
Plant AvailableMn (ppm)	8,45	Adequate
Plant Available Zn (ppm)	0,78	Adequate

Results

Treatments investigated significantly (P<0.05) affected the plant growth parameters in both spring and autumn planting dates depending on the application (Tables 3, 4). Emergence time ranged between 38 - 42 days (Table 3). Emergence was 4 days earlier in SFA+MFA plots and 2 days earlier in MFA plots in spring period in colder soil temperatures (Figure 1, Table 3) whereas there was no advantage in SFA and MFA plots in warmer soil temperatures in autumn planting (Table 3).In the SFA+MFA applied plots, emergence time 3.7 days earlier in both seasons compared with control plots (Table 3, 4). The number of stems did not differ significantly in the spring but it was higher in SFA, MFA and SFA+MFA applied plots than control in autumn planting (Tables 3, 4). No statistically significant difference was recorded for plant height in relation to treatments in both seasons. But on average of both seasons, SFA and SFA+MFA plots produced taller plantsby 3.4-5.0 cm than control plots (Table 3). Leaf number was higher in SFA (47.4), MFA (41.6) and SFA+MFA (52.0) plots than in control (38.6) plots in autumn planting but no statisticallysignificant difference was recorded in spring sowing (Tables 3, 4). On Average of both seasons, number of leaves was higher in SFA (35.8), MFA (37.2) and SFA+MFA (42.3) than control (31.2) plots (Table 3). Leaf SPAD readings were higher in SFA-MFA (40.2), MFA (34.2) and SFA (38.7) than in control plots (33.6). Treatments investigated did

Table 2. Climatic data for long term average (LTA, 1929-2015) and growth period (2016) on the experimental site

Month	Monthly Total Precipitation (mm)		Average Air Temperature (°C)		Maximum Air Temperature (°C)		Minimum Air Temperature (°C)	
	2016	LTA	2016	LTA	2016	LTA	2016	LTA
January	85	231.9	10.4	9.9	19.5	23,9	1.0	-3.4
February	67.4	150.2	14.5	10.4	25.2	25.9	6.1	-4.6
March	54.4	103.2	15.2	12.7	22.4	28.8	9.1	-1.6
April	14.6	55.5	19.1	16.2	29.1	36.4	11.9	1.4
May	25.9	31.4	20.4	20.5	29.2	38.0	12.9	5.7
June	23.4	7.7	26.9	25.3	42.3	44.8	18.9	11.1
July	1.1	2.8	29.9	28.4	41.4	45.0	25.2	14.6
August	0.0	3.1	29.5	28.2	40.7	44.6	24.2	15.3
September	32.3	15.8	26.4	24.8	39.5	42.1	19.0	10.6
October	0	80.1	23.3	20.0	32.2	37.7	14.9	4.9
November	99.2	135.0	17.5	14.9	23.9	33.0	10.9	0.8
December	76.3	257.9	11.2	11.4	21.3	25.4	2.7	-1.9

Table 3. Effect of commercial of *B. subtilis* and *B. amyloliquefaciens* culture broth on the plant growth and yield of potato (*S. tuberosum* L. var. Universia).

Treat.	SE (days)	S (no.)	PH	Leaf (no.)	Leaf SPAD	SD (mm)	AG (t/ha)	Tuber (no.)	ST (g)	TY
					Spring Season					
Control	42.0	2.1	49.5	23.9	33.6	8.5	3.82	5.2	69.89	16.07
SFA	40.0	2.0	54.9	24.2	38.7	9.4	7.08	6.4	90.71	29.75
MFA	40.0	2.8	50.2	32.8	34.2	9.6	4.18	5.1	76.36	17.55
SFA+MFA	38.0	2.8	51.4	32.7	40.2	10.5	5.70	6.1	91.32	23.94
Aver.	40.0	2.4	51.5	28.4	36.6	9.5	5.2	5.7	82.07	21.83
LSD	1.15*	0.47ns	3.21ns	5.54ns	1.58*	0.50**	0.10ns	0.69ns	5.90*	0.38**
					Autumn Season					
Control	42.7	2.4	58.2	38.6	43.7	9.8	4.98	5.1	111.86	24.21
SFA	42.2	3.2	62.8	47.4	49.5	10.2	8.06	4.3	139.53	26.81
MFA	43.5	3.3	56.8	41.6	44.5	10.7	6.66	4.7	132.06	26.56
SFA+MFA	39.2	3.3	63.0	52.0	47.9	12.1	7.42	4.1	130.07	21.72
Aver.	41.9	3.05	60.2	44.9	46.4	10.7	6.78	4.55	128.38	24.825
LSD	1.16**	1.19*	3.75ns	2.73*	0.10ns	0.72*	0.10*	0.58ns	16.72*	0.29*
					Mean of Both Seasons					
Control	42.3	2.2	53.8	31.2	38.6	9.15	4.40	5.1	90.88	20.14
SFA	41.1	2.6	58.8	35.8	44.1	9.80	7.57	5.3	115.12	28.28
MFA	43.5	3.0	53.5	37.2	39.3	10.15	5.42	4.9	104.21	22.06
SFA+MFA	38.6	3.0	57.2	42.3	44.0	11.30	6.56	5.1	110.70	22.83
Aver.	42.3	2.2	3.8	31.2	38.6	9.15	4.40	5.1	90.88	20.14
LSD	0.55*	0.17*	51.70**	2.50**	1.39**	0.384*	2.939NS	0.37*	11.130*	3.148 NS

*Statistically significant at (P<0.05), ** Statistically significant at (P<0.01), NS Statistically not significant.

SE (days): Seedling emergence time; S (no.): Number of stems per plant; PH (cm): Plant height; Leaf (no.): Number of leaves per plant; Leaf SPAD: Leaf SPAD values; SD: Stem diameter (mm); AG (t/ha): Above ground biomass yield; Tuber (no.): Tuber number per plant; ST (g): Single tuber weigh; TY (t/ha): Tuber yield.

Table 4. Percent Advantage (+) or disadvantage (-) of Standard Fertilizer Application (SFA), Microbial Fertilizer Application (*B.subtilis* and*B. amyloliquefaciens)* (MFA) and SFA+MFA over control (no application).

Plant Growth Parameters	Growing Season					
	Spring			Autumn		
	SFA	MFA	SFA+ MFA	SFA	MFA	SFA+ MFA
Seedling Emergence time (day)	5	5	10.52	1.18	-1.73	8.92
Number of stems per plant	-4.77	35.71	33.33	32.65	36.73	36.73
Plant Height (cm)	10.91	1.41	3.83	7.99	-2.41	8.33
Number of Leaves Per Plant	1.04	36.95	36.53	22.77	7.63	34.54
Leaf SPAD values	15.21	1.73	19.61	13.37	1.99	9.7
Stem Diameter (mm)	11.45	13.46	23.73	3.67	9.58	23.24
Above Ground Bio-mass Yield (T/ha)	85.34	9.42	49.21	61.85	33.73	48.99
Tuber Number Per Plant	23.56	-3.07	16.09	-16.12	-9.13	-20.98
Single Tuber Weight (g)	62.03	6.83	52.73	3.25	6.92	-11.42
Tuber yield (t/ha)	85.13	9.21	48.97	10.74	9.71	-10.29

not significantly affect SPAD values in autumn planting. On average of both seasons, the highest SPAD readings were recorded in SFA+MFA plots (40.2) in spring and SFA applied plots (47.9) in autumn planting whereas the lowest values were obtained in control plots (Table 3). Stem diameter was higher only in SFA+MFA plots in both seasons. The above ground biomass yield did not differ significantly between treatments in spring period but all MFA and SFA+MFA plots produced 33.73 and 48.99 % higher above ground biomass yields than control plots in autumn sowing (Table 4). Tuber number per plant was significantly affected by treatments in both seasons (Tables 3, 4). Treatments significantly increased also tuber weight in both seasons (Tables 3, 4).Single tuber weight in MFA plots was higher (76.36 g in spring and 132.06 g in autumn)than in control plots(69.89 g and 111.86 g, respectively), but the highest single tuber weights were obtained in SFA+MFA plots in spring(90.71 g)and SFA plots in autumn (139.53 g)(Tables 3, 4).

Tuber yields ranged between 16.07t/ha and 29.75 t/ha depending on season and treatments (Table 3). Control plots alwaysproduced the lowest tuber yields (Table 3, 4). In spring sowing, SFA plots produced the highest tuber yields (29.75 t/ha) followed by SFA+MFA (23.94 t/ha) and MFA (17.55 t/ha) plots. SFA+MFA treatment yields (23.94 t/ha) were even lower than SFA plots (29.75 t/ha). In autumn sowing, SFA (26.81 t/ha) and MFA (26.56 t/ha) yields were higher than control (24.21 t/ha) and SFA+MFA (21.27 t/ha). SFA+MFA plots produced lower yields than control plots. On average of both seasons, MFA applied plots produced 22.06 t/ha tuber yield compared with control (20.14 t/ha) and SFA plots (28.28 t/ha). SFA+MFA plots produced even lower tuber yields (22.83 t/ha) than SFA plots. MFA applied plots produced more tuber yields by1.48 t/hain spring, 2.35 t/hain autumnand by 1.92 t/ha on average of both seasons when compared with control plots (Table 3).

Discussion

Data obtained from off season field experiments in spring and autumn period showed that the application of *B. subtilis* and *B. amyloliquefaciens* culture shortened emergence time, increased plant growth, produced fewer but larger tubers and increased tuber yield over control treatment. Microbial fertilizer application produced 9.2% more tuber yields in spring and 9.7% in autumn period over control plots. Tuber yield was higher in autumn (26.56 t/ha) than in spring (17.55 t/ha) in MFA plots probably due to warmer soil temperatures in autumn sowing favorable for shoot growth and probably proliferation of bacteria inoculated on tubers in the soil. Although emergence time was the same in spring and autumn planting, average above ground biomass, plant height, number of leaves and stem diameter were higher in autumn than in spring plantings. Warmer soil and air temperatures recorded for autumn were favorable for plant growth and development. Colder soil and air temperatures during January, February and March period as minimum temperatures of 1.0 °C, 6.1 °C and 9.1 °C limited plant growth and probably activity of bacterial growth in spring. Higher tuber yields obtained from microbial fertilizer applied (MFA) plots (26.56 t/ha) in warmer autumn period, which were equal to SFA plots (26.81 t/ha), as opposed to colder spring period (17.55 t/ha), may support the above assumption. MFA plots produced higher tuber yields than control plots in both seasons although spring yields were lower. In this experiment, an equal mixture of *Bacillus* strains were used in order to investigate the possibility of employing environmental friendly alternative fertilization for off-season potato production in coastal areas where relatively warmertemperatures allow plant growth during winter. Further experiment was warranted with more cold tolerant *Bacillus* bacterial strains alone or in mixtures for off season potato production. Selection of bacterial strains that maintain their activity and survive in cold soils may offer new avenue for the use of associative Rhizobacteria in off season potatoproduction. Data here with two seasons indicate that under favorable conditions microbial fertilizers alone produced tuber yields equal to chemical fertilizers. Microbial fertilizers may be also used for organic potato production. SFA ensured higher tuber yields even in colder spring period. Tuber yields were higher in SFA plots in both seasons.Tuber yield was evenhigher in fertilizer applied (SF) plots in spring than in autumn planting.Soil conditions in spring probably favored the slow, effective and longer utilization of nitrogen, phosphorus and potassium applied with chemical fertilizersas opposed to warmer soil temperatures recorded in August planting.This mayalso belinked with lower tuber yields in SFA+MFA plots both in spring and autumn period. Supplementation of chemical fertilizers with microbial fertilizers did not ensure higher tuber yields. Although not measuredin this experiment, competition of bacteria inoculated on tubers for nitrogen, phosphorus and other plant nutrients probably resulted in lower yields in SFA + MFA plots (Madigan et al., 1997; Herrero et al., 2001). In further experiments, survivability of strains and availably of macro nutrients in soil may be followed in order to explain this case.

Plant growth promoting rhizobacteria including *B. subtilis* and *B. amyloliquefaciens* had functions of bioprotection, phytostimulation and biofertilization on potato enhancing plant growth and tuber yields (Aloo et al., 2019). There were plenty of evidence that PGPR had positive effects on potato growth. In our experiment, *B. subtilis* and *B. amyloliquefaciens* culture applied plots had equal yields compared with chemical fertilizer applied plots in warmer August conditions.However, advantage of *B. subtilis* and *B. amyloliquefaciens* application was not seen in colder January plantings. Colder conditions probably restricted activities of *B. subtilis* and *B. amyloliquefaciens*. Psychrophilic bacteria collected from Andean mountains at 4500 above sea level exhibited plant growth promoting properties (Balcazar et al., 2015). Hence, isolation and use of cold tolerant psychrophilic bacteria may offer an alternative strategy for off season potato production during winter period.

Conclusions

A two season experiment with an equal mixture of *B. subtilis* and *B. amyloliquefaciens* strains produced consistently higher tuber yields in off-season potato. Tuber yields in microbial fertilizer applied plots were equal to chemical fertilizer applied plots under more favorable autumn planting, but lower in colder spring planting. Further experiments are warranted with selection and use of psychrophilic bacterial strains that maintain their activity in cold soils for the production of off-season potato production.

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Effects of humus and shading levels in the production of *Lactuca canadensis* L. seedlings

Efectos de niveles de humus y sombreado en la producción de plántulas de *Lactuca canadensis* L.

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Resumen

El trabajo se realizó en la Universidad Federal de Mato Grosso, Cuiabá, Brasil, con el objeto de evaluar diferentes niveles de sombreado y dosis de humus en la producción de plántulas de *Lactuca canadensis* L. (lechuga silvestre). Se utilizó un diseño de bloques completos al azar en esquema factorial 4 x 5 (cuatro niveles de sombreado y cinco dosis de humus). Los diferentes niveles de sombreado fueron obtenidos utilizando tela negra de polietileno con 35, 50 y 70% de sombra y más un tratamiento testigo (pleno sol). Los sustratos fueron obtenidos por la mezcla del producto comercial para hortalizas (Vivatto Slim® plus) más humus de lombriz en las proporciones de 0, 15, 30, 45 y 60%. En contraste con el tratamiento testigo, con sombra de 50% se encontró la mejor emergencia y desarrollo de plántulas. Tanto el sustrato sin adición de humus como con 60% de éste, presentaron el mayor número de hojas (3.14) y contenido de clorofila (32.9%). Con base en la relación beneficio/costo, el sustrato comercial puro puede ser utilizado en la producción de plántulas de *Lactuca canadensis* L.

Palabras clave: hortalizas, humus de lombriz, *Lactuca canadensis* L., sustratos

Abstract

The objective of this study was to verify levels of shade and doses of earthworm humus in the production of *Lactuca canadensis* L. seedlings. The experimental design was completely randomized in a 4 x 5 factorial scheme (four levels of shade and five doses of humus). The different levels were obtained using black polyethylene screen with 35, 50 and 70% shade and in full sun. The substrates were obtained by mixing commercial substrate for vegetables (Vivatto Slim ® plus), with earthworm humus in the proportions of 0, 15, 30, 45 and 60%. There was no seedlings emergence in the full sun and, the treatment with 50% of shade allows the best development. The substrate without humus and with 60% presented superior results but the higher dose stood out for number of leaves (3.14) and chlorophyll content (32.9%), in 50% of shade. For the other variables, there was no difference between the commercial substrate and 60% of humus, so the commercial substrate can be used in the production of *Lactuca canadensis* L. seedlings, considering the ratio benefit/cost ratio.

Key-words: *Lactuca canadensis* L., seedling, substratum, vegetable.

Introduction

Lactuca canadensis L. belongs to the family Asteraceae. It is popularly known as wild lettuce, tall lettuce or Florida blue lettuce (Kinnup and Lorenzi, 2014). This species is biennial and produces leaf rosettes in the first year and tall stems in the second year. It reaches maximum heights of 0.5-2.0 m (Michalska, Szneler and Kisiel, 2013). Native to North America, it is one of the most common species of the genus (Lebeda, Doležalová and Novotná, 2012). *Lactuca canadensis* L. is an unconventional food plant (UFP) (Kinnup and Lorenzi, 2014) which occurs spontaneously. This species has characteristics of rusticity, which allows its economic production. In addition, Michalska, Szneler and Kisiel (2013) have identified compounds in the roots of *L. canadensis* that may have medicinal properties.

Although it presents characteristics desirable to production as a vegetable (resistance and adaptation to diverse environments), there is no information regarding the cultivation of *Lactuca canadensis* L. especially in relation to the production of seedlings. Thus, the study of suitable conditions for the cultivation of this species is essential. Such study aims to obtain an efficient production as already established for other species (Freitas, Silva, Barros, Vaz-de-Melo and Abrahão, 2013; Goés, Dantas, Araújo, Melo and Mendonça, 2011; Hirata and Hirata, 2015; Neves et al., 2016).

An efficient production of seedlings requires adequate conditions to plants since each species presents different levels of adaptation to production methods. Luminosity and temperature exert a great influence on the response of seedlings to substrates. In tropical climate conditions, high luminosities have been limiting the production of leafy vegetable seedlings such as *Rorippa nasturtium-aquaticum* (Hirata and Hirata, 2015). In addition, high luminosity with high temperatures can make the production of seedlings unfeasible. In order to minimize the influence of such factors, shading screens have been widely used in the production process.

Another important factor is the substrate. It should have adequate characteristics, such as good water retention, nutrient availability and root aggregation. The objective of this study is to evaluate the production of *Lactuca canadensis* L. seedlings in a substrate containing different doses of earthworm humus and three levels of shading.

Material and methods

The experiment was carried out from August to September in Cuiabá, Mato Grosso state (15°36’33” S, 56°03’55” W and altitude of 145 m). *Lactuca canadensis* L. seeds were obtained from matrices plant, collected at physiological maturity. At this point, they presented a black coloration and panicle dispersion. Subsequently, the seed water content was determined in subsamples of 0.2 g using the oven method at 105 ± 3 ºC for 24 h with three replications (Brazil, 2009). The average water content was 10%.

The experiment was completely randomized in a 4 x 5 factorial design (four levels of shading and five doses of humus) with four replications. The levels of light were obtained using black polyethylene screens with 35, 50 and 70% shading, plus a treatment in full sun (without shading). The substrates were composed of commercial vegetable substrate (CS) (Vivatto Slim® plus) and earthworm humus at the ratios 0, 15, 30, 45 and 60% (v:v). The commercial substrate was composed of charcoal mill, pine bark and peat, according to the manufacturer. Subsequently, the substrates were distributed into 128 expanded polystyrene trays (depth: 56 mm, width: 35 mm) with 34.6 cm^3 of capacity per cell. Sowing was performed by placing approximately six seeds per cell at a depth of 2 mm. The seeds were covered with a thin layer of expanded vermiculite to avoid direct exposure to the sun, wind and irrigation water. Thinning was carried out at 14 days after sowing. Only the most vigorous seedlings remained in each cell. Irrigation was performed manually using a watering twice a day.

Fourteen useful plants were selected in each plot after discarding the borders. The evaluated characteristics were emergence percentage (EP) by counting the emerged seedlings; mean emergence time (MET) using the equation of Labouriau (1983) by observing the daily number of emerged seedlings, until stabilization; stem diameter (SD) by measuring the stem at ground level with a digital caliper; chlorophyll content (CC) determined in the second younger leaf with a digital chlorophyll meter (chlorofiLOG CFL1030); plant height/stem diameter ratio (H/D); Dickson quality index (DQI) (Dickson, Leaf and Hosner, 1960); number of leaves (NL) by counting the completely expanded leaves; plant length (PL) considering the length from the root end to the leaf end; shoot length (SL) measured from the plant base to the leaf end; fresh (FM) and dry matter (DM) obtained by the difference of plant weight before and after drying in an oven with forced air ventilation for 72 h at 60°C. An analysis of variance was performed and when significant, a Tukey test was carried out ($p < 0.05$), using the software Sisvar 5.6 (Ferreira, 2011).

Results

For the variables seedling emergence, mean emergence time, stem diameter, chlorophyll content, plant height/stem diameter ratio and Dickson quality index, there was interaction between levels of shading and humus doses.

The emergence percentage of *L. canadensis* L. seedlings was generally above 80%. However, it did not differ statistically among levels of shading, except for plants sown at 70% shading in the substrate containing 30% humus. In these conditions, there was an emergence of 75% (Table 1). In treatments without shading (full sun), there was no emergence of seedlings.

Regarding humus doses in each environment, the substrates without humus under 50 and 70% shading allowed the highest emergence percentages. For the mean emergence time, observing the levels of shading in each humus doses, in the substrates with 15 and 60% of humus, the 70% of shading allowed the emergence in a shorter time. Other humus doses did not differ. Comparing the doses of humus and the shading levels, the substrate without humus provided the shortest time for emergence of seedlings at 30% of shading. Under 50 and 70% shading, there were no differences in MET among humus doses (Table 1).

In stem diameter, there was an increasing tendency in function of increases in shading levels. This difference becomes more evident as the dose of humus in the substrate increases (Table 1). Evaluating the humus doses, the 60% dose was superior to the others doses for seedlings under 70% shading.

For the relation between plant height and stem diameter, the plants at 35 and 50% shading did not differ among themselves considering the addition of humus to the substrate. However, the highest values occurred in plants sown on substrates with 0, 15 and 30% humus at 70% shading. There was a higher growth in height, but an increase in stem diameter did not follow.

The chlorophyll content was little influenced by shading levels. There was a difference between the substrates without humus: the 35% shading provided a lower chlorophyll content in leaves (Table 1). The increase in humus doses in the substrate caused an increase in the chlorophyll content at all shading levels.

The Dickson quality index increased at 15, 30 and 45% humus doses under 50% shading (Table 1). In the unfolding of humus doses in function of shading levels, plants at 35 and 70% shading and substrates containing 0 and 60% humus were superior to the others. For plants at 50% shading, there was no difference in DQI.

Regarding number of leaves, plant length, shoot length, fresh and dry matter, the interaction between shading levels and humus doses was not significant. Therefore, there was an isolated effect of factors.

Seedlings under 50 and 70% shading presented a higher number of leaves than those under 35% shading. The same tendency was observed for plant length, shoot length and fresh seedlings

Table 1. Emergence percentage (EP), mean emergence time (MET), stem diameter (SD), plant height and stem diameter ratio (H/D), chlorophyll content (CC) and Dickson quality index (DQI) of *Lactuca canadensis* L. grown in different doses of humus (0, 15, 30, 45, 60%) and shading levels (35, 50 and 70%).

Doses of humus (%)	Shading (%)					
	35	50	70	35	50	70
	EP (%)			MET (days)		
0	93 aA*	95 aA	96 aA	6.5 aA	6.8 aA	6.6 aA
15	88 aA	83 aAB	93 aAB	7.8 bB	7.0 abA	6.6 aA
30	83 abA	91 aAB	75 bC	7.1 aAB	7.0 aA	6.9 aA
45	81 aA	81 aB	84 aBC	7.5 aAB	6.8 aA	7.2 aA
60	81 aA	84 aAB	88 aAB	7.7 bB	7.2 bA	6.2 aA
CV (%)	7.15			7.41		
	DC (mm seedling^{-1})			H/D		
0	1.98 aA	2.24 aA	2.07 aBC	2.70*bA	2.65 bA	3.22 aA
15	1.40 aB	1.73 aA	1.71 aC	2.63 aA	2.51 aA	2.75 aA
30	1.27 bB	2.01 aA	1.91 abBC	2.91 aA	2.43 bA	2.79 abA
45	1.72 bAB	2.13 abA	2.41 aB	2.75 aA	2.57 aA	2.12 aB
60	2.09 bA	2.07 bA	3.10 aA	2.78 aA	2.79 aA	2.22 bB
CV (%)	13.71			9.08		
	CC (%)			DQI		
0	24.5 bC	28.2 aB	27.1 aB	0.90 aA	0.92 aA	0.89 aAB
15	24.9 aC	26.3 aB	25.0 aB	0.82 bB	0.89 aA	0.83 bC
30	25.3 aC	26.8 aB	25.8 aB	0.77 cC	0.89 aA	0.85 bBC
45	28.2 bB	31.4 aA	25.8 cB	0.81 cBC	0.90 aA	0.86 bBC
60	31.4 aA	32.9 aA	30.9 aA	0.89 aA	0.92 aA	0.91 aA
CV (%)	4.63			2.53		

*Means followed by the same lowercase letters in lines and uppercase letters in columns do not differ statistically by Tukey test ($P < 0.05$). CV = Coefficient of Variation.

matter. For dry matter, the of 50% shading allowed the development of plants with a higher mass (Table 2).

As for humus doses, seedlings produced in substrate with 60% humus had a higher number of leaves. However, seedlings produced in a substrate without and with 60% of humus were superior than other humus doses for the remaining variables (length, height, fresh and dry matter) (Table 3).

Discussion

Development of .*Lactuca canadensis* L. seedlings under full sun was influenced by high temperatures potentiated by the high luminosity during the experimental period. This condition did not allow the evaluation of seedlings in treatments without shading, since there was no emergence of seedlings in this condition.

This plant originates from cold regions of the North American continent (Lebeda et al., 2012). In these regions, there is a predominance of mild temperatures at the time of seed dispersal. It can determine the absence of germination at high temperatures, as occurred in the full sun treatment.

Callegari, Santos and Scapim (2001) verified that high temperatures (between 30 and 35ºC) cause a decrease in seedling emergence of lettuce.

Table 2. Number of leaves (NL), plant length (PL), shoot length (SL), fresh (FM) and dry matter (DM) of *Lactuca canadensis* L. in different shading levels (35, 50 and 70%).

Shading (%)	NL	PL	SL	FM	DM
		(cm seedling^{-1})		(mg seedling^{-1})	
35	2.42 b*	12.99 b	4.60 b	166.75 b	16.66 b
50	2.93 a	14.48 a	5.26 a	226.10 a	25.43 a
70	2.83 a	15.30 a	5.72 a	197.27 ab	18.22 b
CV (%)	8.26	8.67	11.70	34.7	17.0

*Means followed by the same letters, in the columns, do not differ statistically by Tukey test (P < 0.05). CV = Coefficient of Variation.

Table 3. Number of leaves (NL), plant length (PL), shoot length (SL), fresh (FM) and dry matter (DM) of *Lactuca canadensis* L. grown in different doses of humus (0, 15, 30, 45, 60%).

Doses of humus (%)	NL	PL	SL	FM	DM
		(cm seedling^{-1})		(mg seedling^{-1})	
0	2.77*b	15.62 a	5.95 a	231.05 ab	26.88 a
15	2.39 c	13.23 c	4.19 c	125.93 c	15.45 b
30	2.54 bc	13.32 c	4.62 bc	163.09 bc	15.79 b
45	2.79 b	14.18 bc	5.08 b	190.43 bc	16.28 b
60	3.14 a	14.90 ab	6.13 a	273.03 a	26.11 a
CV (%)	8.26	8.67	11.70	34.70	17.00

*Means followed by the same letters, in the columns, do not differ statistically by Tukey test (P < 0.05). CV = Coefficient of Variation.

In order to minimize the effects of excess light and high temperatures, shading screens can be used (Hirata and Hirata, 2015; Neves et al., 2016).

For shaded treatments, there was little influence of shading level on seedling emergence. Beltrame, Lopes, Mengarda, Manhone and Freitas (2014) also observed this on the seedling production of *Joannesia princeps* Vell. In relation to the influence of humus doses in the substrate, the substrate composition caused different responses in the emergence of *L. canadensis* L. seedlings. Mauri, Lopes, Ferreira, Amaral and Freitas (2010) evaluated broccoli seeds and obtained variations in germination according to different compositions of substrates.

For mean emergence time and stem diameter, as higher was the shading level, greater was the speed of emergence and the stem diameter. This may be related to the maintenance of substrate moisture during the germination/emergence process because of less water loss from the substrate by evaporation. Costa, Rodrigues, Alves, Santos and Vieira (2009) confirmed the effects of shading on the reduction of water evaporation, generating favorable conditions for the development of yellow passion fruit seedlings.

In general, stem diameter has been used as an indicator of the quality standard of seedlings, that is, seedlings with a smaller and very large stem base diameter are considered of inferior quality due to etiolation (Beltrame et al., 2014). Therefore, the development in height should be accompanied by an increase in the stem diameter, avoiding etiolation and seedlings falling.

For seedlings under 35 and 50% shading, the increase in height and stem diameter was proportional at different humus doses. However, for seedlings under 70% shading, the increase in humus dose caused a better developmental balance due to lower H/D values. Harmony in the morphological development of the seedling allows a balanced growth in relation to height and stem diameter, avoiding falling. Souza, Barros, Silveira Santos and Silva (2013) stated that the balance between base diameter and seedling height is important for the estimation of seedling growth after definitive planting in the field.

As for chlorophyll content, the maximum concentrations of chlorophyll occurred at the highest doses of humus. Therefore, there is an effect related to the addition of humus to the substrate. According to Armond et al. (2016) the increase in chlorophyll content may be associated with increasing doses of organic fertilization and greater availability of nutrients. They are constituent elements of the plant chlorophyll molecule.

The Dickson quality index relates morphological parameters, allowing inferences on the development and obtaining of quality seedlings. The highest values occurred in plants under 50% shading. There was no difference between humus doses at this shading level. In all evaluated treatments, the DQI was higher than 0.20, which is the minimum value Hunt (1990) stipulated. However, seedlings with better development and greater morphological balance present a competitive advantage when taken to the field.

For number of leaves, plant length, shoot length, fresh and dry matter, seedlings under the highest shading levels (50 and 70%) generated plants with a greater vigor possibly due to the conservation of moisture in these substrates, as a consequence of less water loss by evaporation. According to Gomes, Francisco, Gemin, Rossa and Westphalen (2017) the number of leaves is an important factor because it indicates the plant's photosynthetic capacity and, consequently, the capacity to assimilate carbon, increasing vigor and seedling quality.

For humus dose, the substrate without and with 60% of humus were prominent. The substrate with the highest dose presented superior results for number of leaves. However, for the other variables (seedling emergence, mean emergence time, stem diameter, plant height and stem diameter ratio, Dickson quality index, plant length, shoot length, fresh and dry matter) there were no differences. So, the pure commercial substrate can be used considering the benefit/cost ratio.

Conclusions

Shading is essential for the production of *Lactuca canadensis* L. seedlings since sowing under full sun inhibits the emergence. Among the levels studied, the black polyethylene screen with 50% shading allows the best development of seedlings. The commercial substrate without humus and the 60% humus dose favor the production of seedlings of *L. canadensis* L. The addition of humus to the substrate at a dose of 60% also causes an increase in the number of leaves and chlorophyll content of plants.

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Plant growth and yield of butter kale (*Brassica oleracea* L. var. acephala), as influenced by the combined application of bovine manure and rock powder

Efecto de la aplicación combinada de estiércol bovino y polvo de roca en el crecimiento y producción de col rizada (*Brassica oleracea* L. var. acephala)

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Abstract

The supply of organic matter in soil of arid and semiarid in Brazilian regions is very import, as it allows to increase crop yields. However, there are few studies on alternative sources of fertilizer for vegetables. Therefore, the objective was to evaluate the effect of different doses of bovine manure and rock powder (RP) on growth characteristics, concentration of chlorophyll, and physiological indices and production of (*B. oleracea* L. var. acephala). The treatments were arranged in five randomized blocks in a factorial 4 x 4 reference doses of bovine manure (60, 120, 180 and 240 g pit^{-1}) combined with doses of rock powder (6, 12, 18 and 24 g pit^{-1}). Doses of bovine manure and rock powder of 180 and 18 g, respectively, increased the growth of *B. oleracea* by plant height, stem diameter, leaf number. The combination doses of bovine manure 120 g + 12 g rock powder and 120 g of bovine manure + 18 g rock powder, provided, in this order, larger leaf area results for the productivity of *B. oleracea*. The bovine manure promotes greater concentration of CO_2, net photosynthesis and stomatal conductance of *B. oleracea* plant, while rock powder limits these characteristics. Intermediate doses of bovine manure and RP promote higher growth and yield of *B. oleracea*.

Key words: *Brassica oleracea*, agricultural fertilizers, organic matter.

Resumen

El suministro de materia orgánica en suelos áridos y semiáridos en las regiones brasileñas permite aumentar los rendimientos de los cultivos. Sin embargo, hay pocos estudios sobre estas fuentes alternativas de fertilizantes para vegetales. Por tanto, el objetivo del trabajo fue evaluar el efecto de diferentes dosis de estiércol bovino y polvo de roca en el crecimiento, la concentración de clorofila, los índices fisiológicos y la producción de *B. oleracea* L. var. acephala. Los tratamientos fueron dispuestos en cinco bloques aleatorizados en dosis de referencia factoriales de 4 x 4 de estiércol bovino (60, 120, 180 y 240 g por pozo) combinados con dosis de polvo de roca (6, 12, 18 y 24 g por pozo). Las dosis de estiércol bovino y polvo de roca de 180 y 18 g, respectivamente, aumentaron el crecimiento de la *B. oleracea*, la altura de planta, el diámetro del tallo y el número de hojas. Las dosis combinadas de estiércol bovino 120 g + 12 g de polvo de roca y 120 g de estiércol bovino + 18 g de polvo de roca, proporcionan, en este orden, mayores resultados de área foliar para la productividad de la *B. oleracea*. El estiércol bovino promueve una mayor concentración de CO_2, la fotosíntesis neta y la conductancia estomática de la planta de *B. oleracea*, mientras que el polvo de roca limita estas características. Las dosis intermedias de estiércol bovino y polvo de roca promueven un mayor crecimiento y rendimiento de la *B. oleracea*.

Palabras clave: *Brassica oleracea*, fertilizantes agrícolas, materia orgánica.

Introduction

The cultivation of vegetables in Paraíba Agrest is mainly performed in soils with low content of organic matter. Moreover, they are characterized as acidic and poor in nutrients, caused in most cases by intensive agricultural use, which have the need for continued use of mineral fertilizers, mainly resulting limiting carbon content (C) over the years (Conceição et al., 2014).

Even in soils with nutrient levels considered adequate, a supply of organic material becomes necessary, it allows to enhance the chemical, physical and biological properties of the soil, resulting from the action of humic acids present and available by adding organic fertilizer. Associated to those situations soils in semiarid regions present naturally low levels of organic matter, due to weather conditions favorable to decomposition, compromising even more the viability of a successful farm, and there should have application of organic matter that can supply the nutrient requirements of crops indispensably, thus while increasing cation exchange capacity (CEC) decrease evaporation losses, and increase the water storage (Nascimento et al., 2015).

As an alternative in the supply of organic matter to the soil, has been used bovine manure (BM), due to greater availability and lower cost, because its consecutive use tends to increase soil fertility, especially nitrogen (N), phosphorus (P) and potassium (K) (Tejada et al., 2016). Studies using BM to fertilize vegetables in northeastern Brazil have been conducted and proved the beneficial effect on increasing crop productivity. For instance, in the culture of watermelon, Nascimento et al. (2015) and Nascimento et al. (2017) observed that 1.10 kg dose pit^{-1} of BM promoted higher productivity and the increase of BM dose reduced the need for potassium fertilizer to increase plant productivity.

In gherkin culture, Oliveira et al. (2014) observed that the doses of 26.3 and 27.7 t ha^{-1} of BM were responsible for the maximum commercial productivity of gherkin fruits, wherein the authors indicate that the highest values obtained with BM must be due to the larger supply of macronutrients (N, P and K). Silva et al. (2012) also observed that the BM dose of 19.2 t ha^{-1} gave the highest yield of 20.3 t ha^{-1} in commercial yam tubers.

On the other hand, stonemeal technique, a practice that allows you to provide nutrients and increase the cation exchange capacity (CEC) due to the formation of new minerals of clay during the rock change, it is another common practice in the Northeast. The technique consists of rock powder (RP) application directly into the soil, providing nutrients to the soil, moreover, it is a low cost alternative, which provide nutrients for plants in the short, medium and long term (Manning and Theodoro, 2018), and can be used to increase the quality and amount of mineral organic fertilizer raising productivity in horticultural, ensuring economy, since the use of alternative sources may reduce the intake of chemical fertilizers and minimize the environmental impacts, turning essential to the organic production system. Therefore, the objective was to evaluate the effect of different doses of manure and rock powder on growth characteristics, chlorophyll content, and physiological indices and *B. oleracea* production.

Material and methods

The experiment was conducted in experimental, located in the city of Bananeiras-PB, in the geographical coordinates 6°45'4" 35°38'0". According to the Kopen the climate classification is considered the typ As' - Tropical rainy, dry summer, irregular annual rainfall distribution (1174.7 mm), with a maximum annual temperature of 27°C and a minimum of 18,8°C, averaging 22°C yearly. Meteorological data of the experimental period are shown in Figure 1.

The treatments were arranged in five randomized blocks in a factorial 4 x 4 refers to the different doses of bovine manure (BM) (60, 120,

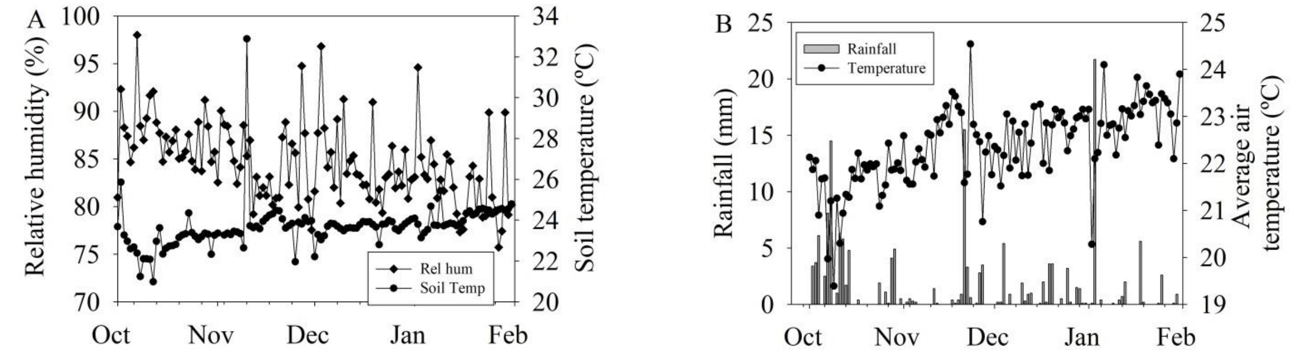

Figure 1. Graphical representation of relative humidity, soil temperature, rainfall and average air temperature in the period from October 2014 to February 2015. Data from the weather station, EFSA, PB, 2015.

180 and 240 g pit^{-1}) combined with rock powder (RP) doses (6, 12, 18 and 24 g pit^{-1}). Each block is composed of three plots of 18 m long and 1 m wide, the bed is composed of six portions and each experimental plot consisted of 14 plants spaced in 0.40 x 0.40 m making a total of 1190 plants, 85 portions and 15 beds in the studied area and 4 beds were used as surrounders.

The soil of the prevailing experimental area corresponds to Yellow Oxisol of medium texture, with gently curly relief, with deep profile, well drained, with moderate moisture retention capacity and Loan clay sandy textural class. Before conducting experiment soil simple samples were collected in the experimental area (0 - 20 cm), being homogenized and processed into composite samples, subsequently performing chemical and physical characterization (Table 1).

The seedlings of *B. oleracea* L. var. acephala cv. Georgia were produced in cups containing substrate composed of humus (60%), sand (30%) and bovine manure (10%). During the production of seedlings carried out a simple bio-fertilizer application at 15 days after sowing, the same being used as a natural defense.

Was used the conventional soil tillage preparation with plowing and harrowing with a subsequent lifting of the beds. Holes were made with 0.20 m depth in the transplanting seedling stage at the time when the fertilizer was also performed with the incorporation of BM and RP (MB-4®) according to the proposed treatments. The BM and RP were analyzed and showed the following characteristics, as can be seen in Table 2. The RP used was of origin of the mother rock (Neoproterosóica Brasiliane s.I- Granitoids) (Santo et al., 2002) composed of 55% sand, 43% silt and 2% clay.

After transplantation and fertilization, the beds were covered with straw *Brachiaria decumbens* in a layer of 2 cm thick, in order to enhance the development of biological activities, control weeds, and maintain soil moisture.

The irrigation was performed by the drip system, according to the water requirements of the *B. oleracea* crop, adopting the 9 h irrigation shift in the initial phase and establishing the cultivation field, continuing up to 1 day in the final stage, corresponding to the last 20 days of cultivation in the field, and use of self-cleaning emitters and self-compensation with a flow of 6.0 L h^{-1}, spaced apart 0.40 m, distributed along the line. The methodology used to calculate the irrigation was based on: $Etc = Kc \times ETo$ (crop evapotranspiration = crop coefficient x reference evapotranspiration (mm day^{-1}).

The growth of kale was evaluated by plant height measurements, stem diameter, leaf number and leaf area at 8, 15, 22, 29, 36, 43, 50 and 57 days after transplanting (DAT), length root system and productivity at 57 days after transplanting.

Physiological parameters were assessed at 57 DAT by determining the carbon internal concentration (Ci), stomatal conductance (gs), transpiration (E), net photosynthetic rate (A), the instantaneous efficiency in the use of water, calculated by relating it to the net photosynthesis to transpiration and instantaneous carboxylation efficiency ($EiC - A/Ci$) from the net photosynthetic rate and the internal concentration of carbon, using infrared gas analyzer (IRGA) + LCpro system model.

Table 1. Physical and chemical soil properties at the beginning of the experiment.

Physical atributes	Values	Chemical attributes	Values
Sand (g kg^{-1})	740	pH in H_2O (1.0:2.5)	6.50
Silt (g kg^{-1})	120	P (mg dm^{-3})	69.5
Clay (g kg^{-1})	140	K^+ (cmol$_c$ dm^{-3})	0.20
CW (g kg^{-1})	78	Ca^{2+} (cmol$_c$ dm^{-3})	3.50
DF (%)	30	Mg^{2+} (cmol$_c$ dm^{-3})	2.20
DI (%)	56	Na^+ (cmol$_c$ dm^{-3})	0.20
Sd (kg dm^{-3})	1.61	H^++Al^{3+} (cmol$_c$ dm^{-3})	1.50
Pd (kg dm^{-3})	2.60	Al^{3+} (cmol$_c$ dm^{-3})	0.00
Pt (m^3 m^{-3})	0.49	SB (cmol$_c$ dm^{-3})	6.1
M (m^3 m^{-3})	0.049	CECpH7 (cmol$_c$ dm^{-3})	7.6
m (m^3 m^{-3})	0.442	EC (%)	0.00
Fhc (m^3 m^{-3})	0.209	V (%)	80.26
Wpt (m^3 m^{-3})	0.134	O.M. (g dm^{-3})	24.96
Aw (g kg^{-1})	0.074	-	-

Table 2. Chemical analysis of bovine manure and rock powder used in the planting fertilizer of *B. oleracea.*

Bovine manure	Values	Rock powder	Values
pH in H_2O (1.0:2,.5)	8.25	pH	6.80
P (mg dm^{-3})	5.06	P (mg dm^{-3})	42.82
K^+ (cmol$_c$ dm^{-3})	0.0018	K^+ (cmol$_c$ dm^{-3})	0.12
Ca^{2+} (cmol$_c$ dm^{-3})	4.00	Ca^{2+} (cmol$_c$ dm^{-3})	5.65
Mg^{2+} (cmol$_c$ dm^{-3})	3.90	Mg^{2+} (cmol$_c$ dm^{-3})	1.95
Na^+ (cmol$_c$ dm^{-3})	1.08	Na^+ (cmol$_c$ dm^{-3})	0.30
H^++ Al^{3+}(cmol$_c$ dm^{-3})	0.495	H^++Al^{3+} (cmol$_c$ dm^{-3})	1.70
Al^{3+} (cmol$_c$ dm^{-3})	0.00	Al^{3+} (cmol$_c$ dm^{-3})	0.00
Ca^{2+}+ Mg^{2+}	7.90	Ca^{2+}+ Mg^{2+}(cmol$_c$ dm^{-3})	7.60
EC (dS m^{-1})	5.48	CEC (cmol$_c$ dm^{-3})	9.72
CEC (cmol$_c$ dm^{-3})	9.47	V (%)	97.77
V (%)	95.10	SB	74.56
SB	9.69	O.M. (g Kg^{-1})	43.85
O.M. (g kg^{-1})	100.82		

EC = electrical conductivity; CEC = cation exchange capacity; V = base saturation; SB = Sum of bases; O.M. = Organic Matter.

Also were evaluated the levels of leaf chlorophyll a, b and total through Clorofilog® chlorophyll (Falker), every 15 days after transplantation in the field. Readings were taken at intermediate leaves of four central plants from the experimental area between 08:00 and 10:00 hours, performing four readings by plants and was analyzed as average data.

The results were subjected to the variance analysis with 'F' test, evaluated by comparison of means by Tukey's test at up to 5% probability and by polynomial regression using the statistical software SAS® 9.2.

Results and discussion

The height of the plants was evaluated at different growth stages presenting significant effect for the interaction of bovine manure (BM) doses and RP to only at 8 (Figure 2A) and 15 (Figure 2B) days after transplanting (DAT) in the field. In others evaluations periods, the rock poder and BM did not differ one to another.

At 8 DAT the treatment that influenced initial development of *B. oleracea* interaction the most was 180 g of BM + 18 g of RP where plants obtained 12.4 cm height, the lowest values being observed in plants fertilized with BM 180 g + 6 g RP (Figure 2A). This limitation of the growth may be due to lower concentrations of RP present in the treatment used, which consequently may have been lower availability of nutrients to the plants, resulting in the commitment of its development in height.

At 15 DAT interaction of doses 240 g BM + 18 g of RP provided for taller plants of *B. oleracea* with maximum values of 16.85 cm statistically different of doses of BM 240 g + 24 g RP where lower values were observed in height, recording up to 14.26 cm (Figure 2B). The dose of 24 g of RP provided the greatest plant height, obtaining the maximum value of 16.91 cm (Figure 2B).

Possibly the largest doses of manure have provided greater levels of nutrients for plants of *B. oleracea* (Table 1), mainly nitrogen, which is the most important macronutrients for plants,

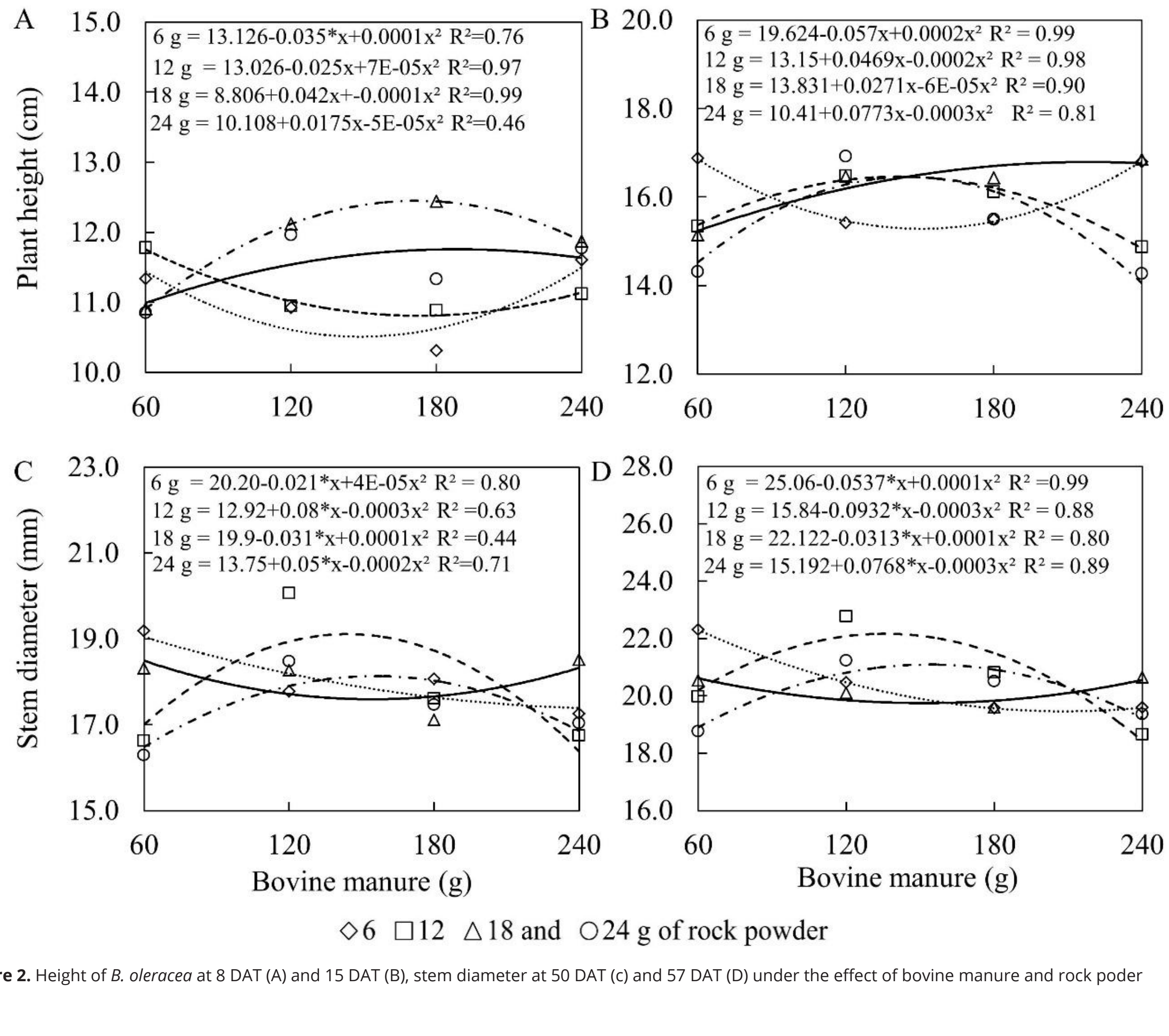

Figure 2. Height of *B. oleracea* at 8 DAT (A) and 15 DAT (B), stem diameter at 50 DAT (c) and 57 DAT (D) under the effect of bovine manure and rock poder

especially at the initial stage of growth as plants absorb nitrogen actively whenever they are in the initial growth phase for the formation of cells, generating more growth in the initial phase of development (Aguilar et al., 2015).

The plant of *B. oleracea* at 50 (Figure 2C) and 57 (Figure 2D) days presented stem diameter of respectively 20.1 and 22.7 mm plant^{-1}, recording the highest values when fertilized with 120 g of BM + 12 g of RP. With the reduction of organic fertilizer even associated to the maximum dose used in RP, 60 g of BM + 24 g of RP, the *B. oleracea* plants presented the greatest reduction in the expansion of stem diameter, and observing 16.2 and 18.7 mm plant^{-1}, in this order at 50 and 57 DAT. The intermediate doses of BM and RP may have provided better balance in soil fertility and generated higher mineralization and solubilization of these matters, and thus a greater availability of nutrients, including nitrogen, providing greater growth in the diameter of stems of plants.

The number of leaves of *B. oleracea* were influenced by the interaction between doses of BM and RP only at 15 DAT (Figure 3A), which in dosages of 60g of BM + 6 g of RP reached 8.6 leaves plant^{-1}. In comparison there was a restriction of leaf on plants fertilized with the maximum doses of 240 g fertilizer BM + 24 g of RP, just promoting the establishment of 7.2 leaves plant^{-1}.

Intermediate doses of cattle manure and rock dust may have provided a better balance in soil fertility and led to greater mineralization and solubilization of these issues, and thus greater nutrient availability, including nitrogen, providing greater growth in stem diameter of the *B. oleracea* plants. In addition, higher doses may have caused phytotoxic effect on buter kale plants (Moura et al., 2018).

The rock powder as an isolated factor influenced at 29 DAT (Figure 3B), and BM at 36 DAT (Figure 3C). In this first period, the doses of 6 and 12 g of RP showed higher number of leaves with 13.8 leaves plant^{-1}. In the second period, the dose of BM provided reduction in the number of leaves, that way, increasing doses resuled in growth restriction, resulting in the largest number of leaves in *B. oleracea* plants fertilized with 60 g of BM (Figure 3D).

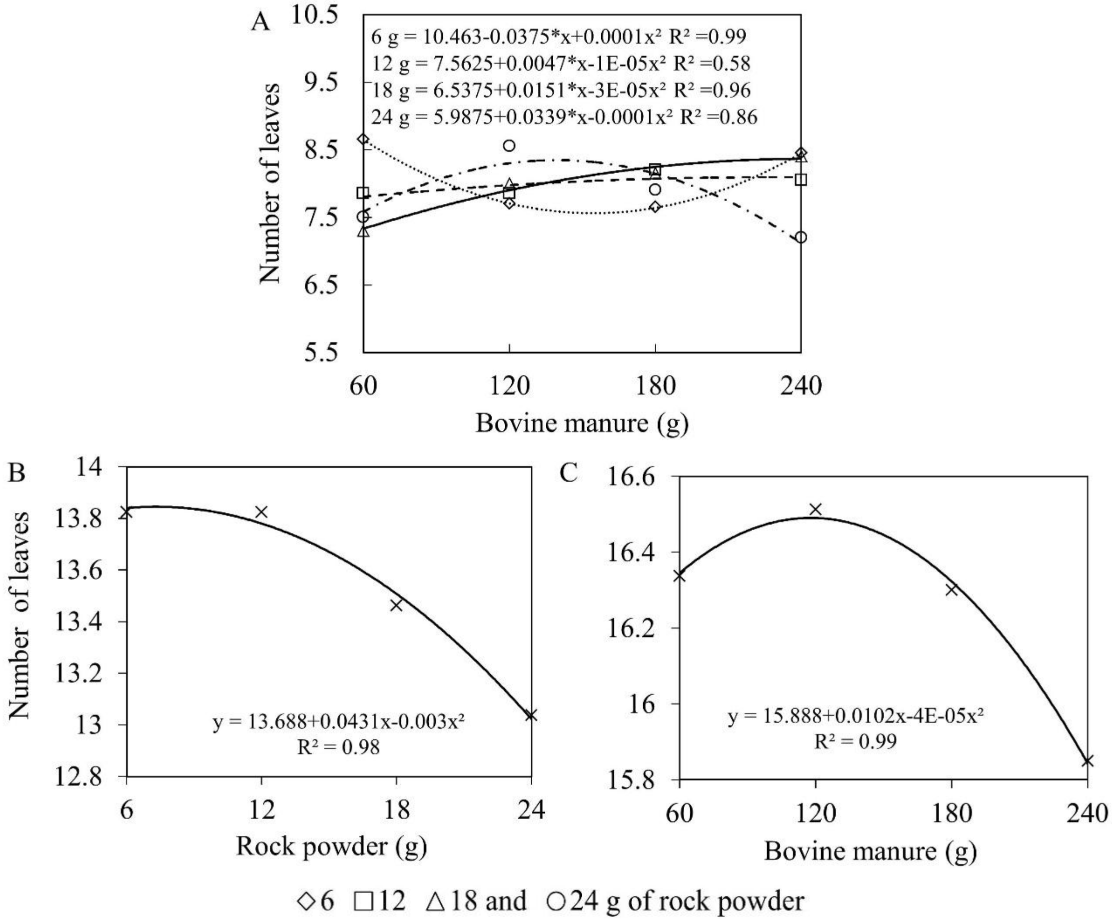

Figure 3. Number of leaves at 15 DAT (A), 29 DAT (B) and 36 DAT (C) of *B. oleracea* fertilized with bovine manure and doses of rock poder

In general, for growth variables (plant height, stem diameter, leaf number) which presented significant difference for the BM doses and RP or the interaction of these factors, the results obtained during the different growth stages can be justified in function of the mineralization process and the amount of these materials applied to the soil. The BM and RP are materials that tend to have a release of nutrients variable, affecting the availability to the plants and plant characteristics. In the case of BM, it has a higher relation C/N ratio when compared to other sources of fertilizer, which ends ultimately providing more slowly minerals for plants. The rock powders, the mineralization and release of the present ions are slow and gradual, they are not available in some cases in the initial phase of crop development (Tito et al., 2019).

However, when applied in appropriate associated form and in amount and particle sizes in a soil that has biological activity and chemical and physical characteristics favorable, the BM and RP can cause effects in a shorter period of time and thus to promote plant growth due to the fertilization (Osterroht, 2003). In this context, is realised that the effects on plants from the practice of fertilizing with these materials can be diverse, affecting them since the establishment phase of the field in culture to the production phase.

The results obtained for plant height, stem diameter, leaf number corroborate with those observed by Tessaro et al. (2012), analyzing agroecologic seedling production and development on field of Chinese kale using different percentages of compound (bovine manure + corn + soy+ wheat), sand and basalt powder, where the height plants obtained 46.7 to 53 cm, stem diameter 16.2 to 25.09 mm plant^{-1} and for leaf number from 30.8 to 52.6 plant^{-1}.

Steiner et al. (2009) evaluated the effect of the organic compound on the production and accumulation of nutrients in the leaves of *B. oleracea*, also observed results for stem diameter variables in function of leaf number in function of alternative fertilizing used, where for these variables were influenced and obtained stem diameter of 16.2 mm to 22 mm and exhibiting 18.8 leaves plant^{-1}.

The leaf area was significantly affected by interactions of BM dosages and RP doses, where plants grown at doses 120 g of BM + 18 g of RP had greater leaf area up to 594 cm^2 plant^{-1} (Figure 4A), the lowest leaf area of 403 cm^2 plant^{-1} was observed in the plants fertilized with doses of BM 180 g + 24 g of RP. It is possible that the response of leaf area to fertilizer applied at intermediate doses is due to the effect of fertilization in a balanced way in the development of culture.

The interaction of doses of BM and RP also affect the productivity of *B. oleracea* plants, verifying expression between plants cultivated with doses 120 g of BM + 12 g of RP (70 t h^{-1}) and treatments with lower yields 120 g of BM + 6 g of RP (43 625 t h^{-1}), 180 g of BM + 6 g of RP (43.750 t h^{-1}) and 240 g of BM + 6 g RP (38.187 t h^{-1}).

The manure used as a source of organic fertilizer had C:N relation of 18/1 being considered optimal mineralization (Schröder et al., 2013). After incorporation of the BM into the soil, the mineralization process is activated by the microorganisms, allowing the release of these nutrients in its mineral form to the soil solution, being able to be absorbed by the plant root system and thus increase the productivity of *B. oleracea*. Thus, the gradual mineralization of bovine manure and solubilization of rock powder added to the soil may have allowed the supply of nutrients that were at low levels in the soil until appropriate levels, thereby generating an increase in productivity *B. oleracea* (Camargo et al., 2012).

Camargo et al. (2012) when took BM doses (0 to 100 t ha^{-1}) test and of basalt powder dose (0 to 6 t ha^{-1}) in the strawberry productivity, also achieved significant results. The evaluated fruits outperformed the association with higher doses of BM with intermediate doses of basalt powder. Even for the variable productivity,

Considering the behavior observed in Figure 4, can be attributed to the supply of BM has increased the absorption of macronutrients by *B. oleracea* plants, contributing to increased productivity since with the addition of organic matter there is increase of CEC, improvement of soil physical structure, factors that are crucial to keep the cationic nutrients such as K in the root absorption zone (Nascimento et al., 2015).

As observed in this study, use of organic sources such as BM and RP positively influence the *B. oleracea*. In semiarid regions, the use of alternative fertilizer is extremely important, since in these regions soil organic matter is oxidized rapidly due to high temperatures. Thus, in semi-arid regions, the cultivation of vegetables such as *B. oleracea* and other crops requires periodic replacement of the soil matters via manure (Medeiros et al., 2017).

Observing the Table 3 and can be verified that higher doses of BM promoted higher internal CO_2 concentration (Ci), net photosynthetic rate (A), stomatal conductance (gs), leaf transpiration (E) and instantaneous efficiency carboxylation (EIC), however, the higher water use efficiency (EiUA) was observed in *B. oleracea* plants fertilized with 60 g of BM.

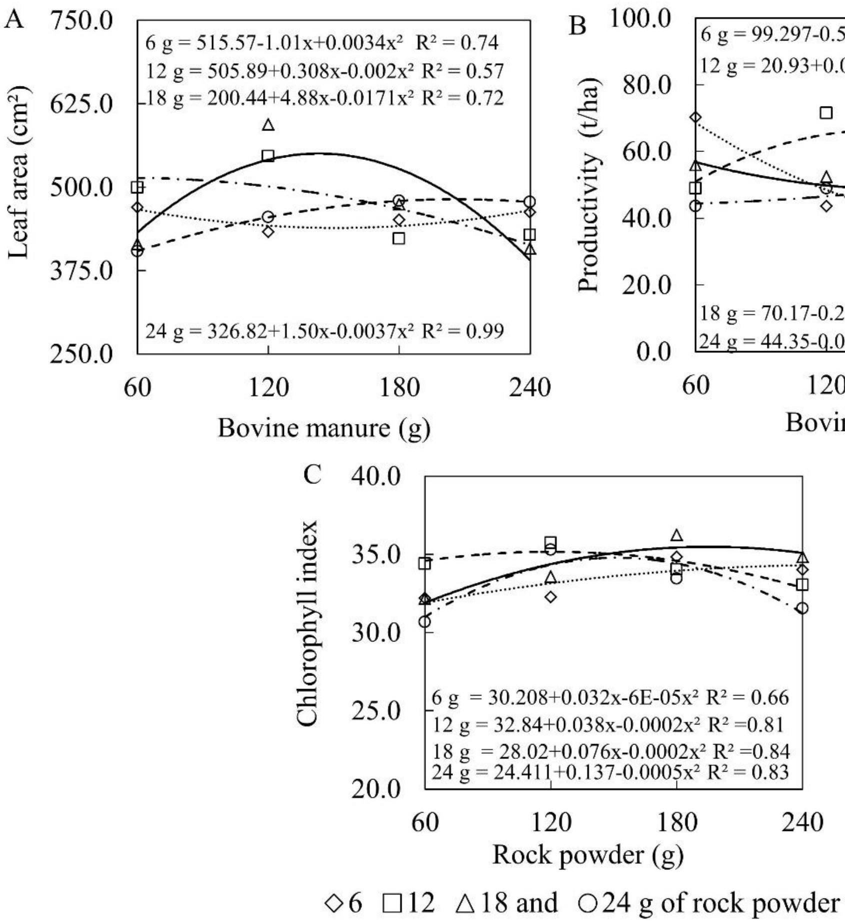

Figure 4. Leaf area (A), yield (B) and chlorophyll content (C) in the *B. oleracea*, depending on bovine manure and rock powder

These results indicate that the BM also positively influence the physiological characteristics of the *B. oleracea* concentrations of net photosynthesis and CO_2 indicate higher photosynthetic activity. The *B. oleracea* plant who received higher doses of BM presented the highest growth, proving that higher photosynthetic rates can be correlated with an increased stomatal opening, and thus greater absorption of CO_2, providing thus higher plant growth (Koyoro et al., 2013).

In beet crop, Gondim et al. (2015) observed that higher net photosynthesis rate (A), transpiration (E), stomatal conductance (Gs) and intercellular concentration CO_2 (Ci) was obtained in plants fertilized with the higher doses of BM, similar to that seen in Table 3 for *B. oleracea* culture. The authors note that these results are due to the abundant supply of essential nutrients for crops growth and development.

Variations referenced in Table 3, may be due to the improvement in the physical characteristics of the soil, since the BM has a high content of organic matter, allowing greater water retention, thereby plants absorb more water, a fact evidenced by the greater stomatal conductance.

Smaller doses of rock powder provided higher net photosynthesis rate, leaf transpiration, efficiency in water use and instantaneous efficiency of carboxylation. On the other hand, although not have shown a significant difference,

Table 3. Medium relating to internal concentration of carbon (Ci), net photosynthetic rate (A), stomatal conductance (gs), transpiration (E), instantaneous efficiency in the use of water (EiUA - A/E) and instantaneous carboxylation efficiency (EiC - A/Ci) B. oleracea plants depending on the dose of bovine manure and rock powder.

Sources dose (g)	Ci	A	gs	E	EiUA	EiC
Bovine manure						
60	286.31b	11.17a	0.307ab	2.51b	4.37a	0.040a
120	297.21ab	8.74b	0.268b	2.39b	3.72a	0.030b
180	370.83a	10.28ab	0.309ab	2.66ab	3.84a	0.035ab
240	280.73b	11.69a	0.370a	2.96a	3.93a	0.042a
ASD	163.88	2.20	0.06	0.32	0.83	0.009
Rock powder						
6	284.32a	12.57a	0.305a	2.76a	4.62a	0.044a
12	296.65a	11.99a	0.331a	2.90a	4.08ab	0.041ab
18	362.48a	9.58b	0.313a	2.59ab	3.68b	0.035bc
24	291.63a	7.74b	0.305a	2.28b	3.48b	0.026c
ASD	163.88	2.20	0.06	0.32	0.83	0.009

ASD = average significant difference. Means followed by the same letter in column are not different by Tukey test at 5% probability.

larger doses of rock powder tended to promote a higher concentration of CO_2, however, the higher concentration of carbon in the carboxylation site was not secured by the rubisco, since the highest concentration of CO_2 coincided with the reduced photosynthetic rate (Table 3).

Within the socio-economic context of agriculture in Brazil, where most low-income vegetable producers, the use of organic sources in agriculture with low purchasing value and high biological quality may be a further alternative for greater producer independence. and offering healthier foods. Thus, the fertilization of vegetables with organic sources becomes of great relevance.

Conclusion

Doses of bovine manure and rock powder 180 and 18 g, respectively, increased the growth of *B. oleracea* L. var. acephala by plant height, stem diameter, number of leaves;

The combination of doses bovine manure 120 g + 12 g rock powder and 120 g bovine manure + 18 g rock powder, provided, in this order, larger results for leaf area and productivity of *B. oleracea* L. var. acephala;

The bovine manure promotes greater concentration of CO_2, net photosynthesis rate and stomatal conductance of *B. oleracea* L. var. acephala plant, while rock powder limits these characteristics.

Intermediate doses of bovine manure and rock powder promote higher growth and yield of *B. oleracea* L. var. acephala.

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Influence of high planting densities and arrangements on yield and fruit development of *Musa* AAA Simmonds

Influencia de altas densidades de siembra y arreglo poblacional en el desarrollo y producción de fruta de *Musa* AAA Simmonds

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Abstract

Banana is one of the most abundantly consumed fruits in the world for its contribution to human nutrition, including an important content of potassium. Given the social contribution of this crop in the cropping areas of Colombia, it is necessary to develop agronomic management strategies that contribute to increasing productivity and offering alternatives that regulate the effects of climate seasonality on banana production in the Urabá region of Colombia. For this purpose, different planting densities (2,000, 2,500, 3,000 and 3,500 plants ha^{-1}) and planting arrangements (triangle, rectangle and double furrow), plus a relative control (1,700 plants ha^{-1} under triangle arrangement), were evaluated in the banana cv. Williams. The experimental design corresponded to randomized complete blocks in a 4 x 3 + 1 (densities x arrangements + control treatment at 1,700 plants ha^{-1}) factorial arrangement. During harvest, the following parameters were monitored: bunch weight, number of hands and fingers per bunch, finger length and diameter, bunch ratio and percentage of loss per bunch. Two analyses of variance were carried out, a general one including the control treatment, and an additional one excluding it. The double furrow planting arrangement favored the productivity of the crop in terms of a greater assimilation and lesser loss records at the bunch level. The strong correlation of the studied variables with the double furrow arrangement became evident.

Key words: Banana, light interception, plant competition, plant population, bunch quality.

Resumen

El banano (*Musa* AAA Simmonds) es una de las frutas más consumidas en el mundo, por su aporte nutricional y fuente de potasio para los seres humanos. Dada su contribución social en las zonas productoras de Colombia, es necesario desarrollar estrategias de manejo agronómico que contribuyan a incrementar la productividad y ofrezcan alternativas para reducir los daños derivados de la estacionalidad del clima sobre la producción de esta fruta en la zona del Urabá, Colombia. Para ello se evaluaron en una plantación de banano cv Williams diferentes densidades de plantación (2000, 2500, 3000 y 3500 plantas/ha) en diferentes arreglos poblacionales (triángular, rectangular y doble surco) más un testigo relativo (1700 plantas/ha) dispuestas en triángulo). El diseño utilizado fue de bloques completos al azar en arreglo factorial de 4 x 3 + 1 (densidades x arreglos) + el testigo de 1700 plantas). En cosecha se midieron el peso, el número de manos y dedos por racimo, largo y diámetro del dedo, radio del fruto y porcentaje de reducción o pérdida de peso por racimo. Se realizó un análisis de varianza general y un análisis de varianza excluyendo el testigo. Los resultados mostraron que, la distribución de plantas en doble surco favoreció la productividad del cultivo debido a un mayor aprovechamiento y una reducción significativa de la pérdida por racimo. En este arreglo se encontró una alta correlación entre las variables evaluadas.

Palabras clave: Banano, Interceptación de luz, competencia entre plantas, población de plantas, calidad de racimo y fruta.

Introduction

Banana contributes 107 million yearly tons of fruit to the world, where in this crop holds an outstanding place among the most important production systems (FAO, 2015). Colombia holds the fifth place at the continental level (FAO, 2017), with a planted area of 49,307 hectares distributed in two zones: Urabá, which is the most important one, with 34,789 ha; and Magdalena and La Guajira regions, which jointly cover 14,518 ha. These three regions produce 98.4 million boxes of banana per year (Augura, 2017; FAO, 2017), which are equivalent to 11.8% of the agricultural exports of the country (Augura, 2016).

However, banana production in Colombia is dominated by climate seasonality through the presence of two marked periods namely, a first dry semester and a second wet semester (Toro et al., 2016), which determine alternate moments of scarcity and oversupply, respectively. In turn, this behavior directly affects the international prices, which actually drop when there is greater supply (Augura, 2012; FAO, 2017), thus constituting a clear disadvantage for the traditional cultivation system.

Plantation density increase in banana crops generates significant plant heigh and higher productivity (Shaikh et al., 1986; Athani et al., 2009) without affecting the organoleptic quality parameters of the fruit, although bunches have certainly exhibited low weight (Palkar et al., 2012; El- Khawaga, 2013). In this regard, Mustaffa and Kumar (2012) indicate that Israeli banana plantations have reached productions of 120 t ha^{-1} under a planting density of 4,091 plants ha^{-1}. However, these authors reported low bunch weight. On the other hand, in Colombia, Belalcázar et al. (2003) have observed plantain production increases ranging from 270% to 345% under planting densities between 3,000 and 5,000 plants ha^{-1}.

The establishment of banana crops under High Density Planting (HDP) is a novel management practice that not only optimizes the use of natural resources, but also allows increasing productivity without affecting fruit quality (Gogoi, 2015; Hanuman et al., 2016). HDP is even more favorable in double furrow systems, wherein a higher number of plants leads to a greater leaf area index (LAI) and an increase in interception of photosynthetically active radiation (PAR), with a positive impact on yield (Rodríguez et al., 2007). In contrast, Benson (2013) states that densities greater than 2,000 plants ha^{-1} have an adverse effect on growth and development as a consequence of root superposition, while self-shading (the result of leaf overlapping) promotes the formation of low-weight bunches (Israeli et al., 1995; Daniells et al. 1984). In addition, a positive effect of HDP on finger size, has been observed by Sarrwy et al. (2012). As, for the Urabá Antioqueño región, it is essential to increase productivity for the first half of the year, due to the best international prices in response to the low supply of fruit, it is estimated the HDP alternative may be viable to achieve this purpose.

Despite inconsist results, HDP has been adopted in plantain production systems of the Colombian regions (Cárdenas et al., 2017) and the coffee zone (Cayón et al., 2004); in Venezuela (Delgado et al., 2008); in Ecuador (Toapanta et al., 2002); and Costa Rica (Smith et al., 2010; Gonçalves and Kernaghan, 2014). Just as well, HDP has been implemented in banana plantations in India (Chaudhuri and Baruah, 2010) and Bangladesh (Khalequzzaman et al., 2009). Thus, this crop management alternative, which has proved successful in plantain, currently needs to be evaluated in banana. Therefore, the objective of this study was to determine the effect of HDP and arrangements on finger yield and quality in *Musa* AAA clone Williams, as a contribution for the Urabá growers to reach greater profits.

Materials and methods

Research was conducted from 2015 to 2016 at La Tagua farm, which belongs to the company Bananeras de Urabá, located in the municipality of Turbo, Antioquia (Colombia). The experimental site was located at 07°53′N 76°41′O, 28 m a.s.l., with an average of maximum and minimum temperatures of 32.39 °C and 22.37 °C respectively, relative humidity of 86.94%, average annual rainfall of 3246.42 mm and average annual solar brightness of 4.2 daylight hours. All these features correspond to a very humid tropical forest climate (Guarin, 2011). The soil exhibited clay loamy texture, pH (5.8), organic matter (OM) content (1.73%), respective Ca and Mg levels (8.9 and 4.11 $cmol_c kg^{-1}$), K (0.41 $cmol_c kg^{-1}$;), P (21.4 mg kg^{-1}), S (5.67 mg kg^{-1}), Zn (2.0 mg kg^{-1}), B (0.54 mg kg^{-1}), Cation Exchange Capacity (CEC) (13.6 $cmol_c kg^{-1}$), and an adequate relation between bases. This information allowed defining the nutrition plan to meet the needs of the plantation, which was set up with in vitro propagated seedlings of the clone cv. Williams, distributed according to the treatment plan (Table 1). Initial soil organic matter and P supplies conformed to crop requirements and soil analysis. The subsequent agronomic management was done according to the technical recommendations for each particular case.

Experimental design. The study was conducted under a randomized complete block design with 4 x 3 + 1 (four planting densities x three population arrangements plus a control treatment) bifactorial arrangement with four planting densities (2,000;

Table 1. Treatments, planting densities and spatial arrangements in a banana plantation of the clone Williams (*Musa* AAA Simmonds). Urabá region, Colombia.

Treatment No.	Planting density (plants ha^{-1})	Spatial arrangements (m)
1	1,700	Control (2.7 x 2.7)
2	2,000	Triangle (2.3 x 2.3)
4	2,500	Triangle (2.15 x 2.15)
5	3,000	Triangle (1.95 x 1.95)
6	3,500	Triangle (1.8 x 1.8)
7	2,000	Rectangle (3.2 x 1.57)
8	2,500	Rectangle (2.8 x 1.30)
9	3,000	Rectangle (2.6 x 1.20)
10	3,500	Rectangle (2.4 x 1.15)
11	2,000	Double row (dp * 1.0 x dr ** 3.3)
11	2,500	Double row (dp * 1.2 x dr ** 3.3)
12	3,000	Double row (dp 1.0 x dr 3.0)
13	3,500	Double row (dp 1.0 x dr 2.8)

* dp: distance between plants (m) ** dr: distance between rows (m).

2,500; 3,000 and 3,500 plants ha^{-1}) and the three arrangements (triangle, rectangle and double furrow), plus the relative control (1,700 plants ha^{-1} under a triangular arrangement) and three replicates. Each experimental unit contained a population of 26 plants, at the center of which the 'useful plot' (made up of 10 plants) was established.

Harvest. The finger bunches were harvested according to their state of commercial maturity (10-12 weeks), based on the quality protocols established by the trader Uniban. In each treatment, four bunches were selected from the plants located in the useful plot, delimited for the data collection planned in the study.

Bunch weight (kg). The gross weight of 10 bunches per treatment was determined on a scale at the time of harvest.

Hands and fingers per bunch (No). They were established by directly counting the units.

Finger length (cm). It was measured from the base of the pedicel to the apex of the finger with a metric tape measure.

Finger diameter (cm). It was estimated with a caliper at the middle part of the three central fingers of the second and last hands of the bunch.

Ratio. Average number of boxes filled with one bunch.

Loss (%). It was evaluated in 10 bunches from each useful plot. For this purpose, the quality defects of the finger (exclusively related to physiological aspects) as identified during the post-harvest process were taken into account.

Data analysis. A SAS 9.13 software was employed to assess the general effect of the treatments on the variables. An analysis of variance only including the two factors under study (planting densities and arrangements) was used to determine their effects on the variables and their interactions. Orthogonal polynomial contrasts were applied to estimate the linear and quadratic effects of the planting density factor. In addition, polynomial regression models were fitted to the data, including the control treatment. A Tukey test at 5% allowed assessing the spatial arrangements. A Pearson correlation analysis was also performed between the variables of production and fruit quality.

Results and discussion

Production. The analysis of variance (excluding the control) showed that the yield variables bunch weight, number of hands and number of fingers were not influenced by the effects of planting arrangement or planting density, although the number of fingers per bunch responded significantly to their interaction. However, when the control treatment was included, these variables showed no response to the effects of these factors, and no differences were detected between the control and the average of the treatments. The absence of effect of the treatments on the variables of production, allows to confirm that the HDP crop system studied for Urabá antioqueño, can be viable because the objective is to obtain a greater production per unit of area, therefore when increasing density is achieved this purpose. These results coincide with those reported by Pujari et al. (2010) and Smith et al. (2010), in the sense of the little influence of spacing on banana yield variables. Still, they contrast with a report by Lanza et al. (2017), who recorded yield increments under increased planting densities of 'BRS Princess' banana in Brazil.

Other studies have revealed yield drops under increasing planting density, and one of the reasons is the competition for radiation. In analyzing the incidence of shading on banana, Israeli et al. (1995) detected bunch weight reductions of 7% for the medium-to-low shading level and of 32% for abundant shade during the first production cycle. In the second cycle, said reductions were 8%, 21% and 55% under the light, medium and heavy shade levels, respectively.

In studies carried out by Athani et al. (2009), bunch weight was observed to drop under increasing planting densities, from 3,086 to 6,250 plants ha^{-1}, although the productivity per ha certainly increased through an augmented number of bunches. According to Gogoi et al.

(2015) bunch weight reduction is due to a higher solar radiation intersection, in turn resulting from increased leaf area. This situation certainly affects the translocation of assimilates (Thippesha et al., 2008), which does not enhance bunch weight, but productivity. In the opinion of Rodríguez et al. (2007) this effect can be dissipated by modifying planting arrangement, which, in their experiment, allowed achieving greater yield through a double furrow system, which facilitated better PAR interception as a result from LAI modifications. However, Hanuman et al. (2016) state that reduced bunch weight is a frequent result of HDP in the *Musaceae*.

In this regard, the average bunch weight values found in the present study ranged from 17.22 to 23.77 kg; the number of hands from 6.11 to 6.89, and the number of fingers from 85.34 to 107.89. These values approximately match those indicated by Rodríguez et al. (2012) for the Urabá region of Antioquia (Colombia).

The number of fingers slightly increased at low planting densities, as observed in the triangle and rectangle arrangements. This result is the likely effect of a higher incidence of solar radiation per unit of leaf area at these planting densities (Table 2). In this sense, Israeli et al. (1995) point out that shading can negatively affect banana yield by up to 55%. On the other hand, the largest numbers of fingers were recorded at 2,500 plants ha^{-1} arranged in triangle and at 3,000 plants ha^{-1} established in double furrow. The remaining densities did not determine any difference between planting arrangements. These results coincide with those of Andrade et al. (2015) regarding the scarce influence of competition between plants on the finger characteristics of banana cv. Angola.

Finger development. The analysis of variance that included the control treatment revealed that only the bunch ratio (number of busches/box) and the percentage of loss (finger not meeting quality standards) were altered by planting arrangement and planting density. For its part, the analysis of variance that excluded the control treatment indicated that the finger length of the last hand and the finger ripeness of the second hand were not affected by planting density, planting arrangement or their interactions. On the other hand, bunch ratio and last hand finger ripeness were influenced by planting arrangement. Likewise, loss was observed to be the result of planting density and arrangement and their interaction.

Table 2. Response of the number of fingers to planting arrangement and density in banana cv. Williams. Urabá region, Colombia.

Planting arrangement	Planting density (plants ha^{-1})			
	2,000	2,500	3,000	3,500
Triangle	108.00 A a*	88.22 B b	89.56 AB b	88.11 A b
Rectangle	95.00 A ab	107.89 A a	85.33 B b	99.37 A ab
Double furrow	91.33 A a	93.00 AB a	105.11 A a	91.33 A a

*Mean values accompanied by similar lower case letters along the same line or capital letter along the same column do not differ significantly according to orthogonal contrasts (F, P ≤ 0.05).

These data allow inferring that HDP had a negative effect on finger development, specifically associated to low radiation interception, higher loss levels and smaller bunch ratios under the triangle, rectangle and row arrangements. However, the double furrow planting arrangement increased the bunch ratio and decreased the percentage of loss (Table3), which is in agreement with prior research. Firstly, Rodríguez et al. (2007) attribute observed productivity rises and optimal bunch disposition on the plant to the double furrow arrangement. Secondly, Andrade et al. (2015) state that the double furrow arrangement probably minimizes the influence of interspecific competition on the characteristics of the finger. According to Kesevan (2002) said effect is only evident at densities higher than 5,000 plants ha^{-1}. Regarding plantain, Prata et al. (2018) have found an association between HDP and productivity increases, which, however, excludes finger quality, as also reported by Cayón et al. (2004), Delgado et al. (2008) and Cortázar et al. (2017).

Although showing no significant differences, the length of the last hand ranged between 20.06 and 21.13 cm, while the diameter of the central finger of the second hand varied from 2.63 to 3.86 cm and the diameter of the central finger of the last hand varied from 2.19 to 2.76 cm. Although these values comply with the quality standards required by international marketers, they are lower than those reported by Díaz et al. (2003) for the Urabá Antioqueño region, and by Vargas and Valle (2011) for Costa Rica. Just as well, there were no significant differences for the degree of ripeness and the diameter of the central finger of the last hand. These results coincide with those of Gogoi et al. (2015) who compared different planting densities, with the work of Rodríguez et al. (2007) on a double furrow system, and with those obtained by Smith et al. (2010) from date banana (*Musa* AA cv. Pisang MAS) planted under HDP. Yet, contrasting results have also been found. Atahi et al. (2008) observed a decrease in finger length and diameter under increasing planting densities. This discrepancy is confirmed by Hanuman et al. (2016) in evaluating decreasing planting densities, which correlated with increased numbers of hands, fingers per bunch and finger diameter.

The bunch ratio was favored by the triangle arrangement at the 2,000 plants ha^{-1} density (Table 3). The triangle arrangement is likely

Table3. Effect of planting arrangements and planting densities on the finger quality variables of the banana clone cv. Williams. Urabá region, Colombia.

Treat. No.	Planting arrangement	Planting density (plants ha^{-1})	LL (cm)	DCFS (cm)	DCFL (cm)	Ratio	Loss (%)
1	Triangle	1,700	20.67	2.97	2.58	0.86 ab*	16.04 ab
2	Triangle	2,000	20.92	2.81	2.49	0.96 a	8.80 b
4	Triangle	2,500	21.03	3.04	2.76	0.90 ab	13.33 ab
5	Triangle	3,000	20.80	2.94	2.63	0.95 a	18.22 ab
6	Triangle	3,500	20.06	2.90	2.45	0.86 ab	15.44 ab
7	Rectangle	2,000	20.67	2.95	2.55	0.83 ab	13.33 ab
8	Rectangle	2,500	20.06	2.95	2.35	0.91 ab	14.60 ab
9	Rectangle	3,000	20.90	2.81	2.53	0.71 b	22,58 a
10	Rectangle	3,500	20.24	2.63	2.19	0.82 ab	16.44 ab
11	Double furrow	2,000	21.13	2.74	2.42	0.90 ab	15.35 ab
11	Double furrow	2,500	20.90	3.07	2.76	0.81 ab	9.49 b
12	Double furrow	3,000	21.00	3.01	2.59	0.93 ab	10.51 b
13	Double furrow	3,500	20.95	3.86	2.69	0.85 ab	9.18 b

*Means followed by the same letter are not statistically different according to the Tukey test (P = 0.05). LL: Last hand's length; DCFS: Diameter of the central finger of the second hand; DCFL: Diameter of the central finger of the last hand; Ratio: Average number of boxes filled with one bunch. Loss: Finger eliminated due to quality defects.

to allow a better leaf area distribution and a higher LAI. However, previous studies indicate that planting arrangement does not affect PAR capture, thus implying that the estimated extinction coefficient $\boldsymbol{k}$ and the average angle of inclination of the leaves do not influence yield, although it has been seen that the double furrow system allows a better location of the bunches (Rodríguez et al., 2007).

Interaction arrays x densities. The interaction between planting arrangement and planting density indicates that loss was greater under the triangle arrangement (18.22%) for the 3,000 plants ha^{-1} density, followed by the records of the 1,700, 2,000 and 2,500 plants ha^{-1} densities for the same parameter (Table 4). For the rectangle arrangement, the highest yield reduction count (22.58%) was recorded under the 3,000 plants ha^{-1} density, overcoming by 69.39% the record of the 2,000 plants ha^{-1} density. Finally, no loss differences were observed under the double furrow arrangement, which registered an average count of 10.84% (Table 4). The aforementioned resonates with a previous analysis by Rodríguez et al. (2007) who point out that yield is more favored by planting density than by spatial arrangement. However, the double groove arrangement under HDP offers better bunch filling advantages.

It can be inferred that the 2,000 plants ha^{-1} density under the triangle arrangement favors productivity by contributing to a better assimilation on the part of the finger, as also observed for the double furrow arrangement at the 2,500 and 3,000 plants ha^{-1} densities. These results correspond with those of Mahmoud (2013) which allows concluding that spacing plants favors larger and heavier bunches, these attributes being beneficial to the finger. In the opinion of Rodríguez et al. (2007) this is due to a better leaf area distribution, which optimizes the use of PAR. Finger length showed no significant differences in response to planting arrangements, thus contrasting with the bunch ratio results (Figure 1).

Table 4. Percentage of loss in banana cv. Williams as a function of planting density and planting arrangement. Urabá region, Colombia.

Planting arrange-ment	Planting density (plants ha^{-1})				
	1.700	2.000	2.500	3.000	3.500
Triangle	12.90 b	7.85 c B*	13.33 b A	18.22 a A	15.44 ab A
Rectangle		13.33 b A	14.60 ab A	22.58 a A	14.11 ab AB
Double furrow		14.19 a A	9.49 a B	10.51 a B	9.18 a B

*Mean values with similar lowercase letters on the same line do not differ statistically according to orthogonal contrasts (F, P ≤ 0.05).

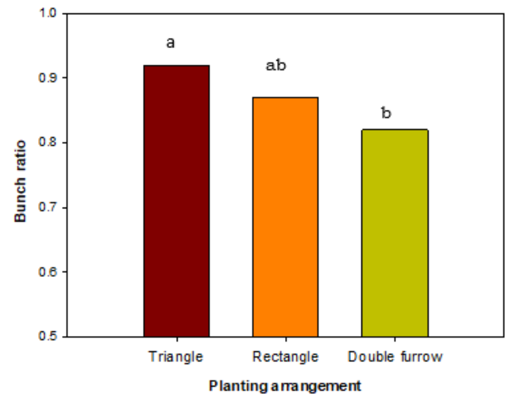

Figure 1. Bunch ratio of banana cv. Williams as a function of planting arrangement. Urabá Antioqueño región, Colombia.

*Mean values with similar lowercase letters on the same column do not differ statistically according to the Tukey test (P=0.05).

In general, from these observations, it can be speculated that low densities combined with the double furrow arrangement allow greater light penetration. This is likely to provide greater availability of assimilates for the bunch filling process, thus increasing finger diameter and favoring bunch ratio.

Contrastingly, Eckstein and Robinson (1995) and Delgado et al. (2008) indicate that, from flowering to harvest, banana plants can fill the finger using a limited amount of leaves because this favors light penetration and air circulation. The latter, in turn, improves CO_2 diffusion and photosynthetic compensation by young and more exposed leaves. These considerations are consistent with those of Rodríguez et al. (2007) and Smith et al. (2010), who state that an increase in planting density linearly reduces bunch weight.

Correlation between yield and finger development. The number of hands per bunch correlated negatively with loss and the diameter of the last hand, while the number of fingers per hand negatively correlated with the diameter of the central finger of the last hand and the percentage of loss (Table 5). These results corroborate the strong association existing between yield and development variables, since assimilation by the last hand is determining to increase productivity and decrease the percentage of loss, which is largely due to the source-sink relationship. In this sense, larger numbers of exposed leaves guarantee a greater source of photoassimilates for the bunch. However, the numbers of hands per bunch and of fingers per hand (i.e., the size of the sink) determine the redistribution of said assimilates. In this way, those bunches that received the most amounts of photoassimilates due to better leaf exposure were favored with the increase of the length and diameter of the fingers and, ultimately, with a better development of the whole bunch. In general, high planting densities were found to negatively affect assimilation by the last hand (Rodríguez et al., 2007).

Table 5. Pearson correlation between yield and finger quality variables in banana cv. Williams under the triangle planting arrangement. Urabá region, Colombia.

Characteristics	LL (cm)	DCFS (cm)	DCFL (cm)	Ratio	Loss (%)
Bunch weight	0.49	0.34	0.36	-0.23	-0.09
Number of hands	-0.19	-0.24	-0.62*	0.33	-0.68**
Number of fingers	0.36	-0.36	-0.50	0.29	-0.66**

*: Significant differences at 5% ($P \leq 0.05$); **: Significant differences at 1% ($P \leq 0.01$).

LL: Last hand's length; DCFS: Diameter of the central finger of the second hand; DCFL: Diameter s of the central finger of the last hand; Ratio: Average number of boxes filled with one bunch. Loss: Discarded finger due to quality defects.

Under the rectangle arrangement there was only a correlation between bunch weight and the length of the last hand (Table 6). That is, as the length of the last hand rises, bunch weight and the number of usable fingers per bunch are also increased.

Table 6. Pearson correlation between yield and finger quality variables in banana cv. Williams under the double furrow planting arrangement. Urabá region, Colombia.

Characteristics	LL (cm)	DCFS (cm)	DCFL (cm)	Ratio	Loss (%)
Bunch weight	-0.29	0.47	0.02	0.76**	-0.46
Number of hands	-0.78**	-0.18	-0.50	0.50	-0.53
Number of fingers	-0.74**	-0.27	-0.72**	0.68*	-0.54

*: Significant differences at 5% ($P \leq 0.05$); **: Significant differences at 1% $P \leq 0.01$).

LL: Last hand's length; TCFS: Diameter of the central finger of the second hand; TCFL: Diameter of the central finger of the last hand; Ratio: Average number of boxes filled with one bunch. Loss: Discarded finger due to quality defects

In the double furrow arrangement there was a positive correlation between bunch weight and bunch ratio. The number of hands correlated negatively with loss and the length. The number of fingers correlated negatively with all the finger quality variables, except for the diameter of the second hand (Table 6). The treatments that favored bunch weight contributed to increase energy assimilation, thus allowing the production of more boxes per bunch. Contrastingly, the rectangle planting arrangement at 3,000 plants ha^{-1} remarkably had the most negative impact on both loss and bunch ratio when compared to the other evaluated arrangements and densities.

HDP showed statistical similarity to the control treatment. These results are in agreement with those reported by Rodríguez et al. (2007), by stating that the double furrow arrangement combined with low densities favor banana yield. Just as well, the findings of El-Khawaga (2013) utter that bunch weight reduction resulting from HDP somehow contributes to increasing the percentage of loss.

Conclusions

The spatial distribution of HDP did not have a significant influence on the quality of the fruit required in international marketing, which allowed for greater use of the bunch (ratio), particularly under the triangle distribution. In addition, it was observed that the arrangement in double furrow, contributed to decrease the losses due to reductions in the bunch, as the density of the plantation was higher, an aspect that favors the productivity of the crop.

None of the studied combinations between HDP and planting arrangements affected bunch weight or the number of hands and fingers per bunch, while finger development, bunch ratio and the percentage of loss increase under the rectangle and triangle arrangements at the 3,000 and 3,500 plants ha^{-1} densities.

The arrangement of plants in double furrow improve the use of solar radiation a benefit that was reflected in a greater length of the fruit, in particular when combined with higher than 3,000 plants ha^{-1} densities. This same arrangement favored crop yield and influenced a lower percentage of loss by the bunch.

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Utilización de cepas de *Bacillus* como promotores de crecimiento en hortalizas comerciales

Plant growth promotion of commercial vegetable crops by *Bacillus* strains

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Resumen

El uso de las bacterias promotoras del crecimiento vegetal (PGPB) es una posibilidad para sustituir parcialmente el empleo de fertilizantes químicos en cultivos comerciales, que no solo encarecen la producción sino que también tienen efectos negativos sobre el medioambiente. El trabajo tuvo como objetivo evaluar el potencial de cepas de *Bacillus* para la promoción del crecimiento vegetal *in vivo* en tres diferentes cultivos hortícolas de importancia económica. Se utilizaron cuatro cepas de *Bacillus*, dos aisladas de los cultivos del maíz (*Zea mays* L.) cultivar híbrido P-7928 y dos de cafeto (*Coffea arabica* L.) cultivar Caturra rojo, para evaluar su efecto en diferentes variables fenológicas y de crecimiento en plantas de maíz, tomate (*Solanum lycopersicum* Mill.) y zanahoria (*Daucus carota* subsp. *sativus* L.) en condiciones de invernadero. Las cepas mostraron un efecto estimulador de la germinación de las semillas de maíz, las cuales presentaron mayor porcentaje e índice de germinación y vigor de plántula que los testigos sin inocular; no obstante no todas las cepas estimularon los mismos indicadores del crecimiento en este cultivo, aunque se destacan las cepas RM5, RC9 y RC15 en la materia seca de raíz. En los cultivos de tomate y zanahoria los resultados fueron variables, por tanto se sugiere profundizar los estudios en estos cultivos.

Palabras clave: bacterias promotoras del crecimiento vegetal, maíz, tomate, zanahoria.

Abstract

The use of plant growth promoting bacteria can substitute partially the chemical fertilizers, which put up the price of agricultural products and have negative effects over the environment. The aim of this work was to evaluate the potentialities of *Bacillus* strains for the plant growth promotion in different economical important crops. Four strains of *Bacillus* were used: two isolated from maize (*Zea maíz* L.) hybrid cultivar P-7928 and two from coffee (*Coffeea arabica* L.) Caturra rojo cultivar. The strains showed positive effect in the seed germination of maize using index such as: germination percentage, germination index, vigor index and germination rate. Following greenhouse assays were carried out in maize, tomato and carrot. The strains stimulated the germination in maize but not all had the stimulation effect in the same indicators in the maize crop, although the strains RM5, RC9 and RC15 were the best in the root dry weight. In tomato and carrot crops, the results were variables, so it is necessary deeper in the study of plant-bacteria interaction.

Key words: carrot, maize, plant growth promoting bacteria, tomato.

Introducción

Un alto porcentaje de los nutrientes aplicados en el suelo en forma de fertilizantes de síntesis química no son aprovechados por las plantas y se pierden por lixiviación o volatilización; además de los daños asociados al uso de productos químicos en la agricultura y los impactos negativos que estos tienen sobre la salud y el medioambiente. En estas condiciones, es necesario investigar alternativas ecológicamente amigables y económicamente posibles para hacer frente a la demanda creciente de alimentos aplicando técnicas de agricultura sostenible (Vejan et al., 2016).

El manejo de la rizosfera representa un área importante para la agrobiotecnología, con el precepto de aumentar el rendimiento y la producción de biomasa con un uso mínimo de agua, fertilizantes y agroquímicos (Ortíz-Castro et al., 2009). El estudio de la interacción planta-microorganismo puede ayudar a comprender los fenómenos que ocurren en ese ecosistema y podría guiar las aplicaciones que resulten en recursos sustentables, con un menor impacto ambiental y la disminución de la contaminación. Esta interacción puede ser empleada para mejorar el crecimiento de las plantas para la producción de alimentos, pero también de fibras, biocombustibles y metabolitos claves.

El empleo de microorganismos con potencialidades para la promoción del crecimiento vegetal es una alternativa para aumentar la producción agrícola. Las Bacterias Promotoras del Crecimiento Vegetal (PGPB) son un grupo de microorganismos promisorios y ampliamente estudiados como una de las formas posibles para reducir los costos de producción en la agricultura moderna (de Souza et al., 2015). Estas bacterias poseen varias estrategias para potenciar el crecimiento de la planta como la solubilización y reciclaje de nutrientes, la producción de hormonas estimuladoras del crecimiento, la fijación de nitrógeno, la inducción de defensa de las plantas, la producción de antibióticos y otras sustancias antimicrobianas, y la desintoxicación del suelo, entre otras (Rai et al., 2017).

El género *Bacillus* es uno de los microorganismos más ampliamente estudiado para la promoción del crecimiento de las plantas (Pérez-Montaño et al., 2014). Se ha demostrado que estas bacterias poseen características que les permiten su utilización como promotores del crecimiento vegetal y antagonistas de fitopatógenos (Grobelak et al., 2015).

El maíz, el tomate y la zanahoria son cultivos de gran importancia para la alimentación humana. En la actualidad, el maíz es uno de los cereales con mayor volumen de producción a nivel mundial, junto con el trigo y el arroz. Además es una fuente de alimento y sustento económico directo para gran parte de la población (Paliwal et al., 2001). El tomate es la hortaliza más difundida en el mundo y la de mayor valor económico, la demanda aumenta continuamente y con ella su cultivo, producción y comercio (InfoAgro, 2020a). El cultivo de la zanahoria ha experimentado un importante crecimiento en los últimos años, tanto en superficie como en producción, ya que se trata de una de las hortalizas más producidas en el mundo (InfoAgro, 2020b).

Estudios *in vitro* realizados en el Laboratorio de Ecología Microbiana de la Facultad de Biología de la Universidad de La Habana, permitieron seleccionar cepas de *Bacillus* provenientes de cultivos de maíz y cafeto con potencial para la promoción del crecimiento de plantas, entre ellas las hortalizas antes mencionadas. Este trabajo tuvo como objetivo evaluar el efecto de cepas de *Bacillus* en la promoción del crecimiento vegetal en plantas de maíz (*Zea mays* L.), tomate (*Solanum lycopersicum* Mill.) y zanahoria (*Daucus carota* subsp. *sativus* L.) en experimentos de interacción planta-microorganismo en condiciones de invernadero.

Materiales y métodos

Cepas bacterianas

En el estudio se utilizaron cuatro cepas de *Bacillus*, obtenidas y seleccionadas en estudios preliminares realizados en el Laboratorio de Ecología Microbiana de la Facultad de Biología, Universidad de La Habana. La cepa RM5 fue aislada de la rizosfera y la EAM5 como endófita de tallos de maíz (*Zea mays* L.), cultivar Híbrido P-7928, cultivado en áreas experimentales del Instituto de Investigaciones Fundamentales en Agricultura Tropical Alejandro de Humboldt (INIFAT) (Rojas et al., 2016). Las cepas RC9 y RC15 fueron aisladas de la rizosfera del cafeto (*Coffea arabica* L.) cultivar Caturra rojo, cultivado en la Estación Experimental de Café y Cacao de Baracoa, Guantánamo (Sánchez, 2018).

Aplicación de las cepas bacterianas en invernadero

Las cepas fueron cultivadas en caldo triptona soya durante 24 h, a 30 ºC y bajo condiciones de agitación en zaranda orbital. La concentración celular se ajustó a 10^8 cel/ml, tomando como referencia el tubo 0.5 de la escala Mc Farland (NCCSL, 2005).

Se utilizaron tres cultivos de importancia económica: maíz (*Zea mays* L.) cv. Francisco mejorado, tomate (*Solanum lycopersicum* Mill.) cv. INIFAT 28, y zanahoria (*Daucus carota* subsp.

sativus L.) cv. New Kuroda. Antes de la siembra las semillas fueron desinfectadas siguiendo el protocolo propuesto por García et al.(2008): inicialmente fueron sumergidas en una solución Tween 80 (500µl/200ml de agua destilada estéril) durante 5 minutos. Seguidamente fueron desinfectadas en etanol al 70% por 30 segundo se hipoclorito de calcio al 25% en una solución Tween 80 (500µl/200ml) por 20 min, uno a continuación del otro. Finalmente fueron lavadas varias veces con agua destilada estéril para eliminar restos del desinfectante.

En invernadero, para el maíz y el tomate se dispusieron dos bandejas de 20 x 15 cm por tratamiento con suelo Ferralítico Rojo Lixiviado Compactado, Gléyico y Nodular Ferruginoso (Hernández et al., 2015) y para zanahoria 10 macetas con capacidad de 2 kg de suelo por tratamiento. La inoculación de los aislados se hizo en el momento de la siembra a razón de 1 ml de cultivo bacteriano por semilla de cada cultivar. Se utilizaron 12 semillas por tratamiento en las bandejas y dos semillas por macetas. El experimento tuvo una duración de 21 días para el maíz y el tomate y de 90 días para la zanahoria. Para mantener la humedad suficiente, en cada cultivo se aplicaron riegos periódicos con agua corriente. En ningún caso se aplicaron nutrientes como fertilizantes a los cultivos. Como testigos absolutos se utilizaron inóculos de medio de cultivo estéril.

Diseño experimental y mediciones

Se utilizó un diseño completamente aleatorizado. La germinación (%) en maíz fue medida en los días 4, 5, 6, 8 y 11 después de la siembra (D.D.S.), igualmente fueron determinadas la tasa de germinación teniendo en cuenta las nuevas semillas germinadas, el índice de germinación que indica cómo ocurre la germinación en el tiempo y el índice de vigor que considera la masa seca de las semillas germinadas y el porcentaje de germinación (Moeinzadeh et al., 2010). Al final del experimento se midieron diferentes variables de acuerdo con cada cultivo. Para maíz se midió la longitud de la raíz más larga (cm) y de la hoja (cm), la altura (cm), la masa fresca (MV) y seca (MS) de la raíz y la parte aérea (g), el número de hojas y el diámetro del tallo (cm). En el caso del tomate se midió la longitud de la raíz más larga (cm), la longitud transversal de la hoja (cm), la masa fresca de la raíz y la parte aérea (g), el número de hojas y el diámetro del tallo (cm). Para la zanahoria se midió la longitud de la raíz más larga y de la raíz más engrosada (cm), las masas fresca y seca de la raíz y de la parte aérea (g), el número de hojas y el diámetro de la raíz (cm).

El procesamiento estadístico de los datos se realizó con el programa Statgraphics Plus versión 5.0, con el que se comprobó la normalidad y la homogeneidad de varianzas, según las pruebas de Kolmogorov-Smirnov, Cochran C, Hartley y Bartlett. Se realizó un Anova de clasificación simple para las variables de crecimiento y cuando existieron diferencias significativas entre los tratamientos, las medias se compararon según la prueba de Duncan al 5% de significación.

Resultados

Efecto de las cepas bacterianas sobre la germinación de maíz

Para analizar el efecto de las cepas sobre la germinación de maíz se utilizaron cuatro índices que indican la calidad y celeridad de la germinación. Se encontró que el porcentaje de germinación fue de 100% para las cuatro cepas, porcentajes que fueron superiores al testigo (50% a los 11 D.D.S.) (Figura 1), no obstante en este caso en especial las semillas continuaron germinando después de esta medición. Por otra parte, todos los tratamientos presentaron tasas de germinación de 0.12 (RC9 y RC15), 0.13 (RM5) y 0.15 (EAM5), siendo menores que el testigo (0.17). La cepa EAM5 mostró el mayor índice de germinación (7.02) con respecto al testigo no inoculado (2.29) y al resto de los tratamientos: RC9 (3.74), RC15 (2.84) y RM5 (3.73). Los valores obtenidos en el índice de vigor en las plantas testigo fue de 3138.33, mientras que para las plantas inoculadas con las cepas de *Bacillus* este índice fue más del doble: RC9: 6876.67, RC15: 6664.54, RM5: 6284.54 y EAM5: 6153.63, aunque la toma de los datos no permitió el análisis estadístico debido a que el conteo fue total y no se hizo por repeticiones.

Evaluación de la promoción del crecimiento

En la Tabla 1 se observa el comportamiento de las variables fenológicas correspondientes al cultivo del maíz. La cepa EAM5 presentó los valores más altos para las variables longitud de raíz y longitud de tallos ($P < 0.05$) en comparación con las demás cepas y el testigo. Para longitud de la hoja, la cepa RC9 mostró diferencias significativas con las cepas EAM5 y RC15 ($P < 0.05$), pero no el testigo no inoculado.

La variable diámetro de tallo solo fue significativa ($P < 0.05$) en el tratamiento con la cepa RC9 vs. el testigo no inoculado. La variable número de hojas en las plantas inoculadas con la cepa RC15 solo difirió ($P< 0.0$) con el tratamiento testigo. Por otra parte, la masa fresca de la raíz y de la parte aérea de las plantas no difirió entre tratamientos ni con relación al testigo. Por el contrario, la masa seca de la raíz en los tratamientos con las cepas RC9,

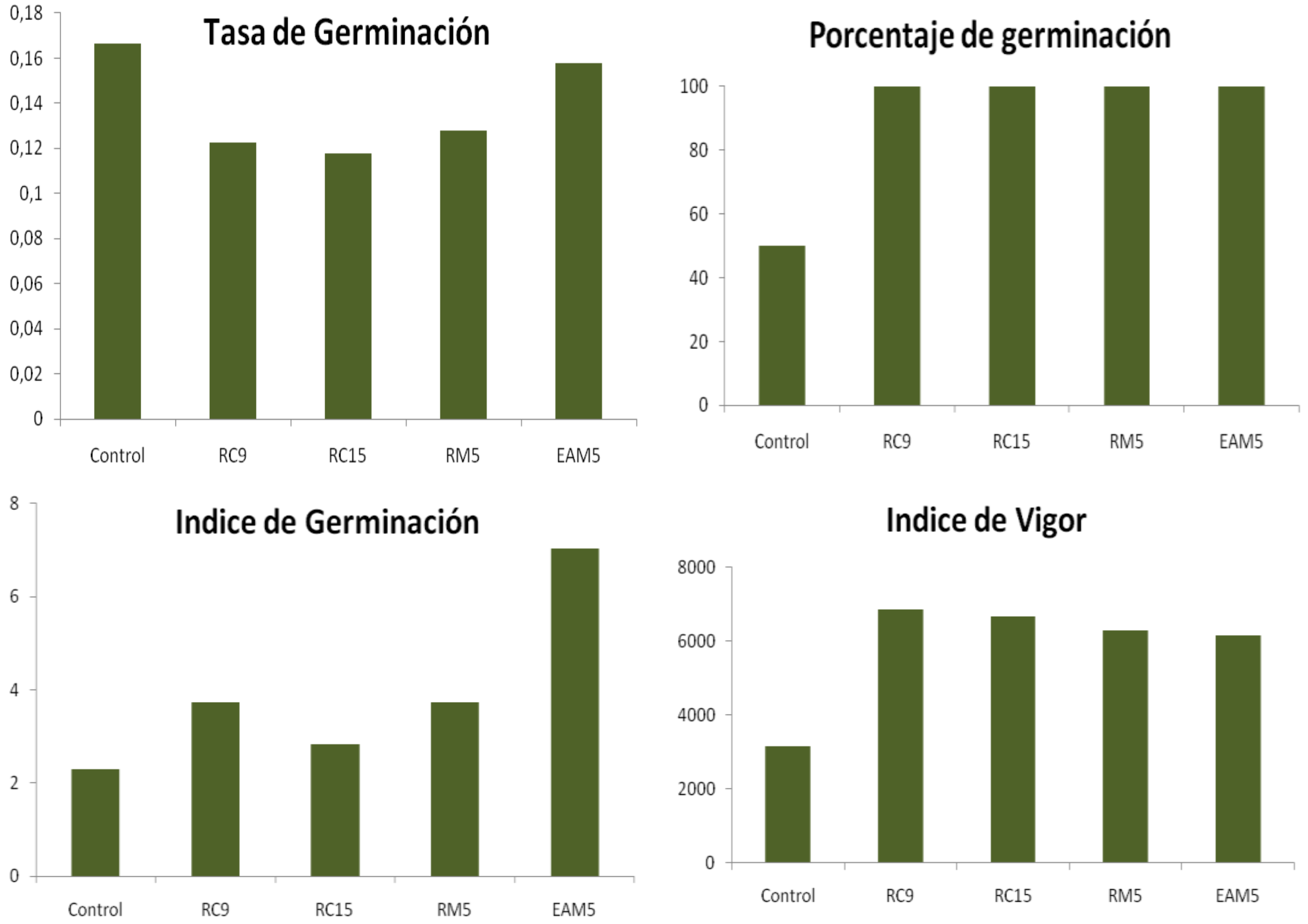

Figura 1. Efecto de las cepas de *Bacillus* sobre la germinación de maíz (*Zea mays* L.) cultivar Francisco Mejorado en suelo Ferralítico rojo Lixiviado Compactado, Gléyico y Nodular Ferruginoso en condiciones de invernadero.

Tabla 1. Influencia de las cepas de *Bacillus* en plantas de maíz (*Zea mays* L.) 21 días días postinoculación cultivadas en suelo Ferralítico Rojo Lixiviado Compactado, Gléyico y Nodular Ferruginoso, en invernadero.

Trat.	Longitud de la raíz más larga (cm)	Altura (cm)	Longitud de hoja (cm)	Número de hojas	Diámetro del tallo (cm)	MV raíz (g)	MV aérea (g)	MS aérea (g)	MS raíz (g)
Control	2.63±1.65b*	3.78±0.64b	15.72±4.78 ab	3.20±0.52 b	0.23±0.04 bc	0.59±0.16 ab	0.53±c0.14 ab	0.09±0.04 ab	0.15±0.07 b
EAM5	5.01±3.86a	5.45±1.61a	17.02±3.42 ab	3.65±0.49 ab	0.25±0.05 ab	0.51±0.17 ab	0.52±0.16 a	0.06±0.02 abc	0.16±0.06 ab
RM5	3.00±2.56b	3.60±0.64 b	14.90±5.06 b	3.35±0.49 ab	0.22±0.05 bc	0.64±0.24 a	0.53±0.24 b	0.08±0.09 c	0.16±0.07 a
RC9	10.07±5.89b	3.97±1.09 b	17.90±5.27 a	3.35±0.93ab	0.25±0.05 a	0.51±0.20 b	0.50±0.30 b	0.09±0.04 a	0.18±0.08 a
RC15	2.85±2.27b	3.91±1.20 b	14.58±3.74 b	3.74±0.70a	0.21±0.02 c	0.51±0.14 b	0.45±0.14 ab	0.06±0.02 bc	0.18±0.07ca
ESx	0.6495	0.2601	1.1147	0.142	0.009	0.0343	0.0531	0.0043	0.0134

*Letras diferentes en la misma columna indican diferencias significativas para la prueba de Duncan (P < 0.05) entre los tratamientos. En cada variable se indica el error estándar de la media y en cada tratamiento la desviación estándar de 12 repeticiones.

RC15 y RM5 mostraron diferencias significativas (P < 0.05) cuando se compraron con el testigo, pero esto no ocurre con con la masa seca de la parte aérea, donde los tratamientos no superaron al testigo y la cepa RM5 mostró valores inferiores. Los cambios en la arquitectura de la raíz de las plantas inoculadas fueron notorios con respecto a las no inoculadas. Sobresalió la estimulación observada en la masa seca de la raíz, producto de la actividadde las cepas RC9, RC15 y RM5 en maíz.

En la Tabla 2 aparecen los resultados de las variables fenológicas medidas en el cultivo de tomate. La longitud de la raíz y el número de hojas fueron similares entre los tratamientos y con el testigo; sin embargo la longitud transversal de la hoja en plantas inoculadas con las cepas RC9 y RC15 fue mayor cuando se comparan con el testigo (P < 0.05), este indicador es importante ya que determina el área foliar y la capacidad fotosintética de la planta. Para diámetro de tallo,

Tabla 2. Influencia de las cepas de *Bacillus* en plantas de tomate (*Solanum lycopersicum* Mill.) 21 días postinoculación cultivada en suelo Ferralítico Rojo Lixiviado Compactado, Gléyico y Nodular Ferruginoso en invernadero.

Trat.	Longitud raíz más larga (cm)	Longitud transversal de la hoja (mm)	Número de hojas	Diámetro del tallo (cm)	MV fresca Raíz (g)	MV fresca aérea (g)
Control	18.0±1.97	49.26±4.96b*	3.83±0.41	0.27±0.03 b	0.73±0.10 ab	1.90±0.44 b
EAM5	17.65±3.87	50.84±2.49ab	4.09±0.30	0.33±0.03 a	0.70±0.17 b	2.41±0.35 ab
RM5	17.50±3.82	47.94±2.80ab	3.73±0.42	0.30±0.05 ab	0.82±0.10 ab	2.12±0.59 ab
RC9	16.37±3.99	51.90±3.00a	4.00±0.00	0.29±0.03 b	0.89±0.18 ab	2.66±0.28 a
RC15	20.41±3.78	52.07±2.82a	3.73±0.65	0.30±0.05 ab	0.98±0.23 a	2.23±0.41 ab
ESx	1.4085	1.3214	0.1561	0.1229	0.0717	0.1576

*Letras diferentes en la misma columna indican diferencias significativas para la prueba de Duncan ($P < 0.05$) entre los tratamientos. En cada variable se indica el error estándar de la media y en cada tratamiento la desviación estándar de 12 repeticiones.

Tabla 3. Influencia de las cepas de *Bacillus* en plantas de zanahoria (*Daucus carota* subsp. *sativum*) 90 días postinoculación cultivada en suelo Ferralítico Rojo Lixiviado Compactado, Gléyico y Nodular Ferruginoso en invernadero.

Trat.	Longitud de la raíz (cm)	Longitud de la raíz más engrosada (cm)	Diámetro de la raíz (cm)	Número de hojas	MV raíz (g)	MV aérea (g)	MS raíz (g)	MS aérea (g)
Control	5.21±2.09	1.72±0.58 ab*	0.34±0.25	4.0±0.95 ab	0.01±0.003 c	0.85±0.64 ab	0.03±0.01ab	0.13±00.08 a
EAM5	5.86±2.76	1.83±0.56 ab	0.35±0.27	3.92±1.32 b	0.10±0.09 bc	0.7±0.38b	0.02±0.01b	0.12±0.07a
RM5	5.51±3.59	1.39±0.63b	0.36±0.25	4.0±1.15 ab	0.20±0.24 ab	0.71±0.55b	0.04±0.03ab	0.06±0.04b
RC9	5.3±3.15	2.06±0.64a	0.41±0.26	4.83±0.93 a	0.24±0.22 ab	1.18±0.62 a	0.05±0.01a	0.15±0.07a
RC15	6.94±3.0	1.84±0.73 ab	0.47±0.23	4.18±0.87ab	0.30±0.24a	0.73±0.37 ab	0.04±0.03a	0.11±0.06ab
ESx	1.0203	0.1873	0.0735	0.3091	0.0600	0.1620	0.0080	0.1997

*Letras diferentes en la misma columna indican diferencias significativas para la prueba de Duncan ($P < 0.05$) entre los tratamientos. En cada variable se indica el error estándar de la media y en cada tratamiento la desviación estándar de 12 repeticiones.

la cepa EAM5 superó los valores obtenidos con el testigo y con el tratamiento RC9. La masa fresca de la parte aérea fue más alta en el tratamiento con la cepa RC9, lo que no ocurrió con la masa fresca de la raíz, donde el resultado fue similar entre los tratamientos y el testigo.

Para las plantas de zanahoria, en la Tabla 3 se observa que el efecto de los tratamientos solo fue significativo en la masa fresca de la raíz, siendo mayor en el caso de las cepas RC9, RC15 y RM5 en comparación con el testigo.

Discusión

Los resultados de las tasas de germinación de maíz coinciden con los obtenidos para *Pseudomonas fluorescens* (Moeinzadeh et al., 2010). Se ha demostrado que *Bacillus subtilis* y *B. megaterium* mejoraron el porcentaje y la tasa de germinación en semillas de rábano (Kaymak et al., 2010). El índice de germinación indica cómo ocurre este proceso en el tiempo. El índice de vigor, por su parte, considera la masa seca de las semillas germinadas y el porcentaje de germinación. Este índice es indicativo no solo de la estimulación de la germinación, sino también de la eficacia del tratamiento sobre la plántula que se obtiene a partir de semillas.

Los resultados en este estudio coinciden con los obtenidos en estudios previos que muestran cómo cepas de *Bacillus* presentan potencialidades para ser utilizadas como promotores de la germinación ('biopriming') y sus capacidades fisiológicas (De Araujo et al., 2012). Además, el uso de este conjunto de índices relacionados con la germinación y el vigor permite realizar una mejor valoración de los resultados y el efecto de las cepas sobre la germinación de las semillas.

La diversidad de las bacterias que habitan como endófitas y rizosféricas en diferentes plantas brinda la posibilidad de seleccionar aquellas con mejores cualidades en la promoción del crecimiento y con un amplio rango de acción (De Araujo et al., 2012); por esta razón en el presente trabajo se evaluaron bacterias provenientes de los cultivos de maíz y cafeto en cultivos de importancia para la alimentación como son maíz, tomate y zanahoria.

Los resultados obtenidos en el cultivo del maíz coinciden con los obtenidos por De Araujo et al. (2012) en la altura de la planta, el número de hojas y la masa seca de la raíz, pero no con la masa seca del tallo. Quizás los mecanismos involucrados en la promoción del crecimiento por las cepas utilizadas en los estudios son diferentes o exista mayor influencia de alguno de ellos, pero que haya un efecto positivo en la masa seca de la raíz es definitorio para el mejoramiento general de la salud de la planta. Además, se ha planteado que generalmente los cambios en la arquitectura de la raíz de las plantas inoculadas van acompañados de cambios en los procesos endógenos de las plantas (Verbon et al., 2016). Además, la raíz provee del anclaje de la planta al suelo y desempeña un papel importante en la toma de agua y nutrientes (Ortíz-Castro et al., 2009), por lo que se considera fundamental el efecto de las bacterias en el desarrollo de las raíces como se ha obtenido en este trabajo. Zhang et al. (2015) demostraron que la cepa SQR9 de *B. amyloliquefaciens* cuando está viva tiene un efecto positivo en plantas de maíz, lo que indica la importancia de la interacción planta-bacteria y el impacto directo desde el punto de vista agronómico (Rogers et al 2015). Como se puede apreciar en los resultados, dos de las cepas en que se observan los mejores resultados no se originaron de este mismo cultivo (RC9 y RC15) y una de las cepas aisladas a partir del cultivo del maíz no promueve el crecimiento, lo que indica que no siempre existe una relación directa entre el origen de la cepa y su efecto positivo en la fitoestimulación.

En el cultivo del tomate no se observó efecto de las cepas en las variables evaluadas. Esto contrasta con resultados de Ajilogba et al. (2013) quienes encontraron un efecto positivo de cepas de *Bacillus* en la altura y el largo de la raíz de plantas de tomate, y en el incremento en el rendimiento de la planta y peso de los frutos (Mena-Violante et al., 2007). Estos autores coinciden en que si bien los cambios producidos por los microorganismos ocurren a nivel de la raíz, pueden estar relacionados con procesos fisiológicos que afectan el desarrollo y la maduración de los frutos.

En la literatura los estudios de la interacción zanahoria-*Bacillus* son escasos. Fall et al. (2004) y Surette et al. (2003) aislaron *B. megaterium* de cultivos de esta hortaliza el cual mostró efectos positivos en el crecimiento de la planta; en contraste, en el presente trabajo no se observaron efectos positivos de la bacteria en el desarrollo de este cultivo. Esto pudiera deberse a la interacción directa entre las cepas y las variedades de zanahoria que se probaron en el estudio, donde existieron efectos diferentes para cada cultivo probado.

Las bacterias utilizadas en este trabajo tienen la capacidad de producir compuestos indólicos como el ácido indolacético, fithormona que estimula el crecimiento del largo de raíces y tallos, solubilizan fosfatos y crecen en medio semisólido libre de nitrógeno (Rojas et al., 2016). Estos pueden ser algunos de los mecanismos involucrados en la estimulación del crecimiento de las plantas que se observó en este trabajo.

Las cepas de *Bacillus* examinadas muestran ser aislamientos promisorios para su evaluación como PGPB. Los criterios utilizados en la selección *in vitro* pueden ser útiles en un programa de prospección de *Bacillus* promotores del crecimiento vegetal, reduciendo el número de cepas para llegar a un estado final de ensayos en las plantas (De Araujo et al., 2012), aunque es necesario entender los mecanismos básicos y la interacción PGPB-plantas y la especificidad de ésta para llegar a obtener un producto comercial a base de estos microorganismos (Rojas et al., 2016), ya que como plantean otros investigadores (Kumar et al., 2014), la aplicación de *Bacillus* como PGPR ofrece una vía amigable con el ambiente y sustentable para potenciar la salud de la planta y la productividad de los cultivos en sentido general.

Conclusiones

A nivel de invernadero las cepas de *Bacillus* provenientes de plantas de maíz y y cafetoejercen efecto estimulador de la germinación de las semillas de maíz y se destacan las cepas RM5, RC9 y RC15 en la masa seca de raíz en el cultivo del maíz. En los cultivos del tomate y la zanahoria, se obtienen resultados variables, por lo que se debe profundizar en el estudio de estas interacciones planta-bacteria.

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Responses of sour passion fruit (*Passiflora edulis* Sims) seeds from the third recurrent selection cycle during storage

Respuestas al periodo de almacenamiento de semillas de maracuyá-agrio (*Passiflora edulis* Sims) del tercer ciclo de selección recurrente

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Abstract

Conflicting responses have been found for the period of conservation of passion fruit seeds. Therefore, this study aimed to evaluate the response of the seeds of passion fruit progenies to a 24-month storage period. The sour passion fruit progenies from the third cycle of Universidade Estadual do Norte Fluminense Darcy Ribeiro - UENF intrapopulation recurrent selection program were grown in Itaocara- RJ and the progenies seeds were evaluated for: germination potential, germination first count, accelerated aging, first count of accelerated aging test, seedling and radicle size and germination speed index. The germination and vigor of the seeds of the full-sib progenies were assessed by analysis of variance and factorial arrangement with 3 progenies and 7 periods of storage and the averages were compared by Tukey test at 5% probability. Progenies 1 and 2 responded better to germination (92% for both progeny) and vigor test of accelerated aging (92% for both progeny), rootlet size (6.4 and 5.8 cm, respectively) and germination speed index (2.91 and 2.85, respectively) during storage. Variations were observed during the storage period in mean of progenies for germination (81-93%) and vigor for all traits, except accelerated aging (84-91%). At eight months of storage, the seeds presented increased vigor; at 16 months, they showed reduced vigor. The passion fruit seeds of the progenies under analysis can be stored for up to 24 months with high and uniform germination rate and no loss in vigor. The seeds from the passion fruit breeding program showed increases in the variables of germination and vigor during the selection process for fruit production variables, proving the efficiency of the breeding program.

Key words: Breeding program, germination, longevity, progeny, vigor, *Passiflora edulis* Sims, sour passion fruit.

Resumen

El largo del periodo de conservación de semillas ocasiona cambios variales y significativos en el desarollo de plantas de maracuya-agrio. En este trabajo se evaluó la respuesta de semillas de progenies de este cultivo a un periodo de almacenamiento de 24 meses. Progenies del tercer ciclo del Programa de selección recurrente por intrapoblación de la Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF fueron cultivadas en Itaocara-RJ y las semillas producidas fueron evaluadas en relación con la capacidad de germinación, primer recuento de prueba de germinación, envejecimiento acelerado, primer recuento del envejecimiento acelerado, tamaño de plántulas y de raíz e índice de celeridad de germinación. Para ello, las semillas de las progenies de hermanos completos fueron evaluadas por germinación y vigor en un arreglo factorial con 3 progenies y 7 periodos de almacenamiento, donde las medias fueron comparadas por la prueba Tukey (P = 0.05). Las progenies 1 y 2 presentaron las mejores respuestas para germinación (92%) y para vigor de envejecimiento acelerado (92%), tamaño de radícula (6.4 y 5.8 cm, respectivamente), índice de celeridad de germinación (2.91 y 2.85, respectivamente) durante el almacenamiento hasta 24 meses. Se encontraron variaciones en germinación (81-93%) y vigor para todas las características, pero no para envejecimiento acelerado (84-91%). El mayor vigor de plántulas se observó en semillas con 8 meses de almacenamiento y el menor, con semillas de 16 meses de almacenadas. Se concluye que, las semillas de maracuyá de las progenies evaluadas pueden ser almacenadas hasta 24 meses, con altas de germinación uniforme y sin pérdida de vigor; las semillas provenientes del programa de mejoramiento del maracuyáreo mostraron incrementos en las variables de germinación y vigor en el proceso de selección para producción de frutos, lo que confirma la eficiencia de este programa.

Palabras clave: Programa de mejoramiento, germinación, longevidad, progenie, vigor, *Passiflora edulis* Sims, maracuyá agrio.

Introduction

The genus *Passiflora* has more than 400 species, being more than 140 native to Brazil. The sour passion fruit (*Passiflora edulis* Sims) originates from the tropical regions of South America (Bernacci et al., 2008) is a multifunctional species whose fruit is of great relevance among the tropical fruit crops. Plantations of this culture help maintaining work force in rural areas, which is a strong social role of this culture (Pires et al., 2011).

The seeds of sour passion fruit are considered orthodox (Posada et al., 2014) and can be stored, since the most widely used alternative of passion fruit dispersal is sexual. However, for some authors, its seed germination is low, irregular and of fast decay. Another problem in the passion fruit production chain is the lack of quality seed commercial suppliers (Alexandre et al., 2004; Freitas, 2009; Oliveira et al.; 2012).

The conservation of seeds by storage is carried out in a place other than their natural habitat and has been performed since agriculture started to be expanded, together with the maintenance of samples of the genetic heritage, because of their importance for the conservation of cultivated crop species and their wild parents (González-Arnao and Engelman, 2013).

The maintenance of seed vigor during storage makes seed available throughout the year for producers, which allows them to produce the fruit to the market in the off season, when better prices can be obtained (de Lima et al., 2019).

The UENF passion fruit breeding program has been developed since 1998 and has assessed the genetic diversity among commercial genotypes of passion fruit and others species of genus *Passiflora* (Ribeiro et al., 2019), estimated the genetic parameters for passion fruit traits and the genetic progress for crop yield traits with the selection of full-sib families, through the recombination of the selected progenies and the registration of the new variety of passion fruit at the Ministry of Agriculture in 2015, the UENF Rio Dourado (Gonçalves et al., 2007; Gonçalves et al., 2009; Silva et al., 2009; Ferreira et al., 2016; Silva et al., 2014). This study aimed to assess how the seeds of passion fruit progenies from the UENF breeding program responded to storage, even these variables were not taken into account in the selection process in a block design with a factorial arrangement with three progenies and seven storage periods.

Material and methods

The seeds were extracted from three full-sib progenies of passion fruit (112x42, 117x19 and 46x14) of the third cycle of the intra-population recurrent selection program developed by the Universidade Estadual do Norte Fluminense Darcy Ribeiro - UENF, implemented in the Unidade Experimental Ilha Barra do Pomba, in Itaocara – RJ, in 2013.

Yellow and yellow-greenish fruits found on the ground were harvested (Negreiros et al., 2006). Each treatment was individually and the seeds were extracted by friction in steel mesh sieve under running water and dried on paper towels for 48 h, at room temperature. Seed moisture was determined by the oven with air circulation, according to Rule for Seed Analysis, with two replicates for each treatment, for 24 h, at 105 ± 3 °C (Brasil, 2009).

The seeds of the three progenies were assessed immediately after being extracted and dried. The remaining seeds were packed in paper bag, stored in the refrigerator, and assessed every four months until the storage completed 24 months. The following variables were measured: (1) Germination test (G), four replications of 50 seeds were placed in three germination paper sheets, wetted with distilled water twice the amount of the weight of the substrate and subjected to alternating temperatures of 20-30 °C in germination chamber. Normal seedlings were recorded at 28 days in percentage (Brasil, 2009); (2) First count of the germination test (FC), evaluated together with the germination test, with record of normal seedlings in percentage at 14 days after the start of the test (Krzyzanowski et al., 1999, adapted from Brasil, 2009); (3) Accelerated aging (AA), the seeds were placed on aluminum screen in mini-chambers represented by a Gerbox containing 40 mL of water and kept for 48 hours at 40 °C (Larré et al., 2007). Then, the same methodology applied for the germination test was carried out and normal seedlings in percentage were recorded at 28 days (Brasil, 2009); (4) First count of the accelerated aging testing (FCA), evaluated together with the accelerated aging test with the record of normal seedlings in percentage at 14 days after the start of the test; (5) Seedling size (SS), implemented in conjunction with the germination test where the 10 seedlings located in the top row of the paper roll were measured in centimeters at the end of test (Negreiros et al., 2008); (6) Rootlet size (RS), implemented in conjunction with the germination test where the rootlets of 10 seedlings located in the top row of the roller were measured in centimeters at the end of the test (Negreiros et al., 2008); and (7) Germination speed index (GSI), performed along with the germination test, with the record of the number of normal seedlings every four days (adapted from Maguire, 1962).

The variables were subjected to analysis of variance, arranged in a factorial design with 3 progenies (112x42, 117x19 and 46x14) and

7 periods of storage (0, 4, 8, 12, 16, 20 and 24 months after harvest), according to the following model (Equation 1):

$$Y_{ijk} = b_j + g_i + a_k + ga_{ik} + e_{ijk} \quad \textbf{Eq. 1}$$

where, *bj* = effect of block *j*; *gi* = effect of progeny *i*; *ak* = effect of storage *k*; *gaik* = effect of the interaction between progeny and storage *ik*; *eijk* = experimental error. The degrees of freedom of the effects of the factors as well as the interaction were unfolded via regression analysis, considering a coefficient of determination of the models above 60%. When this value was not obtained, the means were compared via Tukey test at 5% probability with the aid of the Assistat program, version 7.7 Beta (Silva, 2014).

Results and discussion

The average moisture content of the three progenies assessed immediately after drying was 10%. Fonseca and Silva (2005) found that values of 7-17% of water content helped maintaining the viability of sour passion fruit seeds, regardless of the storage temperature. Catunda et al. (2003) found that the water content of 10% in passion fruit seeds was better than 8% for storing seeds in a permeable container, since they present superior vigor at ten months of storage.

We decided to present the variables of the trend of the points observed by the average test, since the values of the coefficients of determination (R^2) were too low for regression analysis, even in a 4th-order polynomial model.

The analysis of variance showed a significant difference between the means of the progenies for G, AA, RS and GSI. For the averages of the period, it was significant for G, FC, FCA, SS, RS and GSI (Table 1).

The lowest coefficients of variation were for variables G and AA, with values of 4.76 and 6.67%, respectively, comparing the best data dispersion. The variables SS, RS and GSI obtained intermediate values (14.14, 13.82 and 10.37%). The variables FC and FCA obtained the highest values (35.46 and 23.81%) showing that the data set was not as homogeneous, since these vigor tests are more variable than the average (Table 1).

In the progeny interaction with the storage period there was significant difference for FC, RS and GSI (Table 1). Table 2 shows that even though there was a significant difference by the analysis of variance, the means test showed no difference between the three progenies, proving the efficiency of the breeding program in adding favorable characteristics to the seeds of all evaluated progenies.

Table 1. Factorial analysis of variance of the results obtained from the evaluation of the physiological traits of the seeds. Germination (G), first count of germination (FC), first count of the accelerated aging testing (FCA), accelerated aging test (AA), seedling size (SS), rootlet size (RS) and speed germination index (GSI) of three sour passion fruit (*Passiflora edulis* Sims) progenies for seven periods of storage, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 2015.

FV	DF	Mean Square						
		G	FC	FCA	AA	SS	RS	GSI
Block	1	0.9803	32.8896	770.0006	91.2779	42.9020	8.3963	0.0007
Progeny (Pr)	2	377.4621 **	481.0994 ^{ns}	493.3378 ^{ns}	673.0013 **	6.8866 ^{ns}	2.5820 *	0.6340 **
Storage (St)	6	87.9402 **	3162.7739 **	4972.5798 **	44.7037 ^{ns}	34.5226 **	11.8474 **	1.3580 **
Pr x St	12	9.7851 ^{ns}	54.3100 **	310.8619 ^{ns}	15.6643 ^{ns}	0.9546 ^{ns}	0.2158 *	0.0078 **
Error	20	17.9387	286.5955	198.6201	34.0168	2.5756	0.6660	0.0819
Total	41	—	—	—	—	—	—	—
CV%		4.76	35.46	23.81	6.67	14.14	13.82	10.37

** Significant at 1% probability; * Significant at 5% probability; ^{ns} non significant.

Table 2. Physiological traits of the seeds of three sour passion fruit (*Passiflora edulis* Sims) progenies during storage. First count of germination (FC); rootlet size (RS); and germination speed index (GSI), Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 2015.

Storage (months)	FC (%)			RS (cm)			GSI		
	112x42	117x19	46x14	112x42	117x19	46x14	112x42	117x19	46x14
0	52aAB*	58aAB	51aAB	6.6aAB	6.6aAB	6.9aAB	2.97aAB	3.11aAB	2.90aAB
4	40aBC	42aBC	43aBC	3.9aD	4.1aC	3.8aC	2.36aBC	2.62aBC	2.71aBC
8	79aA	87aA	85aA	7.3aA	7.7aA	7.5aA	3.49aA	3.64aA	3.52aA
12	52aAB	66aAB	62aAB	7.3aA	7.3aA	7.6aA	2.83aAB	3.07aAB	2.89aAB
16	9aC	11aC	13aC	4.6aCD	4.4aC	4.3aC	1.99aC	2.09aC	2.09aC
20	28aBC	33aBC	35aBC	5.2aBCD	5.3aBC	5.3aBC	2.37aBC	2.47aBC	2.45aBC
24	48aAB	55aAB	56aAB	5.9aABC	6.3aAB	6.3aAB	2.75aB	2.85aB	2.81aB

*Means followed by the same letter do not differ statistically, lowercase in column and uppercase in row, by Tukey test at 5% probability.

Progenies 1 and 2 provided better responses for G and in vigor evaluation tests AA, RS and GSI, during storage (Table 3). Carvalho and Nakagawa (2012) refer to the longevity of the seeds, which is mainly characterized by the period in which they can live according to their genetic traits, showing the presence of genetic variability for these attributes. These traits were not included in the selection indices developed over the cycles for the selection of the best progenies, but the intrapopulation recurrent selection was effective (Silva and Viana, 2014).

The progenies assessed were obtained by the recurrent selection method, which is an intra-population breeding method with good results in the selection of superior genotypes, through the gradual increase in the frequency of favorable alleles and the maintenance of population genetic variability (Reis et al., 2011), as observed in the results of G, AA, RS and GSI (Table 3).

Finding genetic variation within a species enables the maintenance of heterosis and resistance to certain limiting problems of the culture, especially in the case of an allogamous plant, such as passion fruit, which requires population variability to facilitate cross pollination (Lobo, 2006). The presence of diversity by assessing the physiological seed quality is important for plant breeding, as it increases the number of variables to be evaluated for the identification of superior individuals.

Cardoso et al. (2009) evaluated the genetic divergence to estimate the genetic parameters of traits related to seed physiological quality in a papaya germplasm bank and found high divergence for the variables related to the physiological quality of seeds, which indicates that this can be exploited in breeding programs aimed at improving seed quality. Freitas (2009) and Alexandre et al. (2004) also found differences characterized by the genotype of plants while evaluating, among other variables, the germination of seeds from different half-sib progenies of passion fruit. Torres et al. (2019) showed differences between a segregating population of Pasiflora (*P. edulis* x *P. setacea*) by evaluating physiological and morphological quality traits.

The recurrent selection process increased the frequency of alleles favorable for the production traits under selection in the UENF passion fruit intra-population breeding program (Cavalcante et al., 2019; Silva et al., 2014; Silva et al., 2009) and affected the variables related to the physiological seed quality in progenies 1 and 2 (Table 3).

The variables FC, RS and GSI showed significant difference in progeny interaction and storage periods by analysis of variance (Table 1), but did not show different averages between progenies by Tukey test at 5% probability (Table 2), thus it was made the average of these three progeny in Table 4, for to better display the seven periods responses that were evaluated. Significance was observed for the periods for all variables, except AA, and the responses were presented in each storage period (Table 4).

Since significance was observed for the periods for all variables, except AA, they were grouped by the average value of the results of the variables assessed in the three progenies. The responses were presented in each storage period (Table 4).

At the end of 24 months of storage, the responses to the tests G, FC, SS and RS did not differ statistically from the initial period (zero) when the tests were assessed. The seeds of the progenies responded similarly to AA in all storage periods, and showed uniforme germination, which is proven with 84 to 91% germination vigor in this vigor test, being the lowest value found immediately after the seeds were harvested (Table 4).

Table 3. Physiological traits of the seeds of three sour passion fruit (*Passiflora edulis* Sims) progenies during storage. Germination (G); accelerated aging testing (AA); rootlet size (RS); and germination speed index (GSI), Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 2015.

Progeny	G	AA	RS	GSI
	(%)		(cm)	
1	92a*	91a	6.4a	2.91a
2	92a	91a	5.8ab	2.85a
3	83b	79b	5.6b	2.52b

*Means followed by the same letter do not differ statistically by Tukey test at 5% probability.

Table 4. Physiological traits of the seeds of three sour passion fruit (*Passiflora edulis* Sims) progenies, according to the storage periods. Germination (G), first count of germination (FC), first count of germination of the accelerated aging testing (FCA), accelerated aging testing (AA), seedling size (SS), rootlet size (RS) and germination speed index (GSI), Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 2015.

Storage (months)	G	FC	AA	FCA	SS	RS	GSI
	%				cm		
0	93a*	54ab	84a	51c	13.6a	6.7ab	2.99ab
4	81b	42bc	87a	61bc	9.7bc	3.9c	2.56bcd
8	92a	84a	91a	86ab	13.9a	7.5a	3.55a
12	90a	60ab	89a	89a	13.3a	7.3a	2.93bc
16	88ab	11c	85a	9d	7.7c	4.4c	2.05d
20	89ab	32bc	85a	39c	9.6bc	5.3bc	2.43cd
24	90a	53ab	90a	79ab	11.6ab	6.2ab	2.80bc

*Means followed by the same letter do not differ statistically by Tukey test at 5% probability.

Oliveira et al. (2012) observed in the storage of seeds of several passion fruit progenies, for 24 months, that 21% of them maintained their physiological quality for seedling emergence, when stored in multiwall paper bag at 5 °C, which corroborates the results obtained for germination and vigor and proves that they have no dormancy. Gross and Olsen (2010) reported that, in the species domestication process developed throughout selection, evolutionary traces are observed in the genotypes obtained, such as the loss of seed dormancy. This may have occurred with the passion fruit which had been reported several times for presenting low and disuniform germination (Alexandre et al, 2004; Freitas, 2009; Oliveira et al, 2012).

Seed storage for eight months proved beneficial to the variable GSI, since it reached higher average values and demonstrated high seed vigor for this test in this period (Table 4). Catunda et al. (2003) and Martins et al. (2005) also observed oscillations between the results of germination percentage during storage of passion fruit seeds for up to 10 months. Freitas (2009) found wide difference between the averages of the germination test, first germination count, accelerated aging and electrical conductivity for 26 half-sib progenies of passion fruit from the UENF first cycle of recurrent selection that were recombined by Gonçalves et al. (2007). The results obtained in this study, using seeds from the third cycle of recurrent selection, recombined by Ferreira et al. (2016), present increased uniform germination and vigor evaluation for the population, compared to that of the beginning of the breeding program.

Before storage, by comparing the results of germination and vigor of the seeds from the UENF passion fruit first cycle of recurrent selection, Freitas (2009) observed maximum and minimum germination of 96 and 75%, respectively; and those from the third cycle resulted in 97 e 88%. For FC, the seeds of the first cycle obtained the maximum and minimum values of 97 and 71%; and from the third cycle, 58 and 51%; for AA, the seeds of the first cycle obtained the maximum and minimum values of 91 and 47%; and those of the third cycle, 92 and 82%. Therefore, it is possible to verify that the range between the averages was reduced by the selection of genotypes, and the current population shows increased uniformity.

After 12 months of storage, the lowest and the highest germination percentages were respectively 76 and 91% for seeds from the UENF passion fruit first cycle of recurrent selection (Freitas, 2009) and 84 and 95% for seeds from the third cycle, which generates a range of 15 and 11 for each cycle.

In the first two months of storage, Lima et al. (2010) observed an increased germination percentage, for the first germination count and seed germination speed index for passion fruits purchased from the market. From that period on, a decreased germination was observed, until reaching the six months of storage. They concluded that the seeds presented postharvest dormancy, which was broken after 30 days of storage. In this study, no changes were observed in the germinating potential for the AA stress resistance test, which remained uniform throughout the 24 months of storage (Table 4).

Lots with higher number of normal seedlings in the first count are more vigorous and the higher the germination speed index, the higher the speed of germination of these seeds, which are also considered more vigorous (Krzyzanowski et al., 1999). Accordingly, the seeds have proven more vigorous in the FC, SS, RS and GSI tests after 8 months of storage and less vigorous at 16 months, although no statistical different was found between the average of other periods (Table 4).

Variation was observed in the results for FC, FCA, SS, RS and GSI during the storage periods, as evidenced by Martins et al. (2005). However, for AA, the results of this study disagree with those provided by the authors.

The assessment of stress resistance by accelerated aging testing showed high percentage of normal seedlings for the variable FCA, close to 90%, for the periods of 8 and 12 months of storage and, antagonistically, a very low percentage at sixteen months of storage, below 10%. However, even with reduced vigor, these seeds have reached a percentage of normal seedlings of 85%, which is equal to the averages of the other storage periods. The same happened with the vigor tests FC and GSI, which responded with low values at 16 months, but the germination rate reached 89% (Table 4).

The larger the seedling or its evaluated part, the more vigorous it is, because it is more capable of transforming the reserve supply from the storage tissues into meristematic tissues (Krzyzanowski et al., 1999). Thus, for SS and RS, seedlings of measured progenies were more vigorous during periods 0, 8, 12 and 24 months of storage.

In order to complement the information provided by the variables RS and SS, it is interesting to interpret the seedling size results together with the germination percentages, since vigorous seedlings coming from low percentage germinating seeds are useless. Table 4 shows that at periods 0, 8, 12 and 24 months of storage, the mean SS and RS obtained the same positive response as G.

The highest GSI and consequent increased seed vigor were observed at periods 0 and 8 and the lowest, at 4, 16 and 20 months (Table 4), according to most vigor tests performed in this study. In this sense, the passion fruit seeds in the third recurrent selection cycle bring with them the plant breeding benefits and even the germination and vigor variables were not considered in the selection, they followed the production variables with high values that were maintains up to 24 months of storage.

Conclusions

At eight months of storage, the three progenies seeds presented increased vigor; at 16 months, they showed reduced vigor. The sour passion fruit (*Passiflora edulis* Sims) seeds of the progenies under analysis can be stored for up to 24 months with high and uniform germination rate and no loss in vigor. The seeds of the three evaluated progenies from the passion fruit breeding program showed increase in the germination and vigor variables during the selection process of the fruit production variables, proving the efficiency of the breeding program.

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Efecto de la procedencia sobre el comportamiento productivo de *Prosopis alba* en plantación

Provenance effect on productive behavior of *Prosopis alba* within plantation

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Resumen

Prosopis alba (algarrobo blanco) es una especie forestal nativa utilizada principalmente para aserrío en Argentina. La Ley 27.487/19 de fomento forestal considera las densidades y prácticas adecuadas para el logro de madera de calidad. Además del buen manejo, la correcta selección del origen del material genético es una estrategia importante para la obtención de buenos rendimientos de madera. Con el objeto de evaluar el comportamiento de plantas de *P. alba* de tres procedencias se establecieron ensayos en las localidades de Corrientes y Chaco (Argentina). Se trabajó con plantines logrados a partir de semillas de las procedencias Santiagueña, Chaqueña y Salta Norte. Una vez establecidos en campo se evaluó el índice de respuesta neta y cuatro variables dasométricas (altura total, diámetro a la altura del cuello, volumen de árbol en pie e incremento corriente anual). No se detectó efecto del origen geográfico de las plantas sobre la sobrevivencia. Considerando los parámetros dasométricos estudiados, la procedencia Salta Norte resultó superior respecto a las procedencias Chaqueña y Santiagueña. En Sáenz Peña (Chaco) todas las variables dasométricas evaluadas resultaron estadísticamente superiores, mientras que en Corrientes solamente el diámetro a la altura del cuello y altura total fueron superiores. Se observó que la procedencia Salta Norte se destaca como la de mejor comportamiento en ambas localidades. Por tanto, se concluye que la procedencia del germoplasma de *P. alba* influye en su comportamiento productivo.

Palabras clave: Algarrobo blanco; altura; crecimiento; diámetro; incremento corriente anual; origen.

Abstract

Prosopis alba is one of the most widely used native forest species for sawmilling in Argentina. The forestry development law N° 27.487/19 considers the plantation of this specie taking into account densities and suitable practices for quality wood achievement. Coupled with good management right selection of the origin of the genetic material is a strategy that contributes to obtain good yields. In order to evaluate the behavior of *P. alba* three provenances plants were established in Corrientes and Chaco to assess their performance in these environments. It was worked with seedlings compound by seeds from the provenance of Santiago, Chaco and North Salta. As established on field, net response rate and four dasometric variables (total height, diameter at neck height, standing tree volume and annual current increase) were evaluated. Effect of plants' geographical origin over survival was not detected. In consideration of dasometric studied parameters, North Salta provenance was higher than the ones from Chaco and Santiago. In Sáenz Peña (Chaco) for this provenance all the evaluated dasometric variables were statistically greater, whereas in Corrientes they only were the diameter at neck height and total height. It has been proved that North Salta provenance stands out as the one with the best behavior in both tested locations. Therefore, it is concluded that the geographic origin of *P. alba* germplasm influences its productive behavior.

Keywords: annual current increase; diameter; growth; height; origin; white carob tree.

Introducción

El éxito de una plantación maderera comercial está relacionado con la correcta selección de las especies y sus procedencias, así como el manejo durante su ciclo productivo. Prueba de ello es que los aumentos de productividad dentro del sector forestal responden a resultados obtenidos en programas de investigación enfocados en mejoramiento genético y silvicultura intensiva (Rubilar, Fox, Allen, Albaugh y Carlson, 2008). El mejoramiento de las plantaciones depende en gran medida de la disponibilidad de material adaptado que garantice la sostenibilidad y la productividad del recurso forestal; por tanto, la selección de especies, clones y/o procedencias adaptados a los requerimientos actuales, así como la disponibilidad de variabilidad genética, son fundamentales para afrontar los nuevos desafíos de mercados y producción de madera (Marcó, 2005; White, Adams y Neale, 2007). *Prosopis alba* (Griseb.) o algarrobo blanco es una especie leñosa de amplia distribución natural en Sudamérica. Se encuentra en Argentina, Uruguay, Paraguay, en el pantanal de Brasil, sur de Bolivia y norte de Chile y Perú (Delvalle, Atanasio, Ayala, Svriz y Petkoff, 2003). Muy valorada por su madera, es una de las especies nativas de mayor uso para aserrío en Argentina y, debido a que la totalidad de la madera de algarrobo consumida proviene de bosques nativos, la explotación del recurso resulta insostenible, no sólo desde el punto de vista ambiental sino también económico (Venier et al., 2013).

En este contexto, y dada la potencialidad de la especie para algunas regiones, la Ley Nacional de inversiones para bosques cultivados fomenta la forestación con *Prosopis* spp. en 13 provincias de Argentina, contemplando las densidades de plantación y prácticas de manejo necesarias para el logro de madera de calidad (Boletín Oficial de la República Argentina, 2019).

La correcta selección del material genético es una estrategia básica para la obtención de buenos rendimientos (Callaham, 1964). La determinación de la procedencia geográfica de una especie es importante, ya que las plantas se adaptan mejor en las condiciones ambientales donde crecen por selección natural (White et al., 2007). Si los factores ambientales de los sitios de plantación son diferentes a los de la distribución natural de la especie, las plantaciones tendrán problemas de adaptación, mortandad o simplemente, crecimientos inferiores a los deseados (Sáenz, 2004). En este sentido, los ensayos de procedencia resultan una herramienta útil para evaluar la variación de rasgos adaptativos (plasticidad) entre poblaciones de una misma especie (Nabais et al., 2018) y su principal objetivo práctico es identificar las procedencias (áreas geográficas de origen) cuyas semillas dan lugar a bosques productivos y bien adaptados (Burley, 1969). Tomando en cuenta la importancia de la especie evaluada y la utilidad de la información generada, el objetivo de este trabajo fue evaluar el comportamiento de plantas de *P. alba* de tres procedencias en dos ambientes diferentes.

Materiales y métodos

Material vegetal

Los plantines de *P. alba* utilizados fueron obtenidos de la siembra de semillas provenientes de tres rodales semilleros: (1) Santiagueño: 27°52’44”S-64°9’16”W, a 15 km al sudeste de la ciudad de Santiago del Estero a orillas del río Dulce, con temperatura media anual de 20.7 °C y 579 mm precipitación media anual; (2) Chaqueño: 24°15’58”S-61°54’00”W, en el extremo oeste de la provincia de Formosa a orillas del río Bermejo, temperatura media anual de 22.8 °C y precipitación media anual de 678 mm; y (3) Salta Norte: 22°12’10”S-63°40’33”W, en el extremo norte de la provincia de Salta, con temperatura media anual de 21.9 °C y precipitación media anual de 1054 mm. Los plantines fueron obtenidos según el protocolo recomendado por Fontana et al. (2018).

Áreas de estudio

Se implantaron sendos ensayos en dos localidades diferentes: (1) Estación Experimental Agropecuaria (EEA) INTA Sáenz Peña (ensayo-1), en la localidad de Sáenz Peña, Chaco (26°49’42.62”S-60°26’42.56”W), en un Argiustol údico (serie Chaco) y temperaturas media, máxima media y mínima media de 25, 31.3 y 19 °C en el período estival y de 17.6, 24.2 y 11.7 °C en el período invernal, respectivamente; 1266 mm de precipitación acumulada en el primer período y 663.7 mm en el segundo; y (2) Campo Didáctico Experimental de la Facultad de Ciencias Agrarias (CDEA) (ensayo-2) en la ciudad de Corrientes (27°28’33.3”S-58°47’00.1”W) en un Udipsament árgico (serie Ensenada Grande) y temperaturas media, máxima media y mínima media de 25.7, 31.3 y 20.9 en el período estival y de 19, 23.9 y 14.9 °C en el período invernal; 2688.3 mm de precipitación acumulada en el período estival y 1848.2 mm en el período invernal.

Los datos de clima utilizados fueron tomados en las estaciones meteorológicas automáticas de la EEA INTA Sáenz Peña en Chaco (26°50’23.99”S-60°26’23.99”W) y del Instituto Correntino del Agua y el Ambiente en Corrientes (27°28’18”S-58°49’20”W).

En ambos ensayos la preparación del suelo consistió en dos pasadas de rastra de discos 2 meses antes de la plantación de los materiales.

En ambos lugares, para el control de hormigas se aplicó Formidor®, p.a. Fipronil 0.003% en la dosis especificada por el fabricante, según el género de hormiga identificada en el monitoreo.

La siembra en ambos ensayos se hizo en octubre a una distancia de 4 x 4 m entre plantas, para un total de 625 plantas/ha. El ensayo-1 (S. Peña-Chaco) fue plantado el 13 de octubre de 2015, mientras que el ensayo 2 (Corrientes) se plantó el 23 de octubre del mismo año; en ambos se aplicó un riego inicial y otro 15 días después. El ataque de roedores determinó la reposición de plantas y la consiguiente protección utilizando un soporte de PVC de 50 cm de alto fijado en el suelo.

El ensayo tuvo una duración de 2 años, tiempo durante el cual se hizo control de hormigas y de malezas por método químico mediante aplicación dirigida de herbicida al 2% (Panzer® Gold, p.a. glifosato sal dimetilamina 60.8%) entre plantas y, en las entrelíneas se hizo control mecánico con desmalezadora. En el período de ensayo se realizaron podas de formación selectiva a nivel de árbol, eliminando bifurcaciones y ramas verdes que competían con el eje principal.

Evaluación del comportamiento en campo

El comportamiento de cada procedencia fue evaluado utilizando los parámetros siguientes:

Índice de respuesta neta (IR), propuesto por Armas, Ordiales y Pugnaire, (2004), ecuación 1:

$$IR = (S1 - S2)/(S1 + S2) \quad \textbf{Ec. 1}$$

donde, *S1* y *S2* corresponden a las supervivencias de las distintas procedencias. Este índice varía entre 0 (no hay efecto de la procedencia) y 1 (la supervivencia es 100% superior en una partida que en la otra), siendo insensible a los valores absolutos de supervivencia. La comparación entre las dos procedencias que mostraron el mejor comportamiento se hizo al finalizar el segundo año desde la plantación.

Variables dasométricas:

Altura total: Se utilizó una cinta métrica con precisión de 1 mm para registrar la altura de planta hasta el meristemo apical.

Diámetro a la altura del cuello (DAC): Correspondiente al diámetro a ras del suelo del tronco o tallo de cada árbol. Las mediciones se realizaron con un calibre digital de precisión de 0.1 mm y los resultados se expresaron en centímetros.

Volumen de árbol en pie: Se utilizó el método de segmentación visual de Born y Chojnacky (1985) que consiste en la segmentación visual de tallos y el cálculo del volumen de cada segmento, cuya suma indica el volumen total por árbol. Para la estimación del volumen (*V*) se siguió el procedimiento de Smalian (British Columbia Government, 2011), ecuación 2:

$$V = (AB + ab)/2 * \mathrm{h} \quad \textbf{Ec. 2}$$

donde, *AB* es el área de la sección mayor; *ab* es el área de la sección menor y *h* la longitud de la troza. Las áreas basales (*AB* y *ab*) se determinaron con la ecuación 3:

$$\mathrm{A} = diámetro^2 \ * 0.7854. \quad \textbf{Ec. 3}$$

Incremento Corriente Anual (ICA): Con los volúmenes de 2 años consecutivos se calculó el incremento alcanzado en un año, ecuación 4:

$$ICA = Y_{(t+1)} - Y_t \quad \textbf{Ec. 4}$$

donde, *Y* es la dimensión considerada (volumen) y *t* la edad (12 y 24 meses).

Diseño experimental y análisis estadístico

De acuerdo con las recomendaciones de Burley et al. (1979) y Read y French (1993), el diseño utilizado fue bloques completos al azar con tres tratamientos (procedencias) y tres repeticiones, donde la unidad experimental consistió en 12 plantas/tratamiento. Los datos fueron analizados estadísticamente con el software Infostat (Di Rienzo et al., 2011). El análisis de la varianza se hizo por comparación de las medias de los tratamientos a través de la prueba de Duncan (P <0.05), empleando el modelo siguiente, ecuación 5:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij} \quad \textbf{Ec. 5}$$

donde, μ es la media general, el efecto del i-ésimo tratamiento, β_j el efecto del j-ésimo bloque (*j =1,…,b)* y ε_{ij} es el error aleatorio asociado a la observación Y_{ij}.

Resultados

La supervivencia de las procedencias a través de los valores de índice de respuesta neta (*IR*) no fue significativo en ambos ensayos, siendo para S. Peña-Chaco (P = 0.1501) y para Corrientes (P = 0.2937). En todos los casos los valores de *IR* fueron estadísticamente iguales a cero (0), lo que indica que la procedencia no tuvo efecto sobre la supervivencia de las plantas.

Las medidas de las variables dasométricas registradas durante dos ciclos de crecimiento (Tabla 1) en Salta Norte mostraron valores más altos que en las demás procedencias; asimismo, los coeficientes de variación de las procedencias Chaqueña y Santiagueña fueron mayores, lo que indica mayor heterogeneidad entre plantas respecto a los caracteres evaluados.

El análisis de la varianza mostró que para el ensayo conducido en S. Peña la procedencia Salta Norte resultó diferente y superior a las procedencias Chaqueña y Santiagueña para todas las variables en ambas mediciones (Tabla 2). Por el contrario, en el ensayo de Corrientes, Salta Norte resultó significativamente superior para las variables DAC y altura total en el tiempo de evaluación, pero no así en volumen.

El análisis de la varianza del incremento corriente anual (*ICA*) (Tabla 3) mostró que en el ensayo-1 la procedencia Salta Norte fue diferente y superior a las demás ($P < 0.05$). El *ICA* mínimo de las procedencias Salta Norte fue 2670 y 2841% superior a las procedencias Chaqueña y Santiagueña respectivamente, e igualmente el *ICA* máximo fue 244 y 501% mayor. En Corrientes no se encontraron diferencias estadísticas entre procedencias para el *ICA*; mientras que la procedencia Chaqueña fue la que presentó el valor superior más alto (3491.88 cm^3), aunque algunas plantas no aumentaron el volumen en el transcurso del año.

Tabla 2. Análisis de varianza y prueba de comparaciones múltiples (Duncan) de las medias ± DE para las variables DAC (cm), altura (cm) y volumen (cm^3) de plantas de *Prosopis alba* de tres procedencias durante 2 años de evaluaciones.

		Año 1			Año 2		
	P	**DAC**	**Altura**	**Volumen**	**DAC**	**Altura**	**Volumen**
EEA INTA S. Peña	**Ch**	2.2±0.6[a*]	130.3±43.1[a]	263.5±189[a]	3.6±1.1[a]	198.4±63.9[a]	1324.1±1119.8[a]
	Sg	2.1±0.5[a]	112.2±31[a]	208.3±127.3[a]	3.2±0.8[a]	167.9±39.8[a]	824.9±610.9[a]
	SN	3.2±0.6[b]	193.5±43.9[b]	760.8±384.4[b]	5.6±1.0[b]	288.3±65.1[b]	4751.6±2797.8[b]
	p-valor	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CDEA Corrientes	**Ch**	1.1±0.6[a]	108.4±41.3[a]	52.0±91.3	1.4±0.9[a]	124.8±53.7[a]	305.5±900.1
	Sg	1.0±0.3[a]	104.9±39.2[a]	40.6±49.1	1.4±0.5[a]	126.5±43.6[a]	132.92±128.3
	SN	1.5±0.4[b]	136.9±41.2[b]	90.7±74.0	2.5±0.8[b]	181.6±53.3[b]	491.2±411.0
	p-valor	0.0173	0.047	0.1128	0.0001	0.0034	0.3282

P: procedencia; **Ch**: Chaqueña; **Sg**: Santiagueña; **SN**: Salta Norte; **DAC**: diámetro a la altura de cuello.

*Medias con una letra distinta son significativamente diferentes (p<0.05).

Tabla 1. Variables dasométricas de plantas de *Prosopis alba* de tres procedencias evaluadas durante 2 años en dos localidades.

			Año 1 (2016)					Año 2 (2017)				
		P	**Media**	**D.E.**	**C.V.**	**Mín.**	**Máx.**	**Media**	**D.E.**	**C.V.**	**Mín.**	**Máx.**
EEA INTA S. Peña	**DAC (cm)**	**Ch**	2.2	0.6	27.1	0.8	2.9	3.6	1.2	32.2	1.9	5.8
		Sg	2.1	0.5	22.3	1.3	3.2	3.2	0.9	26.7	2	4.7
		SN	3.2	0.6	18.9	2.1	4.2	5.6	1	18.3	4.1	7
	H (cm)	**Ch**	130.3	43.1	33.1	68	200	198.4	63.9	32.2	90	300
		Sg	112.2	31	27.7	83	190	167.9	39.8	23.7	104	270
		SN	193.5	43.9	22.7	99	250	288.3	65.1	22.6	170	400
	Volumen (cm^3)	**Ch**	263.5	189	71.7	17.5	705.2	1324.1	1119.8	84.6	104.2	4672.6
		Sg	208.3	127.3	61.1	63	500.7	824.9	610.9	74.1	151.5	2570
		SN	760.8	384.4	50.5	156	1517.2	4751.6	2797.8	58.9	1178.5	11721.6
CDEA Corrientes	**DAC (cm)**	**Ch**	1.1	0.6	59.2	0.5	3.1	1.4	0.9	64.5	0.5	4.5
		Sg	1.1	0.4	33.7	0.5	2	1.4	0.5	34.8	0.8	2.4
		SN	1.5	0.4	26.9	0.8	2.2	2.5	0.8	31	0.8	4.3
	H (cm)	**Ch**	108.4	41.4	38.1	53	236	124.8	53.7	43	61	296
		Sg	104.9	39.3	37.4	45	172	126.5	43.6	34.4	53	190
		SN	136.9	41.2	30.1	36	212	181.6	53.3	29.4	45	288
	Volumen (cm^3)	**Ch**	52	91.3	175.7	5.3	405	305.5	900.1	294.6	9.2	3896.9
		Sg	40.6	49.1	121	5	216.1	132.9	128.3	96.5	19.7	380.9
		SN	90.7	74	81.6	1.8	288.8	491.2	411.1	83.7	15.6	1790.4

P: procedencia; **Ch**: Chaqueña; **Sg**: Santiagueña; **SN**: Salta Norte; **D.E.**: desvío estándar; **C.V.**: coeficiente de variación; **DAC**: diámetro a la altura del cuello.

Los coeficientes de variación más bajos fueron los de la procedencia Salta Norte, siendo evidente que la variabilidad de cada material fue mayor en Corrientes con relación a S. Peña.

Tabla 3. Análisis de la varianza y prueba de comparaciones múltiples (Duncan) para el incremento corriente anual (ICA) en volumen (cm^3) de plantas de *Prosopis alba* de tres procedencias evaluadas en dos localidades.

	P	Media	D.E.	C.V.	Mínimo	Máximo
EEA INTA S. Peña	Ch	1060.6[a*]	1035.6	97.6	36.5	4260.1
	Sg	616.6[a]	500	81.1	34.3	2069.4
	SN	3990.8[b]	2537.3	63.6	974.1	10373.5
	p-valor	<0.0001	—	—	—	—
CDEA Corrientes	Ch	268.5[a]	833.6	310.5	0	3491.9
	Sg	92.4[a]	99.1	107.3	3.3	320.1
	SN	400.8[a]	360.6	90	3.6	1501.6
	p-valor	0.3925	—	—	—	—

P: procedencia; **Ch**: Chaqueña; **Sg:** Santiagueña; **SN**: Salta Norte; **D.E.**: desvío estándar; **C.V.:** coeficiente de variación.

*Medias con una letra distinta en cada localidad son significativamente diferentes (p<0.05).

Discusión

La supervivencia como carácter de comportamiento es influenciada por la procedencia, lo cual es debido a que presiones de selección diferentes dan lugar a distintas adaptaciones (Climent, Gil, Pérez y Pardos, 2002) que determinan la capacidad de un germoplasma para establecerse en un ambiente.

Considerando los ambientes en este ensayo, se observa que las temperaturas promedio de ambos sitios están por encima de aquellas en las procedencias. Por otra parte, el régimen pluviométrico en S. Peña fue similar al de Salta y más húmedo que en Santiago del Estero y Formosa; mientras que en Corrientes se presentaron mayores precipitaciones estacionales que en las tres procedencias geográficas en estudio. South Rose y Mcnabb (2001) mencionan que los factores que afectan el establecimiento de las plantas son las condiciones ambientales, el manejo, su morfología y su fisiología, conjuntamente con los factores genéticos modelados por la procedencia (Climent et al., 2002).

Si bien los ambientes en ambos ensayos fueron sensiblemente diferentes, la correspondencia en el *IR* indica que las tres procedencias tienen condiciones que les permiten establecerse en los sitios estudiados, más allá de las diferencias (caracteres morfológicos y calidad de planta) encontradas entre ellas en condiciones de vivero (Fontana, Pérez y Luna, 2018), las cuales tendrían efecto en su sobrevivencia.

En cuanto al éxito del establecimiento de las diferentes procedencias, Bush y Van Auken (1991) sostienen que existen dos etapas clave en el establecimiento de las plantas de algarrobo que se caracterizan por una alta mortalidad; la primera, ocurre en el establecimiento de la plántula y la segunda, en el pasaje de plántula a renoval. No obstante, una vez superada la etapa de plántula la supervivencia es alta, por ello el comportamiento durante las etapas iniciales es un buen indicador de la sobrevivencia y desempeño en campo, lo que se observó en este ensayo.

Al igual que la supervivencia, las variables dasométricas son parámetros que se incluyen en los ensayos de procedencias para estudiar el comportamiento de diferentes poblaciones en ambientes distintos y facilitan la elección del origen geográfico de la semilla más adecuado para cada sitio de plantación (López Lauenstein et al., 2015). Palacios y Brizuela (2005) consideran que la caracterización del material biológico disponible es de gran utilidad en las propuestas de reforestación con algarrobo y debe partir de germoplasma seleccionado por genotipo y productividad.

En 2011, en el marco del programa de Domesticación y mejoramiento de especies forestales nativas e introducidas para usos de alto valor (PROMEF), se establecieron en cinco sitios distintos ensayos de procedencias que incluyeron plantas originadas a partir de semillas de los rodales Salta Norte (Campo Durán, Salta), Chaqueño (Isla Cuba, Formosa) y Santiagueño (Chañar Bajada, Santiago del Estero), además de otros siete materiales (López Lauenstein et al., 2015). Las evaluaciones realizadas en estos ensayos concuerdan con la información generada en el presente trabajo, incluso en la localidad de Corrientes, donde no existen precedentes en dicha temática.

Al finalizar el primer año, en los registros de la red de ensayos (PROMEF) se destaca la procedencia Salta Norte frente a otros sitios de evaluación, tanto por diámetro como por altura de planta, sobresaliendo el sitio Laguna Yema que presentó el mayor crecimiento de las plantas de todos las procedencias, con DAC en el rango de 3.5 a 4 mm para Salta Norte y entre 1.5 y 2 mm para las procedencias Chaqueña y Santiagueña y altura total entre 2 y 2.5 m para Salta Norte y entre 1.5 y 2 m para Chaco y Santiago del Estero (López Lauenstein et al., 2012).

Estos registros son similares a los observados en el presente ensayo en S. Peña para las mismas procedencias y diferentes a los de Corrientes donde todas las variables presentaron menores valores. López Lauenstein et al. (2015) encontraron que, para todos los materiales, los mayores crecimientos ocurren en áreas con precipitaciones entre 700 y 500 mm/año (Laguna Yema –Formosa- y Fernández –Santiago del Estero) y los valores más bajos se ordenan de manera inversamente proporcional al registro pluviométrico del sitio.

Lo anterior explicaría los resultados contrastantes encontrados en el presente trabajo, así, en S. Peña, con 964 mm/año, ocurrieron altas tasas de crecimientos en DAC, altura y volumen; a diferencia, los registros en Corrientes, con 2268 mm/año, son del orden de 50% para DAC y entre 2 y 5 veces menor para altura y volumen. Cabe mencionar que las diferencias encontradas están también asociadas con las características edáficas de los sitios. Vicentini, Pérez y Rhiner (2012) observaron en Formosa que en la primera etapa de plantación el desarrollo y el crecimiento de los árboles son afectados por la textura de los suelos indicando que aquellas finas (arcillosas) resultan desfavorables en periodos secos ya que retardan el desarrollo radical.

De igual forma, Kees et al. (2017) señalan que la altura de los árboles es mayor en suelos con textura ligera. Lo anterior contrasta con los resultados encontrados en el presente trabajo para la localidad S. Peña, donde los mayores crecimientos se lograron en el sitio con suelo de textura más fina (serie Chaco). Probablemente durante los años de evaluación la interacción suelo-régimen pluviométrico posibilitó la disponibilidad de agua necesaria, sin encharcamiento ni endurecimiento del suelo, lo que determinó que en este sitio las plantas se desarrollaran mejor que en Corrientes, donde los suelos son de textura más gruesa, pero las precipitaciones están muy por encima del rango de preferencia (300 a 600, hasta 1200 mm) mencionado por Galera y Bruno (1993).

La revisión bibliográfica mostró que en los estudios consultados no se registraron variables simples como DAC y altura en el segundo año ni en el volumen y su incremento (*ICA*) en un período determinado. Por tanto, no es posible comparar los resultados en el presente trabajo con los de otros autores, no obstante sí coinciden con los hallazgos de Vilela, Brizuela y Palacios (1996) y Ledesma, De Bedia y López (2008) quienes sostienen en base a resultados experimentales, que el diámetro y la altura están correlacionadas con el genotipo de las plantas y las condiciones de crecimiento.

La predominancia de *ICA* de la procedencia Salta Norte en S. Peña es explicable a través del comportamiento en ambientes naturales del algarrobo, que presenta bajas tasas de crecimiento especialmente durante los primeros años de vida, sobre todo cuando las condiciones no son favorables, determinándose que la variabilidad climática es un factor determinante (Villagra, 2000). Bender, Araujo, Perreta y Moglia (2015) sugieren que el factor más limitante es la disponibilidad de agua ya que existe una correlación positiva entre ésta y el crecimiento en altura de la planta (Vilela et al., 1996). No obstante con el exceso de agua por encima de 1200 mm, los incrementos en este índice se reducen.

En ambos ensayos se encontró que los individuos de la procedencia Salta Norte tuvieron mejor desempeño en comparación con las demás. Este origen (Salta Norte, Campo Durán) fue seleccionado como el mejor de acuerdo con los resultados de la Red de ensayos de progenies conducidos desde 2011, y fue seleccionado como prioritario en el proceso de mejoramiento por su alto desempeño en una gran variedad de ambientes (López Lauenstein et al., 2015), entre los que se figuran Sáenz Peña y Corrientes, ambos incluidos en el presente trabajo.

Conclusiones

En el estudio no se encontraron efectos del origen de los materiales en la sobrevivencia de las plantas, siendo similares los índices de respuesta neta en ambos sitios de evaluación.

Los parámetros dasométricos fueron superiores en la procedencia Salta Norte en comparación con las procedencias Chaqueña y Santiagueña. En Sáenz Peña todas las variables dasométricas evaluadas (DAC, altura total y volumen) resultaron estadísticamente superiores, mientras que en Corrientes solamente lo fueron el DAC y altura total de planta.

Los resultados muestran que la procedencia Salta Norte se destaca como la de mejor comportamiento para los parámetros evaluados, por lo que se debe considerar de prioridad en el proceso de mejoramiento de algarrobo por su alto desempeño en diferentes ambientes.

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