Identification of physiological and biochemical markers for salt (NaCl) stress in the seedlings of mungbean [Vigna radiata (L.) Wilczek] genotypes

Hesham F. Alharby a,⇑, Hassan S. Al-Zahrani a, Khalid Rehman Hakeem a,⇑, Muhammad Iqbal b

a Department of Biological Sciences, Faculty of Science, King Abdulaziz University, 21589 Jeddah, Saudi Arabia
b Botany Department, Jamia Hamdard (Deemed University), Hamdard Nagar, New Delhi, India

ABSTRACT

Salt stress, which is dominant among environmental stresses, poses challenges to global agriculture. We studied the role of exogenous application of sodium chloride (NaCl) in three arid and three semi-arid genotypes of mungbean [Vigna radiata (L.) Wilczek] by examining some physiological and biochemical stress indicators. Ten-day old seedlings were subjected to salt stress (00–250 mM) by split application along with the half strength Hoagland’s medium. The salt stress caused a decline in the fresh weight, dry weight, relative water content, photosynthetic pigments (chlorophyll and carotenoids) and glutathione content of the seedlings. On the other hand, it increased the electrolyte leakage, lipoxygenase activity, and the proline, protein and total soluble sugar contents. Osmolyte accumulation was relatively higher in the arid genotypes revealing that they are more tolerant to NaCl stress. The physiological and biochemical screening provides a basic platform for selecting the stress-tolerant genotypes in the absence of suitable salt-tolerance markers in mungbean.

© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The abiotic stresses limit the photosynthetic efficiency rate in plants, thus hampering the biomass production (Grime, 1977) and affecting the plant survival and yield (Qadir et al., 2014). Of these stresses, salinity poses the biggest threat leading to huge economic losses to the tune of 10–14 billion US dollars (Qadir et al., 2014; Shabala, 2013). Salt-stress effects comprise of the adverse effects caused by Na⁺ and Cl⁻ ions on plants (Munns, 2005). The present-day irrigational practices have markedly aggravated the situation of salt stress (Zhu, 2001). As the majority of agricultural crops are salt-sensitive glycophytes (Munns and Tester, 2008), salinity causes tremendous yield losses in agriculture, posing drastic challenges to the world food security (Flowers, 2004; Ozturk et al., 2006; Godfray et al., 2010; Tester and Langridge, 2010; Agarwal et al., 2013).

Plants are able to survive in adverse environments by adapting to the prevailing conditions and/or fine-tuning their metabolic activities with the physiological changes. The regulation of plant adaptations to salinity is acquired by osmoprotectant biosynthesis, which helps plants in controlling the water flux and adjusting the cellular osmosis (Hasegawa et al., 2000; Flowers, 2004; Ashraf and Akram, 2009; Agarwal et al., 2013). The imbalance of ion homeostasis caused by salt stress is compensated by the regulation of ion influx and efflux at plasma membrane and the sequestration of ions by vacuoles (Hasegawa et al., 2000). Additionally, salt stress also causes disturbance in energy supply and redox homeostasis, which are balanced by the rearrangement of primary metabolism and alterations in cell architecture (Chen et al., 2005; Baena-González et al., 2007; Jaspers and Kangasjärvi, 2010; Miller et al., 2010; Zhu et al., 2010). Thus, the salt tolerance of plants is determined by their ability to transport Na⁺ and Cl⁻ ions across the plasma membranes of root cells, vacuolar membranes, and salt accumulation/excretion by the specialized cells. As the world agriculture is faced with the challenge of feeding the ever-increasing human population, it is imperative that salt tolerant genotypes are identified for cultivation on the moderate to above moderate salt-infested soils (Ozturk et al., 1992, 1993, 1997).
Mungbean [Vigna radiata (L.) Wilczek] is a salt-sensitive pulse and an intensive crop due to its short growing period and cultivation worldwide for its protein rich edible seeds (Ashraf et al., 2015). The present study was undertaken to screen and compare the performance of six arid and semi-arid genotypes of mungbean grown under salt stress.

2. Material and methods

2.1. Procurement of genotypes

Seeds of six Mungbean [Vigna radiata (L.) Wilczek] genotypes, AEM-96 (Azri Bhakkar), NCM-1 (NARC-Islamabad) and CM-6 (BARI-Chakwal) from arid region, and NM-12 (NIFA-Peshawar), NM-92 (NIAB-Faisalabad) and NFM-6 (NIFA-Peshawar) from semi-arid region were procured from Pakistan Agriculture Research council (PARC), Islamabad Pakistan.

2.2. Determination of stress tolerance index (STI)

The seeds of each genotype were sterilized with 0.2% HgCl2 solution for 5 min and washed thoroughly with tap water and then with deionized water. These were then put on Petriplates covered with Whatman filter paper for germination and growth. Ten seeds were put on each petriplate (n = 10) including the control and scored for germination. The Petriplates were kept in the dark at 25 °C until germination and later divided into five sets to be treated with different concentrations (0, 100, 150, 200, 250 mM) of sodium chloride. Initially 10 ml of salt concentrations were added to the petriplates. The germination did occur within 48 h, however, the growth of the seedlings was measured after 7 days. Further 5 ml salt solution were added every two days. The experiments were repeated thrice, each with three replicates, and the values presented are the mean of three observations. The root was measured in mm scale in independent experiments. About 2 mm root was considered as the germination. Following Mustafiz et al. (2014), stress tolerance index was calculated as: STI (%) = (Average fresh weight of 10 stressed seedlings/Average fresh weight of 10 control seedlings) × 100%.

2.3. Plant growth and treatment

In the second experiment, the sterilized seeds of each genotype were sown in plastic pots (300 mm diameter) filled with moist 3 kg of acid-washed, autoclaved sand and the pots were moistened (watered) regularly till seed germination. The plants were irrigated with the half-strength Hoagland’s nutrient medium with pH 6.5 (Hoagland and Arnon, 1950). All pots were kept in an environmentally controlled growth chamber at 28 ± 1.5 °C at daytime and at 22 ± 1.5 °C at night. The plants were maintained at 300 μmol m−2 s−1 photosynthetic photon flux density with 60–70% relative humidity. Randomized block design was adapted for the treatments with three replicates and the sampling was completed 20 days after the start of treatments with sodium chloride (0, 200, 250 mM). The sodium chloride treatments prepared in Hoagland’s solution were given in the split application of 50 mM from 1 to 5 days. The course of treatment was started 1 week after germination and the split application of 50 mM was given every day (1–5 days i.e 1–4 days for 200 mM and 1–5 days for 250 mM).

2.4. Measurement of growth parameters

After 20 days of stress imposition, the seedlings were randomly picked from the sets of the control and treated plants. The carefully uprooted seedlings were cleaned systematically with double distilled water for removing the sand particles. Root length, shoot length and fresh weight were then recorded. Dry weight was obtained after drying the material in hot air oven at 65 °C until the weight became constant. The relative water content (RWC) was calculated as: RWC (%) = [(FW – DW)/FW] × 100, as described by Chen et al. (2009).

2.5. Estimation of pigment content

In order to estimate the pigments, 0.2 g of leaf samples, collected 20 days after germination from each of the control and treated plant sets, were homogenized in 80% chilled acetone (10 ml) under dark conditions and the absorbance was measured at 663, 645 and 480 nm. The content of chlorophylls (chl, and chl, ) was quantified by the method of Lichtenthaler (1987). The carotenoid content was determined by using the formula given by Duxbury and Yentsch (1956) and expressed in mg/g FW.

2.6. Electrolyte leakage and LPO measurements

The fresh expanded leaves from the control and treated samples (n = 3) were taken and electrolyte leakage was determined using the formula of Rodriguez-Hernandez et al. (2013), which is: MSI = (EC1/EC2) × 100, where, EC1 and EC2 are the initial and final values of electrical conductivity respectively. Lipid peroxidation of leaves was estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS) as described by Heath and Packer (1968) and modified by Tanveer et al. (2018). The lipid peroxides was expressed as nmol TBARS g−1 fresh weight, using an extinction coefficient for MDA (ε = 155 mM−1 cm−1) calculated by the formula: TBARS content (nmol g−1 FW) = (A532 – A600) × V/1000/W, where V = extraction volume; W = weight of the fresh tissue.

2.7. Determination of osmolytes

The proline content in the control and treated seedlings was estimated according to Bates et al. (1973). Fresh leaves (0.5 g) were homogenized in 3% sulfosalicylic acid (10 ml) followed by centrifugation (10 min) at 10,000 rpm. Incubation (100 °C for 30 min) of 2 ml of supernatant was done with 2 ml each of acid ninhydrin and glacial acetic acid. After cooling the samples were extracted with toluene (4 ml) and their pink colour intensity was recorded at 520 nm against a standard curve of proline. The estimation of soluble sugar was performed according to Dey (1990) by extracting fresh leaves (0.5 g) in hot ethanol (90% v/v). To the ethnolic extract (2 ml), 5% phenol (1.0 ml) and concentrated H2SO4 (5.0 ml) were added. The final volume was adjusted to 10 ml by DDW and the absorbance was recorded at 485 nm. The reduced glutathione (GSH) content was estimated according to Anderson (1985) by homogenizing fresh leaves (0.5 g) in 5% sulfosalicylic acid, followed by centrifugation (4 °C) at 10,000 rpm for 10 min. 1.5 ml reaction buffer and 3 mM 5,5-dithio-bis(2-nitro benzoic acid) were added to 0.5 ml aliquot, and after 1 min, the absorbance was recorded at 412 nm. The total soluble protein content was determined following Bradford (1976) with Bovine Serum Albumin (BSA) taken as standard.

2.8. Statistical analysis

Results are presented as mean ± SE subjected to one-way ANOVA, using the GraphPad Prism 6.0 software. Tukey’s post hoc test was performed to calculate the statistical differences in data at p < 0.05. All experiments were carried out in triplicate (n = 3) excluding for growth parameters (the root & shoot lengths, the fresh & dry weights, and the RWC, where n = 10).
3. Results

3.1. Effect of NaCl on germination and plant growth

Seed germination declined linearly with increase in NaCl concentrations from 100 mM to 250 mM. The effects of NaCl are presented as stress tolerance index (STI) and growth performance with respect to control (Fig. 1a and b). The STI declined in all the genotypes studied, viz. AEM-96 (35.24–5.87%), NCM-1 (37.16–0.00%), CM-6 (29.44–5.63%), NFM-12 (35.22–6.13%), NM-92 (36.89–7.86%), and NFM-6 (22.49–4.78%). At the highest NaCl concentration (250 mM), it declined drastically (4.78–7.86%) in different genotypes, whereas the genotype NCM-1 failed to germinate at this concentration. The shoot and root length were significantly affected under salinity stress, showing a dose-dependent decline (Table 1, Fig. 2). The maximum reduction of shoot length (24.85%...
and 30.17%) was seen in NCM-1 at 200 mM and 250 mM, respectively. All the genotypes taken together, the range of reduction was noted to be 13.15–24.85% at 200 mM and 22.76–30.17% at 250 mM. In semi-arid genotypes (NFM-12, NM-92, NFM-6), the effect was more prominent at 250 mM NaCl (Fig. 2a). The maximum reduction in root length was seen at 200 mM in NM-92 (45.14%) and at 250 mM in AEM-96 (57.44%) as compared to the control. All the genotypes taken together, the range of reduction in the root length varied from 15.81% to 45.14% at 200 mM and from 25.60% to 57.44% at 250 mM (Fig. 2a).

3.2. Effect of NaCl on biomass and relative water content

Fresh weight (FW), dry weight (DW) and relative water content (RWC) of mungbean genotypes were significantly affected by treatments with 200 mM and 250 mM NaCl concentrations (Tables 1 and 2). The fresh weight declined 13.56–29.40% at 200 mM and 23.51–37.69% at 250 mM in all the genotypes studied. The maximum loss was associated with the genotype NFM-6. The range of the dry weight decline was between 6.62% and 47.52% at 200 mM and 28.37–52.30% at 250 mM. The maximum reduction occurred with NM-92 at 200 mM and NFM-6 at 250 mM. The decline in RWC ranged from 14.51% to 29.37% at 200 mM and 21.57% to 36.90% at 250 mM in various genotypes, with the maximum reduction seen in NFM-6 as compared to the control.

3.3. Effect of NaCl on photosynthetic pigment

The chlorophyll and carotenoid contents were significantly affected by salinity, showing a dose-dependent decline in the salt-treated plants as compared to the control (Tables 1 and 3). The mean chlorophyll and carotenoid content was lower in semi-arid genotypes than in the arid ones. Reduction in the chlorophyll content in genotypes was 5.93–49.44% at 200 mM and 7.28–49.11% at 250 mM. The maximum reduction in chlorophyll content was observed in NFM-6, whereas the minimum in CM-6, at both 200 mM and 250 mM treatments. The carotenoid content in the genotypes showed a decline ranging from 7.13% to 46.81% at 200 mM and between 9.55% and 50.33% at 250 mM, with the maximum and minimum reductions seen in NFM-6 and CM-6, respectively, at both concentrations. Moreover, the chlorophyll:carotenoid ratio decreased in all genotypes at 200 mM but the changes were not significant in NFM-6 and CM-6. On the contrary, the ratio increased non-significantly at 250 mM NaCl in NM-92, NFM-6 and CM-6, and significantly in AEM-96 and NCM-1.

![Fig. 2. Effect on shoot length and root length in salt stressed mungbean genotypes. The leaves from control and salt treated seedlings from each genotype were collected and shoot length (a) and root length (b) were recorded and data is presented as mean ± SE (n = 10). Different letters within columns represent significant differences (P < 0.05) between treatments within each genotype respectively. a = **** (highly significant), b = *** (moderately significant), c = ** (less significant) and ns (not significant) with respect to control of each genotype.](image)

| Parameters               | Treatments (NaCl mM) | Genotypes |
|--------------------------|----------------------|-----------|
|                          | AEM-96 | NCM-1 | NM-6 | NFM-12 | NM-92 | NFM-6 |
| Fresh weight (g^{-1} Plant) | 0  | 0.38 ± 0.0016a | 0.42 ± 0.0035a | 0.43 ± 0.0040a | 0.51 ± 0.0031a | 0.47 ± 0.0035a | 0.47 ± 0.0026a |
|                          | 200 | 0.31 ± 0.0036a | 0.36 ± 0.0015a | 0.36 ± 0.0108a | 0.41 ± 0.0022a | 0.36 ± 0.0079a | 0.33 ± 0.0033a |
|                          | 250 | 0.24 ± 0.0031a | 0.34 ± 0.0031a | 0.34 ± 0.0031a | 0.39 ± 0.0033a | 0.39 ± 0.0077a | 0.29 ± 0.0027a |
| Dry weight (g^{-1} Plant)  | 0  | 0.033 ± 0.00048a | 0.043 ± 0.0001a | 0.037 ± 0.0002a | 0.044 ± 0.0004a | 0.034 ± 0.00023a | 0.046 ± 0.00020a |
|                          | 200 | 0.031 ± 0.0016a | 0.037 ± 0.001a | 0.023 ± 0.0005a | 0.033 ± 0.0023a | 0.018 ± 0.0819a | 0.025 ± 0.0006a |
|                          | 250 | 0.022 ± 0.0001a | 0.031 ± 0.00049a | 0.022 ± 0.0002a | 0.028 ± 0.0017a | 0.017 ± 0.00018a | 0.022 ± 0.0005a |
| Relative Water content (%) | 0  | 37.76 ± 1.142a | 41.12 ± 1.402a | 41.78 ± 2.007a | 50.40 ± 0.573a | 46.87 ± 0.1168a | 46.70 ± 0.306a |
|                          | 200 | 30.52 ± 2.072a | 35.15 ± 0.968a | 33.44 ± 1.011a | 40.46 ± 0.207a | 34.98 ± 0.277a | 32.98 ± 0.302a |
|                          | 250 | 23.91 ± 2.468a | 32.25 ± 0.747a | 31.22 ± 1.012a | 39.25 ± 0.308a | 30.77 ± 0.246a | 29.46 ± 1.284a |

Significance of values at P < 0.05, a = **** (highly significant), b = *** (moderately significant), c = ** (less significant) and ns (not significant) with respect to control of each genotype.
3.4. Cell-membrane damage in response to NaCl

The electrolyte leakage and lipid peroxidation (LPO) rates were significantly higher in salt-treated plants than in the control (Table 1). The electrolyte leakage increased 0.18–1.94 fold at 200 mM and 0.43–2.1 fold at 250 mM NaCl concentration. The maximum leakage was observed in NFM-6 whereas the minimum in NM-92 (Fig. 3a). The increase in LPO rate among the genotypes ranged from 6.1% to 23.04% at 200 mM and from 13.10% to 51.76% at 250 mM NaCl respectively, with the maximum levels observed in genotype NM-92 (Fig. 3b).

3.5. Accumulation of osmolytes in response to NaCl

The salt-treated plants had significantly higher levels of osmolytes (proline, total soluble sugar and reduced glutathione) than control plants (Table 1). The proline content of the genotypes increased 0.53–2.86 fold at 200 mM and 0.87–3.38 fold at 250 mM NaCl, with the maximum elevation seen in NM-92 and the minimum in AEM-96 with both 200 mM and 250 mM NaCl treatments (Fig. 4a). The increase in total soluble protein in different genotypes was noted to be 0.05–0.83 fold at 200 mM and 0.16–0.82 fold at 250 mM. The maximum effect was observed in NM-92 and the minimum in CM-6 at both 200 mM and 250 mM NaCl concentrations (Fig. 4b). Likewise, the increase in the total soluble sugar ranged from 0.02 to 1.32 fold at 200 mM and 0.22 to 1.45 fold at 250 mM. The maximum increase was observed in CM-6 at both NaCl concentrations, while the minimum increase at 200 mM was observed in NFM-6 and at 250 mM was observed in AEM-96 (Fig. 4c). The increase in GSH ranged from 0.74 to 1.06 fold at 200 mM and 0.94 to 1.56 fold at 250 mM. The maximum GSH was observed in CM-6 and the minimum in NFM-6 at both the NaCl concentrations used (Fig. 4d).

4. Discussion

4.1. Plant growth response

Seed germination rate declined in all the mungbean genotypes when subjected to different NaCl treatments. Seedling length also decreased in all genotypes, whereas the salinity tolerance index varied among the genotypes (Fig. 1). Plants respond to stress by adapting their morpho-physiological systems to changes in the environment so as to ensure their survival in the changed condition (Shelke et al., 2017). Under salt stress, the plant root system adapts its morpho-physiological characteristics for absorbing nutrients (Hasegawa et al., 2000). The decrease in root length is an adaptation response of plants to avoid and reduce salt absorption (Hasegawa et al., 2000). Salt stress lowers the extracellular water potential and bioavailability of water in the root zone,
causing a low absorption of water and nutrients by plants and this hampers their adequate growth and biomass production (Zhang, 1991; Zhang et al., 2002). Some earlier studies have shown that the effect of salt stress on mungbean is dose dependent (Saha et al., 2010). Salinity stress affects the plant vigor due to reduction in imbibitions resulting in a limited hydrolysis of food reserves from the storage tissues (Ghosh et al., 2015). Our results show that the NaCl stress has a greater effect on roots than shoots with a sudden fall in the root growth (Fig. 2a), which is in accordance with some earlier reports (Saha et al., 2010). The deleterious effect of salinity on mungbean and the genotypic variation of plant response to salt stress has been seen in some earlier works also (Shakeel and Mansoor, 2012).

4.2. Photosynthetic pigment

Chlorophyll is indispensable for photosynthesis and is thus directly correlated with plant growth and health; it acts as an indicator of metabolic state at the cell level. Salt stress caused reduction in chlorophyll and carotenoid contents (Table 3), which might be caused by membrane swelling in chloroplasts and/or excess Na⁺ and Cl⁻ ions in the leaves. The accumulation of ions results in excess ROS production, reducing the photosynthesis and plant growth, as observed in a variety of crop plants such as rice (Saha et al., 2010), soybean (Hakeem et al., 2012), Cucumber (Khan et al., 2013), sweet annie (Qureshi et al., 2013) and mungbean (Ghosh et al., 2015). Usually, there is dominance of chlorophyll a over chlorophyll b, but their values come closer when salinity goes high (Mane et al., 2010).

4.3. Cell membrane damage

The effect of salt stress causing lipid peroxidation (LPO), as observed in the present study, is supposed to lead to increased permeability of membranes causing ion leakage (Zhang et al., 2006), which is likely to exhibit genotypic variation. As the LPO rate is an important index of cell membrane permeability, the lower LPO might be due to elevated levels of antioxidants.

4.4. Accumulation of osmolytes

One of the universal responses to changes in the external osmotic potential is the accumulation of metabolites that act as compatible solutes, which do not inhibit normal metabolic reactions. Accumulation of osmolytes, which facilitates osmotic adjustment by decreasing the internal osmotic potential and hence contributes to tolerance (McCue and Hanson, 1990), is proportional to the external osmolarity (Hasegawa et al., 2000). Plants normally cope with salt stress by accumulating compatible solutes including proline and sugars (Pattanagul and Thitisaksakul, 2008), which help in the osmotic adjustments. Proline is a potential osmolyte for countering the stress and providing resistance (Arshi et al., 2002, 2004); it acts as a source of nitrogen under normal conditions (Tie et al., 2014). The accumulation of proline increased linearly under salt stress concentrations in all genotypes (Fig. 4a) which is in agreement with the previous reports by Hoque et al. (2008) and Misra and Gupta (2006) among others. Sugar accumulation also contributes to osmotic balance permitting the plants to sustain under stressed conditions by maintaining their storage reserves (Smeekens, 2000). The total soluble sugar...
increased with the salt stress in this study, as in many earlier ones (Muscolo et al., 2003). These solutes buffer the redox potential of the cell and protect the cellular structure under stress. Accumulation of these solutes might also involve alteration in the allocation of photo-assimilates. Protein is measured as one of the main indicators of stress in plants (Plata et al., 2009), and the increased protein content during salt stress may be due to enhanced activity of detoxification pathways. The high stress causes a decline of protein content due to protein oxidation. The reduced glutathione has been found to confer tolerance to drought and salt stress in Arabidopsis both endogenously (Cheng et al., 2015, Nahar et al., 2015) and exogenously (Chen et al., 2012). The increased glutathione levels may cause salt-stress tolerance and translational changes. There was an increased GSH content in all the genotypes in this study (Fig. 4d), which reflects an increased demand of GSH-metabolizing enzymes (Thounaojam et al., 2012).

4.5. Genotypic variation

There was a difference in responses of arid and semi-arid genotypes towards salt stress. Semi-arid genotypes exhibited a greater decline in the average root length, relative water content, and photosynthetic pigments, whereas a greater increase in the electrolyte leakage and the corresponding LPO levels in comparison to arid genotypes. Increase in the proline and total soluble protein accumulation under salt stress was more pronounced in arid genotypes than in the semi-arid ones. The sugar and GSH accumulation was also relatively more in arid genotypes. The differential behaviour of arid and semi-arid genotypes in response to salinity stress under uniform growth conditions merits special focus in future investigations.

4.6. Conclusion

In conclusion, salt treatments overall had a negative impact on the growth and survival of the mungbean genotypes studied. The tolerance was ensured by the linear increase in osmolyte concentrations. The arid and semi-arid genotypes displayed a differential response to the salinity stress applied.

Acknowledgement

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. RG-7-130-38. The authors, therefore, acknowledge with thanks DSR technical and financial support.

Conflict of interest

The authors declare no conflict of interest among them.

References

Agarwal, P.K., Shukla, P.S., Gupta, K., Jha, B., 2013. Bioengineering for salinity tolerance in plants: state of the art. Mol. Biotechnol. 54, 102–123. https://doi.org/10.1007/s12033-012-9358-3.
Anderson, M.E., 1985. Determination of glutathione and glutathione disulfides in biological samples. Methods Enzymol. 113, 548–570.
Arshi, A., Abdin, M.Z., Iqbal, M., 2002. Growth and metabolism of Senna as affected by NaCl stress and protease activity of salt-tolerant genotype. Crop. Biomed. 168 (8), 2309–2329.
Arshi, A., Iqbal, M., 2012. Genotypic variability among soybean genotypes under NaCl stress and protease activity of salt-tolerant genotype. Crop. Biomed. 168 (8), 2309–2329.
Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Phys. 51, 463–499. https://doi.org/10.1146/annurev.arplant.51.1.463.
Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–211.
Hochst, D.R., Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72, 248–253.
Hochst, D.R., Arnon, D.I., 1950. The water-culture for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347 (Rev).
Hoff, S., Mita, S., Paul, A., 2015. Physiological studies of chemical chloride on mungbean (Vigna radiata L. Wilczek) and its possible recovery with spermine and glibberellic acid. Sci. World J. 2015, 858016. https://doi.org/10.1155/2015/858016.
Godfrey, H.C., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., et al., 2010. Food security: the challenge of feeding 9 billion people. Science 327, 812–818. https://doi.org/10.1126/science.1185381.
Grime, J.P., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111, 1191–1194.
Hakeem, K.R., Khan, F., Chandna, R., Siddiqui, T.O., Iqbal, M., 2012. Genotypic variability among soybean genotypes under NaCl stress and protease activity of salt-tolerant genotype. Crop. Biomed. 168 (8), 2309–2329.
Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Phys. 51, 463–499. https://doi.org/10.1146/annurev.arplant.51.1.463.
Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–211.
Hogland, D.R., Arnon, D.L., 1950. The water-culture for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347 (Rev).
Haque, M.R., Banu, M.N., Nakamura, Y., Shimoishi, Y., Murata, Y., 2008. Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. J. Plant Physiol. 165, 813–824. https://doi.org/10.1016/j.jplph.2007.07.013.
Jaspers, P., Kangasjärvi, J., 2010. Reactive oxygen species in abiotic stress signaling. Physiol. Plant. 138, 405–413. https://doi.org/10.1111/j.1399-3054.2009.01321.x.
Khan, M.M., Al-Masoudy, R.S.M., Al-Said, F., Khan, L., 2013. Salinity effects on growth, electrolyte leakage, chlorophyll content and lipid peroxidation in cucumber (Cucumis sativus L.). In: International Conference on Food and Agricultural Sciences (ICPBEE). IACSIT Press, Singapore. https://doi.org/10.7763/ICPBEE.
Lichtenhaller, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic membranes. Methods Enzymol. 148, 350–382.
Mame, A.V., Karadge, R.A., Samant, J.S., 2010. Salinity induced changes in photosynthetic pigments and polyphenols of Cymbopogon nardus (L.) Rendle. J. Chem. Pharm. Res. 2, 338–347.
McCue, K.F., Hanson, A.D., 1990. Drought and salt tolerance: towards understanding membranes. Methods Enzymol. 148, 350–382.
Mune, N., Gupta, A.K., 2006. Interactive effects of sodium and calcium on proline metabolism in salt tolerant green gram cultivar. Am. J. Plant Physiol. 1, 1–12. https://doi.org/10.4392/appe.2006.1.12.
Muns, R., 2005. Genes and salt tolerance: bringing them together. New Phytol. 159, 651–681. https://doi.org/10.1111/j.1469-8137.2003.00828.x.
Muns, N., Tester, M., 2008. Mechanisms of salt tolerance. Annu. Rev. Plant Biol. 59, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.
Muscolo, A., Panuccio, M.R., Sidari, M., 2003. Effects of salinity on growth, carbohydrate metabolism and nutritive properties of kikuyu grass (Pennisetum clandestinum Hochst.). Plant Sci. 164, 1103–1110.
Mustafiz, A., Ghosh, A., Tripathi, A.K., Kaur, C., Ganguly, A.K., Bhavesh, N.S., Tripathi, J.K., Pareek, A., Sopory, S.K., Single-Pareek, S.L., 2014. A unique Ni$^{2+}$-dependent and methylglyoxal-inducible rice glyoxalase I possesses a single active site and functions in abiotic stress response. Plant J. 78 (6), 951–963.

Nahar, K., Hasanuzzaman, M., Alam, M.M., Fujita, M., 2015. Glutathione-induced drought stress tolerance in mung bean: coordinated roles of the antioxidant defence and methylglyoxal detoxification systems. AoB Plants 7, plv069. https://doi.org/10.1093/aobpla/plv069.

Ozturk, M., Gemici, M., Yilmazer, C., Ozdemir, F., 1992. Alleviation of salinity stress by GA3, KIN and IAA on seed germination of Brassica campestris L. Turk. J. Bot. 17, 47–52.

Ozturk, M., Gemici, M., Guven, A., 1993. Effects of salt and growth regulators on the stomata of Vignaunguiculata (L) Walp. Ege. Univ. Sci. Fac. J. 15, 33–41.

Ozturk, M., Dogan, Y., Baslar, S., Mert, H.H., 1997. Alleviation of salinity stress in the germination of Erucia sativa Mill. Eucarpia (INRA Rennes) 19, 69–70.

Ozturk, M., Baslar, S., Dogan, Y., Sakcali, S., 2006. Alleviation of salinity stress in the seeds of some brassica species. Kluwer-Springer, Netherlands, pp. 145–156.

Pattanagul, W., Thitisaksakul, M., 2008. Effect of salinity stress on growth and carbohydrate metabolism in three rice (Oryza sativa L.) cultivars differing in salinity tolerance. Ind. J. Exp. Biol. 46, 736–742.

Plata, J.S., Villasante, C.O., Flores-Cáceres, M.L., Escobar, C., del Campo, F.F., Hernández, L.E., 2009. Differential alterations of antioxidant defenses as bioindicators of mercury and cadmium toxicity in Alfalfa. Chemosphere 77 (7), 946–954.

Qadir, M., Qaiser, M., Khan, M., Tahir, M., Hassan, S., et al., 2014. Economics of salt-induced land degradation and restoration. Nat. Resour. Forum 38, 282–295. https://doi.org/10.10111/1477-8947.12054.

Qureshi, A., Niaz, M.Z., Ahmad, J., Iqbal, M., 2013. Effect of long-term salinity on cellular antioxidants, compatible solute and fatty acid profile of Sweet Annie (Artemisia annua L.). Phytochemistry 95, 215–223.

Rodriguez-Hernandez, M.D., Moreno, D.A., Carvajal, M., Ballesta, M.D.M., 2013. Interactive effects of boron and NaCl stress on water and nutrient transport in two broccoli cultivars. Funct. Plant Biol. 40, 739–748.

Shabala, S., 2013. Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. Ann. Bot. 112, 1209–1221. https://doi.org/10.1093/abo/mct205.

Shakeel, D.B., Pandey, M., Nikalje, G.C., et al., 2017. Salt responsive physiological, photosynthetic and biochemical attributes at early seedling stage for screening soybean genotypes. Plant Physiol. Biochem. 118, 519–528.

Smeekens, S., 2000. Sugar-induced signal transduction in plants. Ann. Rev. Plant Biol. 51, 47–81.

Tester, M., Langridge, P., 2010. Breeding technologies to increase crop production in a changing world. Science 327, 818–822. https://doi.org/10.1126/science.1183700.

Thounaojam, T.C., Panda, P., Mazumdar, P., Kumar, D., Sharma, G., Sahoo, L., Panda, S., 2012. Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol. Biochem. 53, 33–39.

Tie, S.G., Zhao, Y.M., Li, W., 2014. Oxidative damage and antioxidant response caused by excess copper in leaves of maize. Afr. J. Biol. 11, 4378–4384.

Zhang, L., Li, J.M., Wang, H.X., 2002. Physiological and ecological responses of wheat (Triticum aestivum L.) root to cadmium stress. Chin. J. Soil Sci. 33 (1), 61–65 (in Chinese).

Zhang, J., 1991. Root signals and the regulation of growth and development of plant in drying soil. Annu. Rev. Plant Physiol. 42, 55–76.

Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci. 6, 66–71.

Zhu, J., Lee, E.H., Dellinger, M., Cui, X., Zhang, C., Wu, S., et al., 2010. A cellulose synthase-like proteins required for osmotic stress tolerance in Arabidopsis. Plant J. 63, 128–140. https://doi.org/10.1111/j.1365-313X.2010.04227.