



Interactive Effects of Salinity and Chilling Stress on the Growth of the Two Forage Species Elephant Grass and Maize

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DURING the winter season in various parts of Saudi Arabia, temperatures decrease up to 14°C, and water salinity levels are affected by irrigation. The purpose of this study was to investigate the interactive effects of salinity stress and chilling temperature on plant growth, F_v/F_m , chlorophyll content and gas exchange in two forage species, *Pennisetum purpureum* and *Zea mays*. The treatments involved daytime/nighttime temperatures of 14/12°C (chilling temperature) and 24/22°C (optimum temperature) and 5 NaCl concentrations (0, 75, 150, 225, 300mM). The findings showed that NaCl levels were higher and growth parameters and gas exchange of the two species lower at chilling temperature than at the optimum temperature. The optimum temperature did not induce any significant decrease in F_v/F_m values of *P. purpureum* at the different salinity treatments, while chilling temperature was significantly reduced the F_v/F_m values for both species at higher salinity. The chlorophyll content was higher at 24°C under increased salinity in *P. purpureum*. In addition, senescence occurred in *Z. mays* grown at 14°C and salinity concentrations of 225 and 300mM within three weeks of emergence. The interaction between salinity and chilling temperature significantly affected most measurements of the two species; however, *P. purpureum* showed high resistance to stress. Therefore, the obtained results will be useful for agricultural application of *P. purpureum* as a reliable crop for saline lands under chilling temperature conditions.

Keywords: Chilling stress, Forage species, Gas exchange, *Pennisetum purpureum*, Salinity, *Zea mays*.

Introduction

Crops that are grown in field conditions regularly experience cycles of abiotic pressure throughout their lives. To alleviate certain adversities resulting from abiotic pressures, plants undergo a sequence of changes in morphology, physiology and biochemistry (Hussain et al., 2018). Abiotic pressures, such as drought, salinity, cold, freezing, and elevated temperatures, can slow the growth and expansion of leaves, decrease plant production, and potentially kill plants in extreme cases (Krasensky-Wrzaczek & Jonak, 2012). Several previous studies have focused on crop plant responses to individual stress factors (Wang et al., 2016; Anjum et al., 2017). However, it is imperative to consider plant reactions to a range of stressors to enhance

plant adaptation under field conditions (Pandey et al., 2015). Salinity stress and chilling temperature are among the most important abiotic stresses that limit the production and geographic distribution of many principal crops, such as maize (Idikut, 2013).

Salinity is a significant challenge for crop production under managed conditions or in the field (Youssef, 2007). Approximately 12 billion USD is lost per year worldwide due to the decline in agricultural production from areas influenced by salinity (Flowers et al., 2010). The conditions in arid and semiarid areas are worse when saline water is used at high temperatures for irrigation systems, resulting in higher salt concentrations in irrigation water due to higher evaporation rates (AbbdElgawad et al., 2016). Most of the crops

that are commercially important are salt-sensitive, and their growth and development are impaired as a result of salinity (Flowers, 2004). Salinity allows various morphological, physiological and biochemical pathways to interact in diverse ways. Oxidative stress can be caused by salinity due to high development of reactive oxygen species (ROS), resulting in the alteration of plant metabolism. This has been found to result in disruption to DNA, proteins, lipids, carbohydrates, and membranes (Hichem et al., 2009). In addition, salinity lowers water flow to the roots and causes the accumulation of ions in certain tissues to reach harmful levels (Munns et al., 2006). Both factors influence the percentage and rate of seed germination, shoot and root length, and fresh weights of roots and shoots (Al-Shoaibi & Al-Sobhi, 2007; Tang et al., 2015). AbbdElgawad et al. (2016) found that salinity caused the greatest decline in the sizes of roots, followed by those of mature and young leaves in maize plants stressed by salt. Salinity also induces stomatal closure and damage to both electron transfer systems and the apparatus of photosynthesis, leading to decreased photosynthesis (Deinlein et al., 2014). A previous investigation of gas exchange in *Atriplex lentiformis* (Torr.) found that high rates of photosynthesis may be achieved at low levels of NaCl salinity (Zhu & Meinzer, 1999). Furthermore, high concentrations of NaCl inhibited the assimilation of CO₂ in this C₄ plant. Photosynthesis inhibition by higher concentrations of NaCl is typically related to a decrease in both the photosynthetic light saturation rate (A_{sat}) and the maximum quantum yield (ϕ) (L'opez-Climent et al., 2007). However, there are no major changes in the overall performance of PSII photochemistry (F_v/F_m) or impairment of the donor or acceptor regions of PSII due to salinity (Chen et al., 2004).

Chilling temperature limits the production and geographical distribution of several commercial crops, such as maize (Erdal, 2012). It has been reported that chilling temperatures account for a 13.8% reduction in the production of agricultural land in the United States (Boyer, 1982). Chilling temperatures influence all stages of plant development, from seed germination, early growth of seedlings to canopy closure, and a wide variety of physiological processes (Tambussi et al., 2004; Hund et al., 2007). In addition, chilling temperatures harm thylakoid membranes, catabolise chlorophyll and reduce the photosynthetic potential of C₄ crops, such as maize (Tambussi et al., 2004).

The decline in photosynthetic potential in leaves of *Z. mays* at chilling temperatures is correlated with decreases in A_{sat} and ϕ (Sowinski et al., 2005; Hund et al., 2007). Chilling temperature also influences the chlorophyll content of the leaves and the proportion of variable to maximum fluorescence of chlorophyll a (F_v/F_m) (Hu et al., 2006). Walker et al. (1991) observed that chilling temperature regulates the rate of photosynthesis, the composition of thylakoid membrane fatty acids, stomatal conductance, and chlorophyll fluorescence of tomato. Additionally, chilling stress is known to cause several anomalies, such as membrane damage, generation of reactive oxygen species (ROS), denaturation of proteins and toxic compound accumulation at different levels of cellular structure (Hamayun et al., 2010).

In various areas in Saudi Arabia, for example, Jaizan, Mecca, Asir, Al-Madinah Al-Munawwarah and Al-Baha, *Pennisetum purpureum* and *Zea mays* are important C₄ crops for feeding cattle (Migahid, 1990). Temperatures in these areas decrease to chilling levels (14°C) during the winter season, with water salinity levels affected by irrigation. Therefore, the purpose of this study is to investigate the interactive effects of salinity and chilling stress on the growth of *P. purpureum* and *Z. mays* to determine the reliable crop for saline lands under chilling temperature conditions.

Materials and Methods

Plant material and growth conditions

Elephant grass rhizomes (*Pennisetum purpureum* Schumacher) were collected from a crop farm in Al-Madinah Al-Munawwarah, while maize seeds (*Zea mays* L. cv. Legacy SU; United States) were commercially obtained from Al-Hilali Agricultural Company, Al-Madinah Al-Munawwarah, Saudi Arabia. The *P. purpureum* rhizomes and *Z. mays* seeds were sown in plastic 2-L pots -litres (12cm x 20cm) each containing 2kg of sandy soil, washed and sterilised. Each pot contained eight seeds of *Z. mays* or four pieces of *P. purpureum* rhizomes. After emergence, plant seedlings were reduced to two per pot. The plants were all grown in a managed atmosphere chamber (JSR 314-240, 40-1 Gumsang-Dong, Gongju City, JS Research Inc, Korea) with a photoperiod of 14-hrs./10-hrs., 60% relative humidity and illumination from halogen and fluorescent spotlights providing 500 μ mol m⁻²s⁻¹. Five concentrations of NaCl (0, 75, 150,

225 and 300mM) were used in full-strength Hoagland nutrient solution. For each treatment, four replications under different concentrations of NaCl were placed in the chamber at daytime/nighttime temperatures of 14/12°C (chilling temperature) and 24/22°C (optimum temperature), resulting in 80 pots for the two species. All pots were watered daily for 7 days to field capacity with Hoagland full-strength nutrient solution. The NaCl treatments began on the 8th day. At the end of the experiment, 60d after planting, the following growth parameters were measured: height of plant, leaf number, and fresh weights of both roots and shoots. To estimate dry weight, shoot and root samples were dried for 48hrs. in an oven at 80°C. The leaf area was measured using a leaf area meter (LICOR Inc., Lincoln, Nebraska, USA).

Photosynthesis and respiration

On the recent fully expanded leaf, the rates of gas exchange were measured for *P. purpureum* and *Z. mays* species using a LICOR 6400XTT Analyser (LI-6400, LICOR Inc., Lincoln, United States of America). The fourth youngest leaves that were completely expanded were used for measurements of photosynthesis and dark respiration. The light response curves were fitted at a leaf temperature of 24°C and an ambient CO₂ concentration of 410 μmol mol⁻¹. Leaves were gradually illuminated with photon flux densities (PPFDs) between 0 and 1500 μmol m⁻²s⁻¹. Von Caemmerer and Farquar equations were used to determine the net photosynthesis per unit leaf area and the concentration of intercellular CO₂ (*c_i*) (Von Caemmerer & Farquar, 1981). The photosynthetic light saturation rate (*A_{sat}*) was estimated at saturating PPFD (1500 μmol m⁻²s⁻¹) and at an ambient CO₂ concentration of 410 μmol mol⁻¹.

Chlorophyll

The fluorescence of chlorophyll was determined using a movable fluorimeter (PEA, Kings Lynn, Hansatech, Norfolk). The initial (*F₀*) and ultimate (*F_m*) fluorescence emissions were measured after adaptation in the dark for twenty minutes, and the ratio of variable to maximum fluorescence (*F_v*/*F_m*) was determined as (*F_m*-*F₀*)/*F_m*, as defined by Öquist & Wass (1988). The values of *F_v*/*F_m* were determined four times for the fourth-recent fully extended leaves of *P. purpureum* and *Z. mays* species, as described by Al-Shoaibi (2008).

On the other hand, a portable chlorophyll meter was used to measure the content of chlorophyll,

which was expressed as the chlorophyll index (CCM-200, Opti-Sciences, United States of America). The fourth-newest leaves with complete expansion were used to determine the chlorophyll four times for each species in the various treatments, and averages of the four measurements for each species were used for analysis.

Statistical analyses

Three-way analyses of variance (ANOVAs) was used to perform statistical analysis of the data collected from the various observations and measurements, generally applying linear models to test primary effects and effects of interactions among the variables examined (salinity, species, temperature). Several comparisons were also made using the Tukey test to measure the significance of differences between levels of variables. All of the analyses were completed using Minitab version 15 (Brandon Court, Unit E1-E2, Progress Path, Coventry CV3 2TE, United Kingdom). For each treatment, four replications were used, and Microsoft Excel 2016 was used to calculate the standard deviations and errors.

Results

Plant growth parameters

The interactive effects of salinity and chilling temperature on parameters of growth in *P. purpureum* and *Z. mays* are presented in Figs. 1-3. All growth parameters were significantly reduced by salinity (*P* < 0.001), chilling temperature (*P* < 0.001) and the interaction effect of salinity and chilling temperature (*P* < 0.001). At 24°C, 75mM salinity did not induce significant decrease in any growth parameters in *P. purpureum* relative to control plants. However, all growth parameters at the different concentration of salinity for *Z. mays* grown at 24°C were significantly lower than those of control plants (*P* < 0.01). The fresh and dry weights of *Z. mays* shoots grown at 24°C and 300mM salinity were 82.1% and 83.5% lower, respectively, than those of the control plants grown at 24°C; these decreases were the highest observed among these indices (Fig. 2 A, B). However, most growth parameters for the two species grown at 14°C were significantly lower than those for plants grown at 24°C at the different levels of salinity (*P* < 0.001). Additionally, senescence actually occurred in *Z. mays* grown at 14°C and salinity concentrations of 225 and 300mM within three weeks of emergence.

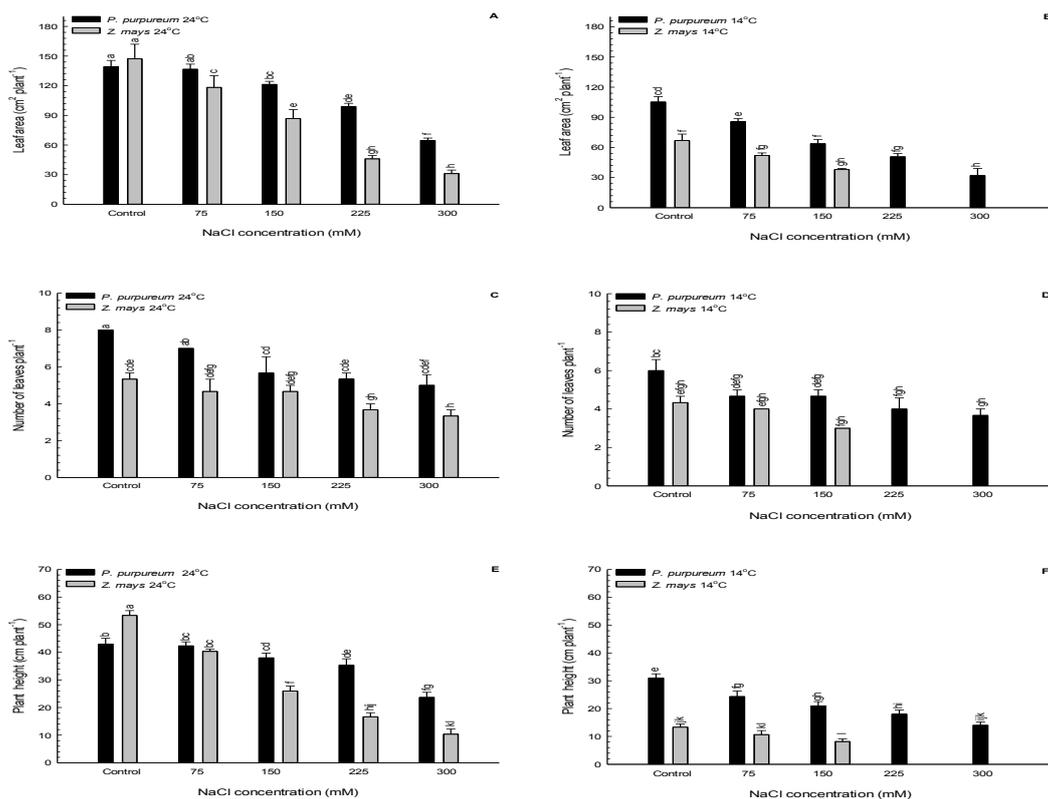


Fig.1. Combined effects of salinity and chilling temperature on the growth parameters of *Pennisetum purpureum* and *Zea mays* (n= 4, mean \pm S.E.). (A) leaf area at 24°C, (B) leaf area at 14°C, (C) number of leaves at 24°C, (D) number of leaves at 14°C, (E) plant height at 24°C, (F) plant height at 14°C [Different letters indicate significance of two-way interactions between chilling temperature and salinity. Means that do not share a letter are significantly different]

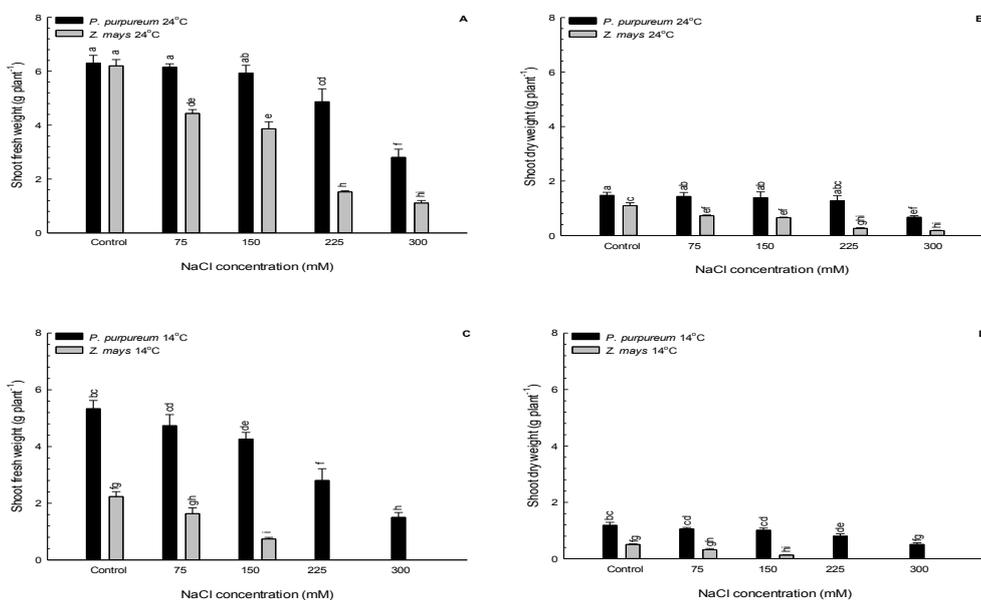


Fig. 2. Combined effects of salinity and chilling temperature on the growth parameters of *Pennisetum purpureum* and *Zea mays* (n= 4, mean \pm S.E.). (A) shoot fresh weight at 24°C, (B) shoot dry weight at 24°C, (C) shoot fresh weight at 14°C, (D) shoot dry weight at 14°C [Different letters indicate significance of two-way interactions between chilling temperature and salinity. Means that do not share a letter are significantly different]

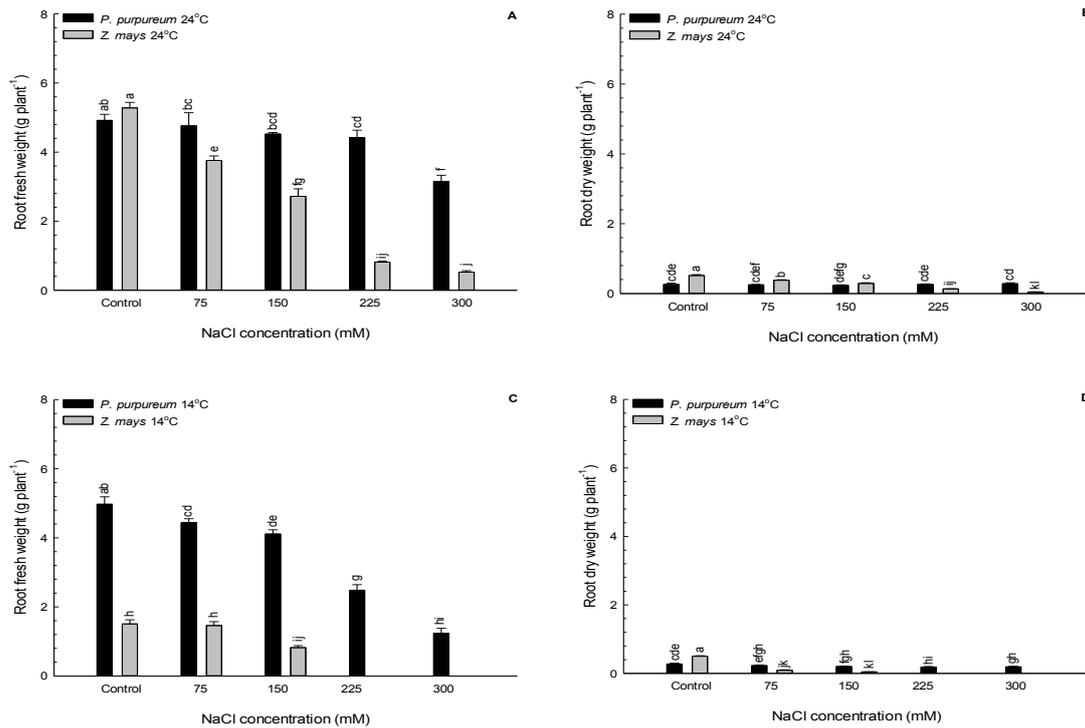


Fig.3. Combined effects of salinity and chilling temperature on the growth parameters of *Pennisetum purpureum* and *Zea mays* (n= 4, mean \pm S.E.). (A) root fresh weight at 24°C, (B) root dry weight at 24°C, (C) root fresh weight at 14°C, (D) root dry weight at 14°C [Different letters indicate significance of two-way interactions between chilling temperature and salinity. Means that do not share a letter are significantly different]

Photosynthesis and respiration

The photosynthetic rates were measured for *P. purpureum* and *Z. mays* (Figs. 4, 5). The results indicated that the interactive effects of chilling temperature and salinity significantly affected the photosynthetic rates of both species ($P < 0.001$). The chilling temperature of 14°C significantly decreased the A_{sat} and ϕ of both species compared to those grown at 24°C at the different levels of salinity ($P < 0.001$). The A_{sat} and ϕ of *Z. mays* grown at 14°C and a salinity concentration of 150mM were 63.7% and 62.5% lower, respectively, than those of the control plants grown at 14°C; these differences were the greatest observed among these indices. In addition, with increasing salinity, the levels of photorespiration in the two species increased in the two temperature treatments (Fig. 4). However, *P. purpureum* exhibited higher rates of photosynthesis in the two temperature treatments under different salinity concentrations (Figs. 4, 5). This indicates that the photosynthetic machinery of *P. purpureum* is more tolerant to chilling temperature and salinity than that of *Z. mays*. The seedlings of *Z. mays* grown at 225 and 300mM NaCl under chilling temperature showed senescence before the

measurements of photosynthesis.

Chlorophyll

The outcomes in Fig. 6 A, B show the interactive effects of salinity and chilling on F_v/F_m for both *P. purpureum* and *Z. mays*. Results indicated that the interactive effects of chilling and salinity significantly affected the F_v/F_m values of both species ($P < 0.001$). At 24°C, salinity of 225 and 300 mM was significantly reduced the F_v/F_m of *Z. mays* relative to the control plants ($P < 0.05$; Fig. 6 A). However, *P. purpureum* grown at 24°C did not induce any significant decrease in F_v/F_m at any salinity level relative to control plants. In contrast, the chilling was significantly decreased the F_v/F_m values of both species at a salinity concentration of 150 mM compared to those of the control ($P < 0.05$; Fig. 6 B). The chlorophyll fluorescence of *Z. mays* grown at 14°C and a salinity concentration of 150 mM was 14.9% lower than that of the control plants grown at 14°C; this difference was the greatest observed among these indices. In addition, *Z. mays* seedlings grown at 225 and 300mM NaCl under chilling temperature displayed senescence before showing effects on F_v/F_m .

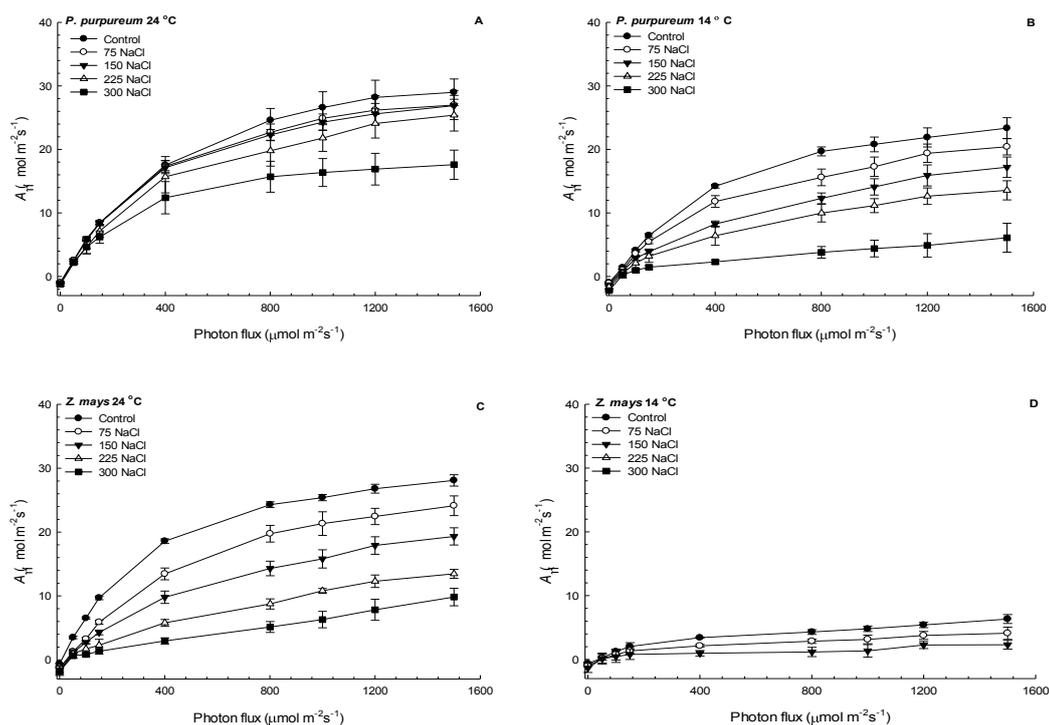


Fig. 4. The Photosynthetic CO_2 absorption response (A) per unit area of the leaf to photon flux (Q) for *Pennisetum purpureum* and *Zea mays*. CO_2 absorption measurements were all made at 24°C and Ca of $410\mu\text{mol mol}^{-1}$ [The data is the mean of leaves ($n=4$, $\text{mean}\pm\text{S.E.}$)]

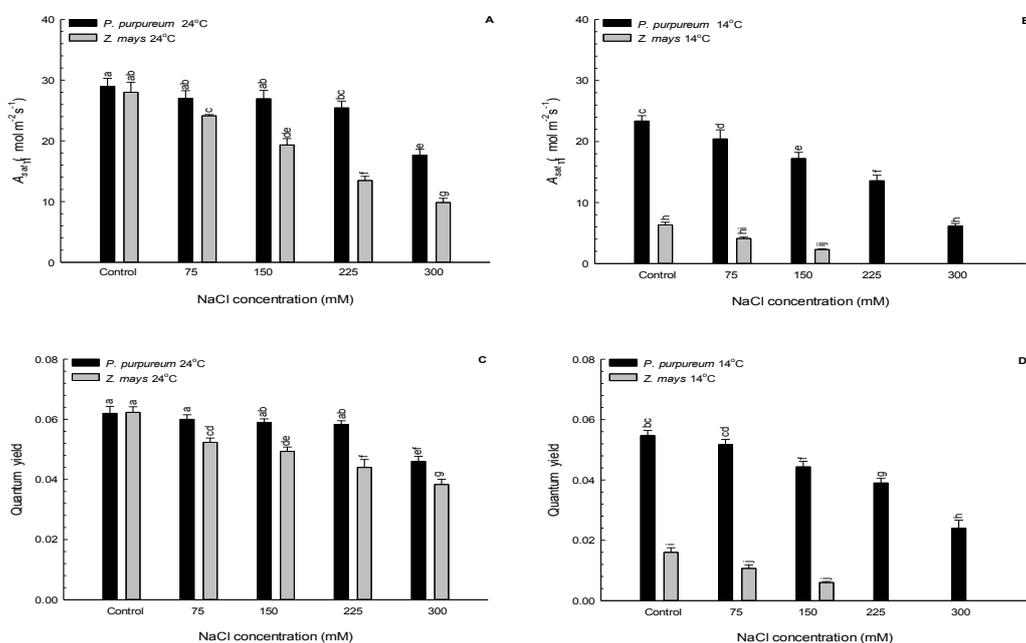


Fig. 5. Combined effects of salinity and chilling temperature on the photosynthesis performance of *Pennisetum purpureum* and *Zea mays* ($n=4$, $\text{mean}\pm\text{S.E.}$). (A) & (B), the photosynthetic light-saturated amount (A_{sat}) per unit area of the leaf for species grown at 24°C and 14°C , respectively. (C) & (D), the mean quantum yield (ϕ) for species grown at 24°C and 14°C , respectively [All the measurements estimated at 24°C and $1500\mu\text{mol m}^{-2}\text{s}^{-1}$. Different letters indicate significance of two-way interactions between chilling temperature and salinity. Means that do not share a letter are significantly different]

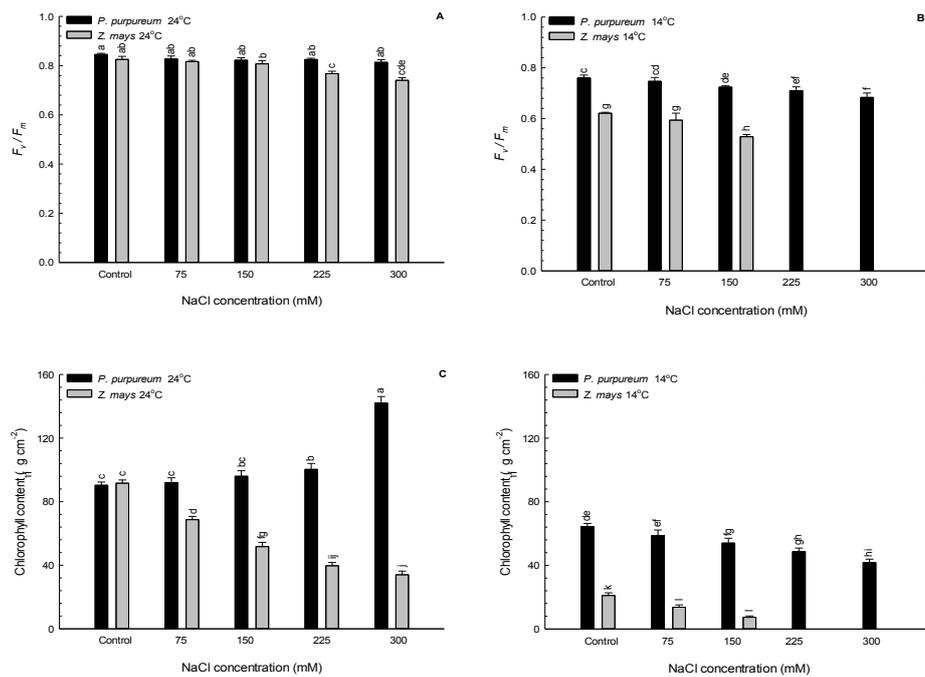


Fig.6. Combined effects of salinity and chilling temperature on chlorophyll content and chlorophyll fluorescence (F_v/F_m) of *Pennisetum purpureum* and *Zea mays* ($n = 4$, mean \pm S.E.). (A) & (B), the chlorophyll content for species grown at 24°C and 14°C, respectively. (C) & (D), the chlorophyll fluorescence for species grown at 24°C and 14°C, respectively [Different letters indicate significance of two-way interactions between chilling temperature and salinity. Means that do not share a letter are significantly different]

The chlorophyll content was determined to estimate the interactive effects of salinity and chilling temperature on the chlorophyll contents of both species. The outcomes indicated that the interactive effects of chilling temperature and salinity significantly affected the chlorophyll contents of both species ($P < 0.001$). At 24°C, the chlorophyll contents of *P. purpureum* increased with increasing salinity (Fig. 6 C). However, the chlorophyll contents of *Z. mays* significantly decreased with increasing salinity levels relative to those of the control plants ($P < 0.01$; Fig. 6 C). On the other hand, the chilling temperature significantly decreased the chlorophyll contents of both species at a salinity concentration of 150mM compared to values for control plants ($P < 0.05$; Fig. 6 B). The chlorophyll content of *Z. mays* grown at 14°C and a salinity concentration of 150mM was 65.1% lower than that of the control plants grown at 14°C; this difference was the greatest observed among these indices. Furthermore, *Z. mays* seedlings grown at 225 and 300mM NaCl under chilling temperature showed senescence prior to the appearance of effects on chlorophyll content.

Discussion

The interactive effects of salinity and chilling stress on plant growth, gas exchange, F_v/F_m and chlorophyll contents in two C_4 species, *P. purpureum* and *Z. mays* was investigated. The growth of young seedlings is more susceptible to salinity than the growth of mature plants. As a result, successful seedling growth is essential for the formation of plant communities (Farooq et al., 2015). The results of this work showed that *P. purpureum* was able to grow at different salinity levels in the two temperature treatments. The ability of plants to grow in a broad range of salinities under the two temperature treatments is important for the growth of plants in various environments (Qu et al., 2008). Additionally, the results showed that the maximum growth parameters for the two species at the different levels of salinity were recorded at 24°C. However, 14°C caused a reduction in most parameters of *P. purpureum* growth at 225 and 300mM salinity, while the seedlings of *Z. mays* were not able to survive at 225 and 300mM salinity (Figs. 1-3). Chilling temperature can inhibit the growth of shoots and roots, resulting in lower yields

(Wahid et al., 2007). Clifton-Brown & Jones (1997) found that the ability of plants to grow at chilling temperature is one of two variables that affect the production of C_4 species in cold climates. Furthermore, the reduction in the growth parameters at 14°C for the two species at higher salinity may have resulted from lower cell elongation rates and numbers of elongated cells (Farooq et al., 2015). Thus, the results reveal a superior resistance of *P. purpureum* seedlings to salinity stress at chilling temperature compared with that of *Z. mays*. The tolerance of *P. purpureum* to salinity at chilling temperature may have resulted from genetic differences between species (Almodares et al., 2007). Related results have been recorded for *P. turgidum* (Al-Khateeb, 2006) and nine medical plant species (Nadjafi et al., 2010). Furthermore, the findings of this study showed that in both species, the combination of salinity and chilling temperature influenced shoots more than roots (Figs. 2, 3). Munns & Sharp (1993) reported that shoots were more susceptible to salt stress than roots.

The performance of photosynthetic apparatus is important for plants that suffer from abiotic stress (Kalisz et al., 2016). Chilling temperature and salinity stress are among the most important abiotic stresses that affect photosynthesis by reducing the transport of electrons, reducing photosystem II efficiency (PSII), harming photosystem I (PSI), varying the cycle of carbon reduction, limiting stomata, degrading chlorophyll and increasing the formation of reactive oxygen species (ROS) (Saibo et al., 2009). In this study, the photosynthetic responses of *P. purpureum* and *Z. mays* to chilling temperature and salt stress were investigated. The results showed that all leaves of *P. purpureum* in the control group and those grown under 75 and 150 mM NaCl had comparable rates of A_{sat} and ϕ at 24°C (Fig. 5). Rates of A_{sat} and ϕ have been previously found to be similar to those in leaves of good health in a variety of NADP-malic enzyme C_4 plants (Ehleringer & Pearcy, 1983). This suggests that *P. purpureum* was not stressed and did not suffer from photoinhibition under earlier temperature and salinity conditions. In contrast, the rates of A_{sat} and ϕ in *Z. mays* plants that had developed at salinity concentrations of 75, 150, 225 and 300mM NaCl and at a temperature of 24°C were lower than those previously found in a number of NADP-malic enzyme C_4 grasses, signifying that *Z. mays* was stressed and suffered from photoinhibition. Thus,

the outcomes indicated that the photosynthetic apparatus of *P. purpureum* is more tolerant to salinity at the optimum temperature of 24°C than that of *Z. mays*. Similar findings have been noted for two sugar beet cultivars (Dadkhah, 2011) and for various cultivars of sorghum (Yan et al., 2012). Furthermore, the 14°C treatment caused substantial declines in A_{sat} and ϕ in *P. purpureum* grown at 225 and 300 salinity relative to those in the control, whereas *Z. mays* seedlings senesced when grown under the same conditions. This reduction in the photosynthetic performance of *P. purpureum* might be a result of stomatal behaviour, toxicity of ions or both (Yan et al., 2012). Additionally, the substantial decrease in the photosynthetic potential of *P. purpureum* at 225 and 300 mM salinity might be a reason for the reduction in chlorophyll content or a pause in leaf growth and expansion (Morales et al., 2020). Another explanation for the decline in the photosynthetic performance of *P. purpureum* may be a decrease in activity bundle sheath cell enzymes, such as Rubisco (Morales et al., 2020).

The findings of this research showed that when *Z. mays* was grown at 24°C, chlorophyll fluorescence parameters were significantly reduced at varying salinity concentrations. On the other hand, the chlorophyll fluorescence parameters were comparable at varying salinity concentrations when *P. purpureum* was grown at 24°C. These excellent F_v/F_m values showed that *P. purpureum* grown at 24°C was resistant to photoinhibition under various salinity treatments. Related findings have been previously described for two wheat cultivars with different salinity tolerances (Arfan et al., 2007). Therefore, the results of this research indicate that the fluorescence parameters of chlorophyll cannot be counted as one of the factors controlling the net CO_2 assimilation rate in *P. purpureum* developing under various salinity treatments at optimum temperatures. The chilling temperature, on the other hand, dramatically decreased the F_v/F_m at 150 NaCl in both species compared to the control. This decrease in F_v/F_m may be due to oxidative damage to photosynthesis devices in plants, particularly when temperatures are low (Yan et al., 2012). According to Turan & Ekmekci (2014), the low F_v/F_m value in PSII suggests the existence of photochemically inactive reaction centres as well as a decreased electron transport power.

According to the results of this study, the chlorophyll content of *P. purpureum* developed at 24°C increased dramatically with increasing salinity levels in contrast to that in the control (Fig. 6 C). Several plants have shown similar results under high salinity (Al-Shoaibi & Al-Sobhi, 2004). The cause for this increase may be attributed to elevated chloroplast numbers in stressed leaves or declines in leaf area (Al-Shoaibi & Al-Sobhi, 2004). In contrast, chilling temperature greatly reduced the chlorophyll content in both species developing under 150mM salinity compared to the control. This reduction in chlorophyll content may be attributed to the lack of grana stacking or to variations in thylakoid composition (Purnama et al., 2018).

Conclusion

In summary, *P. purpureum* displayed greater resistance to salinity than did *Z. mays* for most reported measurements at chilling temperature. *P. purpureum* tends to be a reliable crop for saline agricultural lands under chilling temperature conditions.

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التأثيرات المشتركة لإجهاد الملوحة ودرجة الحرارة التبريد على نمو وتبادل الغازات لنباتي حشيشة الفيل والذرة

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تنخفض درجات الحرارة خلال فصل الشتاء إلى 14 درجة مئوية، مع مستويات ملوحة مرتفعة لمياه الري في مناطق مختلفة من المملكة العربية السعودية. الهدف من هذه الدراسة هو دراسة التأثيرات المشتركة لضغط الملوحة ودرجة حرارة التبريد على نمو النبات، Fv/Fm ، ومحتوى الكلوروفيل وتبادل الغازات في نوعين C_4 ، *Pennisetum purpureum* و *Zea mays*. تضمنت العلاجات درجات حرارة نهائية وليلية تبلغ 12/14 درجة مئوية (درجة حرارة التبريد) و 22/24 درجة مئوية (درجة الحرارة المثلى) و 5 تركيزات كلوريد الصوديوم (0، 75، 150، 225، 300 ملم). أظهرت النتائج أن مستويات كلوريد الصوديوم زادت، وانخفضت معاملات النمو وتناقص الغاز المتبادل بين النوعين، مع تأثيرات قصوى عند درجة حرارة التبريد عن درجة الحرارة المثلى. لم تود درجة الحرارة المثلى إلى أي انخفاض معنوي في قيم Fv/Fm لنبات *P. purpureum* عند تراكيز مختلفة من الملوحة، بينما قلت درجة حرارة التبريد بشكل كبير من قيم Fv/Fm لكلا النوعين عند الملوحة العالية. في المقابل، زاد محتوى الكلوروفيل عند 24 درجة مئوية تحت زيادة الملوحة في *P. purpureum*. بالإضافة إلى ذلك، حدث شيخوخة في *Z. mays* نمت عند 14 درجة مئوية وتركيزات ملوحة 225 و 300 ملم في غضون ثلاثة أسابيع من الانبات. أثر التفاعل بين درجة حرارة الملوحة والتبريد بشكل كبير على معظم قياسات النوعين. ومع ذلك، أظهر *P. purpureum* مقاومة فائقة تجاه الإجهاد. لذلك، يميل *P. purpureum* إلى أن يكون محصولاً موثوقاً به للأراضي الزراعية المالحة تحت معاملات درجة حرارة التبريد.