Can biostimulants and grafting alleviate salinity stress in purple passion fruit (*Passiflora edulis* f. *edulis* Sims)?

¿Pueden los bioestimulantes y la injertación mitigar el estrés por salinidad en gulupa (*Passiflora edulis* f. *edulis* Sims)?

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ABSTRACT

Purple passion fruit crops are affected by salinity conditions in productive systems. The aim of this research was to evaluate the effect of the application of *Ascophyllum nodosum* extract on salinity stress in purple passion fruit plants at the vegetative stage of growth with and without grafting. Eight treatments were evaluated corresponding to the combination of grafting or non-grafting on the *Passiflora maliformis* rootstock, the presence or absence of salt stress, and the application or not of the *A. nodosum* biostimulant. Physiological and growth parameters were evaluated. Salinity significantly decreased (P<0.05) growth and stomatal conductance (g_s), increased contents of photosynthetic pigments and did not affect the chlorophyll *a* fluorescence. The grafted plants presented a positive response (P<0.05) in chlorophyll relative contents (~63 SPAD units) and shoot length (~106 cm); lower g_s (~163 mmol H_2O $m^{-2}s^{-1}$), number of leaves (~43 leaves) and root weight (3.5 g of dry weight), and no change in chlorophyll *a* fluorescence. The biostimulant mitigated the salinity effect on g_s and photosynthetic pigments. In the case of salinity, non-grafted purple passion fruit may present a better performance at the vegetative stage, and the biostimulant can have a slight mitigation effect on salt stress. However, if it is essential to use grafted plants for sanitary reasons, the evaluated salinity does not affect them drastically.

Key words: *Passiflora maliformis*, *Ascophyllum nodosum*, water status, chlorophyll fluorescence.

RESUMEN

En los sistemas productivos de gulupa se presentan condiciones de salinidad que afectan el cultivo. El objetivo de este trabajo fue evaluar el efecto de la aplicación de un extracto de *Ascophyllum nodosum* sobre el estrés salino en plantas de gulupa en etapa vegetativa de crecimiento con y sin injertación. Se evaluaron ocho tratamientos correspondientes a la combinación de la injertación o no sobre el patrón de *Passiflora maliformis*, la presencia o no de estrés salino, y la aplicación o no del bioestimulante *A. nodosum*. Se evaluaron parámetros fisiológicos y de crecimiento. La salinidad disminuyó significativamente (P<0,05) el crecimiento, conductancia estomática (g_s), incrementó los pigmentos fotosintéticos y no afectó la fluorescencia de clorofila *a*. Las plantas injertadas obtuvieron una respuesta positiva (P<0,05) en contenido de clorofila (~63 unidades SPAD) y longitud de la parte aérea (~106 cm), presentaron menor g_s (~163 mmol H_2O $m^{-2}s^{-1}$), número de hojas (~43 hojas) y peso de raíces (3,5 g de peso seco), pero no afectaron fluorescencia de la clorofila *a*. El bioestimulante mitigó el efecto de la salinidad en g_s y pigmentos fotosintéticos. En el caso de salinidad, las plantas de gulupa sin injertar pueden presentar un mejor desempeño en etapa vegetativa, y el bioestimulante puede presentar un leve efecto en la mitigación del estrés salino. Sin embargo, si es indispensable utilizar plantas injertadas por causas sanitarias, la salinidad evaluada no las afecta drásticamente.

Palabras clave: *Passiflora maliformis*, *Ascophyllum nodosum*, estatus hídrico, fluorescencia de la clorofila.

Introduction

The purple passion fruit (*Passiflora edulis* f. *edulis* Sims), is a fruit crop from Brazil, belonging to the Passifloraceae family and cultivated in various Latin American countries due to their edaphoclimatic conditions (Armas Costa *et al.*, 2022). In Colombia, there are suitable conditions for cultivation of this fruit crop at elevations between 1,400 to 2,200 m a.s.l., temperatures from 15°C to 20°C, and minimum annual rainfall of 900 mm (Rodríguez-Polanco *et al.*, 2022). In 2022, Colombia had a national production of 32,353.78 t of the fruits harvested from the area of 2,059.47 ha (Agronet, 2023), with exports of 14600 t, representing a commercial value of USD 45.8 million FOB (Free on board). In 2023, this crop was the fifth most highly exported fruit from Colombia (ANALDEX, 2023). Currently, it is an important product in the international market due to its nutraceutical properties, presenting significant contents of vitamins A, B3, B12, and C, ascorbic acid, minerals, carbohydrates, proteins, and antioxidants (Armas Costa *et al.*, 2022).

Changes in climatic conditions resulting from climate change have a strong impact on the increase of soil salinity

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(Corwin, 2021). Soil salinization is the accumulation of salts in soil due to mineral deposition, capillary rise of groundwater, or agricultural and industrial practices (Eswar *et al*., 2021; Okur & Örçen, 2020). This condition has an adverse impact on the productivity and quality of purple passion fruit, which can be sensitive to salinity (Moura *et al*., 2019). In addition, purple passion fruit is cultivated in greenhouses with plastic coverings and in semi-covered conditions, using a fertigation system (Ocampo *et al*., 2020). These cultivation techniques increase salinity problems in this production system. Among the physiological impacts that are generated in plants under saline stress are osmotic stress, ionic stress, nutritional imbalance, and oxidative stress (Okon, 2019; Ullah *et al*., 2021). Collectively, these effects lead to a decrease in the hormone contents of auxins, gibberellins, cytokinins and to an increase in abscisic acid and ethylene, which reduces cell expansion, increases leaf abscission and stomatal closure, reduces nitrogen assimilation, decreases biomass of shoots and roots, and affects growth, development, and yields (Ahmad *et al*., 2022; Okon, 2019; Ullah *et al*., 2021).

Soil is saline if its electrical conductivity (EC) is greater than 4.0 dS m^{-1}, which is equivalent to 40 mM of NaCl. Na^+ is the most common ion that generates soil salinity, along with, but to a lesser extent, other ions such as Cl^- and SO_4^{-2} (Ahmad *et al*., 2022; Ullah *et al*., 2021). It has been established that plant tolerance to soil salinity decreases when EC exceeds 2.0 dS m^{-1} (Ahmad *et al*., 2022; Ullah *et al*., 2021). In *Passiflora*, saline stress with an EC greater than 1.4 dS m^{-1} in irrigation water is a limiting factor during the formation of seedlings and decreases growth, photochemical efficiency, photosynthetic pigment contents, and biomass production (Andrade *et al*., 2022; Bezerra *et al*., 2016; Nascimento *et al*., 2017).

Various strategies have been evaluated to mitigate the adverse effects generated by salinity in crops (Khalid *et al*., 2023). Among these strategies is the use of biostimulants, which are substances other than nutrients that benefit plants and can increase yield and quality of crops, photosynthetic activity, availability and absorption of soil nutrients, and tolerance to biotic and abiotic stress factors. Biostimulants can be classified depending on their mode of action and active component (Ahmad *et al*., 2022; Rakkammal *et al*., 2023). These biostimulants can be obtained from plants and/or algae extracts, including, in the case of vascular plants, leaves, roots, stems, and other organs (Ahmad *et al*., 2022). One kind of biostimulant is produced in algae, which are autotrophic organisms that can synthesize a large number of secondary metabolites. The extract of the *Ascophyllum nodosum* algae has been reported as a promoter of growth and yield increments in crops. Its action is based on triggering specific metabolic pathways in treated plants and providing organic compounds that may have various effects on plant metabolism (Ahmad *et al*., 2022; Carillo *et al*., 2020). *Ascophyllum nodosum* acts through various metabolic pathways that enable the activation of mechanisms of tolerance to salinity. These can include increases in enzymatic and non-enzymatic antioxidants, essential amino acids, K/Na ratio, beneficial mineral elements, and growth parameters (Carillo *et al*., 2020).

The use of biostimulants in various crops generates favorable effects on salinity tolerance. This includes the use of biostimulants elaborated from marine plants and algae on *Lactuca sativa* cultivated under saline conditions (40 mM NaCl), where increases of 9% and 18% of fresh weight under saline and non-saline conditions were obtained, respectively (Rouphael *et al*., 2022). Additionally, the application of hydrolyzed proteins together with *Ascophyllum nodosum* in *Solanum lycopersicum* plants generated a 31.8% increase in fruit yield under saline conditions and 16.9% increase under non-saline conditions (Ikuyinminu *et al*., 2022). There are some studies on physiological responses and impacts under saline stress in various passion fruit crops (Lima *et al*., 2023; Moura *et al*., 2020; Souza *et al*., 2018), but these studies are scarce for purple passion fruit.

Grafting is a rapid and non-chemical alternative to overcome the adverse effect of salinity (Mozafarian *et al*., 2023). In this respect, autografted *P. edulis* under salt stress conditions develops vital mechanisms that attenuate the effects of salinity (Moura *et al*., 2020). The purple passion fruit is commercially grafted on a rootstock of *P. maliformis* as a strategy to tolerate *Fusarium oxysporum* f. sp. *passiflorae* (Forero *et al*., 2015; López *et al*., 2023). It has been reported that water deficit reduces the growth of purple passion fruit grafted on *P. maliformis* (Jiménez-Bohorquez *et al*., 2024), but the effect of grafting on the growth of purple passion fruit under saline stress is almost unknown.

Given the above, the objective of the present study was to evaluate the effect of the application of an *Ascophyllum nodosum* extract on saline stress in grafted and non-grafted purple passion fruit plants (*Passiflora edulis* f. *edulis* Sims) at the vegetative stage of growth, considering variables of growth and physiology.

Materials and methods

Establishment of the experiment and plant material

The experiment was conducted at the greenhouses of the Faculty of Agricultural Sciences of the Universidad

Nacional de Colombia, Bogota (4°35'56" N, 74°04'51" W) at an altitude of 2650 m a.s.l., with temperature and average relative humidity of 19.8°C and 66.4%, respectively. The plant material consisted of 3-month-old purple passion fruit seedlings propagated by seeds (*Passiflora edulis* f. *edulis* Sims). Additionally, purple passion fruit plants were grafted as a terminal graft on *Passiflora maliformis* rootstock (propagated by seeds), both species being 3 months of age and grafted 45 d after germination. *Passiflora maliformis* is the main rootstock used in Colombia for the control of *Fusarium oxysporum* f. sp. *passiflorae* (Forero *et al*., 2015; Rodríguez *et al*., 2020). The plants were transplanted into polyethylene pots with dimensions of 20 cm x 20 cm x 20 cm containing a mixture of soil and Pindstrup® peat in a ratio 2:3 (v/v). The properties of these substrates are presented in Table 1.

Water was supplied to the plants using the gravimetric method adapted from Segura-Castruita *et al*. (2011), and fertilization was carried out with Nutriponic® (Walco S.A.S, Bogotá, Colombia) in a 5% v/v solution twice per week before the start of treatments, with a dose per plant of 15 ml during the first 10 d after transplant (DAT), 32 ml per plant between 10 and 20 DAT and 50 ml up to 30 DAT. Nutriponic® has the following composition: 40.3 g L^{-1} of NO_3^-, 4.0 g L^{-1} of NH_4^+, 20.4 g L^{-1} of P_2O_5, 50.6 g L^{-1} of K_2O, 28.8 g L^{-1} of Ca, 11.4 g L^{-1} of Mg, 1 g L^{-1} of S, 1120 mg L^{-1} of Fe, 112 mg L^{-1} of Mn, 12 mg L^{-1} of Cu, 26.4 mg L^{-1} of Zn, 106 mg L^{-1} of B, 1.2 mg L^{-1} of Mo, 0.36 mg L^{-1} of Co. After the start of the treatments, fertilizations were carried out twice a week with Nutriponic® and each plant was additionally fertilized with 50 ml of $MgSO_4$ and KNO_3 solution, where 47.8 g and 93.6 g were diluted, respectively, in 1 L of water.

Experimental design and treatments

A completely randomized design was used, with eight treatments (Tab. 2) that corresponded to the combination of the two levels of grafting (without grafting and with grafting), two levels of saline stress (without stress, application of saline solution at 5.0 dS m^{-1}), and the application or non-application of biostimulant (based on *Ascophyllum nodosum* at a dose of 1.5 g L^{-1}). The *Ascophyllum nodosum* extract corresponded to a product with registration number 451-F-AGR-P of the Acadian Plant Health and QSI Ecuador S.A. (Ecuador). Each treatment had 5 replicates, giving a total of 40 experimental units, each composed of one plant.

The saline stress consisted of subjecting plants to a constant stress period at 36 DAT and applying a dose of NaCl to maintain a constant electrical conductivity of 5.0 dS m^{-1} in

TABLE 1. Physicochemical characteristics of soil and composition of peat and biostimulant.

Parameter	Soil	Pindstrup® peat *	Biostimulant
Dry matter	-	55-75 g L^{-1}	-
Texture	Sandy loam	-	-
C organic	7.83%	-	-
Organic matter	-	70%	50%**
ECEC	7.48 meq 100 g^{-1}	-	-
pH	5.31	5.5-6	-
EC	-	1.0 mS cm^{-1}	-
N-total	0.66%	120 g	1%
N-ammonium	-	50 g	-
N-nitric	-	70 g	-
P	12 mg kg^{-1}	140 g	1%
K	0.83 meq 100 g^{-1}	240 g	20%
Ca	4.7 meq 100 g^{-1}	-	1%
S	90.1 mg kg^{-1}	-	-
Mg	0.99 meq 100 g^{-1}	23 g	1%
B	0.73 mg kg^{-1}		-
Cu	0.70 mg kg^{-1}		-
Mn	1.08 mg kg^{-1}	Traces	0.9%
Fe	10 mg kg^{-1}		0.01%
Zn	0.46 mg kg^{-1}		0.01%
Na	0.23 meq 100g^{-1}	-	-
Al	0.73 meq 100g^{-1}	-	-

*Nutrient content in 300 L of peat. **100% Pure and natural seaweed extract (*Ascophyllum nodosum*).

the solution. The application of the *Ascophyllum nodosum* extract began at 14 DAT. This consisted of 4 applications as a preventive measure before the start of saline stress, followed by continued application every 7 d from 32 DAT to the end of the experiment, with a dose of 1.5 g L^{-1}, 60 ml per plant in drench + foliar application with the adjuvant Mixel Top® until reaching total coverage.

TABLE 2. Description of treatments.

Treatment	Description
1	Non-grafted - salinity + *A. nodosum*
2 (control)	Non-grafted - salinity - *A. nodosum*
3	Non-grafted + salinity + *A. nodosum*
4	Non-grafted + salinity - *A. nodosum*
5	Grafted - salinity + *A. nodosum*
6	Grafted - salinity - *A. nodosum*
7	Grafted + salinity + *A. nodosum*
8	Grafted + salinity - *A. nodosum*

The growth variables and physiological variables were measured at the end of the experiment, that is 53 d after the salinity treatments began.

Growth variables

The aerial part length, the number of leaves, the fresh weights of roots, aerial part and whole plant were measured. Additionally, the leaf area (LA) was calculated using the foliar area meter LI-3100 (LI-COR Inc., Lincoln, NE, USA).

Physiological variables

Stomatal conductance

Stomatal conductance (mmol H_2O m^{-2} s^{-1}) was measured with a leaf porometer (SC-1, Decagon Devices Inc., Pullman, WA, USA). The measurements were made between 9:00 am and 10:00 am on leaves of the middle part, which were different from those used for fluorescence and chlorophyll contents.

Contents of chlorophylls and carotenoids

The relative chlorophyll content was measured with a chlorophyll meter (SPAD 502 plus, Konica Minolta, Japan) on 10 completely expanded leaves per plant from the middle strata. The extraction and quantification of total carotenoids and total chlorophylls were performed with acetone according to López-Gómez *et al.* (2015) and Wellburn (1994), using 300 mg of leaf tissue (M) and a final volume of 20 ml (V). Absorbance (A) reading was done with a spectrophotometer (Spectronic BioMate 3 UV–vis, Thermo, Madison, WI, USA) at wavelengths of 647 and 663 nm. To calculate the content of chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), and total chlorophyll (Chl Total), Equations 1, 2, and 3 were applied, respectively.

$$\text{Chl a (mg g}^{-1}) = \frac{[(12.25 \times A_{663})-(2.79 \times A_{647})]\times V}{1000\times M} \quad (1)$$

$$\text{Chl b (mg g}^{-1}) = \frac{[(21.5 \times A_{647})-(5.1 \times A_{663})]\times V}{1000\times M} \quad (2)$$

$$\text{Chl Total (mg g}^{-1}) = \frac{[(7.15 \times A_{647})+(18.7 \times A_{663})]\times V}{1000\times M} \quad (3)$$

The results were expressed in mg g^{-1} of fresh weight (FW). For the quantification of total carotenoids, a calibration curve was carried out with different concentrations of β-carotene (Sigma-Aldrich Co., Nueva York, USA) and was measured in absorbance at 450 nm. Then, Equation 4 was used to establish the total carotenoid content.

$$\text{Total carotenoids } (\mu\text{g g}^{-1}\text{ FW}) = \frac{(A_{450} - b) \times V}{m \times M} \quad (4)$$

Chlorophyll a fluorescence

Chlorophyll *a* fluorescence was determined with the fluorometer JUNIOR-PAM (Walz®, Germany), on a fully expanded leaf from the middle third of the plant, subjected to 30 min of darkness, evaluating the electron transport rate (ETR), the photochemical quenching (QP) and non-photochemical quenching (QNP), and the maximum quantum efficiency of the photosystem II (*Fv/Fm*).

Statistical analysis

The data obtained were evaluated for normality and homoscedasticity through the Shapiro-Wilk and Levene tests, respectively. The statistical analysis was carried out using one-way analysis of variance and the treatment comparisons were carried out using the LSD test ($P<0.05$). All analyses were performed in the R 4.3.1 software.

Results and discussion

Growth parameters

Grafted plants without biostimulant applications and under salinity presented a greater length ($P<0.05$), while non-grafted plants without salinity and with and without *A. nodosum* generated the lowest response (Fig. 1A). On the other hand, non-grafted plants under salinity with biostimulants obtained a greater number of leaves ($P<0.05$), while the opposite trend occurred with plants with biostimulants and without salinity.

Regarding leaf area, non-grafted plants under salinity and without the application of *A. nodosum* presented a greater leaf area ($P<0.05$), while grafted plants with salinity and without application of *A. nodosum* presented a lower leaf expansion. Intermediate results were obtained for the control plants (Fig. 2).

For the weight of the aerial part, grafted plants without salinity and with the application of *A. nodosum* presented the greatest response (P<0.05), in contrast to the non-grafted plants with salinity and the application of *A. nodosum,* which obtained lower fresh weight of the aerial part. Intermediate results were obtained for the control plants (Fig. 3B). Regarding root fresh weight, the plants with the highest fresh weight (P<0.05) were those not grafted, regardless of the presence of salinity or *A. nodosum*, while those grafted had lower root fresh weights (P<0.05) (Fig. 3A). On the other hand, the non-grafted plants with salinity and without application of a biostimulant had a higher root dry weight (P<0.05) than the plants under the same treatment with grafting (Fig. 3C).

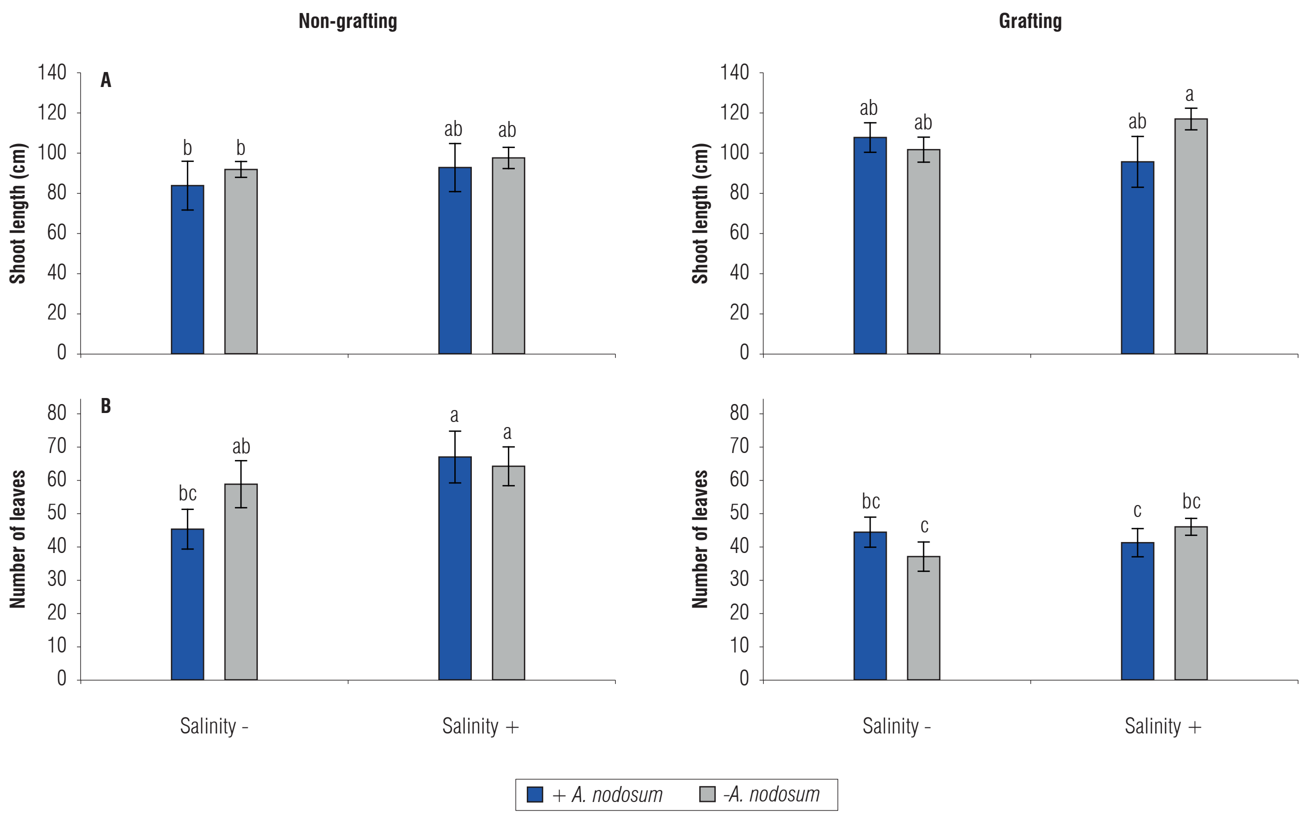

FIGURE 1. Effects of grafting and application of *Ascophyllum nodosum* on purple passion fruit plants subjected to salinity on (A) shoot length and (B) number of leaves. Averages followed by different letters indicate statistical differences according to the LSD test (P<0.05). Vertical bars in each column indicate the standard error (n=5).

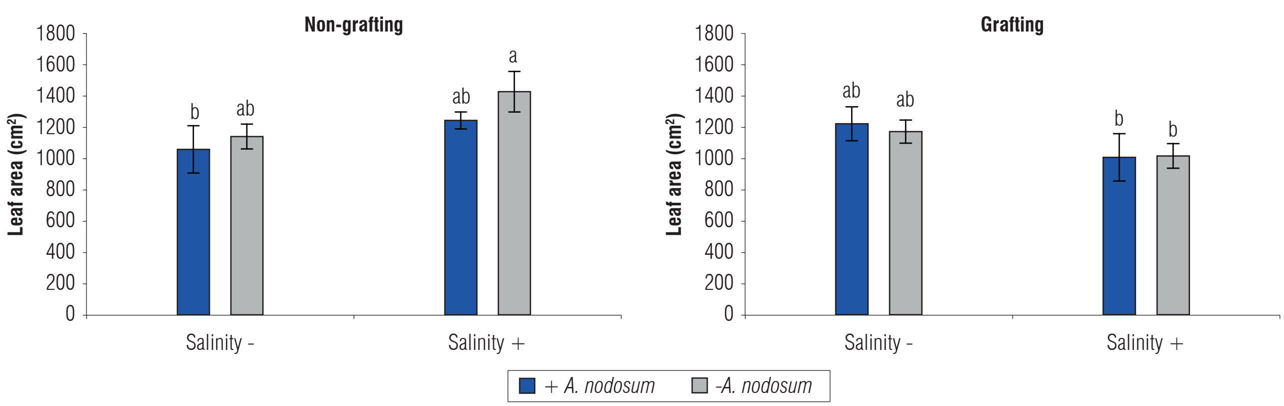

FIGURE 2. Effects of grafting and application of *Ascophyllum nodosum* on leaf area in purple passion fruit plants subjected to salinity. Averages followed by different letters indicate statistical differences according to the LSD test (P<0.05). Vertical bars in each column indicate the standard error (n=5).

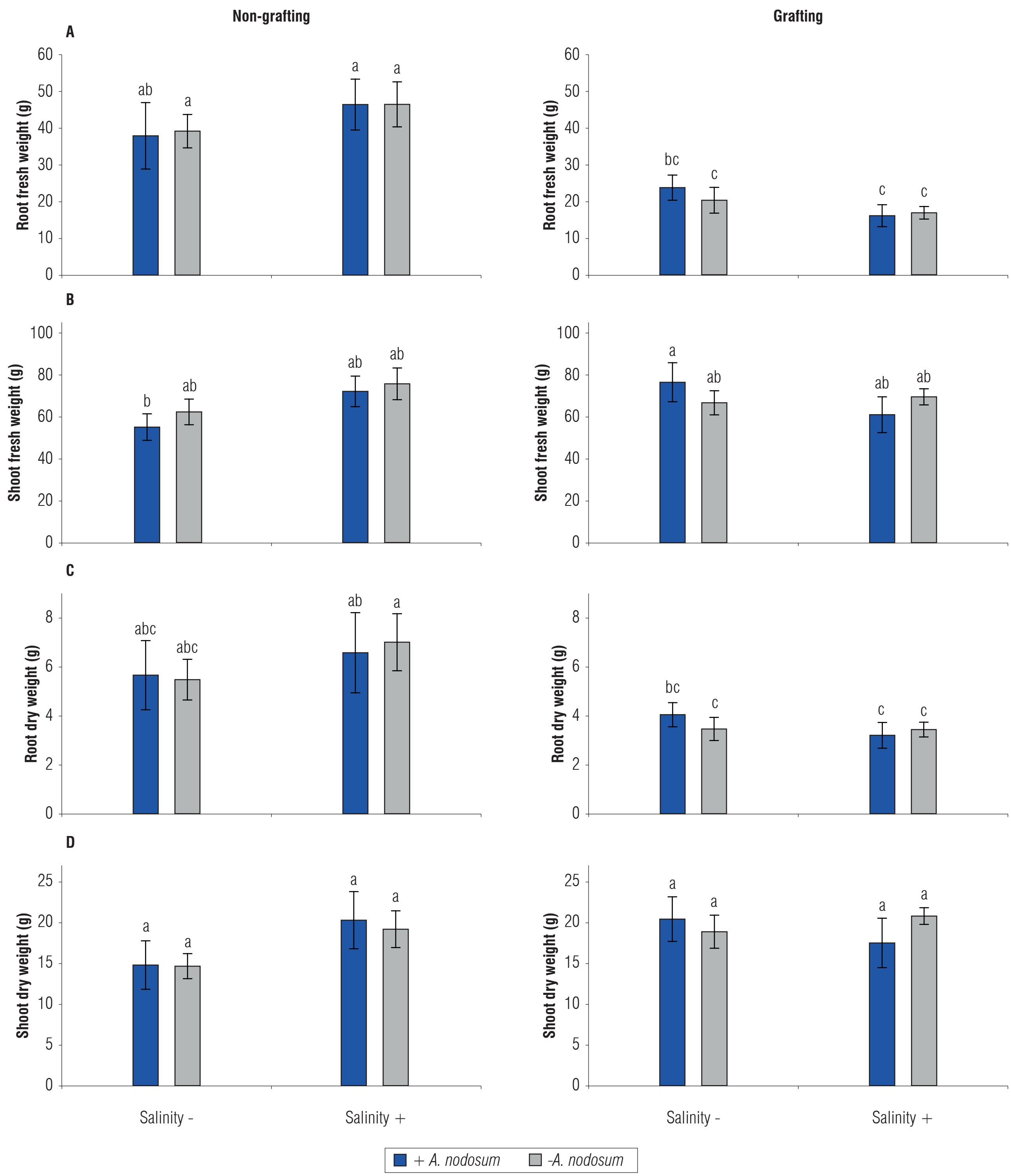

FIGURE 3. Effect of grafting and application of *Ascophyllum nodosum* in purple passion fruit plants subjected to salinity on (A) root fresh weight, (B) shoot fresh weight, (C) root dry weight, and (D) shoot dry weight. Averages followed by different letters indicate statistical differences according to the LSD test ($P<0.05$). Vertical bars in each column indicate the standard error (n=5).

A notable effect of grafting on shoot length was found. Apparently, the pattern of the *P. maliformis* generates a positive response in growth even in salinity conditions and without *A. nodosum* application. This is crucial to consider, as this rootstock also presents resistance to *Fusarium oxysporum* f. sp. *passiflorae,* and it was for this reason that it started to be used commercially as a rootstock in Colombia (Forero *et al*., 2015; López *et al*., 2020). However, the mechanism by which *P. maliformis* generates this response is unknown. These results differ from those obtained by Moura *et al*. (2020), where no significant differences were established for this variable in grafted plants of different passion fruit species under saline stress. However, a reduction in plant height was present at a concentration of 4.5 dS m^{-1}, indicating a differential response to salinity between passion fruit (*P. edulis*) and purple passion fruit (*P. edulis* f. *edulis* Sims).

Regarding the number of leaves, it was established that salinity, together with *A. nodosum,* increases the number of leaves in non-grafted plants, and that the non-grafted plants presented a greater number of leaves than the grafted plants (Fig. 1B). This response indicates a noticeable effect of the rootstock, leading to fewer leaves, which is an aspect that should be studied in greater depth. It has been found earlier that salinity in non-tolerant plants impacts plant growth by altering transpiration and stomatal conductance and generating high concentrations of Na and Cl that accumulate in the cytosol, affecting the expansion of mature leaves. Moreover, there is early senescence, abscission and reduction in formation of young leaves and lateral buds, which reduces the number of leaves (Carillo *et al*., 2020; Okon, 2019). This occurred in the purple passion fruit plants grafted on *P. maliformis*, while *P. edulis* plants have shown tolerance under electrical conductivity up to 4.7 dS m^{-1} in irrigation (Moura *et al*., 2016). Moura *et al*. (2020), for autografted *P. edulis* plants, found an increase in the number of leaves under salinity since the graft attenuated the salinity effects. On the other hand, Bonomelli *et al*. (2018) established that the application of *A. nodosum* on avocado plants under salt stress increased the plant height and number of leaves, in contrast to treatments with salinity. *A. nodosum* may promote plant growth under salinity by stimulating antioxidant responses and increasing the K/Na ratio in plants (Carillo *et al*., 2020).

Plants grafted under salinity presented the lowest leaf area, which correlates with the number of leaves (Fig. 2), but a higher shoot length (Fig. 1A), indicating greater internode length. The leaf area is decreased in non-tolerant plants to reduce water loss through transpiration and also to reduce the consumption of photosynthates, metabolic production (Moura *et al*., 2020), and accumulation of toxic ions in shoots (Lima *et al*., 2020). This coincides with what was reported by Bezerra *et al*. (2019), where passion fruit plants subjected to salinity presented a reduction in leaf area. Meanwhile, the larger leaf area found in non-grafted plants under salinity (Fig. 2) may be due to a possible beneficial effect of Na, a result not found in grafted plants. It is recommended to study this in more detail. In this case, *A. nodosum* had no mitigating effect on salinity, nor did it stimulate leaf area in plants without salinity.

While the highest shoot fresh weight was obtained with the application of *A. nodosum* in grafted plants without salinity (Fig. 3B), the fresh and dry weight of the root was higher in non-grafted plants than in grafted plants (Fig. 3A, 3C). This indicates a greater growth potential in purple passion fruit roots compared to *P. maliformis*, a result that warrants further attention in future studies. On the other hand, salt stress generates lesser growth and, therefore, reduced shoot and root weight (Rakkammal *et al*., 2023) due to the osmotic stress, stimulation of synthesis and accumulation of aminocyclopropane 1-carboxylic acid generated from the accumulation and absorption of toxic ions and the decrease in the foliar content of cytokinins and indole-3-acetic acid (Okon, 2019). Moura *et al*. (2019) found no significant differences in the dry weights of the aerial part and the root in *P. edulis* plants under salt stress. Likewise, Sá *et al*. (2018) found an increase in the fresh weight of the aerial part in passion fruit plants subjected to salt stress with biostimulant application.

Stomatal conductance

The grafted plants subjected to salinity and application of *A. nodosum* presented a lower stomatal conductance g_s (P<0.05) than non-grafted plants under biostimulant treatment without salinity (control), which had higher stomatal conductance (Fig. 4B). Low water availability and osmotic stress generate stomata closure, preventing water loss through transpiration (Jiménez-Bohórquez *et al*., 2024; Lozano-Montaña *et al*., 2021). This occurred in grafted plants under salinity, gradually increasing their temperature (Andrade *et al*., 2022; Okon, 2019). Similarly, Lima *et al*. (2020) and Lima *et al*. (2023) found a reduction in stomatal conductance in *P. edulis* plants subjected to salt stress. In contrast, Al-Ghamdi and Elansary (2018) obtained an increase in stomatal conductance in asparagus plants subjected to salt stress with applications of *A. nodosum* due to an increase in phenolic content, antioxidant activities, chlorophyll, sugars, and proline. The grafted purple passion fruit showed the lowest stomatal conductance, possibly because the rootstock (*P. maliformis*) induces some effect on the stomatal closure of the graft to avoid excessive water loss due to transpiration, potentially increasing ABA levels. This response is evident in plants with and without salinity.

Photosynthetic pigments

Grafted plants had higher relative chlorophyll contents ($P<0.05$) than non-grafted plants with and without salinity or *A. nodosum* (Fig. 4). Regarding photosynthetic pigments, higher concentrations of Chl a, Chl b, total Chl and total carotenoids ($P<0.05$) were observed in grafted and non-grafted plants subjected to salinity and with applications of *A. nodosum* (Fig. 5A).

Under salt stress, a negative effect on photosynthetic pigments is expected (Arif *et al*., 2020; Okon, 2019); however, our results show the opposite. Chlorophyll content can be considered a biochemical marker of tolerance, with plants that maintain or increase their chlorophyll content under high salinity concentrations being tolerant (Moura *et al*., 2020). This possibly occurred in non-grafted plants, whereas in grafted plants, increasing chlorophyll content may be a "concentration effect" because these plants have a lesser leaf area (Fig. 2). The biostimulant *A. nodosum* contains high levels of K, Ca, and proline, and generates greater activity of antioxidant enzymes, leading to reduced osmotic stress and salt stress through the elimination of reactive oxygen species (ROS), thus, allowing the plants to improve their physiological response and resistance to salinity (Rakkammal *et al*., 2023).

Moura *et al*. (2020) reported that grafted *P. edulis* plants subjected to salt stress had a reduction in chlorophyll contents. Likewise, Andrade *et al*. (2022) found a reduction in chlorophyll *a* concentration and an increase in chlorophyll *b* and total carotenoids in passion fruit plants subjected to salt stress. Furthermore, Al-Ghamdi and Elansary (2018) obtained an increase in photosynthetic pigments in asparagus plants subjected to salt stress with *A. nodosum*.

Chlorophyll *a* fluorescence

Regarding the electron transport rate (ETR), non-photochemical quenching (QNP), and maximum quantum efficiency of photosystem II (*Fv/Fm*), there were no significant differences between the treatments (Fig. 6), while the photochemical quenching (QP) presented a greater value ($P<0.05$) in the grafted plants without salinity and

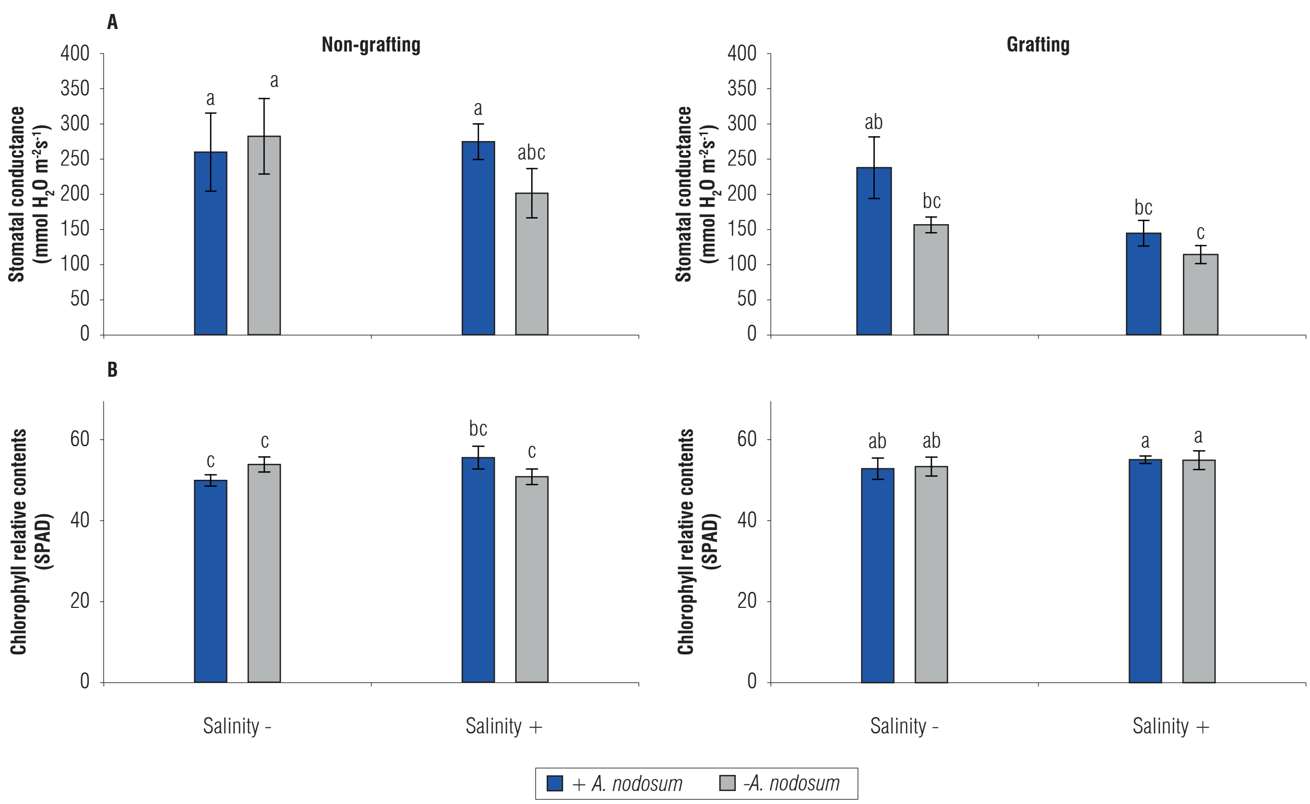

FIGURE 4. Effect of grafting and application of *Ascophyllum nodosum* in purple passion fruit plants subjected to salinity on (A) stomatal conductance (g_s), and (B) chlorophyll relative contents (SPAD units). Averages followed by different letters indicate statistical differences according to the LSD test ($P<0.05$). Vertical bars in each column indicate the standard error (n=5).

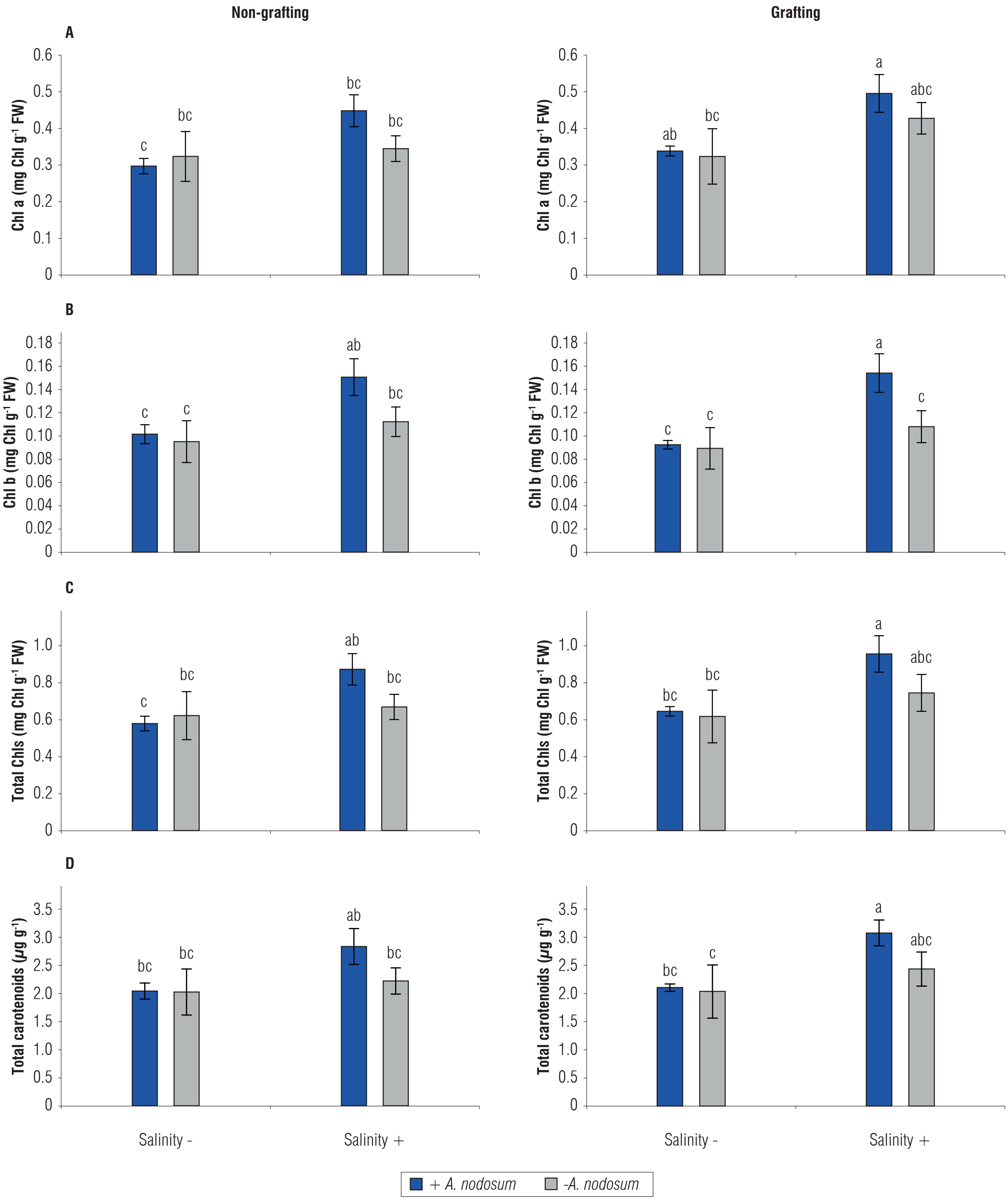

FIGURE 5. Effect of grafting and application of *Ascophyllum nodosum* in purple passion fruit plants subjected to salinity on (A) chlorophyll *a* (Chl a), (B) chlorophyll *b* (Chl b), (C) total chlorophylls (Total Chls), and (D) total carotenoids. Averages followed by different letters indicate statistical differences according to the LSD test ($P<0.05$). Vertical bars in each column indicate the standard error (n=5).

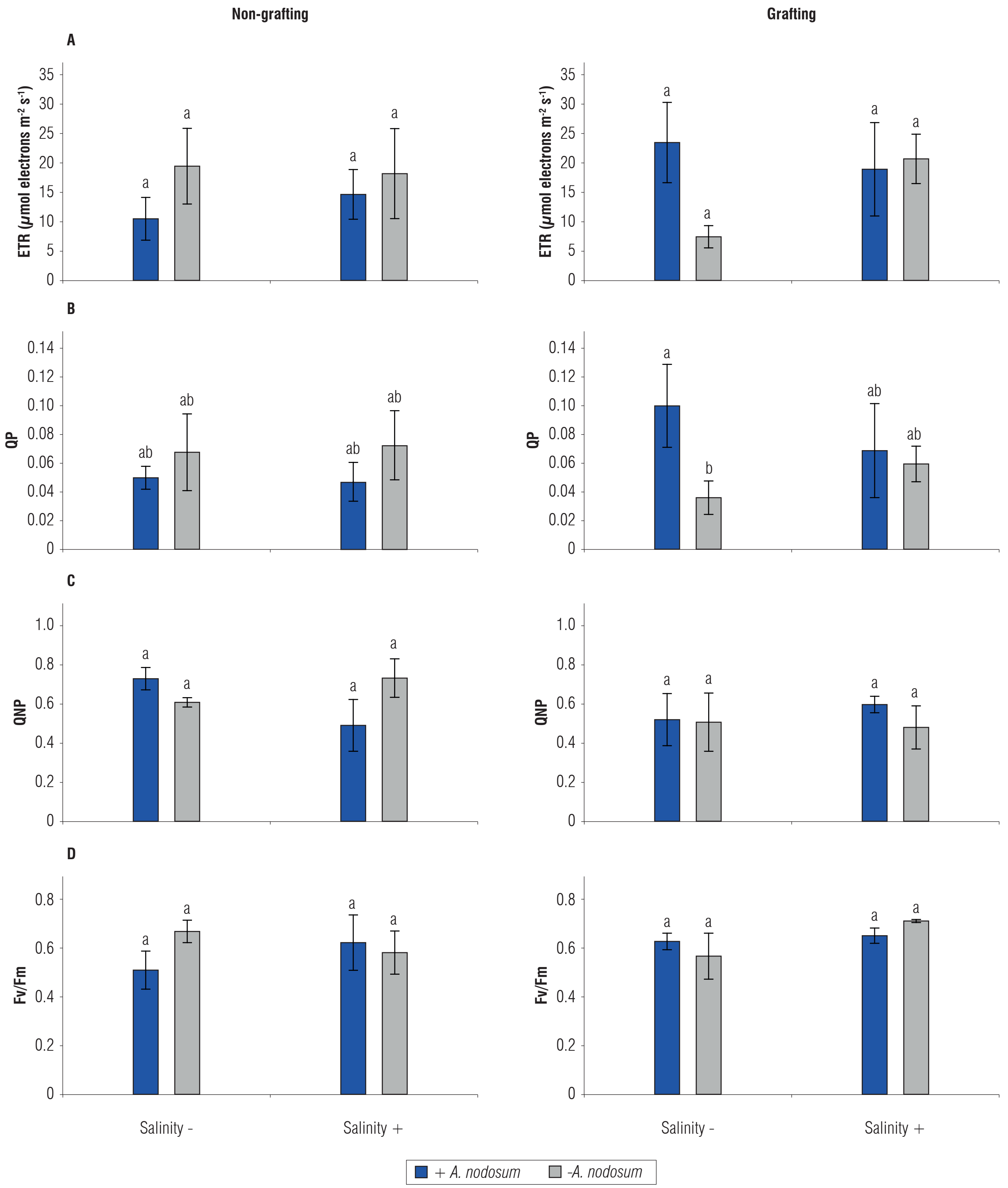

FIGURE 6. Effect of grafting and application of *Ascophyllum nodosum* in purple passion fruit plants subjected to salinity on (A) electron transport rate (ETR), (B) photochemical quenching (QP), (C) non-photochemical quenching (QNP), and (D) the maximum quantum efficiency of the photosystem II (*Fv/Fm*). Averages followed by different letters indicate statistical differences according to the LSD test ($P<0.05$). Vertical bars in each column indicate the standard error (n=5).

with applications of *A. nodosum* than in the grafted plants without salinity and without *A. nodosum*. The control plants showed an intermediate response (Fig. 6B).

The reductions in the fluorescence parameters occur due to damage in the PSII of the plants. In this case, the salt stress applied to purple passion fruit does not alter PSII, indicating adequate functioning of PSII and stability of the photosynthetic apparatus under salt stress (Guedes *et al*., 2023). Interestingly, in grafted plants, the biostimulant tended to increase QP, which may be due to antioxidant mechanisms induced by *A. nodosum*. This is consistent with Guedes *et al*. (2023), there was an increase in QP and ETR and a non-significant increase in *Fv/Fm* in passion fruit plants subjected to salt stress with the application of fertilizers.

Conclusions

These results suggest that purple passion fruit without grafting exhibit tolerance to salt stress (5.0 dS m^{-1}). This plant showed good performance in growth parameters such as leaf number, leaf area, shoot and root weights, and g_s (Fig. 7). Meanwhile, the grafted plants with *A. nodosum*

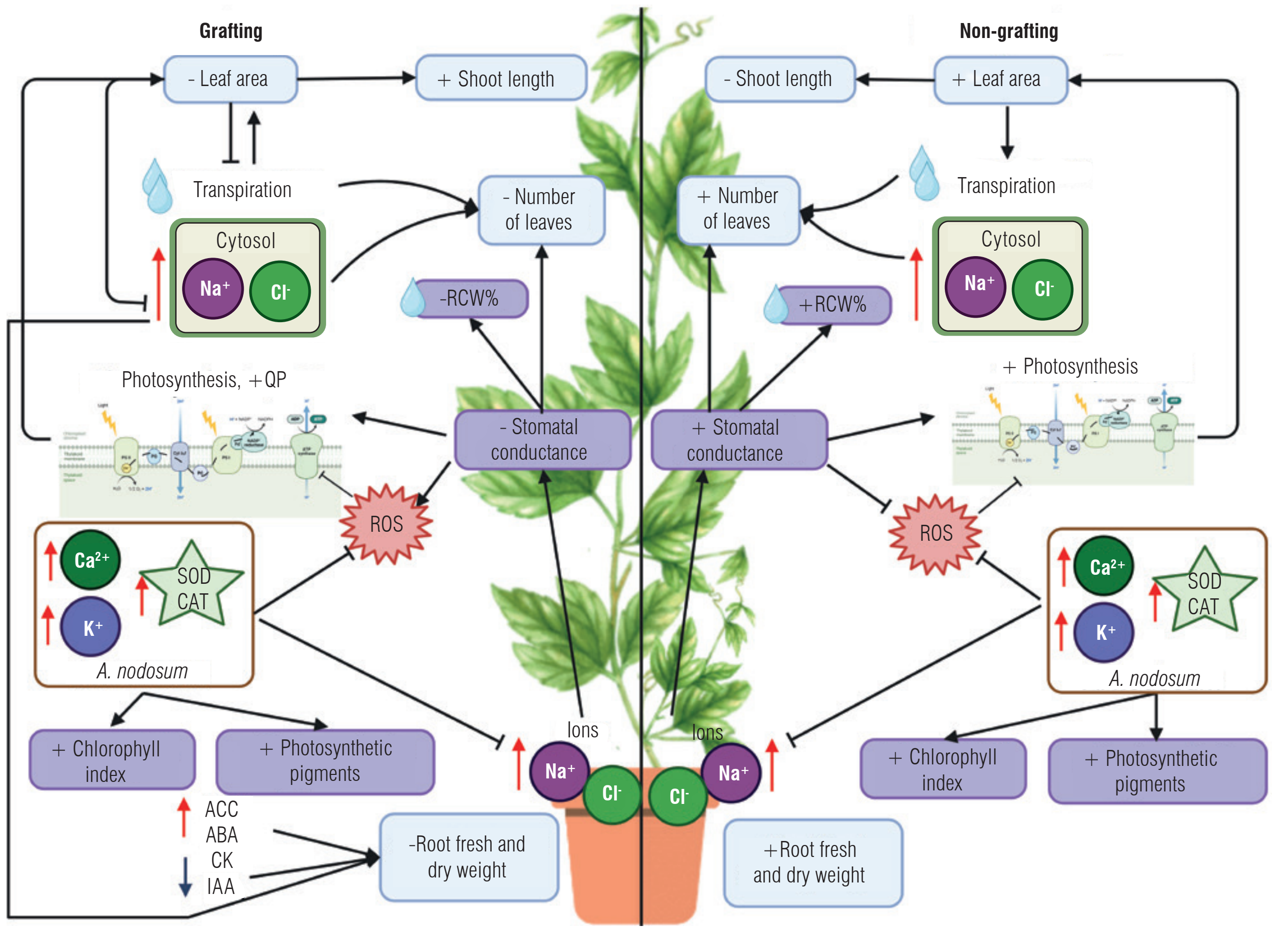

FIGURE 7. Model of the effect of grafting and application of *Ascophyllum nodosum* on purple passion fruit plants subjected to salinity. Left) Plants grafted on *P. maliformis*, Right) plants without grafting. In both cases, salinity initially generated osmotic stress, reducing water availability due to a decreased osmotic potential in soil solution. This caused a lower stomatal conductance in the grafted plants compared to the non-grafted plants, leading to a gradual increase in temperature in non-grafted plants. However, non-grafted plants presented a higher relative water content (RCW) and a lower inhibition of photosynthesis than the grafted plants. Subsequently, ionic stress was generated that harmed leaf expansion, increased leaf abscission, and impaired photosynthesis, especially in grafted plants. This stress indirectly benefits the plants by reducing water loss through transpiration. Lesser leaf emission is also related to the stimulation of synthesis of abscisic acid (ABA), and aminocyclopropane carboxylic acid (ACC) and to the decrease in the foliar content of cytokinins (CK) and indole acetic acid (IAA). Oxidative stress may be induced by the increased reactive oxygen species (ROS) production, impacting the permeability of the plasma membrane and photosynthesis. Finally, photosynthetic pigment contents were reduced, possibly due to the nutritional imbalance. The biostimulant *A. nodosum*, with its proline content and greater antioxidant enzyme activity, likely reduced osmotic and salt stress by eliminating ROS, thereby enhancing physiological response and resistance to salinity in non-grafted plants. In grafted plants, *A. nodosum* increased the contents of chlorophyll and photosynthetic pigments. SOD – superoxide dismutase, CAT – catalase, QP - photochemical quenching.

applications had significantly higher concentrations of photosynthetic pigments and QP. However, if grafting is required for sanitary reasons (e.g., management of *Fusarium oxysporum* f. sp. *passiflorae*), the evaluated salinity does not affect them drastically. Further research is recommended to confirm and establish the tolerance pathways of this species to salt stress. Additionally, evaluating different doses of *A. nodosum* on *P. edulis* under field conditions and varying salinity conditions will be necessary.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author´s contributions

AMOR: conceptualization, research, writing of original draft, visualization, writing, and editing. JECB: conceptualization, research, writing of original draft. HEBL: conceptualization, visualization, writing, and editing. All authors have read and approved the final version of the manuscript.

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