

## Mitigating the Toxic Effects of Salinity in Wheat through Exogenous Application of Moringa Leaf Extract

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### ABSTRACT

Allelochemicals have emerged as an important player in inducing the abiotic stress tolerance. The experiment included three components: different levels of salinity stress (SS: control, 6 dS m<sup>-1</sup>, 12 dS m<sup>-1</sup>), seed priming with moringa leaf extract (MLE: 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%), and saltwater-tolerant and salinity-sensitive wheat cultivars (Faisalabad-2008, Galaxy-2013). Results showed that salinity lowered photosynthetic pigments, photosynthesis, transpiration, internal carbon, and stomatal conductance while causing poor and delayed germination, inconsistent seedling growth, and increased hydrogen peroxide accumulation. However, hydro-priming and MLE priming enhanced emergence dynamics, growth, biochemical and enzymatic characteristics, and physiological aspects. The cultivar Faisalabad-2008 (wheat) performed well, but at high salinity levels, the hormetic impact of moringa leaf extract was more obvious, enhancing the germination and growth of cultivar Galaxy-2013, which was salinity-sensitive. Wheat cultivars' germination and seedling growth improved most when primed with 2% MLE (Faisalabad-2008) and 2.5% MLE (Galaxy-2013). This demonstrated that moringa possesses growth-promoting compounds that efficiently mitigate the toxic impacts of salinity.

**Keywords:** antioxidant, allelochemicals, salinity, seed priming, wheat.

### INTRODUCTION

The intensity of abiotic stresses is continuously increasing which is a posing serious threat to crop productivity. The global staple crops of wheat, rice, and maize supply calories and protein (Kizilgeci et al., 2021). The most domesticated and significant grain crop worldwide is wheat (Iqbal et al., 2021). Although it is cultivated on most of the arable land (38.8%), its production is lesser (Loke et al., 2016). Abiotic stresses have grown due to climate change, which may further lessen it. Climate models predict that stressful events could result in a 6% decrease in

productivity of wheat (Asseng et al., 2015). Approximately 20% of the world's arable land is experiencing worsening salt stress because of human activities and climate change (Arora, 2019). Salinity has the potential to account for 50% of output losses (Acquaah 2009). By 2050, the food supply has to be increased by 70% to keep up with population growth, which would put pressure on food supply (FAO 2009). One-third of people on the planet consume wheat. 20% of the calories and 55% of the carbohydrates in the globe come from wheat. Salinity impairs the development and production of wheat (Royo et al., 2003). Salinity stress causes both osmotic stress and ion

toxicity which cause serious growth and yield reduction (Arif et al., 2020). Salinity alters the cellular ultrastructure, prevents photosynthesis from occurring, damages membrane structures, generates reactive oxygen species, and inhibits enzyme activity, among other consequences on crop development and productivity. Salinity tolerance in plants is polygenic, which means that several different genes regulate it (Arzani et al., 2016) and thus progress attained over the several decades has been far less than anticipated. The generation of an explosion of knowledge and technology related to genetics and genomics over the last few decades is promising in providing powerful tools for future development of salinity-tolerant cultivars. Despite a major progress in defining the underlying mechanisms of salinity tolerance, there are still major challenges to be overcome in translating and integrating the resultant information at the molecular level into plant-breeding practices. Various approaches have been suggested to improve the efficiency of plant breeding for increasing plant productivity under saline environments. In this context, breeding for salinity tolerance in crops largely depends upon the availability of genetic resources of tolerance, reliable screening techniques, identification of genetic components of tolerance, and successful genetic manipulation of desired genetic backgrounds. The efficiency of selection and breeding in the stressful environments can be improved through marker-assisted selection. To date, this is almost exclusively applied to major genes, but this requires to be extended to quantitative trait loci (QTLs). When plants are grown in salty conditions, their antioxidant and immune systems, as well as an increase in  $K^+$  and a decrease in  $Na^+$  ions, all aid in crop development (Rahman et al., 2005).

Wheat is sensitive to salt stress. Recent research has demonstrated that the ability of wheat genotypes to adjust to salt depends on changes in their stem and leaf morphology (Nassar et al., 2020). The breakdown of proteins, reabsorption of mineral nutrients, and loss of photosynthetic pigments are the three main physiological processes that are examined during the senescence of leaves (Sytar et al., 2018). Managing salt-affected soils can decrease salinity's adverse effects. Although well-defined measures like amendments and good drainage can be employed for this objective, it is difficult to manage salinity because of expensive additives, low soil permeability, and a lack of high-quality water (Hussain et al., 2019).

Natural growth regulators known as bio-stimulants increase crop output by improving nutrient uptake, rhizosphere activity, and tolerance to biotic and abiotic stress (Shakirova et al., 2012). To regulate crop development and function, bio-regulators work in conjunction with endogenous phytohormone groups (Wajid et al., 2019). These bioregulators promote biomass production, blooming, and growth. Phyto phenols neutralise free radicals, acting as antioxidants. Numerous plant extracts include bioactive substances that, in small amounts, promote growth (Bhattacharya 2021). Low-concentration allelochemicals increase seedling growth, abiotic stress resistance, and germination (Maqbool et al., 2017). Priming enhances seed vigor, seedling growth, and plant development in stressful environments, such as those with high or low saline levels (Shah et al., 2021). Abiotic stresses in agriculture can be reduced by allopathic aqueous crop extracts (Huang et al., 2021). The fast-growing, salt-tolerant moringa tree (*Moringa oleifera*) leaves contain a lot of phenolics, and antioxidant (Pakade et al., 2013). Zeatin, a cytokinin derivative, vitamins, minerals K, Ca, and Fe are all present in the plant (Moyo et al., 2011). Extract from moringa leaves can increase agricultural yields by 20 to 35% (Abbas et al., 2017). By changing metabolic pathways, moringa leaf extract (MLE) can increase germination, growth, and production of salinity stressed plants (Yasmeen et al., 2013). Moringa leaf extract has been reported to improve seedling vigor, and seedling growth (Ali et al., 2011), and growth and yield of crops (Afzal et al., 2012) KCl (2% w/v). Nonetheless, the role of MLE in wheat physiological and biochemical response is unknown. Thus this study was performed to determine the role MLE seed priming to mitigate the toxic effects of salinity stress.

## MATERIALS AND METHODS

This study was University of Agriculture, Faisalabad (31°-44' N, 73°-06' E) during 2018. Seeds of wheat cultivars (Faisalabad-2008; Galaxy-2013) were taken and sterilized with sodium hypochlorite (2.63%) prior to sowing. The wheat varieties were selected on the basis of salt tolerance. No priming and priming of seeds with water and (0.5, 1, 1.5, 2, 2.5, and 3%) MLE were the treatments, and salinity levels of soils in pots were maintained at (normal, 6, and 12  $dSm^{-1}$ ) using NaCl. The concentration of MLE was selected on

the basis of previous studies findings. The study was performed in CRD with factorial combination having three replications. The pots were daily visited to record the number of germinated seeds and emergence time (ET), was calculated by the methods of TeKrony (1983). On the other time to 50% emergence (TFE) and mean emergence time (MET) were calculated with methods of Farooq et al. (2005) and Ellis et al. (1981) respectively. Physiological parameters such as net photosynthetic rate, transpiration rate, carbon flux, and stomatal conductance were recorded one day before harvesting by using a photosynthetic system (Lci 4225). Chlorophyll contents were measured by following the procedure of Arnon, (1949). For photosynthetic pigments; 0.5 g fresh leaves were homogenized in 80% acetone solution and extract was obtained. Then absorbance was noted at 663, 645, and 480 nm to determine concentration of chlorophyll a, b and carotenoids, respectively. The activity of superoxide dismutase (SOD) was determined with procedures of Dhindsa et al. (1981) lipid peroxidation, and activities of superoxide dismutase (SOD while standard procedures of Aebi, (1984) the enzymic decomposition of H<sub>2</sub>O<sub>2</sub> is a first-order reaction, the rate of which is always proportional to the peroxide concentration present. Consequently, to avoid a rapid decrease in the initial rate of the reaction, the assay must be carried out with relatively low concentrations of H<sub>2</sub>O<sub>2</sub> (about 0.01 M were used to determine CAT activity. Before the experiment, 100 mM H<sub>2</sub>O<sub>2</sub> + 50 mM phosphate buffer (pH 7.8) was heated to 25 °C. In a 10 ml tube, 0.2 ml phosphate buffer and 0.2 ml enzyme solution were heated for 3 minutes. In 10 ml tube, 0.3 mL (100 mM H<sub>2</sub>O<sub>2</sub>) was introduced. The enzyme solution in the control tube was boiled for 5 minutes. After mixing, 240 nm absorbance was measured every minute. 4 minutes of perseverance 1 U of enzyme activity = 0.1 A<sub>240</sub> reduction in 1 min. For determination of POD the reaction mixture was contained, 10 mM guaiacol, 5 mM H<sub>2</sub>O<sub>2</sub>, and 50 mM phosphate buffer (pH 7.0). Then this mixture was allowed to heat and absorbance was noted to determine POD activity. The concentration of H<sub>2</sub>O<sub>2</sub> in plant samples was determined by methods of Velikova et al. (2000). All the collected information on the emergence index, plant development, physiological, and biochemical factors was statistically analyzed by ANOVA (Statistix 8.1) and Tukey's Honestly significant difference (HSD) was used to separate significance among means. Sigma Plot 10.0 was used for the graphical presentation, and Microsoft Excel 2016 was used for the calculations.

## RESULTS

### Time to start emergence (days)

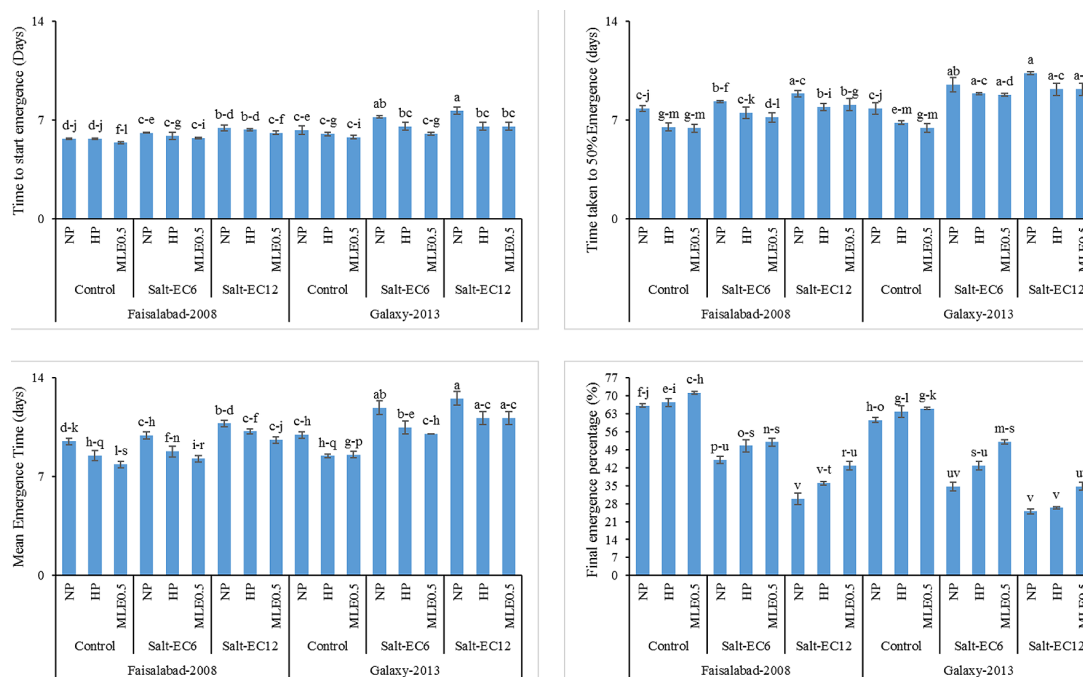
Figure 1 displays data on the variances in the timing of the emergence of primed and unprimed seeds of two wheat cultivars at various salinity levels. A significant difference in the timing of the onset of emergence of both wheat cultivars (C) was seen (S). Data showed that when non-primed seeds from the Faisalabad-2008 crop were exposed to salt the emergence time was increased by 7 and 13%. The emergence time of Galaxy-2013 increased by 15 and 22% in accordance. The emerging time of Faisalabad-2008 was dramatically shortened by hydro priming and priming with various concentrations of MLE, where the emergence time was reduced by 5–38%, 3–77%, and 1–24%, respectively. The Galaxy-2013 wheat cultivar displayed the same behavior, with emergence times decreasing by 4–40% (control), 9–34% (6 dS m<sup>-1</sup>), and 15–32%. (12 dS m<sup>-1</sup>). However, in Faisalabad-2008 and Galaxy-2013, respectively, seeds primed with 2% and 2.5% MLE showed a marked reduction in ET.

### Time taken to 50% emergence (days)

Seed priming with MLE and water reduced the TFE and non-primed seeds of Faisalabad-2008, and Galaxy-2013 extended took more time 50% emergence by 6–13% and 21–32% at salinity levels of 6–12 dS m<sup>-1</sup>, respectively as compared to their respective control (non-primed seeds grown under no-stress conditions). Hydro priming and seed priming with MLE substantially T<sub>50</sub> emergence. Maximum reduction in time taken to 50% emergence was observed in Faisalabad-2008 at 2% MLE and in Galaxy-2013 at 2.5% MLE. In control conditions (no salt stress), all priming treatments in both cultivars (Faisalabad-2008 + Galaxy-2013) reduced T<sub>50</sub> by 16–33% and 13–24% over control (non-primed seeds).

### Mean emergence time (days)

Mean emergence time varied significantly ( $p \leq 0.05$ ) among cultivars (C), salinity levels (S), and seed priming treatments (SP). The two-way interaction of (C×S) and (C×SP) were found significant. Exposure to salt stress in non-primed seeds considerably increased the MET of both cultivars (Figure 1). Maximum increase in MET was observed at 12 dS m<sup>-1</sup> followed by 6 dS m<sup>-1</sup>. When



**Fig.1.** Interactive effect of different priming techniques on time to start emergence, time taken to 50% emergence, mean emergence time and final emergence percentage of two wheat cultivars

compared to the control, salinity increased the MET of Faisalabad-2008 by 4% (at 6 dS m<sup>-1</sup>) and 13% (at 12 dS m<sup>-1</sup>) in non-primed seeds. Similar increase in MET of Galaxy-2013 was observed by 19% (at 6 dS m<sup>-1</sup>) and 25% (at 12 dS m<sup>-1</sup>) in non-primed seeds. The increasing concentration of MLE significantly reduced the MET of both cultivars under salinity as compared with their respective controls (Figure 1). Among dilutions, MLE 2% and MLE 2.5% were the most effective owing to the maximum reduction in MET of Faisalabad-2008 and Galaxy-2013 respectively. At moderate salt stress, MET 8 was decreased by 16–30% and 12–29% in Faisalabad-2008 and Galaxy-2013, respectively. Such reductions in MET of Faisalabad-2008 and Galaxy-2013 were observed by 5–27% and 11–28% respectively under higher SS.

### Final emergence percentage

A significant variation was observed in the final emergence percentage of both wheat cultivars (C) after 15 days. Salinity stress substantially reduced the EP of all the tested cultivars. However, both the cultivars showed improvement in final emergence percentage under all priming treatments (Hydro-priming + MLE). Maximum improvement in final emergence percentage was observed at 2% and 2.5% MLE in Faisalabad-2008 and Galaxy-2013 respectively. All priming treatments significantly

maximized the final emergence percentage of Faisalabad-2008 by 2–36% (control), 12–70% (6 dS m<sup>-1</sup>), and 19–112% (12 dS m<sup>-1</sup>) over control (non-primed seeds). The corresponding improvement in the final emergence percentage of Galaxy-2013 was recorded by 4–39% (control), 22–87% (6 dS m<sup>-1</sup>), and 5–125% (12 dS m<sup>-1</sup>) as compared with their respective control (non-primed seeds).

### Physiological parameters

#### Rate of photosynthesis ( $\mu\text{mol Co}_2 \text{ m}^{-2} \text{ s}^{-1}$ )

The statistically significant ( $p \leq 0.5$ ) effects of hydro-priming and seed priming with different concentrations of MLE on rate of photosynthesis of two wheat cultivars after 30 days under different SS (Figure 1). Salt stress (6 dS m<sup>-1</sup>+12 dS m<sup>-1</sup>) in non-primed seeds considerably reduced the rate of photosynthesis of Faisalabad-2008 (30%+57%) and Galaxy-2013 (47%+74%) as compared with non-primed seeds grown under control conditions (no stress).

#### Rate of transpiration ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )

Significant variation was observed in the rate of transpiration of wheat cultivars with MLE priming, HP and NP under diverse SS levels (Figure 2). It is revealed that exposure of non-primed seeds of cultivars Faisalabad-2008 to both SS levels showed a



reduction of 28% and 57% in transpiration rate (no salinity). The corresponding decrease in the rate of transpiration of Galaxy-2013 was 45 and 75%. Hydro priming and priming with different concentrations of moringa leaf extract improved the transpiration rate of the wheat cultivar (Faisalabad-2008) by 9–68% in control (no salinity) while such improvements ranged from 2–88% and 113–122% under SS. The same behavior was noticed for the wheat cultivar Galaxy-2013 wherein the rate of transpiration was improved by 1–49% (control), 4–109% (6 dS m<sup>-1</sup>) and 27–183% (12 dS m<sup>-1</sup>).

### Internal carbon (μmol Co<sub>2</sub> mol air<sup>-1</sup>)

The maximum internal carbon concentration was observed in non-primed seeds of Galaxy-2013 followed by Faisalabad-2008 (56%) at a salinity level of 12 dS m<sup>-1</sup>. At 6 dS m<sup>-1</sup> salinity maximally suppressed the internal carbon concentration of Galaxy-2013 by 45% followed by Faisalabad-2008 by 28% (Figure 2). All the priming treatments showed a significant influence in improving the internal carbon concentration of both the cultivars which was increased at seed priming level of 2% MLE in Faisalabad-2008 and at 2.5% MLE in Galaxy-2013 under all salinity levels. Hydro-priming and seed priming with different concentrations of MLE significantly improved the internal

carbon concentration of Faisalabad-2008 by 9–68%, 2–88%, 13–121% in control, moderate, and higher SS levels respectively. A similar improving trend in internal carbon concentration of Galaxy-2013 was observed by 1–49%, 4–108%, 27–183% in control, moderate, and higher SS levels respectively.

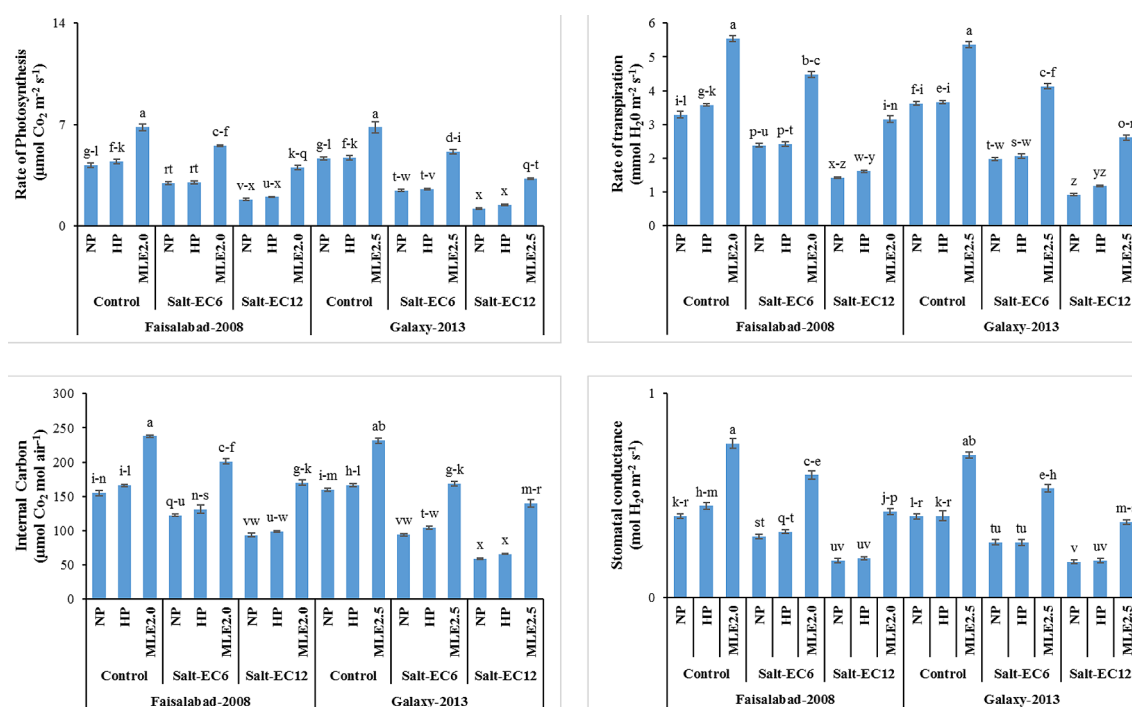
### Stomatal conductance (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)

Stomatal conductance was significantly varied at different salinity levels by varying wheat cultivars and MLE concentration. Figure 2 shows the effect of salinity on primed and non-primed seeds of both cultivars. SS significantly reduced stomata conductance of non-primed seeds of Faisalabad-2008 (21% at 6 dS m<sup>-1</sup>, 40% at 12 dS m<sup>-1</sup>) and Galaxy-2013 (41% at 6 dS m<sup>-1</sup>, 63% at 12 dS m<sup>-1</sup>). Under unstressed conditions, seed priming with distilled water + MLE enhanced the stomatal conductance of Faisalabad-2008 and Galaxy-2013 up to 53% and 44% respectively. At 6 dS m<sup>-1</sup> MLE maximized the stomatal conductance of Faisalabad-2008 and Galaxy-2013 up to 65% and 79% than NP.

## Biochemical parameters

### Chlorophyll a (mg/g)

Significant variations (Figure 3) were observed in chl wheat cultivars under the effect of



**Fig.2.** Interactive effect of different priming techniques on rate of transpiration, internal carbon and stomatal conductance of two wheat cultivars

MLE at different salinity levels. Data (Figure 3) revealed that exposure of non-primed seeds of cultivars Faisalabad-2008 to salinity levels of 6 dS m<sup>-1</sup> and 12 dS m<sup>-1</sup> decreased the chl a content by 27 and 55% as compared with control (no salinity). Hydro priming and priming with 2% (Faisalabad-2008) and 2.5% (Galaxy-2013) moringa leaf extract improved the chl a of wheat cultivars by 15–68% in control (no salinity) while such improvements ranged from 11–68% and 29–126% under moderate and higher SS level. The same behavior was noticed for the wheat cultivar Galaxy-2013 wherein chl a content was improved by 12–60% (control), 6–90% (6 dSm<sup>-1</sup>) and 26–188% (12 dS m<sup>-1</sup>).

#### Chlorophyll b (mg/g)

Data represent the effect of salinity on non-primed and primed seeds of both wheat cultivars. Salinity levels 6 dS m<sup>-1</sup> and 12 dS m<sup>-1</sup> restricted the chl b of non-primed seeds of Faisalabad-2008 by 19–47% and Galaxy-2013 by 41–67% as compared with chl b of non-primed seeds grown under control conditions. Hydro priming and seed priming with 2% MLE in Faisalabad-2008 improved the chl b by 6–62% (in control), 25–48% (at 6 dS m<sup>-1</sup>) and 19–87% (at 12 dS m<sup>-1</sup>). Similarly, the maximum improvement in chl b of Galaxy-2013 was recorded

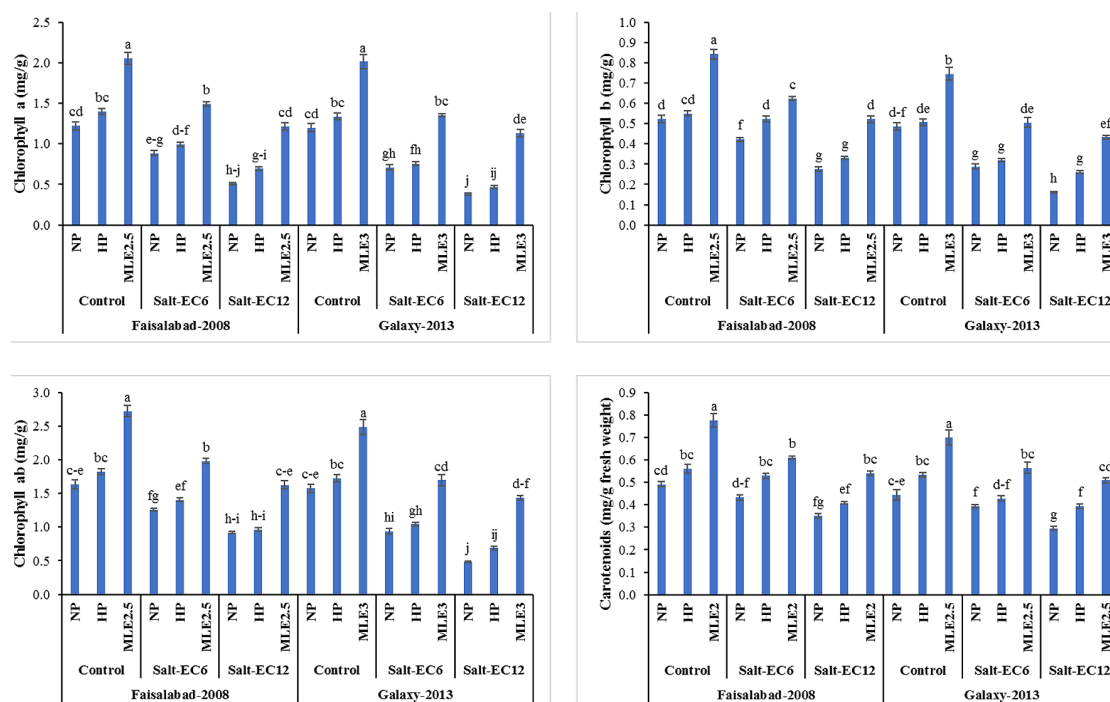
with 2.5% MLE under control, 6 dS m<sup>-1</sup> and 12 dS m<sup>-1</sup> by 4–52%, 11–75%, 63–170% respectively.

#### Total chlorophyll content (mg/g)

Non-primed seeds of both cultivars showed a marked decline in total chlorophyll content at 6 and 12 dS m<sup>-1</sup> (Fig. 3). When compared to control, salinity retarded the chlorophyll of non-primed seeds of both cultivars (Faisalabad-2008 by 22–43%) (Galaxy-2013 by 40–69%) with increasing level of salinity at 6–12 dS m<sup>-1</sup> respectively. Priming treatments (Hydro-priming+ 2–2.5% MLE) under study statistically improved the total chlorophyll of Faisalabad-2008 (11–57% at 6 dS m<sup>-1</sup> and 4–77% at 12 dS m<sup>-1</sup>) and Galaxy-2013 (12–82% at 6 dS m<sup>-1</sup> and 41–193% at 12 dS m<sup>-1</sup>) as compared with their control. Although Faisalabad-2008 showed 16% more chlorophyll but maximum improvement in total chlorophyll was seen in Galaxy-2013 (salt sensitive cultivar) as compared with Faisalabad-2008 (salt tolerant crop).

#### Carotenoids (mg/g fresh weight)

Salinity restricted the carotenoids of non-primed seeds of Faisalabad-2008 and Galaxy-2013 at Ec level of 6 dS m<sup>-1</sup> (11%, 20%) and 12 dS m<sup>-1</sup> (28%, 40%) respectively. However, priming treatments (Hydro-priming + 2% MLE) significantly enhanced



**Fig.3.** Interactive effect of different priming techniques on chlorophyll a, chlorophyll b, total chlorophyll content and carotenoids of two wheat cultivars

the carotenoid contents of Faisalabad-2008 under control (15–58%), 6 dS m<sup>-1</sup> (23–42%) and 12 dS m<sup>-1</sup> (17–54%) whereas in Galaxy-2013, hydro-priming and seed priming with 2.5% MLE maximized the carotenoids under control (9–43%), 6 dS m<sup>-1</sup> (10–45%) and 12 dS m<sup>-1</sup> (31–70%). The data further revealed that salt sensitive variety (Galaxy-2013) showed more improvement in carotenoids at high salinity levels under the hermetic effect of MLE as compared with Faisalabad-2008

**Catalase content (U/g/min fresh weight)**

Significant variation was observed in catalase the content of both wheat cultivars (C) under the influence of seed priming (SP) with moringa leaf extract at different salinity levels (S). Figure 4 revealed that exposure of non-primed seeds of cultivars Faisalabad-2008 and Galaxy-2013 to salinity levels of 6 dS m<sup>-1</sup> increased catalase content by 107% and 31% as compared with control (no salinity). The decrease in catalase content of Faisalabad-2008 and Galaxy-2013 at a salinity level of 12 dS m<sup>-1</sup> was observed by 17% and 35% respectively over control. Hydro-priming and priming with 2% of moringa leaf extract improved the catalase content of wheat cultivar (Faisalabad-2008) by 76–122% in control (no salinity) while such improvements ranged from 29–55% and 36–136%

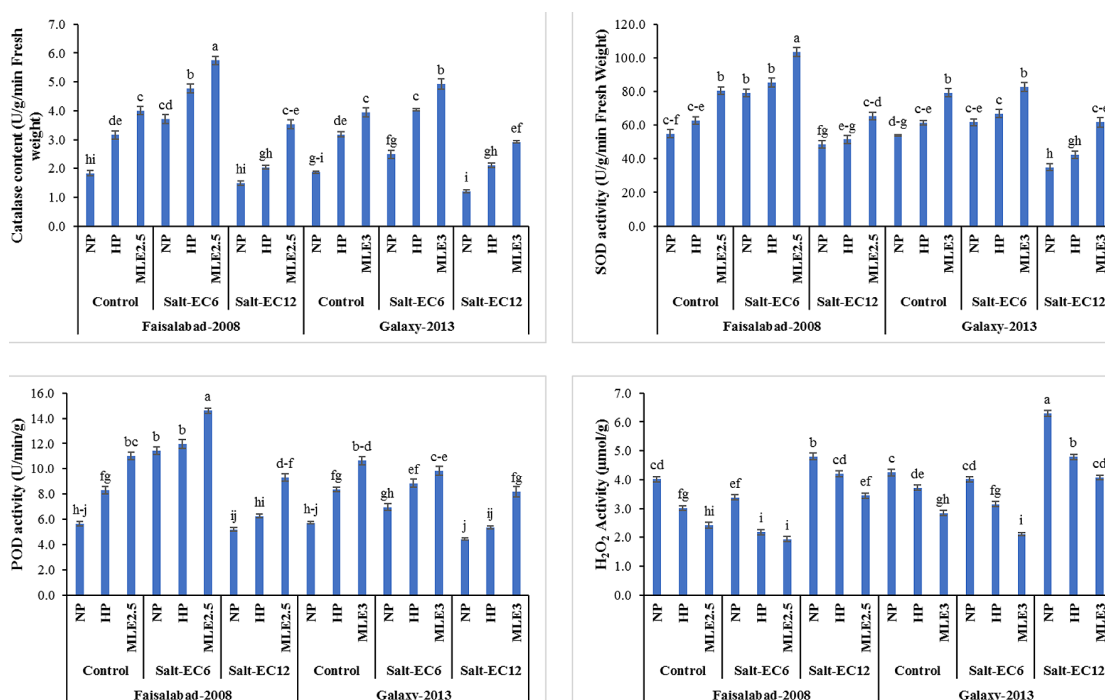
under salinity levels of 6 dS m<sup>-1</sup> and 12 dS m<sup>-1</sup> respectively. The same behavior was noticed for the wheat cultivar Galaxy-2013 wherein catalase content was improved by 67–108% (control), 61–97% (6 dSm<sup>-1</sup>), and 76–144% (12 dSm<sup>-1</sup>).

**SOD activity (U/g/min fresh weight)**

Primed as well as non-primed seeds of both cultivars showed different behavior under different salinity levels (Figure 4). Salinity increased the superoxide dismutase activity of non-primed seeds of Faisalabad-2008 and Galaxy-2013 at Ec level of 6 dSm<sup>-1</sup> (44%, 14%) and decreased the activity at 12 dS m<sup>-1</sup> (12%, 35%) respectively. However, priming treatments (Hydro-priming + 2% MLE) significantly enhanced the activity of superoxide dismutase of Faisalabad-2008 under control (14-46%), 6 dS m<sup>-1</sup> (8–31%) and 12 dS m<sup>-1</sup> (6-34%) whereas in Galaxy-2013, hydro-priming and seed priming with 2.5% MLE maximized the superoxide dismutase under control (13–47%), 6 dS m<sup>-1</sup> (8–34%) and 12 dS m<sup>-1</sup> (21–76%).

**POD activity (U/min/g)**

The statistically significant (p≤0.5) effect of hydro-priming and seed priming with 2–2.5% MLE on peroxidase activity of two wheat cultivars under different salinity levels is shown in Figure



**Fig.4.** Interactive effect of different priming techniques on CAT, SOD, and POD activity and H<sub>2</sub>O<sub>2</sub> concentration of two wheat cultivars

4. Data revealed that exposure of non-primed seeds of cultivars Faisalabad-2008 and Galaxy-2013 to salinity levels of 6 dS m<sup>-1</sup> increased the peroxidase contents by 91% and 16% as compared with control (no salinity). At 12 dSm<sup>-1</sup> decrease in peroxidase contents of Faisalabad-2008 and Galaxy-2013 was observed by 13% and 26% as compared to control. Hydro priming and priming with 2% of moringa leaf extract improved the peroxidase content of wheat cultivar (Faisalabad 2008) by 39–83% in control (no salinity) while such improvements ranged from 9–33% and 25–87% under moderate and higher SS levels. The same behavior was noticed for the wheat cultivar Galaxy-2013 at 2.5% MLE wherein peroxidase contents were improved by 39–78% (control), 26–41% (6 dSm<sup>-1</sup>), and 34–104% (12 dSm<sup>-1</sup>).

#### *H<sub>2</sub>O<sub>2</sub> activity (μmol/g)*

A significant variation was observed in hydrogen peroxide of both wheat cultivars (C) after 30 days under the influence of seed priming (SP) with moringa leaf extract at different salinity levels (S). Data (Figure 4) revealed that exposure of non-primed seeds of cultivars Faisalabad-2008 and Galaxy-2013 to salinity levels of 6 dS m<sup>-1</sup> decreased the hydrogen peroxide content by 15% and 21% as compared with control (no salinity). An increase in hydrogen peroxide content of Faisalabad-2008 and Galaxy-2013 at a salinity level of 12 dS m<sup>-1</sup> was observed by 20% and 24% respectively over control. Hydro priming and priming with 2% of moringa leaf extract decrease the hydrogen peroxide content of wheat cultivar (Faisalabad-2008) by 25–40% in control (no salinity) while such decrease ranged from 33–42% and 12–28% under salinity levels of 6 dS m<sup>-1</sup> and 12 dS m<sup>-1</sup> respectively. The same behavior was noticed for the wheat cultivar Galaxy-2013 wherein hydrogen peroxide content was decreased by 12–32% (control), 21–47% at moderate and higher SS levels.

## DISCUSSION

Salinity impairs seedling germination by delaying the onset of the process, reducing seedling growth, dispersing germination events, and decreasing seedling metabolism, all of which lead to a reduction in plant growth (El Sabagh et al., 2020) Safety and nutritional quality of food is to raise crop production per unit area without compromising

the sustainability of agricultural resources and environmental security. Along with environmental constraints, soil salinization has become one of the major threats that restricts agricultural potential and is closely related to mishandling of agricultural resources and overexploitation of water resources, particularly in arid regions. The effect of salinity on the quality of various agricultural crops has not yet been much explored. Presently, this information is very important due to the increasing use of saline water for irrigation worldwide which has given rise to as soil salinity has become a critical around the world and the situation has been worsening over the last 20 years in arid and semi-arid regions particularly in Mediterranean area. Salinity stress significantly affect the nutritional properties and quality traits of crops due to physiological and biochemical alterations in plants at different growth stage. During salinity stress, plants tend to activate different physiological and biochemical mechanisms to cope with the stress through altering their morphology, anatomy, water relations, photosynthesis, protein synthesis, primary and secondary metabolism and biochemical adaptations such as the antioxidative metabolism response. Therefore, it is important for breeders and producers to understand the influence of salinity on the composition of crops, for improvement of protein and oil quality (amino and fatty acid. An essential approach to be used is to build an understanding of how plants react to salt stress. According to Arzani et al. (2016) and thus progress attained over the several decades has been far less than anticipated. The generation of an explosion of knowledge and technology related to genetics and genomics over the last few decades is promising in providing powerful tools for future development of salinity-tolerant cultivars. Despite a major progress in defining the underlying mechanisms of salinity tolerance, there are still major challenges to be overcome in translating and integrating the resultant information at the molecular level into plant-breeding practices. Various approaches have been suggested to improve the efficiency of plant breeding for increasing plant productivity under saline environments. In this context, breeding for salinity tolerance in crops largely depends upon the availability of genetic resources of tolerance, reliable screening techniques, identification of genetic components of tolerance, and successful genetic manipulation of desired genetic backgrounds. The efficiency of selection and breeding in the stressful environments can be improved



through marker-assisted selection. To date, this is almost exclusively applied to major genes, but this requires to be extended to quantitative trait loci (QTLs, there are two unique phases to the way that plants respond to salinity. In the first stage, salt causes the soil water potential to drop, which causes plants to experience osmotic stress (James et al., 2006). Depending on how severe the salinity is, the second phase, which involves the buildup of  $\text{Na}^+$  ions in various plant tissues, may start within a few days or weeks. This stage may cause the plant to produce less or possibly die (Munns et al., 2008). Salinity stress also negatively affects the production of leaves, root growth, water and nutrient uptake and water use efficiency which leads to a reduction in plant growth (El-Hendawy et al., 2019; Sorour et al., 2019). A high salinity level can prevent seeds from germinating in one of two ways: either it creates ionic toxicity, or it causes osmotic stress, which prevents the seeds from taking in water. These effects, taken as a whole, have the combined effect of inhibiting cell proliferation and expansion, modulating the activity of many important enzymes, and, as a result, lowering the use of seed stores (El-Hendawy et al., 2019). Ahmad et al. (2013) found that the early maturation of wheat brought on by saline stress decreased crop height and leaf area. They also found that the early stages of plant growth were when plumule length was most responsive. Currently, diverse bio-stimulants are being used to improve plant growth under diverse abiotic stresses. The use of MLE got appreciable attention to enhance plant growth under optimal and stress conditions. MLE seed treatments considerably improved the salinity tolerance in wheat cultivars. The corresponding improvement in wheat was recorded in seeds primed with 2% MLE in Faisalabad-2008 and 2.5% MLE in Galaxy-2013.

## CONCLUSIONS

Wheat growth was greatly affected by salinity, however, HP and MLE priming mitigated the adverse impacts of salinity on wheat growth. The effects of priming on growth and germination were consistent. It worked well to prime wheat seeds with 2% MLE (Faisalabad-2008) and 2.5% MLE (Galaxy-2013). Stomatal conductance, SOD, POD, Catalase activity, photosynthetic pigment content, internal carbon content, transpiration, and photosynthesis were improved with priming of seeds. The present

study did not document any negative impacts of selected concentration of MLE. However, in future studies must be done to explore the toxic effects of MLE at higher concentration. Further, this study was conducted in control conditions, therefore, more field studies are direly needed before making it recommendation for farming community.

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## REFERENCES

1. Abbas, T., Nadeem, M. A., Tanveer, A., Chauhan, B. S. 2017. Can hormesis of plant-released phytotoxins be used to boost and sustain crop production? *Crop Protection*, 93, 69–76.
2. Acquaah, G. 2009. Principles of plant genetics and breeding. John Wiley and Sons.
3. Afzal, I., Hussain, B., Basra, S.M.A., Rehman, H. 2012. Priming with moringa leaf extract reduces imbibitional chilling injury in spring maize. *Seed Science and Technology*, 40(2), 271–276.
4. Ahmad, M., Shahzad, A., Iqbal, M., Asif, M., Hirani, A.H. 2013. Morphological and molecular genetic variation in wheat for salinity tolerance at germination and early seedling stage. *Australian Journal of Crop Science*, 7(1), 66–74.
5. Turan, M.A., Elkarim, A.H.A., Taban, N., Taban, S. 2009. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. *African Journal of Agricultural Research*, 4(9), 893–897.
6. Ali, Z., Basra, S.M.A., Munir, H., Mahmood A., Yousaf, S. 2011. Mitigation of drought stress in maize by natural and synthetic growth promoters. *Journal of Agriculture and Social Sciences*, 7(2), 56–62.
7. Arif, Y., Singh, P., Siddiqui, H., Bajguz, A., Hayat, S. 2020. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry*, 156, 64–77.
8. Arora, N.K. 2019. Impact of climate change on agriculture production and its sustainable solutions. *Environmental Sustainability*, 2(2), 95–96.
9. Arzani, A., Ashraf, M. 2016. Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. *Critical Reviews in Plant Sciences*, 35(3), 146–189.
10. Asseng, S., Ewert, F., Martre, P., Rötter, R.P., Lobell, D.B., Cammarano, D., Kimball B.A., Ottman

- M.J., Wall G.W., White J.W., Reynolds M.P., Alderman P.D., Prasad P.V.V., Aggarwal P.K., Anothai J., Basso B., Biernath C., Challinor A.J., De Sanctis G., Doltra J., Fereres E., Garcia-Vila M., Gayler S., Hoogenboom G., Hunt L.A., Izaurralde R.C., Jabloun M., Jones C.D., Kersebaum K.C., Koehler A.K., Müller C., Naresh Kumar S., Nendel C., O’Leary G., Olesen J.E., Palosuo T., Priesack E., Eyshi Rezaei E., Ruane A.C., Semenov M.A., Shcherbak I., Stöckle C., Stratonovitch P., Streck T., Supit I., Tao F., Thorburn P.J., Waha K., Wang E., Wallach D., Wolf J., Zhao Z., Zhu Y. 2015. Rising temperatures reduce global wheat production. *Nature Climate Change*, 5(2), 143–147.
11. Basra, S.M.A., Iftikhar, M.N., Afzal, I. 2011. Potential of moringa (*Moringa oleifera*) leaf extract as priming agent for hybrid maize seeds. *International Journal of Agriculture and Biology*, 13(6).
  12. Basra, S.M.A., Iftikhar, M.N., Afzal, I. 2011. Potential of moringa (*Moringa oleifera*) leaf extract as priming agent for hybrid maize seeds. *International Journal of Agriculture and Biology*, 13(6).
  13. Bhattacharya, A., Bhattacharya, A. 2021. Role of plant growth hormones during soil water deficit: a review. *Soil Water Deficit and Physiological Issues in Plants*, 489–583.
  14. Chamorro, D., Luna, B., Ourcival, J.M., Kavgacı, A., Sirca, C., Mouillot, F., Arianoutsou M., Moreno, J.M. 2017. Germination sensitivity to water stress in four shrubby species across the Mediterranean Basin. *Plant Biology*, 19(1), 23–31.
  15. Chaves, M.M., Maroco, J.P., Pereira, J.S. 2003. Understanding plant responses to drought – from genes to the whole plant. *Functional Plant Biology*, 30(3), 239–264.
  16. Pakade, V., Cukrowska, E., Chimuka, L. 2013. Comparison of antioxidant activity of *Moringa oleifera* and selected vegetables in South Africa. *South African Journal of Science*, 109(3), 1–5.
  17. Dhindsa, R.S., Plumb-Dhindsa, P.A.M.E.L.A., Thorpe, T.A. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany*, 32(1), 93–101.
  18. El-Hendawy, S., Elshafei, A., Al-Suhaibani, N., Alotabi, M., Hassan, W., Dewir, Y. H., Abdella, K. 2019. Assessment of the salt tolerance of wheat genotypes during the germination stage based on germination ability parameters and associated SSR markers. *Journal of Plant Interactions*, 14(1), 151–163.
  19. El Sabagh, A., Hossain, A., Barutçular, C., Iqbal, M.A., Islam, M.S., Fahad, S., Sytar O., Çiğ F., Meena R.S., Erman, M. 2020. Consequences of salinity stress on the quality of crops and its mitigation strategies for sustainable crop production: an outlook of arid and semi-arid regions. *Environment, Climate, Plant and Vegetation Growth*, 503–533.
  20. Ellis, R.H., Roberts, E.H. 1981. The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology (Netherlands)*, 9(2).
  21. Farooq, M., Basra, S.M.A., Ahmad, N., Hafeez, K. 2005. Thermal hardening: a new seed vigor enhancement tool in rice. *Journal of Integrative Plant Biology*, 47(2), 187–193.
  22. Giraldo, P., Benavente, E., Manzano-Agugliaro, F., Gimenez, E. 2019. Worldwide research trends on wheat and barley: a bibliometric comparative analysis. *Agronomy*, 9(7), 352.
  23. Hasanuzzaman, M., Alam, M., Rahman A., Hasanuzzaman M., Nahar K., Fujita M. 2014. Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced. *BioMed Research International*, 2014:757219.
  24. Huang, P., He, L., Abbas, A., Hussain, S., Hussain, S., Du, D., Hafeez, M.B, Balooch, S, Zahra N, Ren X, Rafiq M., Saqib, M. 2021. Seed priming with sorghum water extract improves the performance of camelina (*Camelina sativa* (L.) crantz.) under salt stress. *Plants*, 10(4), 749.
  25. Hussain, S., Shaukat, M., Ashraf, M., Zhu, C., Jin, Q., Zhang, J. 2019. Salinity stress in arid and semi-arid climates: effects and management in field crops. *Climate Change and Agriculture*, 13, 201–226.
  26. Imran, S., Afzal, I., Basra, S., Saqib, M. 2013. Integrated seed priming with growth promoting substances enhances germination and seedling vigour of spring maize at low temperature. *International Journal of Agriculture and Biology*, 15(6).
  27. James, R.A., Munns, R., Von Caemmerer, S., Trejo, C., Miller, C., Condon, T. 2006. Photosynthetic capacity is related to the cellular and subcellular partitioning of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> in salt-affected barley and durum wheat. *Plant, Cell and Environment*, 29(12), 2185–2197.
  28. Khan, S., Basra, S.M.A., Afzal, I., Nawaz, M., Rehman, H.U. 2017. Growth promoting potential of fresh and stored *Moringa oleifera* leaf extracts in improving seedling vigor, growth and productivity of wheat crop. *Environmental Science and Pollution Research*, 24, 27601–27612.
  29. Kizilgeci, F., Yildirim, M., Islam, M.S., Ratnasekera, D., Iqbal, M.A., Sabagh, A.E. 2021. Normalized difference vegetation index and chlorophyll content for precision nitrogen management in durum wheat cultivars under semi-arid conditions. *Sustainability*, 13(7), 3725.
  30. Kumari, A., Kaur, R. 2018. Evaluation of benzyl-butyl phthalate induced germination and early growth vulnerability to barley seedlings (*Hordeum vulgare* L.). *Indian Journal of Ecology*, 45(1), 174–177.
  31. Maqbool, N., Sadiq, R. 2017. Allelochemicals as

- growth stimulators for drought stressed maize. *American Journal of Plant Sciences*, 8(5), 985–997.
32. Mbarki, S., Sytar, O., Zivcak, M., Abdelly, C., Cerda, A., Brestic, M. 2018. Anthocyanins of coloured wheat genotypes in specific response to salstress. *Molecules*, 23(7), 1518.
33. Moyo, B., Masika, P.J., Hugo, A., Muchenje, V. 2011. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology*, 10(60), 12925–12933.
34. Munns, R., Tester, M. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59, 651–681.
35. Nassar, R.M., Kamel, H.A., Ghoniem, A.E., Alarcón, J.J., Sekara, A., Ulrichs, C., Abdelhamid, M.T. 2020. Physiological and anatomical mechanisms in wheat to cope with salt stress induced by seawater. *Plants*, 9(2), 237.
36. Nishida, K., Khan, N.M., Shiozawa, S. 2009. Effects of salt accumulation on the leaf water potential and transpiration rate of pot-grown wheat with a controlled saline groundwater table. *Soil Science and Plant Nutrition*, 55(3), 375–384.
37. Rahman, M.A., Chikushi, J., Yoshida, S., Yahata, H., Yasunaga, E. 2005. Effect of high air temperature on grain growth and yields of wheat genotypes differing in heat tolerance. *Journal of Agricultural Meteorology*, 60(5), 605–608.
38. Royo, A., Abió, D. 2003. Salt tolerance in durum wheat cultivars. *Spanish Journal of Agricultural Research*, 1(3), 27–35.
39. Shah, T., Latif, S., Saeed, F., Ali, I., Ullah, S., Alsahli, A.A., Jan S., Ahmad, P. 2021. Seed priming with titanium dioxide nanoparticles enhances seed vigor, leaf water status, and antioxidant enzyme activities in maize (*Zea mays* L.) under salinity stress. *Journal of King Saud University-Science*, 33(1), 101207.
40. Shakirova, F.M., Avalbaev, A.M., Bezrukova, M.V., Fatkhutdinova, R.A., Maslennikova, D.R., Yuldashev, R.A., Allagilova, C.R., Lastochkina, O. V. 2012. Hormonal intermediates in the protective action of exogenous phytohormones in wheat plants under salinity. *Phytohormones and Abiotic Stress Tolerance in Plants*, 185–228.
41. Sorour, S., Aiad, M. A., Ahmed, A. A., Henash, M. I. A., Metwaly, E. M., Alharby, H., Bamagoos, A., Hossain, A., Barutcular, C., Saneoka, H.El., Sabagh, A. 2019. Yield of wheat is increased through improving the chemical properties, nutrient availability and water productivity of salt affected soils in the North Delta of Egypt. *Applied Ecology and Environmental Research*, 17(4).
42. Sytar, O., Mbarki, S., Zivcak, M., Brestic, M. 2018. The involvement of different secondary metabolites in salinity tolerance of crops. *Salinity Responses and Tolerance in Plants, Volume 2: Exploring RNAi, Genome Editing and Systems Biology*, 21–48.
43. TeKrony, D.M. 1983. Seed vigor testing – 1982. *Journal of Seed Technology*, 55-60.
44. Velikova, V., Yordanov, I., Edreva, A.J.P.S. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant science*, 151(1), 59–66.
45. Wajid, M., Khan, M.A., Shirazi, M.U., Summiya, F. 2019. Seed priming modulates germination potential, osmoprotectants accumulation and ionic uptake in wheat seedlings under salt stress. *International Journal of Agriculture and Biology*, 22(3), 594–600.
46. Yasmeen, A., Basra, S. M., Wahid, A., Farooq, M., Nouman, W., Hussain, N. 2013. Improving drought resistance in wheat (*Triticum aestivum*) by exogenous application of growth enhancers. *International journal of Agriculture and Biology*, 15(6).
47. Zheng, Y., Wang, Z., Sun, X., Jia, A., Jiang, G., Li, Z. 2008. Higher salinity tolerance cultivars of winter wheat relieved senescence at reproductive stage. *Environmental and Experimental Botany*, 62(2), 129–138.