Journal of Agricultural Science and Technology (JAST), 29(1)
In Press, Pre-Proof Version

Morpho-physiological and metabolic response of maize (*Zea mays* L.) cultivars under salt stress

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ABSTRACT

The aim of this study was to investigate the impact of salinity on the morpho-physiological response of maize. The experiment was conducted in a glazed greenhouse located at the Higher Institute of Agronomy of Chott Mariem, Sousse, Tunisia, with two maize cultivars (Aristo, Arper), and four salinity levels (0, 3.4, 6.8, 10.2 dS m^{-1}). A total of 30 traits were evaluated, including agronomic, gas exchange, leaf anatomy, osmotic adjustment (OA), photosynthetic pigments, nitrogen (N) and soluble carbohydrates by gas chromatography. Results revealed significant decline of fresh and dry weight of leaves, stems and roots with salinity. The higher growth, OA, carbohydrates level, leaf water content (LWC), and reduced cuticular transpiration (CT) have contributed to salinity tolerance in Arper cultivar. The findings highlight the 34 Mm salinity tolerance threshold in maize. The reduction of stomatal aperture, stomatal exchange surface (SE), stomatal conductance (gs) and photosynthesis (Pn) by salinity revealed that closing pores when internal $[CO_2]i$ is high is a key function for maize to conserve water under salt stress.

Keywords: Carbohydrates, Maize, Photosynthesis, Salt stress, Stomata.

INTRODUCTION

Salinity is a widely occurring environmental stress that restricts the sustainability of the agricultural sector, and causes serious yield losses in important crop species (Cao *et al*. 2023). It is estimated that more than 1000 Mha, representing 6%–7% of the world's total land area, is affected by soil salinity (Ivushkin *et al*. 2019). The combined effects of global warming, extensive use of low-quality water and overfertilization, has given rise to soil salinization notably in arid and semiarid regions (Liu *et al*. 2023). For these reasons, it is pressing to elucidate the mechanisms of crop salt tolerance and use salt-tolerant species. Salt stress could affect the essential biological processes including photosynthesis, water relationships and nutrient uptake (Parihar *et al*. 2015). It is also recognized that high concentrations of salts in the soil lowered the plant growth by dipping the leaf and root development, causing cell toxicity and physiological disorders (Parihar *et al*. 2015). In summary, significant decreases in

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seedling growth and significant differences in stress responses occur depending on salinity levels (Zulkadir, 2025).

Maize (*Zea mays* L.), is a worldwide food and forage crop with 200 Mha planting area and more than 1000 million tons yield (**Idikut *et al*., 2021**; Wang and Hu 2021). With the growing world population, change in human diets and strong demand for animal products, maize yields are expected to double by 2050 (Cao *et al*. 2023). However, abiotic stresses like drought and salinity caused by global climate change and water shortage threaten the future maize supply (Wang *et al*. 2024). Maize is moderately sensitive to salinity (Cao *et al*. 2023). **Salt stress affects negatively the plant's growth rate**, osmotic status, transpiration, ion transport, photosynthetic activity and leaf senescence (Zhao *et al*. 2018; **Idikut *et al*., 2021**). It has been proved that 10 dS m^{-1} of soil salinity caused a reduction in maize yield of up to 55% (Satir and Berberoglu 2016). Around 70% of maize is produced in arid and semiarid regions where salt stress threatens the agriculture and the agricultural soils (Jiang *et al*. 2021). In Tunisia, however, maize production remains limited, largely due to various abiotic stresses like drought and salinity. Grown primarily as livestock fodder, the average cultivated area for maize in Tunisia is about 1,453 hectares (Kthiri *et al*. 2024). In 2021, this area sharply declined to 1,150 hectares due to severe climatic conditions (Kthiri *et al*. 2024). Planting salt tolerant maize cultivars would increase the yield on these lands and increase the area of the arable land.

At present, the relationship between maize and salinity is very immense, especially in terms of **morpho-physiological response and yield**. Liu *et al*. (2023) declared that photosynthesis is a fundamental physiological process for producing maize material, since it is **very** disposed to environmental changes. Salt stress decreased significantly the leaf area, the chlorophyll content and the **photosynthetic capacity** of maize leaves, leading to growth and yield reduction (Li *et al*. 2020). Numerous studies have examined the relationships between salt stress and the light response curve (Ali *et al*. 2019; Pappert *et al*. 2025). In fact, the optimization of light spectra and intensity constitute a mean of maximizing **the** photosynthesis and comprehend the processes of photosynthetic mechanisms (Taiz *et al*. 2015). **The photosynthetic performances in leaves depended on the light environment, leaves canopy and photosynthetic function of the plant (Han-yu et al. 2022). It is reported that shading mature leaves induced lower photosynthetic capacity and strengthened high light sensitivity in them, but also in new developed leaves (Li *et al*. 2020). In a line, Han-yu *et al*. (2022) reported that the light environment of mature leaves influences the stomatal development, morphological characteristics and photosynthetic function of new leaves.**

In view of the rising salt stress, plants would respond by deploying various strategies like the extrusion or compartmentalization of toxic ions, the establishment of an enhanced level of osmolyte biosynthesis and the activation of reactive oxygen species (ROS) scavenging systems (Sarker *et al*. 2020). Additional salt stress effects, include stomatal closure that limits water loss and carbon dioxide (CO_2) fixation, the production ROS which may leads to enzyme deactivation, protein degradation, and DNA damage (Dikobe *et al*. 2021). Plants can resort to different morphological alterations in order to regulate their water losses, such as changes in the epidermis and **cuticle** water tightness, cuticular transpiration and thickness, ultrastructure, and chemical composition systems (Gašparovic *et al*. 2021). **Additionally, physiological drought may occur due to salt stress. Stomata and leaf trichomes affect water loss through transpiration, thus differing under stress conditions and helping to increase plant tolerance (Idikut *et al*., 2021).**

The current study focused on different traits associated with salinity tolerance in maize, including morphological, physiological and metabolic performances. Our hypothesis argues that maize response may exhibit variations in response to salinity level among **cultivars** and leaf canopy. **The aim of this study was to investigate the impact of salt stress on the morpho-physiological mechanisms of maize plants and to identify if there is possible tolerance or sensitivity of studied cultivars** to assessed salinity levels.

MATERIAL AND METHODS

Plant material and growth conditions

The study was carried out in a glazed greenhouse located **at** the Higher Institute of Agronomy of Chott Mariem, Sousse, Tunisia **during 2020** where the temperature for day/night was 35/23°C, the relative humidity was 60–80%, and the average photosynthetically active radiation (PAR) was 500 **µmol m^{-2} s^{-1}** with a 14 h/day photoperiod **(Hajlaoui et al., 2009)**. **The factorial experimental layout included two factors, the first was cultivars with two levels (Arper and Aristo) and the second was saline treatments with four levels (0, 34 mM/3.4 dSm^{-1}, 68 mM/6.8 dSm^{-1} and 102 mM/10.2 dS m^{-1}). The two silage maize cultivars were provided by the Domanial Earth Office of Kairouan (Tunisia) and were of early maturity group (80-100 days). The assessed cultivars showed variable response to salt stress as Aristo was more susceptible to salinity (Hajlaoui et al., 2009).** Seeds of equal size and fullness were germinated in Petri dishes covered with half strength Hoagland's nutrient solution **(Hoagland and Arnon, 1950)**. After germination, each container was planted with three seeds.

Around 10 days after seeding, they were thinned to one plant per container and the plants were grown in soil containers measuring 35 ×35 × 40 cm (length × width × height), filled with a peat/perlite mixture in a 2:1 ratio (v:v).

Saline treatments were applied 20 days after planting (DAP). Firstly, saline irrigation was done every 7 days. After reaching the full leaf development saline irrigation was done every 3 days. Sodium chloride was added to Hoagland and Arnon (1950) nutrient solution to reach the final saline treatments concentrations. **Plant water requirements were determined using the formula proposed by Kalaji et al. (2014).** All measurements were made 6 weeks after saline stress, when plants achieved a steady state.

Plant growth attributes

Five plants per treatment and per cultivar were randomly selected and divided into leaves, stems and roots to determine the fresh and dry weight. The leaf fresh weight (*LFW*), stem fresh weight (*SFW*), aerial part fresh weight (*APFW*: *LFW*+*SFW*), and root fresh weight (*RFW*) were determined immediately at harvest. **To remove the roots from soil we have drawn a square around the plant with 28 cm length side as the root area. Then we have dig out the soil with roots from 30 cm depth and put it into a mesh bag. After washing away the soil and the impurities we have measured the *RFW*.** The different plant parts were then oven dried at 80°C until constant weight was achieved and the leaf dry weight (*LDW*), stem dry weight (*SDW*), root dry weight (*RDW*) were determined. The measurements were then used to calculate the *APDW*/*RDW*.

Measurement of photosynthetic characteristics and Leaf Water content

Gas exchange measures were analyzed when the majority of green shoots had produced around 12 and 14 internodes. The net photosynthesis (*Pn*), the transpiration rate (*E*) and the stomatal conductance (*gs*), of a sun exposed leaf (8^{th} leaf from the base) of five plants from each cultivar under each saline treatment were measured. Measurements were recorded with an infrared gas analyzer (LI-6400, LI-COR, Lincoln, USA), made up of a ventilated leaf clamp equipped with a temperature and a photosynthetically active radiation (PAR) sensors. The temperature, the CO_2 concentration and the PAR intensity in the leaf chamber were kept at 25 °C, 390 µmol mol^{-1} and 1600 µmol photons m^{-2} s^{-1} respectively. The light response curve (Variations in Pn as function of PAR) were measured by a light source with the leaf clamp linked to a computer CIRAS-III portable photosynthesis system (PP System, Hansatech, UK) on sunny and windless day. To ensure that the gas-exchange conditions were stable, the

measurements for light curves were made after waiting at least 5 min, and up to 20 min, between each light intensity change (Jia *et al*. 2023). The PAR was taken at 2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400 and 200 µmol m^{-2} s^{-1}. The light response curve was fitted using a modified rectangular hyperbolic model: $Pn= \alpha (1-\beta PAR)/(1+\gamma PAR) PAR - R_d$ (Ye and Yu 2008).

The water use efficiency (*WUE*) was calculated as follows (Sun *et al*. 2018): *WUE*= *Pn*/*E*, and expressed in µmol CO_2 $mmol^{-1}$ H_2O.

Cuticular transpiration (CT) was measured in the detached leaves according to Schuster et al. (2017) method. The detached leaves were saturated with water for 3 h, thus ensuring stomata closure. Then, the leaves were transported into a dark chamber, 25 °C temperature and app. 30% humidity with weak green light to have a negligible effect on the plant's stomata. Afterward leaves were placed on filtration paper, and the leaf drying curves were measured gravimetrically at regular intervals (30 min) for ~3 h. The water loss was recalculated per leaf area and then calculated for all time intervals in g h^{-1} cm^{-2}. When the water loss in individual leaves became steady, the values of three subsequent intervals were averaged, representing an estimate of CT (in g h^{-1} cm^{-2}).

For Leaf Water Content (*LWC*) characteristic, measurements were taken on three canopy positions (Upper, Intermediate and Lower). The upper position corresponds to the youngest fully expanded leaf, leaf 14 (L14). The intermediate and lower canopy includes leaf 10 (L10) and leaf 7 (L7) respectively. To determine the *LWC*, we used the leaf fresh weight (*LFW*) measured immediately at harvest, and the leaf dry weight (*LDW*) measured after drying to constant weight at 80 °C. The *LWC* was calculated with the following equation: $LWC = ((LFW-LDW)/LFW)\times100$ (Song *et al*. 2021).

Characterization of the leaf anatomy

The leaf anatomy was analyzed on the same leaves used for photosynthetic characteristics and *LWC*. A transparent fine nail polish peel print was made of an approximately 1 × 1 cm area of the upper side and the underside of leaves, close to the petiolar sinus between the main vein and the right left lateral vein and allowed to dry (d'aMBrogio de argüeSo 1986). Then it was peeled off using a scalpel and preserved between slide and coverslip until use. Observations were made using a Nikon-104 optical microscope. The number of stomata in the visual field (0.196 mm^2) was counted at three different points to determine the stomata density (SD) in the upper side (SD_{US}) and the lower side (SD_{LS}) of leaves.

Values of maximum length and width of the stomatal pore were determined and used to calculate the ostiole section (*So*, µm^2) and the stomatal exchange surface (*SE* %). The *So* was calculated using the following formula: *So* (µm^2) = π × (*Length* / 2) × (*Width* / 2) (Denden et al., 2005). The *SE* was calculated as follows: *SE* (%) = *SD* × *So* × 100 (Denden *et al*. 2005).

Regarding the **Cuticule thickness (CTh)** measure, small leaf pieces (<2 mm) were embedded in resin then cut into 3 µm-thick slices using a manual microtome (Leica, Wetzlar, Germany, RM2125). The sections were then dried at 47°C and stained with Sudan IV dye (Sigma-Aldrich, USA) to highlight the cuticle. Subsequently, the samples were examined under a bright-field optical microscope (Leica, Wetzlar, Germany, DM 2500), and the CT was determined using Fiji software version 2.9.0 (Schindelin *et al*. 2012).

Measurement of osmotic potential and osmotic adjustment

Five plants per treatment were selected, and their L14, L10 and L7 leaves were frozen at -20 °C, then thawed and the cell sap was extracted. The leaf cell sap was used for the determination of leaf osmotic potential (Ψs) using an osmometer (Wescor 5500, Artisan Technology Group 101 Mercury Drive Champaign, IL 61822). The osmotic adjustment (*OA*) was calculated by formula: $OA=\Psi s_{\text{non stressed plants}}-\Psi s_{\text{stressed plants}}$ (Pradhan *et al*. 2012).

Biochemical traits

Photosynthetic pigments (**Chlorophyll a: Chl a, Chlorophyll b: Chl b, Car: carotenoids, Chl a/b ratio**) in the L14, L10 and L7 leaves were determined according to the protocol of Sobrino-Plata *et al*. (2013). For that, 20 ml of acetone 80% were added to 1g of fresh leaves. Then, the mixture was filtered, and the optical density (OD) of leaf extracts was measured at 470 nm, 647 nm and 663 nm. Pigment contents were calculated using the equations described by Lichtenthaler (1987):

$Chla=12{:}25A_{Abs663}–2.79A_{Abs647}$

$Chlb=21.5A_{Abs647}–5.1A_{Abs663}$

$ChlT = Chla + Chlb$

Car = $(1000A_{470} - 1.90C_a\ 64.14C_b)/214$.

Protein extraction was performed following to the method of Ashoub et al. (2013) and the protein concentrations were determined using the Bradford method (Bradford 1976). For the estimation of nitrogen (N) content, samples of dry leaves, stems and roots were grounded to a fine powder and digested with H_2SO_4 as per the method given by Sarkar *et al*. (2020). Nitrogen in the digested samples was estimated using the Kjeldhal method as described

by Bremner and Keeney. (1966). Nitrogen Use Efficiency (*NUE*, g DWg^{-1} leaf N) and Photosynthetic Nitrogen Use Efficiency (*PNUE*, µmol CO_2 mol^{-1} leaf N s^{-1}) were calculated as follow (Debez *et al*. 2006):

$$NUE = plant\ DW / leaf\ N$$

$$PNUE = Pn / Leaf\ N$$

Soluble sugars were also determined in leaves, stems and roots. Extraction was done using the alcohol extraction method Bartolozzi *et al*. (1997). Extracts were dried and converted into trimethylsilyl (TMSi) ethers with a silylation (Pyridine, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS)). An aliquot of 1 µl of each silylated leaf extract was analyzed using a gas chromatograph (Hewlett-Packard 5890 series II) equipped with flame ionization detection (FID system) and a capillary column (HP-5MS, 30 × 0.25 mm). The temperature of capillary column was programmed to increase from 180 to 300 ˚C at a rate of 5 ˚C min^{-1}. Identification of individual carbohydrates was achieved by use of the relative retention times in comparison with that of the standards. The quantification of each compound was performed using the internal standard calculation method.

Data analyses

The effects and the interactions between the two main experimental factors (Maize **cultivar**: V and exposure to salt stress: S) were analyzed through a two-way analysis of variance (ANOVA) using the SPSS Statistics 20. **The least significant difference (LSD) test at 5% significance level was used to find out significant differences among saline treatments.** Pearson correlation analysis was performed to test for associations among the Plant dry weight, PNUE, NUE parameters.

RESULTS

Plant growth attributes

The two-way ANOVA results showed that salinity level significantly affected the LFW, LDW, SFW, SDW, RFW and RDW (Figure 1, Table 1). The LFW, LDW, SFW, SDW, RFW and RDW characteristics differed significantly among **cultivars**, while showing highest values in Arper. The extent of decrease in response salinity was specific on maize **cultivar**, as revealed the significant effect of the V×S interaction. In fact, Arper plants subjected to 102 mM salinity level had their LFW, LDW, SFW, SDW, RFW, RDW, 49%, 46%, 76%, 81%, 59%, 50% shorter than plants from control treatment. The extent of decrease of these same traits in Aristo was respectively by 55%, 46%, 82%, 81%, 83%, 74%. For the APDW/RDW ratio, we found a much

smaller difference between control and 102 mM saline treatment of only 42% in Arper. In contrast, the APDW/RDW of Aristo cultivar have increased under 68 mM and 102 mM.

Photosynthetic activity

From figure 2, the order of the Pn, E and gs among saline treatments in each cultivar was as follows: 0 > 34 > 68 > 102 mM. Different trend in the WUE in both cultivars was registered. In fact, comparable WUE values were noticed in the 0 mM, 34 mM and 68 mM treatments, although least WUE was attributed to the 102 mM. At the same salinity level, Arper plants exhibited higher Pn and gs as compared to Aristo, significant effect of the S and V factors and their interaction. The ANOVA results for the CT and CTh revealed significant effect of the cultivar. Indeed, the rate of the CT in Arper leaves was comparable in the 0, 34 and 68 saline treatments. In contrast, Aristo had higher CT under 0 and 34 mM treatments then 68 and 102 mM. In the other hand, the CTh in Arper leaves showed a gradient decrease with the increase of salinity level while Aristo, had higher values under 0 and 34 mM treatments as compared to 68 and 102 mM. Our results indicate less water loss by CT in Arper leaves. This was accompanied with higher CTh in Arper (9.7µm-5.6µm) compared to Aristo (8.2µm-4.2µm). Overall, the difference in gas exchange characteristics suggested that Arper had more favorable situation for the production of photosynthetic products. Moreover, the observed trends in the light response curves per treatment and per cultivar revealed higher values in Arper compared to Aristo. Hence, Arper can maintain its Pn above 4 µmol m^{-2} s^{-1} at 600 µmol CO_2 m^{-2} s^{-1} PAR intensity. In contrast, Aristo could conserve Pn ≥ 4 at higher light intensity (800µmol CO_2 m^{-2} s^{-1}).

Leaf anatomy

The two-way ANOVA analyze showed that the V×S interaction affected significantly the SD in the upper and the lower surface of leaves ($P \leq 0.05$; Table 3). In fact, the least SD_{US} and SD_{LS} values in Arper were noticed under 68 and 102 mM treatments. In contrast, Aristo do not show significant variation in the SD_{US} and SD_{LS} among saline treatments. In addition, the So and Se traits of both cultivars, decreased gradually with the increase of salinity level, while showing highest values in Arper ($p \leq 0.05$ for V factor) (Figure 4). Furthermore, the response of maize plants at the level of stomatal aperture length and width was negatively affected by saline stress (ANOVA, $p \leq 0.05$). Least values of stomatal aperture length and width were registered under 102 mM in both cultivars.

Osmotic potential and osmotic adjustment

Both salt stress and the interactions between S×LC, affected significantly the Ψs and OA characteristics (Figure 4). Indeed, the Ψs of Aristo plants subjected to 0 and 34 mM salinity level do not differed significantly among the assessed leaf canopy age. In contrast, the distinction between the studied leaf canopy in Arper was significant from the 34 mM saline treatment, whilst showing highest values in the L7 (-1 bar). Likewise, the OA values revealed **different cultivar** response among studied leaves canopy. Seeing that Arper' OA was higher in the L10 and L14 then L7 in all salinity levels. Meanwhile, the difference in OA in Aristo was registered within 68 mM and 102 mM. It was also remarkable that OA values were higher in Arper as compared to Aristo. **Changes in in soluble protein contents revealed that as compared to 0 and 34 mM, salinity at 68 and 102 mM reduced significantly the protein content in both cultivars. Additionally, Arper exhibited higher proteins concentrations then Aristo.**

Photosynthetic pigments and Leaf Water Content

The photosynthetic pigments results showed that the effect of S and LC factors were significant (P≤0.01) on Tot Chl, Chl a, Chlb and **Car** content (Figure 5). In fact, the least amounts of Tot Chl, Chla, Chlb and **Car** in the two studied varieties were obtained in the 102 mM saline treatment and the L14 leaf canopy. Regarding Chl a/Chlb parameter, the registered values were not affected neither by S, V and LC aspects. In the interim, the LWC was not affected by the LC factor and decreased gradually in response to salinity. Likewise, the least LWC in Arper leaves was obtained in the 102 mM salinity level, intermediate values were recorded similarly for the 34 mM and 68 mM although, highest LWC was registered under 0 mM. In the interim, Aristo exhibited less LWC. In addition, the level of LWC in Aristo decreased gradually with the increase in salinity level. **Whereas Chlorophyll synthesis is tightly controlled by plant's water state, this may explain the reduction in photosynthetic pigments by saline stress in this study.**

Nitrogen content and usage efficiency

Salt stress had significant effect on N content in leaves, stems and roots (Figure 6). Accordingly, **the least N concentrations in leaves, stems and roots were observed under 102 mM salinity level. It is also remarked that N was more accumulated in young leaves. Moreover, Arper had higher N content in leaves, stems and roots**. For both **cultivars** the plant DW was positively correlated with the PNUE and NUE.

Soluble carbohydrates

Total carbohydrates content and composition was measured in leaves, stems and roots of the two maize **cultivars** (Table 3). Fructose, Glucose and Xylose were the most abundant sugars in maize leaves. Xylose and Fructose are the most identified sugars in the stems and the roots. In general, the concentrations of the total carbohydrates were higher in leaves than in stems and roots of both **cultivars**. Excepting glucose and sucrose, that showed highest values respectively in Aristo leaves and Arper leaves. Likewise, significant difference in carbohydrate concentrations occurred between **cultivars**. Since, highest carbohydrates content was registered in Arper leaves, stems and roots. In the other hand, the concentration of major sugars in response to saline treatments differed significantly between the identified sugars, plant organs and **cultivars**. Thus, the highest concentrations Fructose and Glucose in leaf tissues were recorded for the 102 mM salinity level. Contrarily, Fructose and Glucose in stems and roots decrease in response to salinity. Xylose showed a contrary response as it decrease in the leaves of both **cultivars** by saline stress and increased in stems and roots in a salinity level dependent manner. In the interim, Galactose decreased by saline stress in leaves and stems of both **cultivars** whereas, it accumulates in Aristo roots as the salinity level increase. In contrast, no significant difference among saline treatments in Galactose content in Arper roots. Regarding Inositol it increased by saline stress similarly in leaves, stems and roots of the two studied **cultivars**. Mannitol and sacharose decrease in Aristo leaves and increase in Arper leaves.

DISCUSSION

Like other environmental stresses, salinity affects many physiological and metabolic processes within the plant. As the photosynthetic capacity is linked to pigment accumulation, water status, osmotic adjustment and metabolic capacity, we wondered how these processes are regulated in different leaf age canopies, **different** saline treatments and maize **cultivars**. Data of the current study revealed that salt stress reduced significantly the maize growth (leaves, stems and roots) in a salinity level dependent extent. **These results agree with Jiang *et al.* (2021) who explained this effect by the restriction of metabolic, molecular and cell division processes**. Indeed, salt stress hampers the plant growth by i) reducing water absorption due to the osmotic effect of salts, ii) impeding the nutrient uptake and iii) affecting the leaf transpiration rate (Semiz *et al.* 2012). **Niamat *et al.* (2019) reported similar results and explained the salinity-induced growth reduction by leaf senescence and reduction of photosynthetic capacity.** The less chlorophyll content is an additional consequence resulting

from the inhibition of chlorophyll synthesis, and/or the activation of its degradation by chlorophyllase enzyme standing out as a mechanism of photoprotection (Hu *et al*. 2017).

In this study, the less chlorophyll pigments (Tot Chl, Chl a and Chlb) under 102 mM salinity level and in aged leaves (L14) point to the less membrane stability (Carpici *et al.* 2010). In a line, Morteza *et al*. (2013) explained that the structure and the function of chloroplasts changed in aged leaves, and if the chloroplast membranous system suffers from destruction, the plant metabolism and the physiological functions will be hindered (Morteza *et al*. 2013). **Additionally, the low LWC of aging leaves (L14) compared younger leaves (L7) point to the fact that chlorophyll synthesis was tightly controlled by plant water state** (Soltabayeva *et al*. 2021). **In accordance with the current work,** Han-yu *et al*. (2022) attributed the decrease in chlorophyll pigments and photosynthetic **capacity of aged leaves in maize plants** to the reduced light intensities in the lower canopy. Beside the higher LWC of Arper, this **cultivar** had superior OA ability under salt stress compared to Aristo. This underlines Arper's aptitude to minimize salinity effects by the osmoregulation process. In the interim, the similarity of N content between L7 and L10 leaves canopy in the 0 and 34 mM saline treatments, may elucidate the less sensitivity of maize plants to 34 mM salinity level, particularly in young leaves. Such effect may be attributed to the dynamic changes in leaf N partitioning among the photosynthetic pools (Liu *et al*. 2023). Likewise, the robustness (r^2>0.8) of the linear correlation between Plant DW vs. PNUE and Plant DW vs. NUE indicated an enhanced nitrogen allocation to chlorophyll synthesis and therefore dry matter accumulation (Lu *et al*., 2020). Meanwhile, the salinity-induced N reduction may reflect a decrease in N translocation ability to dry matter accumulation.

In the same way, Hu *et al*. (2017) revealed that salinity reduced **gas exchange**, electron transport chain, and thus chlorophyll synthesis in plants. Data from this work revealed that E, gs, WUE, CT and **CTh** rates in response to salinity follow trends in leaf N content and were comparable between 0 and 34 mM treatments in both **cultivars**. This underlines the convenient effects of lower salinity on plant physiology (Hassan *et al*. 2017). In addition, the 34 mM>68 mM>102 mM arrange point to the less sensitivity of maize to 34 mM salinity level. In the counter part, the less E, gs, WUE, CT and **CTh** values got under 68 and 102 mM may be explained by reduced water and nutrient uptake (Jiang *et al*. 2021). In the other part, the less water loss by CT in Arper, could expound the efficient water saving startegy of this **cultivar** as compared to Aristo (Gašparovic *et al*. 2021). It can be assumed that this is why the results of this study revealed higher LWC in Arper. Another details of this result is also the higher **CTh**

of Arper compare to Aristo. In this way, Petcu *et al*. (2009), **demonstrated** that water loss by cuticular transpiration shows a very significant effect of the genotype. Additionally, Arper maintain the **Pn**, adapting to weak light from 400 µmol CO_2 m^{-2} s^{1}. In the intervening, the photosynthetic parameters (Pn, E, gs) appeared to follow trends in **CTh** and **were more elevated in Arper**.

The degradation of the chlorophyll pigments by salinity may be allied to destruction of chlorophyll structure due to **ROS generation** and the substitution of essential elements by Cl^- and Na^+, because of increased osmotic pressure in the surrounding soil (Amjad *et al*. 2020). To overcome this, the plant must enhance its osmotic pressure by accumulating compatible solutes and sugars are among the osmotically active substances (Amombo *et al*. 2023). Interestingly, there is 10 % gain in soluble carbohydrates in Aristo and Arper leaves. This response could therefore be an osmotic stress adaptive mechanism. In this line, Amombo *et al*. (2023) found a positive association of SS and water content and **explained that sugars can be used as a phenotypic marker for salt tolearance in maize**. The differences in sugars concentrations observed among **cultivars** suggest that Arper is a high-sugar- **cultivar**. **Moreover**, **the high carbohydrates** content in leaves than in stems and roots of both revealed their osmotic function (Turner 2018). Beside that sugars are required for plant response to biotic and abiotic stress they play an integral role in biomass accumulation and constitute key aspects to consider when selecting excellent fodder for optimum livestock production (Aluko *et al*. 2021).

CONCLUSIONS

Salinity hampers the physiological functions of maize while showing **different cultivar response**. Maize can tolerate 34 mM salt stress, beyond which plant performance declines depending on **cultivar**. **Arper was more effective in maintaining higher Pn, gs, SD, Se, So under saline circumstance, providing an elucidation for its tolerance to salinity compared to Aristo. Moreover, the elevated CTh** of Arper compensated for the less water loss by CT. We observed other physiological **changes** that would have facilitated salinity tolerance, such as the reduction **of stomatal aperture** (length and width), the effective OA, **the increase in carbohydrates and N content**, all of which may be considered as important characteristics for **salinity resistance. Growth results indicated as well the sensitivity of Aristo**. Furthermore, i) the reduced LWC, Ψs, N and chlorophyll pigments in the lower canopy, **ii) the increase** of ostiole dimension and Pn with PAR, suggest that the light environment in the upper canopy influences greatly the stomatal development, morphological characteristics and the

photosynthetic function of maize. **To extend the results of the present experiment to maize growth and production under salt stress condition in the field, we must bridge the controlled lab conditions to field variability by testing promising hormones, biostimulants and nutrients treatments in actual saline field's conditions using field-specific densities, irrigation, soil types to validate effectiveness.**

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Table 1. Summary of the ANOVA analysis for growth parameters in maize plants, and statistical differences between saline treatments and **cultivars**.

Effect	LFW	LDW	SFW	SDW	RFW	RDW	APDW/RDW
S	72.46^{***}	2.55^{*}	2.46^{*}	2.28^{*}	10.26^{***}	8.66^{***}	2.01^{ns}
V	114.72^{***}	0.00^{*}	0.01^{*}	5.12^{*}	0.86^{*}	2.97^{*}	0.17^{ns}
S× V	10.94^{**}	0.07^{*}	1.23^{*}	0.72^{*}	0.85^{*}	0.56^{*}	1.75^{ns}

Note: S: Salt treatment; V: **Cultivar**; * Significant at P < 0.05; **Significant at P < 0.01; ***Significant at P < 0.001; ns non-significant.

Table 2. Summary of ANOVA analysis for the **gas** exchange, osmotic potential (Ψs), osmotic adjustment (OA) in maize plants, and statistical differences between saline treatments and **cultivars**.

Effect	Pn	E	gs	WUE	CT	CW	SD_{US}	SD_{LS}	So	SE
S	20.35^{***}	16.60^{***}	7.57^{**}	0.82^{ns}	16.58^{***}	16.60^{***}	12.97^{***}	11.51^{ns}	6.80^{**}	2.25^{*}
V	1.03^{*}	4.53^{ns}	1.56^{*}	0.36^{ns}	4.44^{*}	4.53^{*}	1.56^{*}	0.36^{*}	4.44^{*}	4.53^{*}
S×V	1.14^{*}	1.06^{ns}	0.02^{*}	0.28^{ns}	1.22^{ns}	1.06^{ns}	2.33^{*}	3.61^{ns}	0.46^{ns}	6.05^{**}

Note: * Significant at P < 0.05; **Significant at P < 0.01; ***Significant at P < 0.001; ns non-significant. Pn: Net photosynthesis, E: Transpiration, gs: Stomatal conductance, WUE: Water use efficiency; CT: Cuticule transpiration; **CTh: Cuticule width**.

Table 3. Soluble sugars concentrations in leaves, stems and roots of maize **cultivars** following saline treatments.

Soluble Sugars (µg mg^{-1} DW)	Salinity level (mM)	Aristo			Arper		
		Leaves	Stems	Roots	Leaves	Stems	Roots
Xylose	0	7.62 ± 0.71^{a}	11.96 ± 0.34^{c}	32.29 ± 0.12^{c}	13.95 ± 0.12^{a}	13.75 ± 0.32^{c}	18.13 ± 1.02^{b}
	34	5.15 ± 0.15^{b}	35.26 ± 0.56^{a}	51.15 ± 0.09^{b}	9.99 ± 0.02^{b}	23.88 ± 0.11^{b}	30.65 ± 0.84^{a}
	68	2.01 ± 0.20^{c}	34.54 ± 0.37^{a}	53.07 ± 0.16^{b}	1.07 ± 0.02^{c}	40.32 ± 0.14^{a}	30.16 ± 0.47^{a}
	102	1.70 ± 0.16^{c}	32.11 ± 0.64^{b}	58.18 ± 0.25^{a}	0.91 ± 0.07^{c}	41.99 ± 0.23^{a}	30.56 ± 1.24^{a}
Fructose	0	25.80 ± 0.35^{c}	30.75 ± 1.82^{a}	18.34 ± 0.11^{a}	20.93 ± 0.09^{c}	30.08 ± 0.62^{a}	17.21 ± 0.95^{a}
	34	28.99 ± 0.83^{b}	24.25 ± 0.52^{b}	10.07 ± 0.07^{b}	20.37 ± 0.12^{c}	26.54 ± 0.45^{b}	6.58 ± 1.20^{b}
	68	32.63 ± 0.32^{a}	18.05 ± 0.26^{c}	5.42 ± 0.10^{c}	25.73 ± 0.73^{b}	10.66 ± 0.21^{c}	3.84 ± 0.54^{c}
	102	32.93 ± 0.28^{a}	18.00 ± 0.36^{c}	2.66 ± 0.01^{d}	35.36 ± 0.60^{a}	3.46 ± 0.11^{d}	2.70 ± 0.27^{c}
Galactose	0	1.19 ± 0.22^{a}	4.78 ± 0.07^{a}	4.40 ± 0.09^{b}	18.60 ± 0.21^{a}	3.56 ± 0.11^{a}	1.28 ± 0.33^{a}
	34	0.91 ± 0.09^{a}	4.40 ± 0.12^{a}	7.75 ± 0.08^{b}	7.68 ± 0.18^{b}	3.46 ± 0.09^{a}	1.21 ± 0.24^{a}
	68	0.81 ± 0.08^{b}	3.92 ± 0.04^{a}	9.65 ± 0.11^{a}	1.69 ± 0.06^{c}	2.40 ± 0.07^{b}	0.64 ± 0.08^{b}
	102	0.57 ± 0.15^{c}	1.58 ± 0.09^{b}	9.52 ± 0.13^{a}	1.47 ± 0.14^{c}	0.86 ± 0.02^{b}	0.67 ± 0.06^{b}
Glucose	0	45.58 ± 0.45^{d}	21.47 ± 0.08^{a}	24.22 ± 0.22^{a}	30.81 ± 0.52^{c}	34.02 ± 1.27^{a}	50.33 ± 0.68^{a}
	34	49.62 ± 0.45^{c}	10.99 ± 1.03^{b}	6.71 ± 0.07^{b}	33.44 ± 0.23^{b}	25.41 ± 0.32^{b}	8.58 ± 0.06^{b}
	68	53.42 ± 0.58^{b}	12.56 ± 0.12^{c}	5.79 ± 0.12^{a}	39.09 ± 0.14^{a}	15.67 ± 0.67^{c}	6.32 ± 0.08^{c}
	102	55.37 ± 0.60^{a}	12.69 ± 0.04^{c}	5.28 ± 0.05^{c}	39.50 ± 0.21^{a}	14.35 ± 1.12^{c}	4.72 ± 0.12^{d}
Mannitol	0	3.34 ± 0.23^{a}	5.74 ± 0.14^{a}	7.33 ± 0.08^{a}	4.65 ± 0.02^{c}	4.20 ± 0.09^{c}	5.49 ± 0.10^{c}
	34	2.98 ± 0.12^{a}	5.51 ± 0.22^{a}	4.93 ± 0.12^{b}	12.68 ± 0.24^{b}	14.39 ± 0.32^{b}	26.88 ± 0.72^{b}
	68	2.01 ± 0.20^{a}	5.49 ± 0.09^{a}	3.86 ± 0.09^{b}	13.29 ± 0.17^{a}	14.92 ± 2.51^{a}	28.56 ± 0.45^{a}
	102	1.32 ± 0.02^{b}	5.01 ± 0.11^{a}	3.17 ± 0.10^{b}	13.69 ± 0.10^{a}	16.05 ± 1.74^{a}	29.02 ± 0.34^{a}
Inositol	0	2.22 ± 0.22^{c}	18.39 ± 0.02^{b}	13.84 ± 0.13^{b}	3.25 ± 0.31^{c}	14.13 ± 0.42^{b}	5.33 ± 0.11^{b}
	34	3.35 ± 0.36^{b}	18.18 ± 0.10^{b}	13.17 ± 0.09^{b}	7.72 ± 0.42^{b}	14.86 ± 0.21^{b}	20.12 ± 0.12^{b}
	68	3.82 ± 0.38^{a}	23.84 ± 0.23^{a}	16.40 ± 0.05^{a}	9.19 ± 0.17^{a}	16.40 ± 0.78^{a}	23.39 ± 0.32^{a}
	102	4.30 ± 0.43^{a}	29.28 ± 0.17^{a}	17.98 ± 1.02^{a}	9.38 ± 0.22^{a}	23.05 ± 1.23^{a}	23.48 ± 0.27^{a}
Sacharose	0	6.21 ± 0.05^{a}	6.88 ± 0.04^{a}	9.54 ± 0.07^{a}	7.79 ± 0.40^{b}	0.18 ± 0.04^{c}	2.19 ± 0.10^{c}
	34	5.02 ± 0.10^{b}	1.37 ± 0.08^{b}	6.20 ± 0.10^{b}	9.07 ± 0.16^{a}	0.24 ± 0.17^{b}	5.95 ± 0.32^{b}
	68	5.26 ± 0.02^{b}	1.57 ± 0.07^{b}	5.79 ± 0.02^{c}	9.91 ± 0.25^{a}	0.59 ± 0.22^{a}	7.05 ± 0.18^{a}
	102	5.70 ± 0.07^{c}	1.29 ± 0.14^{b}	3.17 ± 0.13^{c}	9.65 ± 0.31^{a}	0.41 ± 0.03^{a}	7.81 ± 0.22^{a}
Total	0	91.90 ± 0.06^{c}	99.97 ± 0.07^{a}	109.96 ± 0.14^{a}	99.98 ± 0.15^{b}	99.82 ± 0.41^{a}	99.96 ± 0.21^{a}
	34	96.02 ± 0.21^{b}	99.96 ± 0.12^{a}	99.98 ± 0.21^{a}	100.95 ± 0.23^{b}	108.78 ± 0.22^{a}	96.21 ± 0.32^{b}
	68	99.96 ± 0.09^{a}	99.97 ± 0.21^{a}	99.98 ± 0.13^{a}	99.97 ± 0.07^{b}	100.96 ± 0.31^{a}	99.96 ± 0.08^{a}
	102	101.89 ± 0.11^{a}	99.96 ± 0.03^{a}	78.81 ± 0.42^{b}	109.96 ± 1.21^{a}	100.17 ± 0.46^{a}	98.96 ± 0.45^{a}

Note. Means ± SE of three independent measurements. Values in each column sharing different letters indicates significant differences (P≤0.05) among saline treatments.

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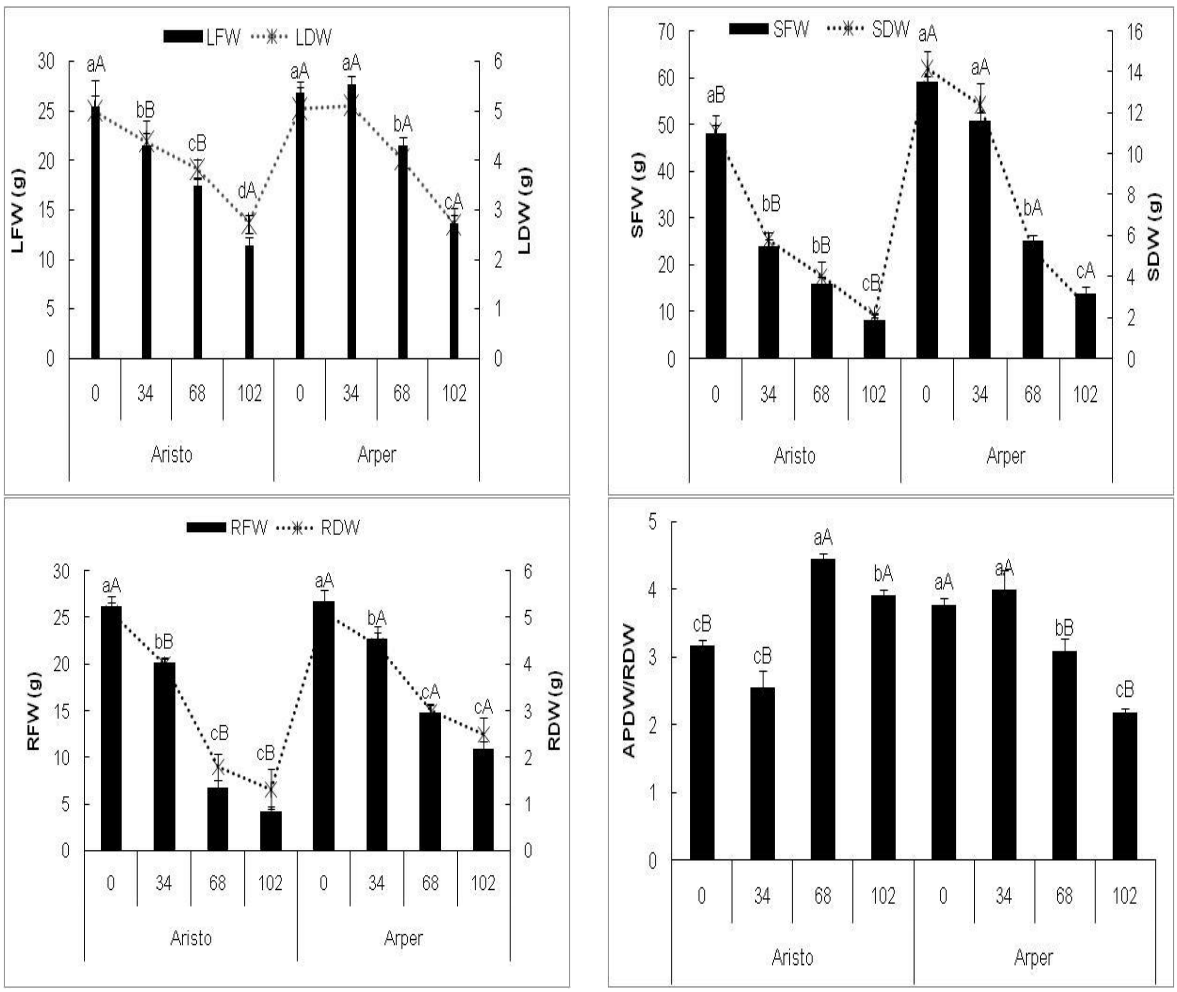

Fig. 1 Effect of salt stress on leaf fresh weight (LFW), leaf dry weight (LDW), stem fresh weight (SFW), stem dry weight (SDW), root fresh weight (RFW), root dry weight (RDW) and aerial part dry weight/root dry weight (APDW/RDW) of Aristo and Arper **cultivars**. Bars being annotated with different lowercase and uppercase letters indicate they are significantly different among saline treatments and cutivars.

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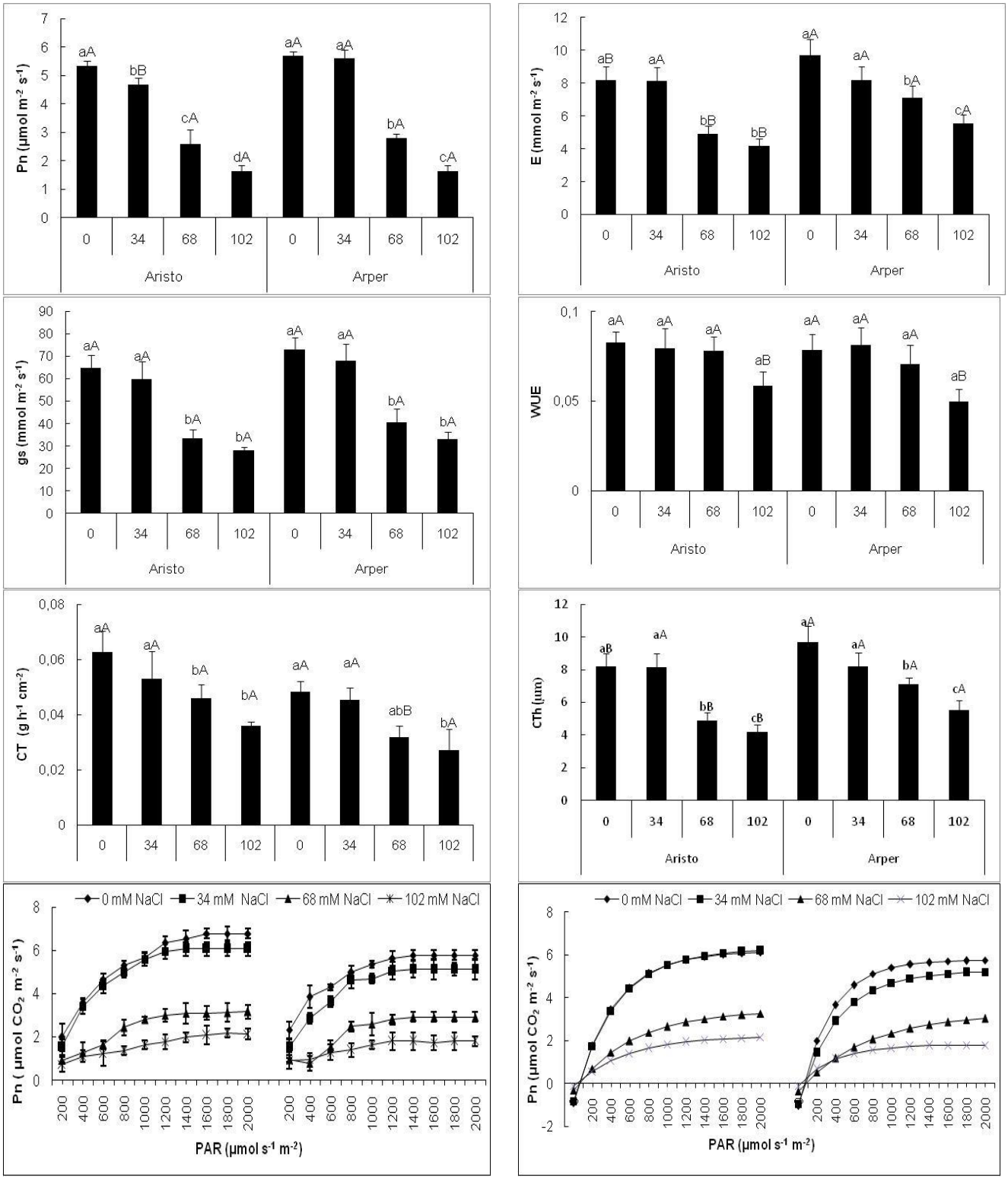

Fig. 2 Effect of salt stress on net photosynthesis (Pn), stomatal conductance (gs), transpiration (E), water use efficiency (WUE), cuticular transpiration (CT), **cuticule thickness (CTh)** and the dynamic changes in Pn as function of photosynthetically active radiation (PAR) of Aristo and Arper **cultivars**. Bars being annotated with different lowercase and uppercase letters indicate they are significantly different among saline treatments and cultivars.

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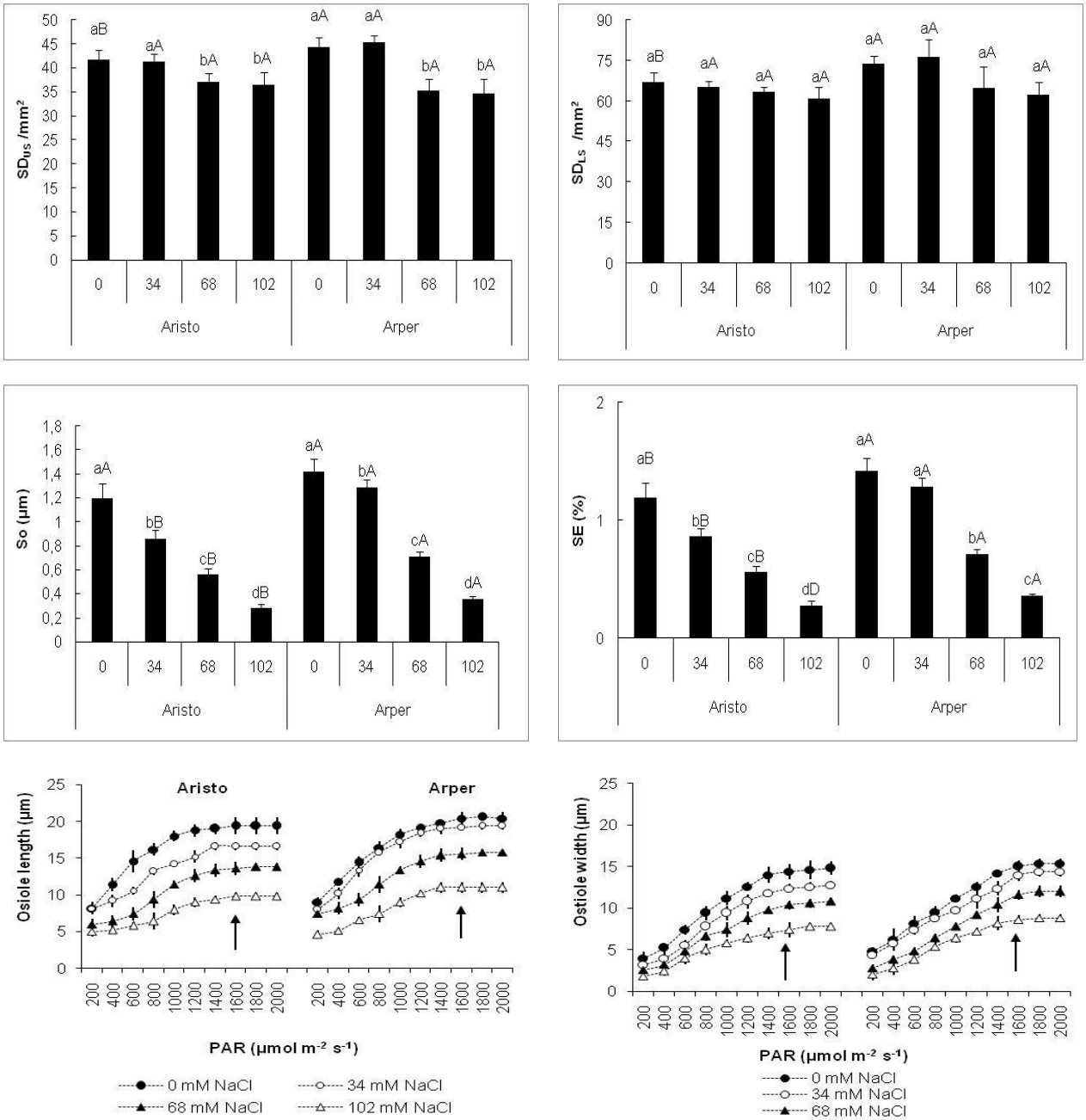

Fig. 3 Effect of salt stress on stomata density in the upper surface (SD_{US}) and lower surface (SD_{LS}) of leaves, **stomatal aperture** section (So) and the stomatal Exchange surface (SE) of Aristo and Arper **cultivars**. Bars being annotated with different lowercase and uppercase letters indicate they are significantly different among saline treatments and cultivars

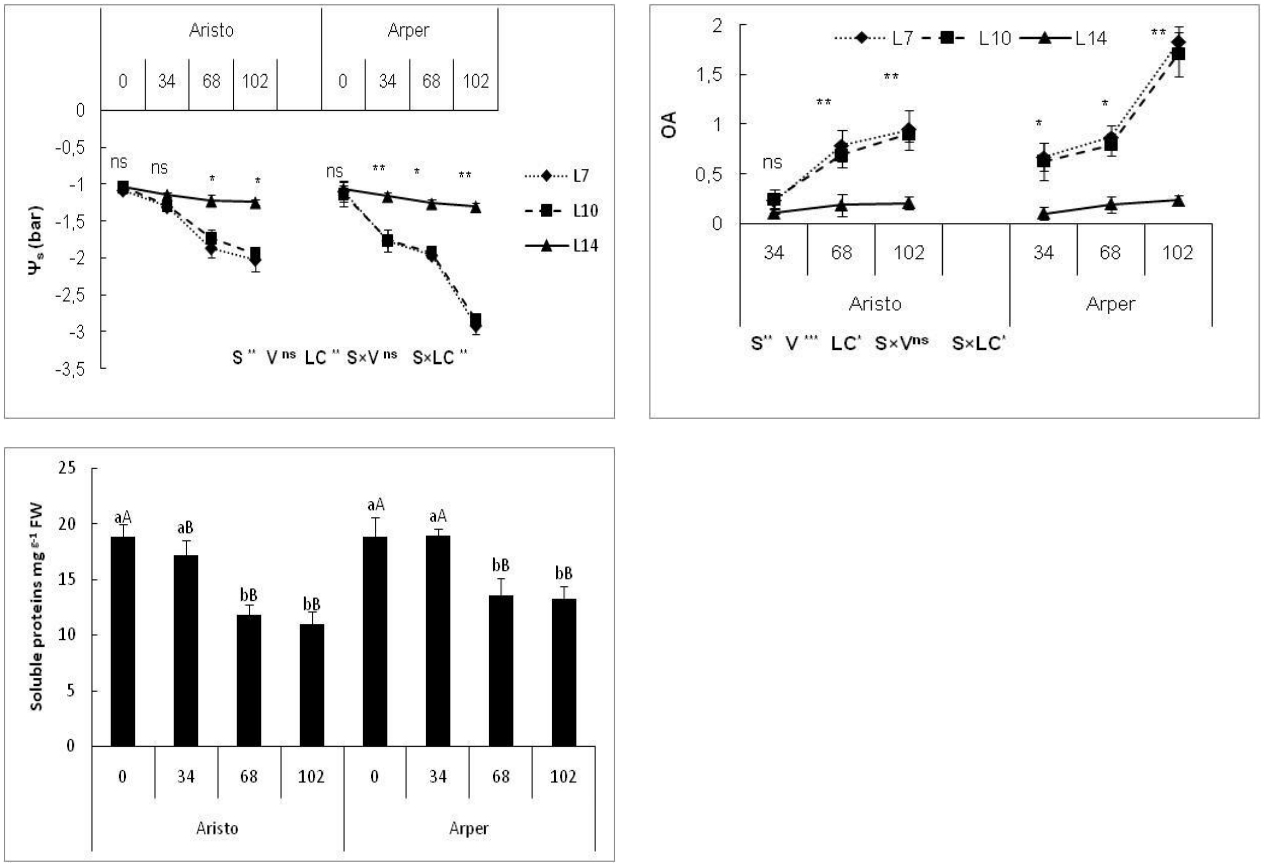

Fig. 4 Effect of salt stress on osmotic potential (Ψs), osmotic adjustement (OA) **and soluble proteins** of Aristo and Arper **cultivars**. * Significant at $P < 0.05$; ** Significant at $P < 0.01$; ns non-significant, amongleavescanopy at $P \leq 0.05$. L7: Leaf 7; L10: Leaf 10; L14: Leaf 14; LC: Leaf canopy; S: Salt stress; V: **Cultivar**

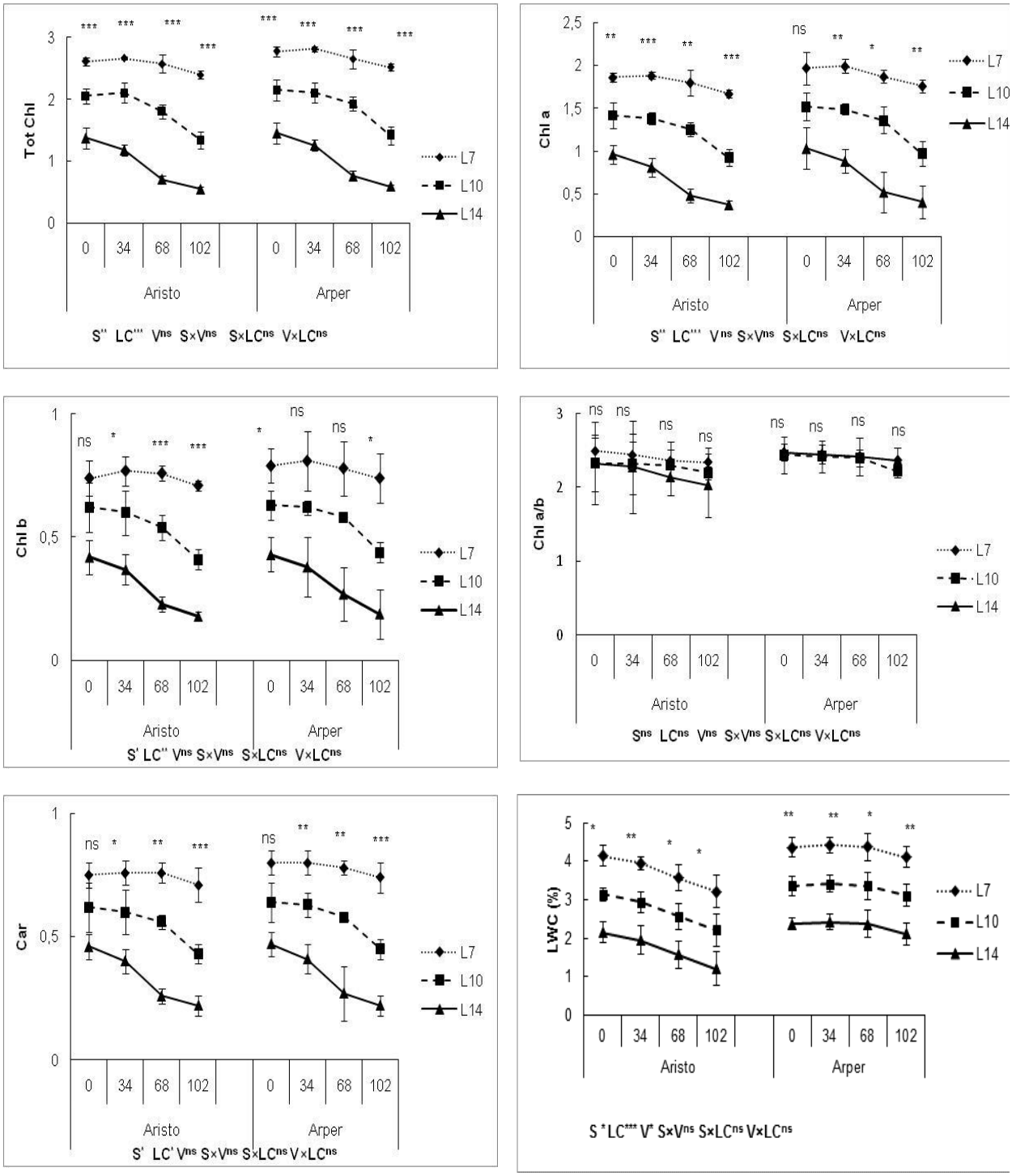

Fig. 5 Effect of salt stress on total chlorophyll (TotChl), Chlorophyll a (Chl a), Chlorophyll b (Chlb), Chl a/b, carotenoids (**Car**) and leaf wáter content (LWC) of Aristo and Arper **cultivars**. * Significant at $P < 0.05$; **Significant at $P < 0.01$; ns non-significant, among leaves canopy. L7: Leaf 7; L10: Leaf 10; L14: Leaf 14; LC: Leafcanopy; S: Salt stress; V: **Cultivar**

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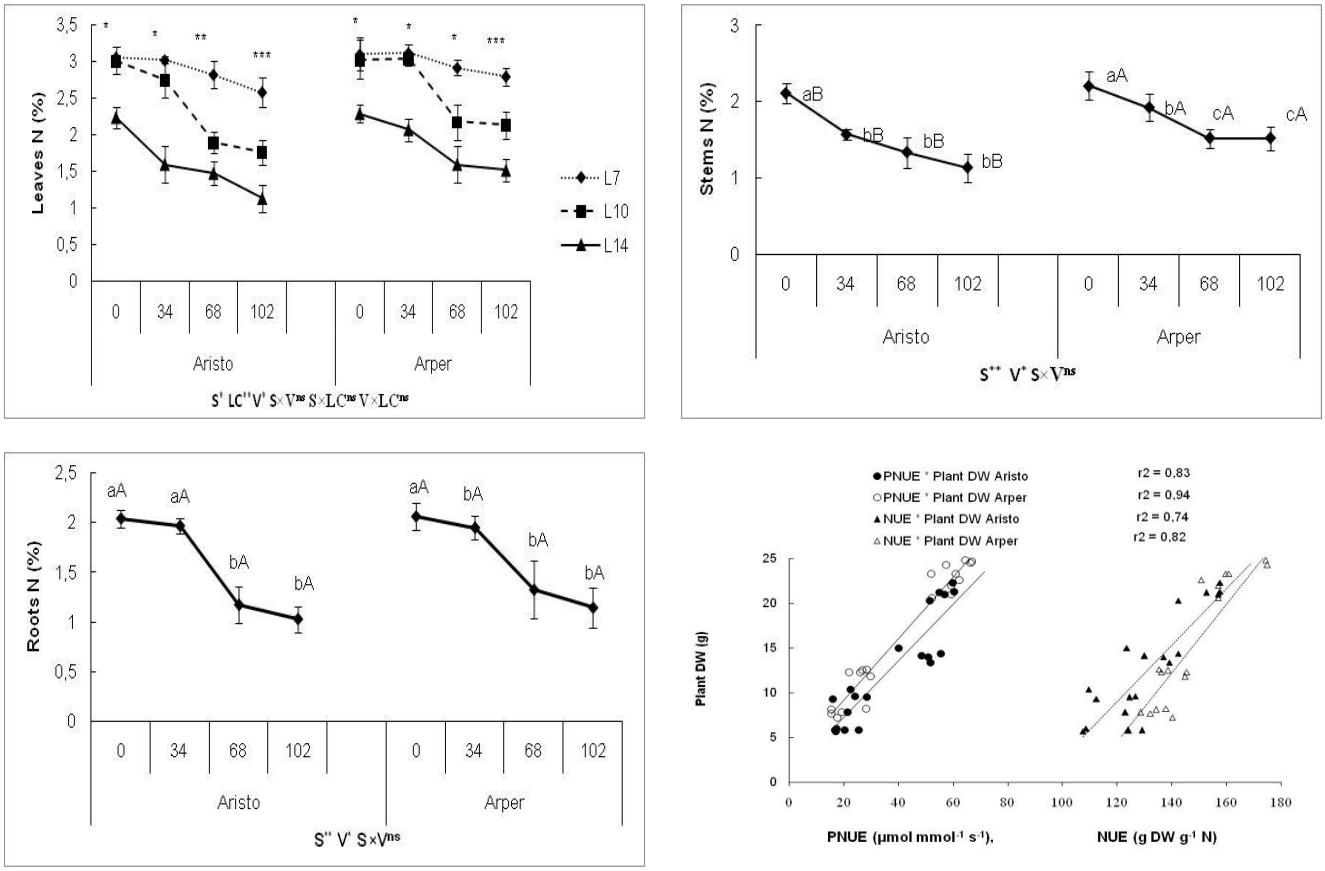

Fig. 6 Effect of salt stress on nitrogen (N) content in leaves, stems and roots and plant dry weight (Plant DW) in correlation with nitrogen photosynthetic use efficiency (PNUE) and nitrogen use efficiency (NUE). Different lowercase and uppercase letters indicatethey are significantly different among saline treatments and **cultivars**. L7: Leaf 7; L10: Leaf 10: L14: Leaf 14; LC: leaf canopy; S: Salt stress; V: **Cultivar.**

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