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Differential response of pea (*Pisum sativum* L.) genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes

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Abstract

The response of nine pea (*Pisum sativum*) genotypes, with varying salt tolerance potential, was studied under salt stress. Salt stress significantly ($p \leq 0.05$) reduced the growth (internodal distance, plant fresh/dry biomass and number of leaves), physiological attributes (photosynthesis rate, stomatal conductance, transpiration rate, chlorophyll contents) and cell membrane stability index (MSI) while elevated antioxidant enzymes, i.e. superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), organic solutes (proline, glycinebetaine and total free amino acids), lipid peroxidation (LPO), hydrogen peroxide (H_2O_2) and leaf abscisic acid (ABA) in tested genotypes. However, root/shoot sodium (Na^+) was increased with increasing salinity levels, which enhanced the Na^+: K^+ ratio and seemed to affect the bioenergetic processes of photosynthesis. Whereas, root and shoot of tested genotypes exhibited a considerable reduction in phosphorus (P) and potassium (K) contents. Based on % increase or decrease in above mentioned attributes, the tested genotypes were categorized into salt tolerant and salt sensitive categories. Therefore, Climax, Samarina zard and 9800-5 were found to be salt tolerant, whereas 2001-20, Meteor and Euro observed as highly salt sensitive. Tolerant genotypes (Climax, Samarina zard and 9800-5) were successful in maintaining the maximum dry matter, low Na^+, while high P and K^+ under saline conditions. Since, genotypes with high concentration of organic osmolytes (proline, glycinebetaine and amino acids) and high antioxidant activity (SOD, POD, CAT) had high salt tolerance, so it is also concluded that salt tolerance potential of pea is highly associated with concentration of osmolytes and antioxidant enzymes.

Keywords: Salt stress, abscisic acid, antioxidants, glycinebetaine, photosynthesis, proline, stomatal conductance.
Abbreviations: ABA-abscisic acid; MSI-membrane stability index; SOD-superoxide dismutase; POD-peroxidase; CAT-catalase; H_2O_2-hydrogen peroxide; PAR-photosynthetic active radiation; LPO-lipid peroxidation; HSD-honestly significant difference

Introduction

Plant growth and productivity is adversely affected by nature's wrath in the form of various abiotic stress factors. Plants are frequently exposed to a plethora of stress conditions such as salinity, drought, heat, flooding and heavy metal toxicity among others, where the various anthropogenic activities have accentuated the existing stress factors (Allakhverdiev et al., 2000; Siringam et al., 2012). Among these stresses, salinity is a serious problem in worldwide agriculture areas because it limits plant growth and productivity (Yildirim et al., 2009; Qin et al., 2010). Salt condition under irrigation water affects physiological process negatively including water relations and gas exchange attributes (Maeda and Nakazawa, 2008), nutritional imbalance (Yang et al., 2008), and disturbing the stability of membranes (Dogan et al., 2010). Salt tolerance could be affected by a number of factors, such as type of salts in the soil solutions, growth conditions (environmental and management), age and plant genotype (Moisender et al., 2002, Sheekh-El et al., 2002). Salts induce the ionic and osmotic stress which alters the morpho-physiological and biochemical processes at tissue and cell level (Murphy and Durako, 2003). Excessive salts in soil solution lower the soil water potential as compared to the potential within plant and this difference in water potential between soil and plant

prevents the root to absorb water (Lloyd et al., 1989). This hindrance in the absorption of available water under saline condition causes the cell dehydration which ultimately leads to cell death. In saline soils, mostly Na^+ and Cl^- are dominant ions and high concentration of both ions in saline soil leads to specific drastic effects in non-salt tolerant plants i.e. non-availability of water to plant and nutritional imbalance (García-Sánchez et al., 2002). Under these conditions, the high concentration of these toxic ions may also interfere with the assimilation of other essential nutrients resulting in nutrient imbalance such as less availability of potassium, magnesium and calcium (Hasegawa et al., 2000). An excessive amount of toxic ions (Na^+ and Cl^-) in plant tissues unstable the cellular membranes by displacing the K^+ and Ca^{2+} (Grattan and Grieve, 1992) and affect their permeability. These variations in cell membrane permeability due to the toxic ions i.e. Na^+ and Cl^- results in the disturbances in various physiological processes (Kao et al., 2003, Sayed, 2003). Salt stress can reduce the leaf photosynthetic activity by affecting stomatal and non-stomatal factors. Lose turgor by osmotic effect can cause stomata closure which lowers the supply of CO_2 to leaves. But salinity can also reduce photosynthetic activity by affecting the non stomatal attributes such as destruction of green pigments, lowering the leaf area or by decreasing the activity of photosynthetic enzymes in calvin cycle (Misra et al., 1997). The photosynthetic activity declination by salt stress is associated with an increase in reactive oxygen species (ROS) which thereby accelerate toxic reactions like lipid peroxidation, protein degradation and DNA mutation (McCord, 2000). The mechanisms of salt and/or drought tolerance in many plants may involve in striking a delicate balance between ion accumulation, osmotic adjustment, production of organic solutes, maintenance of pressure potential and growth. Osmotic adjustment is the improvement in cell water balance due to the accumulation of inorganic and organic osmolytes such as proline, betaines and/or sugars. However, aside from its role as an osmolyte for osmotic adjustment, these chemicals such as sugars (trehalose), sugars alcohols (sorbitol and mannitol), amino acids (proline) and betaines are also found to be particularly effective at protecting cytoplasmatic proteins and cell membranes from desiccation. For example, proline contributes in stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions (Balal et al., 2012). To alleviate the stress induced oxidative effects, plants generate different kinds of antioxidants like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Shahid et al., 2011). Pea (*Pisum sativum*) is a very important commercial vegetable around the world including China, India, USA, France and Egypt, but its production is limited by salt stress because it is a salt sensitive plant. Pea crop can be considered as a vital cool season vegetable which is utilized for various purposes like fresh peas, dry pulses and edible podded type. Nutritional value of peas cannot be denied as these are an excellent source of protein, carbohydrate (Hussein et al., 2006), water-soluble fibers, vitamins (vitamin B_1), and antioxidants (Mukerji, 2004). The present study was therefore conducted to i) evaluate salinity tolerance of different peas genotypes under controlled conditions and to categorize commonly used pea genotypes into salt tolerant and salt sensitive on basis of morphological parameters and ii) establish that physiological and biochemical mechanisms is related with the salt tolerance in pea plants.

Results

Plant growth parameters

Data regarding the plant biomass revealed that salt tolerant cultivars exhibited maximum internodal distance, higher fresh and dry plant weights (Fig. 1) and number of leaves per plant (Fig. 1) as compared to the sensitive ones under salt stressed conditions. Climax exhibited the highest salt tolerance potential by maintaining maximum number of leaves, branches, internodal distance, fresh and dry weights per plant while Euro showed maximum susceptibility in this regard.

Leaf gas exchange and chlorophyll content

Salinity treatments significantly ($P \leq 0.05$) reduced the gas exchange attributes (Fig. 1 and 2). All salinity treatments induced a significant reduction ($P \leq 0.05$) in Pn but most prominent reduction was observed under salinity level of 75 mM (Fig. 1). At this high salinity level (75 mM), maximum reduction was noted for Euro (40%) while minimum for Climax (17%) with respect to the control (non saline). Salinity progressively reduced both E and g_s in all tested pea genotypes but at higher salinity level of 75 mM maximum reduction was observed in Euro (47% and 61%) and 2001-20 (34% and 61%). On the other hand, Climax and 9800-5 showed high salt tolerance by exhibiting the lowest reduction in E and g_s under saline conditions (Fig. 2). Chlorophyll contents were reduced in all the nine pea genotypes grown under salt stress (Fig. 2). From the results it is evident that chlorophyll contents were decreased with the increasing salinity level and maximum inhibiting effect was recorded at high salt stress (75 mM). On the basis of reduction in chlorophyll contents, the genotypes Climax and Samarina zard can be categorized as salt tolerant while Euro and Meteor as salt sensitive.

Membrane stability index, lipid peroxidation, hydrogen peroxide and abscisic acid

Membrane stability index (MSI) decreased under salt stress in all the tested pea genotypes at all NaCl treatments but maximum reduction was noted under 75 mM (Fig. 2 and 3). Among the genotypes MSI was high in Climax and Samarina zard while it was low in Euro and Meteor at high salt stress in relative to the non saline control (Fig. 2). Since, membranes damage increased with increase in salinity level so MSI can be considered as very significant tool for evaluating the salt tolerance potential in pea genotypes. Among all the tested pea genotypes, Samarina zard and Climax had no considerable increase in lipid peroxidation while Euro and 2001-20 showed maximum ratios of lipid peroxidation (Fig. 4). The high lipid peroxidation in Euro and 2001-20 is the indication of their high salt sensitivity. Likewise, it was observed that increasing salt stress significantly enhanced the generation of hydrogen peroxide in all the genotypes but Climax, Samarina zard and Early green showed an excellent performance in terms of no considerable increase in lipid peroxidation. Regarding the abscisic acid, again Samarina zard and Climax showed the best performance by exhibiting less percent increase in ABA with respect to the non saline control (Fig. 4). However, the genotypes Euro and 2001-20 proved to be highly salt sensitive because of maximum percent increase in ABA in relative to the control (non saline).

Table 1. Effect of salt stress on sodium (Na^+) contents (mg g^{-1} D.Wt.) of both leaves and roots

	Leaf				Root			
	m*M* NaCl							
Genotypes	control	25	50	75	control	25	50	75
Samarina Zard	2.42	3.05 (26.03)	3.32 (37.19)	3.46 (42.98)	1.96	4.56 (132.65)	5.28 (169.39)	6.1 (211.22)
Olympia	2.15	3.48 (61.86)	3.74 (73.95)	4.16 (93.49)	4.09	7.74 (89.24)	8.19 (100.24)	7.94 (94.13)
Early Green	1.93	2.88 (49.22)	3.07 (59.07)	3.23 (67.36)	3.23	6.45 (99.69)	6.87 (112.69)	7.55 (133.75)
Climax	2.32	2.89 (24.57)	3.01 (29.74)	3.21 (38.36)	1.04	2.46 (136.54)	2.68 (157.69)	3.24 (211.54)
2001-20	2.16	3.83 (77.31)	6.14 (184.26)	6.29 (191.20)	2.11	3.45 (63.51)	3.6 (70.62)	3.87 (83.41)
Meteor	3.06	5.45 (78.10)	6.27 (104.90)	7.26 (137.25)	1.17	2.18 (86.32)	2.27 (94.02)	2.52 (115.38)
Euro	1.32	2.45 (85.61)	3.12 (136.36)	4.96 (275.76)	1.96	3.34 (70.41)	3.52 (79.59)	4.12 (110.20)
9200-1	2.22	3.26 (46.85)	3.67 (65.32)	4.19 (88.74)	3.45	6.56 (90.14)	7.14 (106.96)	7.67 (122.32)
9800-5	1.78	2.31 (29.78)	2.42 (35.96)	2.53 (42.13)	2.16	5.34 (147.22)	5.88 (172.22)	6.46 (199.07)
HSD ($P \leq 0.05$, n=5) (Tukey Test)								
Genotypes	**	**						
Salinity	**	**						
Salinity x Genotype	**	**						

** Significant; Figures in parenthesis indicate the % increase in Na^+ over control (non saline)

Table 2. Effect of salt stress on potassium (K^+) contents (mg g^{-1} D.Wt.) of both leaves and roots

	Leaf				Root			
	m*M* NaCl							
Genotypes	control	25	50	75	control	25	50	75
Samarina Zard	22.56	20.75 (18.34)	18.34 (17.06)	17.06 (24.38)	17.85	17.14 (3.98)	15.88 (11.04)	15.32 (14.17)
Olympia	22.36	18.69 (16.72)	16.72 (14.61)	14.61 (34.66)	16.54	14.44 (12.71)	13.76 (16.82)	12.92 (21.86)
Early Green	21.42	18.96 (17.13)	17.13 (15.47)	15.47 (27.78)	17.84	16.41 (8.05)	16.14 (9.56)	14.82 (16.96)
Climax	23.16	22.06 (20.81)	20.81 (19.39)	19.39 (16.25)	15.25	14.64 (4.04)	13.91 (8.81)	13.33 (12.63)
2001-20	22.01	17.45 (16.93)	16.93 (13.04)	13.04 (40.75)	18.53	15.52 (16.28)	14.11 (23.88)	12.48 (32.68)
Meteor	20.7	17.30 (14.76)	14.76 (13.45)	13.45 (35.02)	17.37	14.73 (15.23)	14.23 (18.11)	12.76 (26.57)
Euro	24.07	18.46 (16.31)	16.31 (15.73)	15.73 (34.65)	19.15	16.24 (15.21)	15.01 (21.59)	13.24 (30.87)
9200-1	22.83	19.34 (17.73)	17.73 (15.55)	15.55 (31.89)	15.96	14.68 (8.02)	14.40 (9.73)	12.98 (18.67)
9800-5	21.33	19.32 (17.45)	17.45 (16.05)	16.05 (24.75)	16.43	15.29 (6.98)	14.71 (10.51)	14.01 (14.77)
HSD ($P \leq 0.05$, n=5) (Tukey Test)								
Genotypes	**	**						
Salinity	**	**						
Salinity x Genotype	**	**						

** Significant; Figures in parenthesis indicate the % decrease in K^+ over control (non saline)

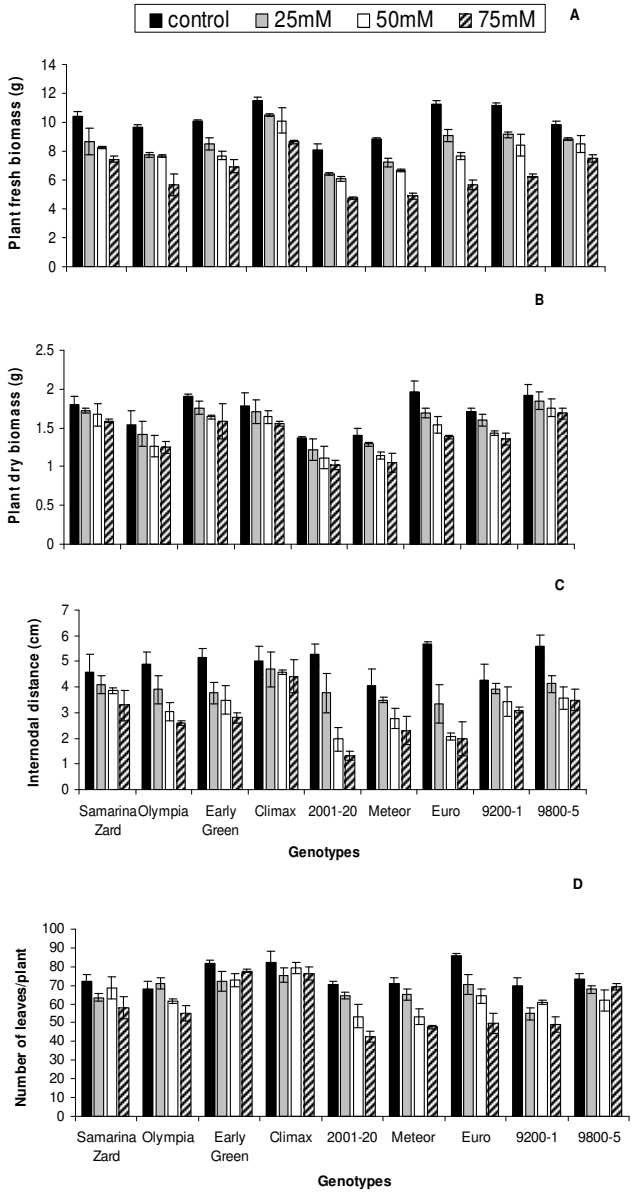

Fig 1. Effect of salt stress on plant fresh biomass (A), plant dry biomass (B), internodal distance (C) and number of leaves plant^{-1} of nine pea genotypes with varied salt tolerance potential. Each value is the mean of five replicates and the vertical bars give the standard error (SE) of the mean. In all figures, HSD (Tukey's test) for genotypes and treatments were significant at P=0.05 unless otherwise stated.

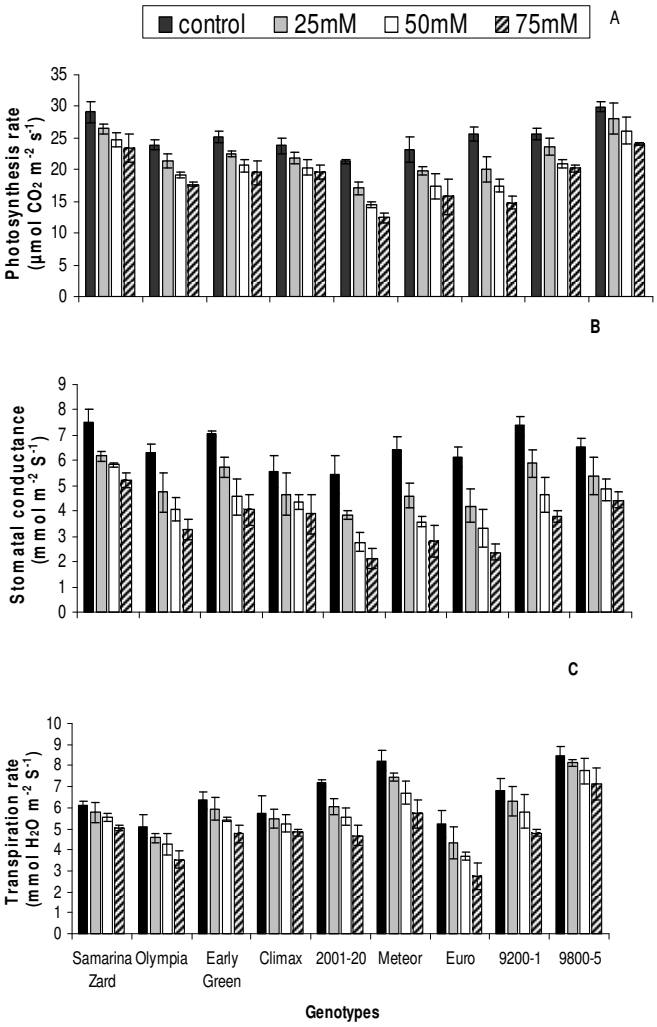

Fig 2. Effect of salt stress on photosynthesis rate (A), stomatal conductance (B) and transpiration rate (C) of nine pea genotypes with varied salt tolerance potential. Each value is the mean of five replicates and the vertical bars give the standard error (SE) of the mean.

Antioxidant enzymes and osmolytes

Analysis of variance of data for antioxidant enzymes (SOD, POD and CAT) indicates that various salinity levels had a significant ($P \leq 0.05$) enhancing effect on the activities of antioxidant enzymes of all the tested pea genotypes (Fig.3). But maximum increase was noted for Samarina zard and Climax as compared to the remaining genotypes. Similarly, in case of osmolytes (amino acid, proline and glycinebetaine) the genotypes Samarina zard and Climax showed the highest salt tolerance potential in terms of maximum accumulation of osmolytes (Fig.3). However the genotypes, Euro, Meteor and 2001-20 gave very poor performance by exhibiting little increase in osmolytes (Fig.3).

Leaf and root mineral nutrition

Sodium concentration in leaves increased with increasing NaCl concentration (Table 1). The genotype that accumulated high ratios of Na^+ in leaves was Euro while Climax accumulated the least sodium in leaves. Under 75 mM salinity level, Climax, 9800-5 and Samrina zard exhibited the lowest percent increase (38, 42 and 42% respectively) in Na^+ but maximum by Euro (275%) and 2001-20 (191%) (Table 1). For instance tolerant cultivars (Climax and 9800-5) had less Na^+ in their leaves than did sensitive like Euro and 2001-

20. Root Na^+ concentrations also tend to increase under all salinity levels (Table 1) but genotypes Climax and Samarina zard gave an excellent performance by accumulating the least amount of Na^+ in roots. Increasing salinity in irrigation water from 0 to 75 mM NaCl decreased both the leaf and root K^+ concentration in all tested pea genotypes (Table 2). The lowest reduction percentage was recorded for Climax and Samrina zard while Meteor and 2001-20 exhibited maximum reduction in K^+ in leaf and root respectively, as compared to their respective controls. Although, salt stress also caused a significant reduction in root/shoot phosphorus (P) of all tested pea genotypes (Table 3) but maximum reduction percentage was recorded in Euro and Meteor. Regarding the P content, Climax and Samarina zard showed high salt tolerance potential by maintaining the high ratios of P under saline conditions. On the basis of results regarding the Na^+, K^+, Cl^- and P, the genotypes Climax and Samarina zard are ranked as salt tolerant, Euro and Meteor as non tolerant and remaining genotypes in between of tolerant and non tolerant.

Discussion

Reduction in internodal distance and number of leaves per plant is a common phenomenon under salinity stress in various plant species (Zhu et al., 2001). Among the investigated pea genotypes, Climax was least influenced by salinity so had maximum number of leaves and internodal distance under saline regimes. This reduction in internodal distance and number of leaves may be due to the reduction in turgor potential, necessary for cell elongation (Iqbal and Ashraf, 2005) and turgor pressure, which were reduced under salt stress (Ashraf and Harris, 2004). In current study, salt stress enhanced the abscisic acid (ABA) concentration in leaves, which act as senescing agent thus, reduction in number of leaves in tested pea genotypes may also be associated with the production of ABA under salt stress. Albacete et al. (2008), Ghanem et al. (2008), Hassine and Lutts (2010) and Bakht et al. (2011) investigated the *Solanum tubersum*, *Atriplex halimus* and *Zea mays*, respectively under saline conditions and found a marked reduction in number of leaves and internodal distance in response to salt stress. So, these reports are in accordance with the findings of current investigation. However, the reduction in plant biomass under salinity may be due to many reasons such as lack of maintenance of turgor, sodium/chloride ion toxicity and disturbances in metabolic pathways. Since these factors disturb the functioning of gas exchange attributes which ultimately leads to decline in activity of photosynthetic apparatus. Thus, the reduction in plant biomass in present study could have been due to reduced photosynthetic activity. A positive correlation was recorded between plant dry biomass and physiological attributes (Pn and g_s) because the genotypes maintaining maximum dry biomass showed higher rates of Pn and g_s. It is reported that salt stress reduced the plant biomass in sunflower (Noreen and Ashraf, 2008) and wheat (Ashraf et al. 2010), so these findings are in agreement with the results of current investigation regarding plant biomass. In current investigation, Pn was decreased due to the elevated level of toxic ions (Na^+ and Cl^-) in different plant parts which induced deleterious effects on physiological processes in plant especially on stomatal functioning. Similarly, Huang and Fu (2000) and Bano (2010) also recorded a decrease in Pn, g_s and *E* of perennial grasses and rice plants, respectively submitted to saline conditions.As results depicted that tolerant genotypes (Samarina zard and Climax) showed the least reduction in Pn and g_s than non tolerant ones (Euro and Meteor) with respect to control, so these physiological attributes can be used as screening tool for salt tolerance in pea genotypes. Centritto et al. (2003), Filella et al. (2004), Noreen and Ashraf (2008) and Nishimura et al. (2011) studied the salt stressed olive, Mediterranean shrub, sunflowers and rice, respectively and observed significant variations in various physiological attributes i.e. Pn, g_s and *E* therefore, claimed that these aspects can be considered as potential indicators of salt stress. Since, salt stress affected the stomatal functioning by disturbing the turgidity of guard cells so the reduction in *E* in tested pea genotypes may be due to the reduction in turgidity of guard cells, which is very common in almost all stresses (Stepin and Klobus, 2006). The present investigation confirmed the findings of Tezara et al. (2002), who studied the sunflower under drought conditions and found a considerable decrease in *E*. As salt stress limits the availability of various nutrients especially potassium (Najafi et al., 2007) that maintain the turgidity of guard cells (Burman et al., 2003) therefore, the disturbances in guard cells turgidity may also be the cause of reduced *E* and g_s in salinized pea plants of tested pea genotypes. The genotypes which maintained the efficient *E* and g_s under adverse conditions were successful to adjust them osmotically by accumulating some osmolytes and osmoprotectants i.e. proline, glycinebetaine and amino acids. So, it is obvious that high salt tolerance potential of tolerant genotypes (Samarina zard and Climax) was due to high accumulation of osmolytes (proline, glycinebetaine and amino acids) in their tissues. Increase in accumulation of compatible solutes under stressed conditions has previously been reported in *Salicornia europaea* and *Suaeda maritima* (Moghaieb et al., 2004), *Phragmites australis* (Pagter et al., 2009), *Zea mays* (Hajlaoui et al., 2010) and *Brassica nupus* (Heidari, 2010). In present investigation, the reduction in chlorophyll contents could have been due to the displacement of Mg^{2+} by toxic Na^+ ions, which caused the degradation of green pigments. Similar kind of findings had reported by Loggini et al. (1999) and Meloni et al. (2003) in drought stressed wheat and salt stressed cotton plants respectively. A positive correlation was observed between chlorophyll contents and plant biomass production. Reactive oxygen species (ROX) species like hydrogen peroxide (H_2O_2) are considered as the indicator of stress (Miller et al. 2008; Wang and Song, 2008). In present study, salt stress significantly enhanced H_2O_2 and lipid peroxidation (LPO), but it was lower in tolerant genotypes than non tolerant ones. From H_2O_2 and LPO values, it is depicted that salt stress disintegrated the membranes to a lesser extent in tolerant genotypes as compared to sensitive ones. Low ratios of H_2O_2 and LPO in tolerant genotypes (Samarina zard and Climax) are also indication of high membrane stability index (MSI). Since, tolerant genotypes showed lower H_2O_2 and LPO but higher MSI than sensitive genotypes, therefore these three attributes (H_2O_2, LPO and MSI) can be used as the measure of salt injury and salt tolerance potential in pea genotypes. Similarly, Noreen and Ashraf (2009) submitted the various pea genotypes to different salinity levels and noted an elevation in H_2O_2 and LPO level with the increasing salt stress. Plants detoxify the ROX species by maintaining the high activities of antioxidant enzymes i.e. SOD, POD and CAT (Sekmen et al., 2012). Literature depicts that salt tolerance potential is highly linked with the maximum ratios of antioxidant enzymes (Bor et al., 2003; Shahid et al., 2011; Balal et al., 2012). In agreement with the current investigation, a consistent trend in enzymatic activities of antioxidant enzymes such a SOD, POD and CAT

Table 3. Effect of salt stress on phosphorus (P) contents (mg g^{-1} D.Wt.) of both leaves and roots.

	Leaf				Root			
	m*M* NaCl							
Genotypes	control	25	50	75	Control	25	50	75
Samarina Zard	1.71	1.61 (5.85)	1.57 (8.19)	1.50 (12.28)	3.64	3.45 (5.22)	3.17 (12.91)	3.03 (16.76)
Olympia	2.03	1.83 (9.85)	1.79 (11.82)	1.63 (19.70)	3.94	3.54 (10.15)	3.29 (16.50)	2.97 (24.62)
Early Green	1.4	1.30 (7.14)	1.28 (8.57)	1.19 (15.00)	4.02	3.66 (8.96)	3.48 (13.43)	3.28 (18.41)
Climax	1.87	1.76 (5.88)	1.73 (7.49)	1.69 (9.63)	4.58	4.33 (5.46)	4.23 (7.64)	4.11 (10.26)
2001-20	1.46	1.27 (13.01)	1.21 (17.12)	1.16 (20.55)	1.96	1.73 (11.73)	1.52 (22.45)	1.37 (30.10)
Meteor	1.4	1.27 (9.29)	1.21 (13.57)	1.05 (25.00)	3.13	2.68 (14.38)	2.36 (24.60)	2.53 (19.17)
Euro	1.59	1.38 (13.21)	1.29 (18.87)	1.15 (27.67)	3.25	2.79 (14.15)	2.5 (23.08)	2.25 (30.77)
9200-1	1.92	1.78 (7.29)	1.69 (11.98)	1.58 (17.71)	3.39	3.08 (9.14)	2.74 (19.17)	2.72 (19.76)
9800-5	2.06	1.92 (6.80)	1.87 (9.22)	1.79 (13.11)	3.94	3.76 (4.57)	3.65 (7.36)	3.51 (10.91)
HSD ($P \leq 0.05$, n=5) (Tukey Test)								
Genotypes	**	**						
Salinity	**	**						
Salinity x Genotype	**	**						

** Significant; Figures in parenthesis indicate the % decrease in P over control (non saline)

Table 4. Effect of salt stress on Na^+:K^+ of both leaves and roots.

	Leaf				Root			
	mM NaCl							
Genotypes	control	25	50	75	Control	25	50	75
Samarina Zard	0.11	0.15 (37.03)	0.18 (68.76)	0.20 (89.06)	0.11	0.27 (142.29)	0.33 (202.81)	0.40 (262.62)
Olympia	0.10	0.19 (93.64)	0.22 (132.63)	0.28 (196.13)	0.25	0.54 (116.76)	0.60 (140.70)	0.61 (148.53)
Early Green	0.09	0.15 (68.58)	0.18 (98.90)	0.21 (131.73)	0.18	0.39 (117.09)	0.43 (135.10)	0.51 (181.38)
Climax	0.10	0.13 (30.78)	0.14 (44.39)	0.17 (65.26)	0.07	0.17 (146.39)	0.19 (182.52)	0.24 (256.41)
2001-20	0.10	0.22 (123.65)	0.36 (269.55)	0.48 (391.52)	0.11	0.22 (95.22)	0.26 (124.06)	0.31 (172.33)
Meteor	0.15	0.32 (113.11)	0.42 (187.36)	0.54 (265.14)	0.07	0.15 (119.72)	0.16 (136.83)	0.20 (193.20)
Euro	0.05	0.13 (142.01)	0.19 (248.82)	0.32 (474.98)	0.10	0.21 (100.94)	0.23 (129.13)	0.31 (204.03)
9200-1	0.10	0.17 (73.35)	0.21 (112.87)	0.27 (177.10)	0.22	0.45 (106.72)	0.50 (129.38)	0.59 (173.36)
9800-5	0.08	0.12 (43.28)	0.14 (66.18)	0.16 (88.89)	0.13	0.35 (165.65)	0.40 (204.05)	0.46 (250.73)
HSD ($P \leq 0.05$, n=5) (Tukey Test)								
Genotypes	**				**			
Salinity	**				**			
Salinity x Genotype	**				**			

** Significant; Figures in parenthesis indicate the % increase in Na^+/K^+ over control (non saline)

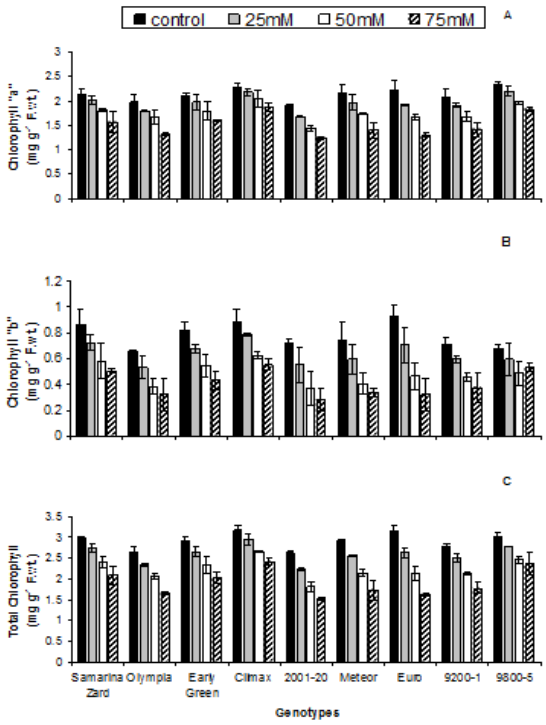

Fig 3. Effect of salt stress on chlorophyll 'a' (A), chlorophyll 'b' (B) and total chlorophyll contents (C) of nine pea genotypes with varied salt tolerance potential. Each value is the mean of five replicates and the vertical bars give the standard error (SE) of the mean.

was observed (Fig. 3). Due to the high antioxidant activities salt tolerant pea genotypes showed the low ratios of H_2O_2, which indicates the presence of strong negative correlation between ratios of antioxidant enzymes and H_2O_2. Since, tolerant genotypes (Samarina zard and Climax) maintained high plant biomass, which was possibly due to their better ability to maintain high antioxidant enzyme activities under saline conditions. These antioxidant activities eliminated the ROX species by converting them into oxygen and water, ultimately alleviated the osmotic stress induced ROX species. In this study, increase in Na^+ while decrease in K^+ and P was observed, but with significant ($P \leq 0.05$) differences between the genotypes. However, the existence of correlation between increased Na^+ accumulations, reduced K^+ and P and salt sensitivity led to a point that mineral nutrition of pea genotypes is associated with their salt tolerance potential. The results are in agreement with the findings of Zeid and El-Semary (2001), Meneguzzo et al. (2000), who investigated the maize and wheat plants submitted to salt and drought stress, and recorded a significant increase in Na^+ contents while reduction in K^+ and P contents. Since, salt tolerant genotypes (Samarina zard and Climax) exhibited the high ratios of Na^+ and low K^+ and P in their roots, so it can be considered as an adaptation to withstand saline conditions by limiting the upward movement of toxic ions in above ground plant parts (shoots and leaves). Similar kind of adaptation was recorded by Maggio et al. (2004) in salinized tomato plants. The antagonistic effect of Na^+ on K^+ may be the reason of reduction in K^+ contents of leaves and roots. Since, the tolerant genotypes with high K^+ contents in leaves also had maximum plant biomass and photosynthesis rate than sensitive ones, thus K^+ can be used as screening criteria for evaluating salt tolerance potential of pea genotypes. From the findings of current investigation it can be extracted that salt stress negatively influence the growth of pea and salt tolerance potential is highly associated with the concentration of organic (proline, glycinebetaine, amino acids etc.) and inorganic osmolytes (Ca, Mg, P) that play a vital role in osmotic adjustment under stressed conditions. Secondly, antioxidant activities are also closely linked with the salinity tolerance so, higher the antioxidant activities then higher will be the stress tolerance potential of a plant genotype. Therefore, the exogenous application of organic and inorganic osmolytes can be utilized to induce or enhance the salt tolerance capacity of commercially important crops especially the vegetables.

Material and methods

Plant material and growth conditions

Seeds of nine different genotypes (Samarina zard, Euro, Early green, Climax, 2001-20, Meteor, Olympia, 9200-1, and 9800-5), with varying salt tolerance potential, were sown in plastic pots filled with fine sand as growth medium. Eight seeds per pot were sown and after 15 days of germination, the plants were thinned to five. The experiment was carried out in the green house of University of Agriculture Faisalabad, Pakistan, with five replications (one pot was considered as one replicate). Plants were grown in Hoagland solution under non saline conditions for 30 days after germination. Afterwards, salt treatment was initiated. Sodium chloride (NaCl) was dissolved in double distilled water to obtain final concentration of 0 (Control), 25, 50 and 75 mM. These salinity levels were screened from a range of salinity treatments in a separate preliminary experiment, and three levels i.e., low salinity (25 mM), intermediate salinity (50 mM) and high salinity (75 mM) was created in current investigation. In this way, a clear performance of tested pea genotypes were evaluated under three saline regimes i.e. low, intermediate and high salt stress. To avoid the osmotic shock the desired salinity levels i.e. 25, 50 and 75 mM were created by gradually increasing the salinity level (25 mM) after one day interval until final concentrations (50 and 75 mM) were reached after three days. These salinity levels were maintained throughout the required duration the experiment by regularly noting the electrical conductivity (E_C) and pH of the rooting medium. The increase or decrease in E_C and pH was adjusted with the help of buffer or salt solution of desired concentration. Plants were grown for 15 days under salt stressed conditions. Plants were irrigated with half strength Hoagland solution, 250 mL per pot. The plants were usually watered with Hoagland solution after one day interval but sometime this interval was varied according to the moisture of the rooting medium (sand).

Growth attributes

Internodal distance in each plant was measured with the help of measuring tape in centimeters. Fresh weight of each plant was taken with the help of electric balance. Average of fresh weight was calculated for each treatment. Dry weight of whole plant was measured after keeping it in an oven at 70ºC

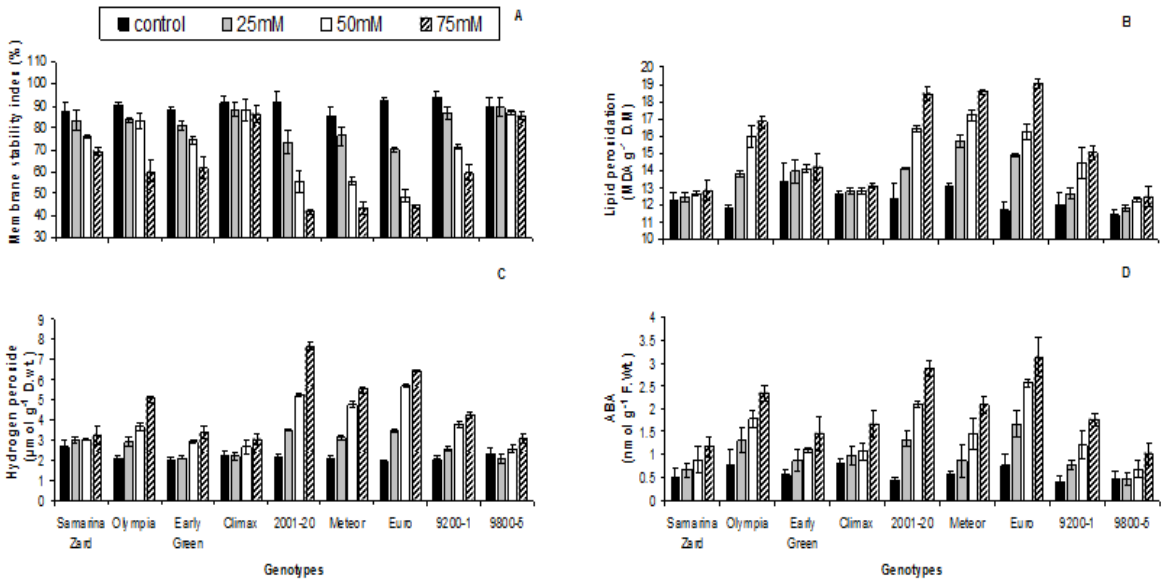

Fig 4. Effect of salt stress on membrane stability index (A), lipid peroxidation (B) hydrogen peroxide (C) and abscisic acid (ABA) (D) of nine pea genotypes with varied salt tolerance potential. Each value is the mean of five replicates and the vertical bars give the standard error (SE) of the mean.

for 72 hours. Dry weights were taken using digital electric balance and means were calculated for each treatment.

Gas exchange and chlorophyll contents

Gaseous attributes were determined by using an Infra-red Gas Analyzer (Analytical Development Company, Hoddesdon, England) (Shahid et al., 2011). The photosynthetic activity (Pn), transpiration rate (E) and stomatal conductance (g_s) were determined on intact fully matured leaves (Shahid et al., 2011; Balal et al., 2012). Measurements were performed from 9.00 to 11.00 a.m. with following specifications/adjustments: molar flow of air per unit leaf area 403.3 mM $m^{-2}s^{-1}$, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 6.0 to 8.9 mbar (PAR) at leaf surface was maximum up to 1711 (mol m^{-2} s^{-1}), temperature of leaf ranged from 28.4 to 32.4°C, ambient temperature ranged from 22.4 to 27.9°C, ambient CO_2 concentration was 352 mol mol^{-1}. Chlorophyll contents were estimated according to the method by Arnon (1979) with the help of following formulae

$$Chlorophyll\ a = [12.7\ (OD\ 663) - 2.69\ (OD\ 645)] \times \frac{volume}{1000} \times weight$$

$$Chlorophyll\ b = [22.9\ (OD\ 645) - 4.68\ (OD\ 663)] \times \frac{volume}{1000} \times weight$$

$$Total\ chlorophyll = Chlorophyll\ a + Chlorophyll\ b$$

Membrane stability index (MSI)

Membrane stability index (MSI) was calculated by taking the electrical conductivity of leaf leachates in double distilled water at 40 and 100°C by following the method of Sairam (1994). Mature leaf were cut into small pieces and then taken (0.5 g) in test tubes having 10 mL of double distilled water in two sets. One set was kept at 40ºC for 30 min and another set at 100ºC in boiling water bath for 15 min and their respective electric conductivity's C_1 and C_2 were measured by conductivity meter (Adawa-260, Germany).

$$Membrane\ stability\ index\ (MSI) = 1 - \frac{C1}{C2} \times 100$$

Antioxidant enzymes and osmolytes

The activity of superoxide dismutase (SOD) was analyzed following the protocol of Giannopolitis and Ries, (1977). Catalase (CAT) and peroxidase (POD) activities were measured by the procedure of Chance and Maehly (1955). The proline was estimated according to the method of Bates et al. (1973) from homogenized fresh leaf tissue while glycinebetaine was determined by the method of Grieve and Gratan (1983). Total free amino acids were estimated by the protocol of Hamilton and Van Slyke (1973).

Lipid peroxidation, hydrogen peroxide and abscisic acid

Lipid peroxidation (LPO) was estimated by measuring the concentration of malondialdehyde (MDA) and thiobarbituric acid (TBA) method of Heath and Packer, (1968). Hydrogen peroxide content (H_2O_2) was estimated by measuring the absorbance of titanium-hydroperoxide according to the protocol of Mukherjee and Choudhari (1983). Abscisic acid (ABA) concentration was calculated by the method of Djilianov et al. (1994).

Na^+, K^+ and P determination

The digested root samples were analyzed for Na^+ and K^+ by flame photometer (Jenway PFP-7, UK). A graded series of standards (ranging from 10 to 100 mg L^{-1}) of Na^+ and K^+ was prepared and standard curves were drawn. The values of Na^+

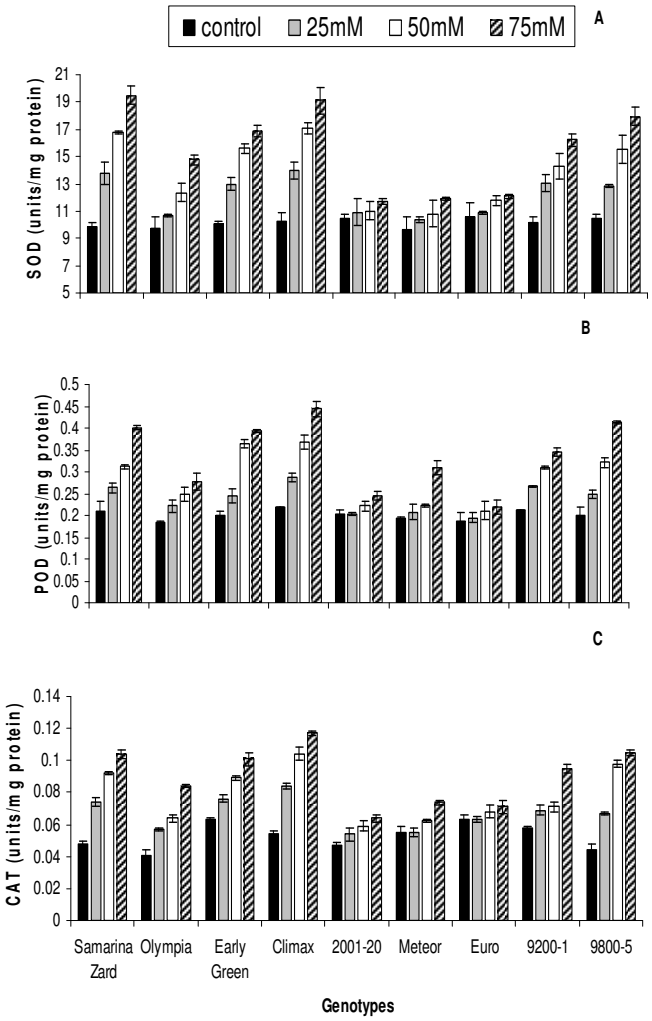

Fig 5. Effect of salt stress on superoxide dismutase-SOD (A), peroxidase-POD (B) and catalase-CAT (C) of nine pea genotypes with varied salt tolerance potential. Each value is the mean of five replicates and the vertical bars give the standard error (SE) of the mean.

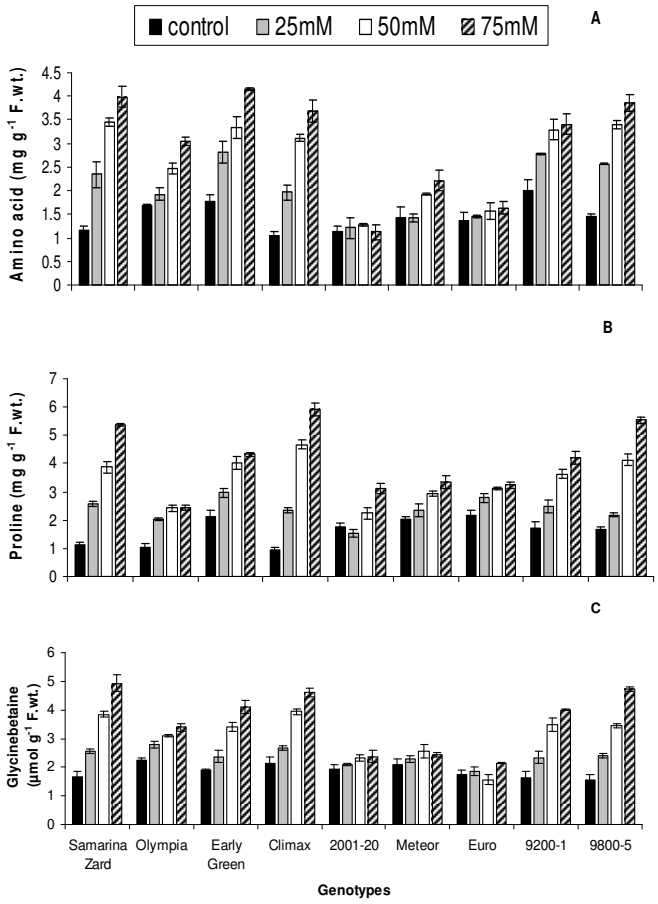

Fig 6. Effect of salt stress on amino acid (A), proline (B) and glycinebetaine (C) contents in the leaves of nine pea genotypes with varied salt tolerance potential. Each value is the mean of five replicates and the vertical bars give the standard error (SE) of the mean.

and K^+ from flame photometer were compared with standard curve and original quantities were computed. Phosphorus (P) was determined on a spectrophotometer by the method of Jackson (1962).

Statistical analysis

The experiment was laid out in two factors (salinity and genotypes) factorial arrangement under Completely Randomize Design (CRD).The data was analyzed statistically by using two-way analysis of variance with the statistical software (Statistix 8.1) and comparisons with *P*-values ≤ 0.05 were considered significantly different by using HSD values (Tukey's Test). Data were presented as mean ± SE at the top of each column in figures while ionic contents were presented in tables as means of five replicates with % increase or decrease over control in parenthesis.

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References

Albacete A, Ghanem ME, Martínez-Andujar, Acosta M, Sanchez-Bravo J, Martinez V (2008) Hormonal changes in relation to biomass partitioning and shoot growth impairments in salinized tomato (*Solanum lycopersicum* L.) plants. J Exp Bot 59:4119-4131.

Allakhverdiev SI, Sakamoto A, Nishijama Y, Inaba M, Murata N (2000) Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. Plant Physiol 123:1047-1056.

Arnon DI (1979) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24:1-10.

Ashraf M, Haris PJC (2004) Potential Biochemical indicators of salinity tolerance in plants. Plant Sci 166:3-16.

Ashraf MA, Ashraf M, Ali Q (2010) Response of two genetically diverse wheat cultivars to salt stress at different growth stages: leaf lipid peroxidation and phenolic contents. Pak J Bot 42:559-565.

Bakht J, Shafi M, Jamal Y, Sher H (2011) Response of maize (*Zea mays* L.) to seed priming with NaCl and salinity stress. Span J Agric Res 9:252-261.

Balal RM, Khan MM, Shahid MA, Mattson NS, Abbas T, Ashfaq M, Garcia-Sanchez F, Ghazanfer U, Gimeno V, Iqbal Z (2012) Comparative studies on the physiobiochemical, enzymatic, and ionic modifications in salt tolerant and salt sensitive Citrus rootstocks under NaCl stress. J Am Soc Hor Sci 137:1-10.

Bano A (2010) Root-to-shoot signal transduction in rice under salt stress. Pak J Bot 42:329-339.

Bates LS, Waldron RP, Teaxe WI (1973) Rapid determination of free proline for water stress studies. Plant and Soil 39:205-207.

Bor M, Ozdemir F, Turkan I (2003) The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet (*Beta vulgaris* L.) and wild beet (*Beta maritima* L.). Plant Sci 164:77-84.

Burman U, Garg BK, Kathju S (2003) Water relations, photosynthesis and nitrogen metabolism of Indian mustard (*Brassica jucea*) grown under salt and water stress. J Plant Biol 30:55-60.

Centritto M, Loreto F, Chartzoulakis K (2003) The use of low CO_2 to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt stressed olive saplings. Plant Cell Environ 26:585-594.

Chance M, Maehly AC (1955) Assay of catalases and peroxidases. Methods Enzymol 2:764-817.

Djilianov D, Gerrits MM, Ivanova A, Van Onckelen HA, De Klerk GJM (1994) ABA content and sensitivity during the development of dormancy in lily bulblets regenerated *in vitro*. Physiol Plant 91:639-644.

Dogan M, Tipirdamaz R, Demir Y (2010) Salt resistance of tomato species grown in sand culture. Plant Soil Environ 56:499-507.

Filella I, Penuelas J, Llorens L, Estiarte M (2004) Reflectance assessment of seasonal and annual changes in biomass and CO_2 uptake of Mediterranean shrub land submitted to experimental warming and drought. Remote Sens Environ 90:308-318.

Garcia-Sanchez F, Jifon JL, Carvaial M, Syvertsen M (2002) Gas exchange, chlorophyll and nutrient contents in relation to Na^+ and Cl^- accumulation in 'Sunburst' mandarin grafted on different rootstocks. Plant Sci 162:705-712.

Ghanem ME, Albacete A, Martinez-Andujar C, Acosta M, Romero-Aranda R, Dodd IC (2008) Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). J Exp Bot 59:3039-3050.

Giannopolitis CN, Ries SK (1977) Superoxide dismutase I. Occurrence in higher plants. Plant Physiol 59:309-314.

Grattan SR, Grieve CM (1992) Mineral element acquisition and growth response of plants grown in saline environment. Agric Ecosyst Environ 38:275-300.

Grieve CM, Gratan SR (1983) Rapid assay for the determination of water soluble quaternary ammonium compounds. Plant and Soil 70:303-307.

Hajlaoui H, Ayeb NE, Garrec JP, Denden M (2010) Differential effects of salt stress on osmotic adjustment and solutes allocation on the basis of root and leaf tissue senescence of two silage maize (*Zea mays* L.) varieties. Ind Crops Prod 31:122-130.

Hamilton PB, Van-Slyke DD (1973) Amino acid determination with ninhydrin. J Biol Chem 150:231-233.

Hasegawa PM, Bressnan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463-499.

Hassine AB, Lutts S (2010) Differential responses of saltbush *Atriplex halimus* L. exposed to salinity and water stress in relation to senescing hormones abscisic acid and ethylene. J Plant Physiol 167:1448-1456.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stochiometry of fatty acid peroxidation. Arch Biochem Biophy 125:189-198.

Heidari M (2010) Nucleic Acid Metabolism, Proline Concentration and Antioxidants Enzyme Activity in Canola (*Brassica nupus* L.) Under Salinity Stress. Agri Sci China 9:504-511.

Huang B, FU J (2000) Photosynthesis, respiration and carbon allocation of two cool season perennials grasses in response to surface soil drying. Plant Soil 227:17-26.

Hussein M, Nadia EL, Gereadly HM, EL-Desuki M (2006) Role of puterscine in resistance to salinity of pea plants (*Pisum sativum* L.). J App Sci Res 2:598-604.

Iqbal M, Ashraf M (2005) Changes in growth photosynthetic activity and ionic relations in spring wheat. Plant Growth Regul 60:41-52.

Jackson ML (1962) Soil chemical analysis. Constable and company, England.

Kao WY, Tsai TT, Shih CN (2003) Photosynthetic gas exchange and chlorophyll a fluorescence of three wild soybean species in response to NaCl treatments. Photosynthetica 41:415-419.

Lloyd J, Kriedmann PE, Aspinall D (1989) Comperative sensitivity of Prior Lisbon lemon and Valencia orange trees to foliar sodium and chloride concentrations. Plant Cell Environ 12:529-540.

Loggini B, Scartazza A, Brugnoli E, Navari-Izzo F (1999) Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol 119:1091-1099.

Maeda Y, Nakazawa R (2008) Effects of the timing of calcium application on the alleviation of salt stress in the maize, tall fescue, and reed canary grass seedlings. Biol Plant 52:153-156.

Maggio A, Pascale SD, Angelino G, Ruggiero C, Barbeiri G (2004) Physiological response of tomato to saline irrigation in long-term salinized soils. Eur J Agron 21:149-159.

McCord JM (2000) The evolution of free redicals and oxidative stress. Am J Med 108:652- 659.

Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot 49:69-76.

Meneguzzo S, Navari-Izzo F, Izzo R (2000) NaCl effects on water relations and accumulation of mineral nutrients in shoots, roots and cell sap of wheat seedlings. Braz J Plant Physiol 16:59-65.

Miller G, Shulaev V, Mittler R (2008) Reactive oxygen signaling and abioticstress. Physiol Plant 133:481-489.

Misra AN, Sahu SM, Misra M, Singh P, Meera I, Das N, Kar M, Shau P (1997) Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. Biol Plant 39:257-262.

Moghaieb REA, Saneoka H, Fujita K (2004) Effect of salinity on osmotic adjustment, glycinebetaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, *Salicornia europaea* and *Suaeda maritima*. Plant Sci 166:1345-1349.

Moisender PH, Mcclinton E, Paerl HW (2002) Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microbiol Ecol 43:432-442.

Mukerji KG (2004) Fruit and vegetable diseases. Hingham, MA, USA: Kluwer Academic Publishers, p. 145.

Mukherjee SP, Choudhari MA (1983) Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol Planta 58:116-170.

Murphy KST, Durako MJ (2003) Physiological effects of short term salinity changes on *Ruppia maritima*. Aquat Bot 75:293-309.

Najafi F, Khavari-Nejad RA, Rastgar-Jazii F, Sticklen M (2007) Growth and some physiological attributes of pea (*Pisum sativum* L.) as affected by salinity. Pak J Biol 10:2752-2755.

Nishimura T., Cha-um S, Takagaki M, Ohyama K, Kirdmanee (2011) Survival percentage, photosynthetic abilities and growth characters of two indica rice (*Oryza sativa* L. spp. *indica*) cultivars in response to iso-osmotic stress. Span J Agri Resh 9:262-270.

Noreen S, Ashraf M (2008) Alleviation of adverse effects of salt stress on sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid: growth and photosynthesis. Pak J Bot 40:1657-1663.

Noreen Z, Ashraf M (2009) Assessment of variation in antioxidative defense system in salt- treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. J Plant Physiol 166:1764-1774.

Pagter M, Bragato C, Malagoli M, Brix H (2009) Osmotic and ionic effects of NaCl and Na_2SO_4 salinity on *Phragmites australis*. Aquat Bot 90:43-51.

Qin J, Dong WY, He KN, Yu Y, Tan GD, Han L, Dong M, Zhang YY, Zhang D, Li ZA, Wang ZL (2010) NaCl salinity-induced changes in water status, ion contents and photosyn thetic properties of *Shepherdia argentea* (Pursh) Nutt. seedlings. Plant Soil Environ 56:325-332.

Sairam RK (1994) Effect of moisture stress on physiological activities of two contrasting wheat genotypes, Indian J Exp Biol 3:584-593.

Sayed OH (2003) Chlorophyll fluorescence as a tool in cereal crop research. Photosynthetica 41:321-330.

Sekmen AH, Turkan I, Tanyolac ZO, Ozfidan C, Dinc A (2012) Different antioxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblanceolata* Bark. Environ Exp Bot 77:63-76.

Shahid MA, Pervez MA, Balal RM, Mattson NS, Rashid A, Ahmad R, Ayyub CM, Abbas T (2011) Brassinosteroid (24-epibrassinolide) enhances growth and alleviates the deleterious effects induced by salt stress in pea (*Pisum sativum* L.). Aust J Crop Sci 5: 500-510.

Sheekh-EL MM, Omar HH (2002) Effect of high salt stress on growth and fatty acids content of the unicellular green algae Chlorella vulgaris. Am J Microbiol 55:181-191.

Siringam K, Juntawong N, Cha-um S, Boriboonkaset T, Kirdmanee C (2012) Salt tolerance enhancement in *indica* rice (*Oryza sativa* L. spp. *indica*) seedlings using exogenous sucrose supplementation. Plant Omics J 5:52-59.

Stepien P, Klobus G (2006) Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. Biol Plant 50:610-616.

Tezara W, Mitchell V, Driscoll SP, Lawlor DW (2002) Effects of water deficit and its interaction with CO_2 supply on the biochemistry and physiology of photosynthesis in sun flower. J Exp Bot 53:1781-1791.

Wang P, Song CP (2008) Guard-cell signalling for hydrogen peroxide and abscisic acid. New Phytol 178:703-718.

Yang CW, Wang P, Li CY, Shi DC, Wang DL (2008) Comparison of effects of salt and alkali stresses on the growth and photosynthesis of wheat. Photosynthetica 46:107-114.

Yildirim E, Karlidag H, Turan M (2009) Mitigation of salt stress in strawberry by foliar K, Ca and Mg nutrient supply. Plant Soil Environ 55:213-221.

Zeid IM, Al-Semary NA (2001) Response of two differentially drought tolerant varieties of maize to drought stress. Pak J Biol Sci 4:779-784.

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci 6:66-72.