Australian Journal of Crop Science AJCS
AJCS 6(9):1324-1331 (2012) ISSN:1835-2707

Screening of pea (*Pisum sativum* L.) genotypes for salt tolerance based on early growth stage attributes and leaf inorganic osmolytes

M. A. Shahid*[1], M. A. Pervez[2], R. M. Balal[1], T. Abbas[1], C. M. Ayyub[2], N. S. Mattson[3], A. Riaz[2], Z. Iqbal[4]

[1]Department of Horticulture, University College of Agriculture, University of Sargodha, (40100) Punjab, Pakistan
[2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad (38040) Punjab, Pakistan
[3]Department of Horticulture, College of Agriculture and Life Sciences, Cornell University, Ithaca, New York, USA
[4]Department of Plant Pathalogy, University College of Agriculture, University of Sargodha, (40100) Punjab, Pakistan

***Corresponding author: adnanshahid@uaf.edu.pk**

Abstract

In order to investigate the screening attributes that can be used to determine early growth stage sensitivity of pea plants to salt stress, 30 pea genotypes (*Pisum sativum*) were grown in trays containing fine sand as growth medium and subjected to five different salinity levels, i.e. 0, 2.5, 5.0, 7.5 and 10 dS m^{-1} NaCl. The Hoagland's solution was applied as the base nutrient solution. The germination (%) and emergence (%) was investigated in Petri dishes and plastic trays, respectively. Salt stress significantly reduced the germination (%), emergence (%), root and shoot fresh weight, root and shoot dry weight, root and shoot length and leaf inorganic osmolytes (K^+ and Ca^{2+}). While the leaf Na^+ content increased in all the genotypes, tolerant genotypes exhibited the lowest leaf Na^+ content under saline conditions. High leaf Na^+ accumulation indicated genotypes which are particularly sensitive to salt damage while high leaf K^+/Na^+ and Ca^{2+}/Na^+ ratio indicated genotypes with lower leaf injury. Compared with control, all added salinity levels induced an injurious effect on the above mentioned attributes but high salt stress (7.5 and 10 dS m^{-1}) resulted in considerable variation in tested genotype. A significant correlation was found between the root/shoot dry weight and leaf K^+/Na^+ and Ca^{2+}/Na^+ ratios. Based on the percent increase or decrease over control for the measured attributes, the tested pea genotypes were categorized into less salt tolerant, intermediate and high salt tolerant groups at seedling stage.

Keywords: pea, salt stress, genotypes, Na^+, K^+, K^+/Na^+, Ca^{2+}/Na^+.
Abbreviations: SC-standard curve; HSD-Honestly significant difference.

Introduction

Salinity refers to the buildup of soluble salts, which forms saline soils (Levy and Syvertsen, 2004; Ibraheem et al., 2011). It is well documented that besides other stresses, exposure to saline conditions limits plant growth and productivity (Abbas et al., 2010; Bhantana and Lazarovitch, 2010; Siringam et al., 2012). The increase in land area affected by salinity in arid and semi-arid lands is an issue of great concern in agriculture, because it resulted in gradual decline in crop productivity. In arid and semiarid regions, soil salinization occurs when there is insufficient irrigation, regular use of saline water or with improper cultural practices such as excessive fertilization. It is estimated that worldwide 831 x 10^6 hectares of land is affected by salinity (Beltran and Manzur, 2005). It is important to sustain the soil fertility and quality of water resources to fulfill the food, feed and fiber demand of an ever-growing world population. Salinity limits the production of nearly over 6% of the world's land (FAO, 2008) and 20% of the irrigated land (15% of total cultivated areas) (Munns, 2005) and negatively impacts agricultural yield throughout the world. Salinity is regarded as a grave situation globally because it has been estimated that it will affect 30% of cultivated world land area within the next 25 years and about 50% of land area by the end of this century (Wang et al., 2003). Salinization of the underground water resources is another related major problem affecting the agricultural productivity (Hasaneen et al., 2009). In Pakistan, a loss of about 20 billion rupees (351 million US$) annually has been estimated from salt-affected irrigated areas of the Indus Basin on account of decrease in crop yield. Salinization of good arable land in Pakistan is creating problem with immense socio-economic losses. The loss of an excellent natural resource is another problem, because population depends for its livelihood on these lands, which are gradually dwindling through the spread of salinity (IAEA, 1995). Soil salinity can be attributed to many ions present in the soil solution but adverse effects of salinity have been particularly attributed to chloride and sodium ions (Zekri, 2004) in different plant parts hence these ions produce conditions that impair plant survival by disturbing several physiological and biochemical mechanisms (Raveh, 2005; Grieve et al., 2007).

Excessive salts in the root zone or in soil solution may enter within the plant, resulting in plant death by ionic imbalance and osmotic stress (Mahajan and Tuteja, 2005). This ionic and osmotic imbalance leads to many morpho-physiological and biochemical abnormalities within the plant tissues (Pessarakli and Tucker, 1988). Salinity influences the physio-biochemical processes such as photosynthesic rate (Hayat et

al., 2010), transpiration rate (Cambrolle et al., 2011), stomatal conductance (Perez-Perez et al., 2009), water use efficiency (Grewal, 2010), sugars (Noreen and Ashraf, 2009), osmolytes proteins and water relations. Mainly there are three ways by which plants are affected under saline environments (Gama et al., 2007). These include reduced water absorption potential due to increased water potential, toxicity due to accumulation of specific ions such as Na^+ and Cl^- (Ashraf and Foolad, 2007) and reduction in nutrient transport resulting in nutritional imbalance (Tester and Davenport, 2003; Apse and Blumwald, 2007). Crops vary significantly in their threshold limits above which salt stress causes reduction in growth and productivity (Khan et al., 2006). Various approaches have been applied to alleviate the drastic effects of salinity. Among these strategies, the exploitation of genetic differences of available germplasm has greatest significance because it helps to identify the genotypes that can be readily adopted to perform well under saline conditions (Ashraf et al., 2006). Screening of crops for tolerance can strengthen the breeding programs by identifying genotypes with high salt tolerance and yield potential. This strategy involves the investigation of various morphological, physiological, biochemical, enzymatic and ionic responses at different developmental stages under salt stress as reported in various crops (Azhar and Ahmad, 2000; Ali et al., 2002; Khan et al., 2003a; Khan et al., 2003b; Kamal et al., 2003). There are many pitfalls to such approach i.e., climatic variation, varied physio-chemical properties of soil and amount of precipitation, which reduces the effectiveness of screening of crops for salt tolerance under field conditions. So, there is a dire need to screen the available germplasm of all horticultural crops especially the vegetable for salinity tolerance under controlled conditions. In this way we can easily investigate the salt tolerance potential of a genotype, by minimizing the above said factors that limits the effectiveness of screening procedure. Pea (*Pisum sativum*) is a vital cool season vegetable crop. It is cultivated across the globe, including China, India, USA, France, and Egypt. It is utilized for various purposes such as fresh peas, edible pods, and as dry pulses. Peas are an excellent source of protein, carbohydrates (Hussein et al., 2006), water-soluble fibers, vitamins (vitamin B_1), and antioxidants (Mukerji, 2004). Salinity and its allied factors limit the production and growth of various legumes, including pea globally (Najafi et al., 2007) as well as in Pakistan. Therefore, there is a dire need to screen available pea genotypes for salinity tolerance. Keeping in view the importance of pea and the drastic effects of salinity, current investigation was organized with the purpose to screen 30 available and commonly cultivated pea genotypes at seedling stage for salinity tolerance under five different salinity levels. As many researchers did that kind of investigation on various crops but present investigation was first time applied in pea, using fine sand as neutral growth medium in place of soil. The use of this neutral medium (no nutrients) was very helpful in eliminating the errors/variation in tested attributes that can be produced by the use of soil as rooting medium. In this way, we purely evaluated the toxic effects of salinity on various growth and nutritional aspects, which be not so pure in case of soil as growth medium. So, the investigation at seedling stage coupled with use of fine sand as growth medium differentiated the present study from previous studies and can be considered as novelty of this investigation. Several responses were measured i.e. the deleterious effects of salt stress on the seed germination, emergence, plant fresh/dry biomass, shoot/root length and leaf ionic contents of pea genotypes. Tolerant pea genotypes, screened in present investigation can be recommended for cultivation in marginal salt affected areas.

Results

Effect of salt stress on germination percentage

Pea genotypes significantly responded to increasing salinity levels. Although, salt stress decreased the germination percentage in all tested genotypes (Supplementary data 1) but maximum reduction in germination was recorded in seeds submitted to high salinity levels, i.e., 7.5 and 10 dS m^{-1} NaCl. The genotypes, samarina zard, 2001-35, climax, 9800-10 and 9800-5 performed well by maintaining the highest germination percentage under saline conditions while 9200-1, ambassidar, azad-P1, 2001-40, PF-400 gave the poorest response in this regard. The least mean % reduction in germination was recorded for samarina zard (4 %), 2001-35 (4 %), climax (8 %), 9800-10 (8 %), 2001-55 (11 %), K2P-6121 (11 %) and 9800-5 (11 %) while it was maximum for PF-400 (48 %), 2001-40 (48 %), azad P1 (43 %), ambassidar (43 %) and 9200-1 (39 %) with respect to control (0 dS m^{-1} NaCl) (Supplementary data 1). Interaction between salinity levels and pea genotypes was found to be significant.

Effect of salt stress on emergence percentage

Emergence percentage was also significantly reduced with increasing salt stress. The genotypes maintaining the highest emergence percentage under stressed conditions were placed under the tolerant category, while those having the lowest emergence percentage were categorized as salt sensitive ones. Emergence percentage indicated in (Supplementary data 2) revealed that samarina zard, climax, 2001-35 and 9800-5 had the lowest % reduction in emergence over the control, but it was maximum for ambassidar, PF-400, 9200-1 and 2001-40. The seeds grown under high salinity level of 10 dS m^{-1} NaCl, showed maximum reduction in emergence percentage (Supplementary data 2).

Effect of salt stress on plant biomass

Salt stress significantly reduced the plant fresh and dry biomass in all tested genotypes (Supplementary data 3). But the genotypes, samarina zard, climax and 9800-5 showed maximum salt tolerance potential in terms of having the least % reduction in plant fresh weight at all imposed salinity levels (2.5, 5.0, 7.5 and 10 dS m^{-1} NaCl). However, the genotypes PF-400, 9200-1, and 2001-40 proved to be highly salt sensitive due to the highest % decrease in plant fresh weight at 2.5, 5.0, 7.5 and 10 dS m^{-1} NaCl with respect to control (Supplementary data 3). Similarly, the plants subjected to salinity stress exhibited the significant decrease in plant dry weights (Supplementary data 4) but maximum reduction was caused by 7.5 and 10 dS m^{-1} NaCl stress. The genotypes, samarina zard (10 %), climax (15 %), 2001-35 (17 %), 9800-5 (20 %), 9800-10 (27 %) and 2001-55 (28 %) had the least mean % decrease in plant dry weight over the control, whereas PF-400 (55 %), ambassidar (52 %), 9200-1 (50 %), 2002-40 (48 %) and K2P-6185 (45 %) found to be the most severely affected genotypes in this regard. The genotypes with the least % reduction in plant fresh and dry weights were categorized as salt tolerant genotypes while those showing the greatest % decrease were categorized as salt sensitive (Table 2). The shoot and root length of

Table 1. Grouping of pea genotypes on the basis of cluster analysis.

Less salt tolerant		Intermediate salt tolerant		Highly salt tolerant	
No.	Genotypes	No.	Genotypes	No.	Genotypes
1	Samarina zard	9	K2P-6121	19	Tere-2
2	Climax	10	FS-2187	20	PF-450
3	2001-35	11	Juras-555	21	Green arrow
4	2001-55	12	K2P-6173	22	K2P-5196
5	9800-5	13	Olympia	23	F-16
6	9800-10	14	2001-20	24	GRW-45
7	Sprinter	15	Sitra Gold	25	K2P-6185
8	Meteor	16	Premume	26	Azad P1
		17	Neptune	27	2001-40
		18	K2P-5180	28	9200-1
				29	Ambassidar
				30	PF-400

(data used are the salt treatment 10 dS m^{-1} relative to control treatment and expressed as %)

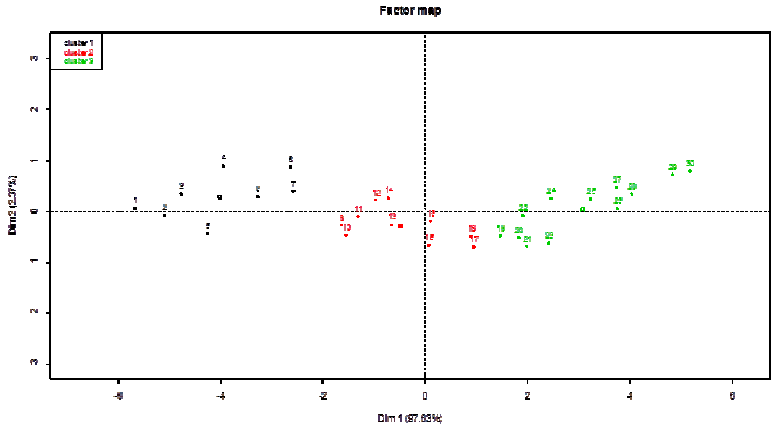

Fig 1. Clustring of tested 30 pea genotypes based on scores of the first principal components (PC1) (data used are the salt treatment 10 dS m^{-1} relative to control treatment and expressed as %.

investigated pea genotypes was significantly decreased under salt stress (Supplementary data 5 & 6). The interaction between salinity and genotypes was significant. Based on the percentage reduction values of shoot and root lengths, climax, 2001-35, samarina zard, 9800-5 and 2001-55 were classified as salt tolerant genotypes while ambassidar, PF-400, 9200-1, 2001-40 and azad-P1 were severely affected in terms of root and shoot length so classified as salt sensitive genotypes.

Effect of salt stress on inorganic osmolytes

Salt stress caused a significant increase in leaf Na^+ contents of all the genotypes grown under saline conditions (Supplementary data 7). The highest % increase was observed at 10.0 (67 %) followed by 7.5 (52 %), 5.0 (33 %) and 2.5 dS m^{-1} (15 %). Climax, samarina zard, meteor, 2001-35, 2001-55 and 9800-10 were the genotypes that accumulated the lowest concentration of Na^+ in their leaves. The genotypes, 9200-1, ambassidar, green arrow, PF-400, F-16, azad-P1 and PF-450 accumulated the highest amount of Na^+ in their leaves so categorized as salt sensitive. Salinized plants had significant decline in leaf K^+ in response to increasing salt stress (Supplementary data 8). The genotypes 2001-55, 9800-10, samarina zard, 2001-35 and climax exhibited the least % reduction in leaf K^+ and were thus categorized as salt tolerant. The genotypes, F-16, 9200-1, PF-400, and ambassidar were classified as salt sensitive ones, due to their high % reduction in leaf K^+ as compared to the control. All the tested genotypes exhibited a significant reduction in leaf Ca^{2+} concentration under saline conditions (Supplementary data 9). The genotypes showing the greatest % decrease in leaf Ca^{2+} were placed under salt sensitive category while those having minimum % reduction were categorized as salt tolerant genotypes. Based on % reduction in leaf Ca^{2+}, samarina zard, climax and 2001-35 proved to be highly salt tolerant while ambassidar, 9200-1 and PF-400 as salt sensitive.Since salt stress enhanced the leaf Na^+ contents which resulted in decrease of K^+/Na^+ and Ca^{2+}/Na^+ ratio in all tested pea genotypes (Supplementary data 10 & 11). The genotypes 2001-55, 9800-10, samarina zard, 2001-35 and climax performed well by maintaining higher K^+/Na^+ and Ca^{2+}/Na^+ ratios, while the genotypes PF-400, ambassidar, 9200-1, azad P1, and PF-450 exhibited the lowest leaf K^+/Na^+ and Ca^{2+}/Na^+ ratios, therefore classified as salt sensitive. The principal component analysis of correlation matrix of investigated attributes, permitted two principal components to be identified, which exhibited 97% of the total variance of the original attributes (Table 2). The first component showed the relative growth/plant biomass of the tested plants while the second principal component exhibits a contrast between relative growth/plant biomass against the accumulation of inorganic osmolytes (Table 2). Therefore, if the investigated pea genotypes show higher scores relative to another, it is due to t high relative values of inorganic osmolytes accumulation. On the basis of the values of two principal components, the investigated pea genotypes can be grouped as less salt tolerant, intermediate and high salt tolerant (Fig. 1; Table 1).

Table 2. Eigenvectors in two principal components (PC1 and PC2) of values relative to the control, for emergence, shoot fresh weight, shoot dry weight, shoot length, root length, root fresh weight, root dry weight, germination, K/Na and Ca/Na of pea genotypes.

Eigenvectors		
Characteristics	PC1	PC2
Emergence percentage	0.32	-0.34
Shoot fresh weight	0.31	0.51
Shoot dry weight	0.32	0.08
Shoot lenght	0.32	-0.008
Root lenght	0.32	-0.008
Root fresh weight	0.31	0.33
Root dry weight	0.32	0.27
Germination percentage	0.31	0.05
K/NA	0.31	0.56
Ca/Na	0.32	0.32
λ	4.34	1.86
CVA (%)	59	97.34

λ = Eigenvalue of the correlation matrix. CVA = Cumulative variance.

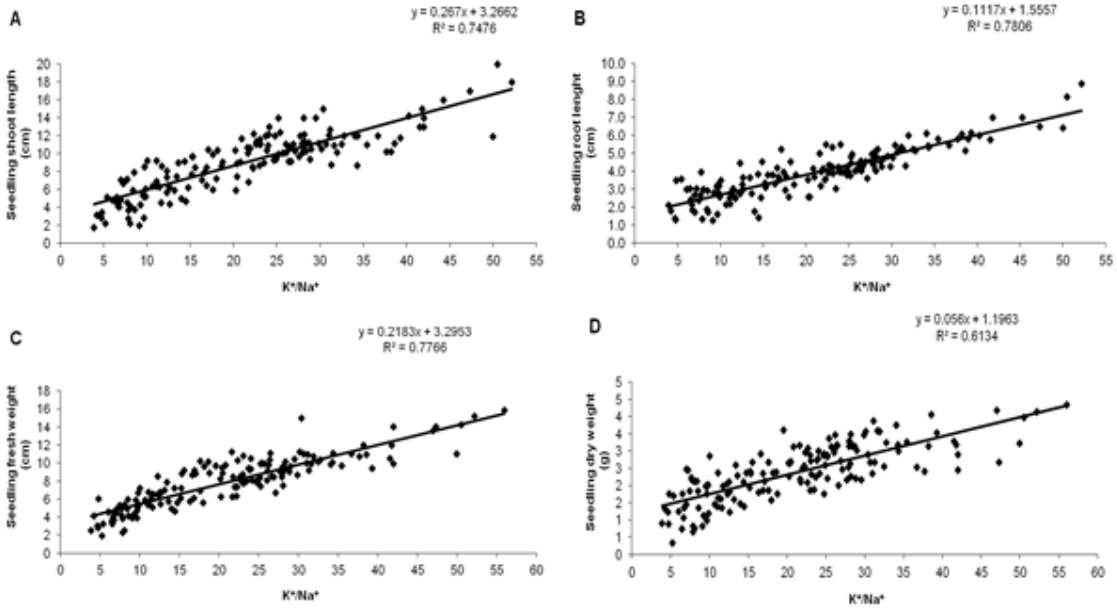

Fig 1. Correlation between growth attributes and inorganic osmolytes (K^+/Na^+); seedling shoot length/ K^+/Na^+ (A), seedling root length/ K^+/Na^+ (B), seedling fresh weight/ K^+/Na^+ (C), seedling dry weight/ K^+/Na^+ (D).

Discussion

Salt stress significantly affected the germination and early seedling growth so these are regarded as indicators of salt stress. On the basis of these important indicators of salt stress, genotypes can be categorized as tolerant and sensitive ones. High seed germination and vigorous seedling growth contribute to the plant's potential to resist the salt stress, therefore indirectly playing a vital role for better growth and productivity (Carpici et al., 2009). Various reports indicate that salt tolerance characteristics are specific to developmental stage; one may be drastically affected while another may exhibit tolerance to salts (Lauchli and Epstein, 1990; Johnson et al., 1992). Therefore, in various plant genotypes, the screening for salt tolerance should be carried out at initial growth stages as well as at the germination stage (Alian et al., 2000; Al-Karaki, 2000). Screening for salinity tolerance at germination and seedling stage has many advantages such as being less laborious, quick, and inexpensive as compared to investigations at mature growth stages (Dasgan et al., 2002). From the work of Tlig et al. (2008) and Guan et al. (2009) it can be concluded that germination test is a useful tool for screening under stressed environments. However, there are various factors such as age of seed, climatic conditions at the time of harvest, and seed storage conditions that may be involved in seed germination so it is reported that germination cannot be considered an authentic marker for screening under saline conditions (Ashraf and Oleary, 1996; Rubio-Casal et al., 2003). In the present investigation we attempted to eliminate the above mentioned factors by taking seeds of uniform size, age andfrom similar storage conditions so the findings observed in this study were real rather than apparent. In the current

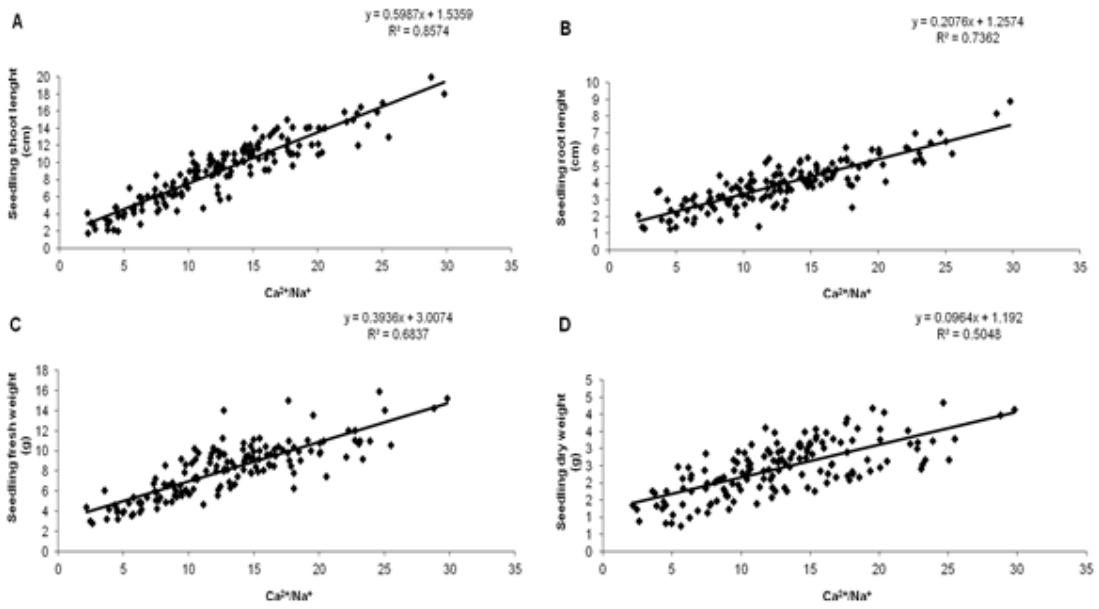

Fig 1. Correlation between growth attributes and inorganic osmolytes (Ca2+/Na+); seedling shoot length/ Ca2+/Na+ (A) seedling root length/ Ca2+/Na+ (B), seedling fresh weight/ Ca2+/Na+ (C), seedling dry weight/ Ca2+/Na+ (D).

investigation salt stress markedly reduced the germination percentage in all the tested pea genotypes (Supplementary data 3). These variations in germination percentage could be due to excessive deposition of Na^+ and Cl^- ions in seed tissues that compromises germination metabolism by affecting mobilization of mineral and organic reserves along with the recommencement of respiration. On the other hand, salt stress can decrease in water potential of growing medium thereby limiting water uptake by germinating seeds ultimately leading to a decline in germination. It was also observed that high salt stress makes the seeds to shrink within a few days and later seeds became non-viable. Salt stress negatively influences the protein hydration (Kramer, 1983) and disturbs many enzymatic activities (Dubey and Rani, 1990; Garg et al., 1993) within the tissues of germinating seeds; thus, seeds may fail to germinate under saline conditions. A strong correlation was observed between germination percentage and salinity levels. There are various reports, which indicate that salt stress has an inhibitory effect on germination percentage, seedling emergence percentage and seedling growth (Song et al., 2008; Tlig et al., 2008; Guan et al., 2009; Ahmad and Khan, 2010; Li et al., 2010). The effects of salt stress not only vary within genotypes but also within species. The pea genotypes which experienced the least decrease in emergence were grouped as tolerant (samarina zard, climax, 2001-35, 9800-10 and 9800-5) while those that exhibited the highest decrease were categorized as sensitive ones (PF-400, 2001-40, azad P1, ambassidar and 9200-1).Since the decreasing trend of pea genotypes in this study under saline conditions was very clear so it should also be claimed as an indicator of salt tolerance. A strong negative correlation existed between emergence percentage and concentration of sodium chloride. The inhibition of seedling emergence may be due to the decrease in osmotic potential of the root zone under the effect of excessive salts, which reduces the ability of the seed to absorb moisture for the expansion of the embryo (Al-Niemi et al., 1992) so it ultimately leads to the delayed seedling emergence. On the other hand, toxic ions (Na and Cl) also retarded the growth of radical so seedling failed to emerge properly (Malcolm et al., 2003; Qu et al., 2008). The results regarding the seedling emergence are in accordance with the findings of Patel and Pandey (2007), Patel et al. (2009), Patel et al. (2010) and Goodman et al. (2011). Growth attributes such as seedling fresh and dry weight, root fresh and dry weight, seedling shoot length, seedling root length showed a strong negative correlation with increasing salt stress. The high salt stress decreases the water potential of the growth medium which leads to the reduction in cell turgor. This low cell turgor inhibits the cell elongation and cell division so that plant growth slows. As significant differences were observed between non saline and saline regimes for above mentioned growth attributes in all thirty pea genotypes, they can be used as a successful tool for screening under stressed conditions. Likewise, the presence of positive correlation between shoot and root biomass and root and shoot lengths witnessed that these growths attributes can be authentic and effective markers for evaluating salt tolerance in pea genotypes. A relatively moderate reduction in shoot length, root length, shoot fresh weight and root fresh weight of tolerant genotypes (samarina zard, climax, 2001-35, 9800-5 and 9800-10) could have been due to their successful maintenance of cell turgor under saline conditions while non tolerant genotypes (ambassidar, PF-400, 2001-40 and azad P1) may not maintain the proper cell turgor. These results confirmed the findings of Ashraf et al., (2008). Toxic ions like Na^+ and Cl^- also limits cell elongation and cell differentiation which may lead to the reduction in plant biomass, root and shoot lengths in salinized plants of tested pea genotypes. A strong correlation was observed between the leaf Na^+ and root/shoot length, and leaf Na^+ and root/shoot dry weight (Fig.). Similarly, leaf Ca^{2+} also showed the correlation with plant biomass and root/shoot length (Fig. 1). Since, tolerant genotypes maintained higher K^+/Na^+ and Ca^{2+}/Na^+ ratios this may also be a reliable indicator of salt response.

A strong correlation was established between plant root/shoot biomass and K^+/Na^+ and Ca^{2+}/Na^+ (Fig. 1). Salinity also has a significant impact on the nutritional status of the plant. Therefore, nutrient regulation is a vital process which is very closely linked with the salt tolerance potential. It is well documented that salt stress elevates the Na^+ concentration in plant parts while suppresses the concentration of cations K^+ and Ca^{2+} (Dasgan et al., 2002; Akram et al., 2010). Both K^+ and Ca^{2+} are key ions necessary for various physiological mechanisms but under saline conditions Na^+ ions replace these ions ultimately causing reduced plant performance. In the present investigation, all the tested pea genotypes exhibited an increase in the leaf Na^+ and reduction in leaf K^+ under increasingly saline conditions. However, the genotypes, climax, samarina zard, 9800-5, 9800-10, 2001-55 and 2001-35 showed minimum ratios of Na^+ and maximum K^+ in their leaves. While, on the other hand, genotypes such as ambassidar, PF-400, 2001-40, 9200-1, azad-P1 and F-16 had the highest leaf Na^+ and the lowest leaf K^+ concentration So it is concluded that there is a negative correlation between leaf Na^+ and K^+ and salt tolerance is highly associated with ratios of these ions. This difference in Na^+ and K^+ of pea genotypes may be due to their genetic variability and root permeability for these ions. The salt tolerant plants transport fewer amounts of toxic ions like Na^+ to the upper parts (leaf and shoot) because they store maximum ratios of these ions in their roots, it is an adaptation to withstand saline conditions while salt sensitive plants do not have such an adaptation. So, it may also be the reason of reduced leaf Na^+ in climax, samarina zard, 9800-5, 9800-10, 2001-55 and 2001-35. Similar kind of observations was noted by Balal (2010) in salinized citrus rootstocks. As salt tolerance potential is highly associated with the concentration of inorganic osmolytes (Na^+, K^+, Ca^{2+}) so these can be efficiently used as screening tools for salinity in pea genotypes. Various reports indicated that Na^+ and K^+ can be used as screening tools under saline regimes (Dasgan et al., 2002; Akram et al., 2010; Khayat et al., 2010).

Materials and methods

Plant material and growth conditions

Seeds of 30 different pea genotypes with varied salt tolerance potential were collected from Ayyub Agriculture Research Institute, Faisalabad, Punjab (Pakistan). The selected genotypes had same growth habit and growth season. Seeds were sown in plastic trays placed in the lath house of the Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Twenty seeds per tray were sown and each treatment was comprised of five trays having Astatula fine sand (hyperthermic, uncoated typic quartzipsamments) individually as a growth medium. The sand had pH of 6.0-6.5, with field capacity 7.2% and incipient wilting at 1.2 % (Volume basis), respectively. Half strength Hoagland's solution was used as the nutrient solution. The seeds were watered with 250 ml per tray.

Salinity applications

Sodium chloride (NaCl) at different concentrations (0, 2.5, 5.0, 7.5 and 10.0 dS m^{-1}) was applied 20 days after sowing. To avoid osmotic shock, the seedlings were adjusted to their final NaCl level by imposing the salinity treatment in two days intervals while the control was without salt stress and irrigated only with half strength Hoagland's solution.

Measurement of germination, emergence and growth attributes

For the determination of seed germination in response to salt stress seeds of the 30 pea genotypes were sown in plastic dishes. Each dish was comprised of 20 seeds and there were five dishes per treatment. After disinfecting the seeds with 10% sodium hypochlorite, they were placed in Petri dishes on Whatman filter paper wetted with desired saline solutions (0, 2.5, 5.0, 7.5 and 10 dS m^{-1}). The dishes were placed in a growth chamber at 20 to 22 $^{\circ}$C. Germination was calculated after five days. The germination percentage was calculated by the methods of Shahid et al., (2011). For the emergence and growth attribute experiment half strength Hoagland's solution was used as a nutrient medium. Desired salinity levels (0, 2.5, 5.0, 7.5 and 10 dS m^{-1}) were created before the sowing of seeds. Seeds were sown in trays with sand. Shoot and root samples were collected for the estimation of various indicators of salt stress ten days after salinity application. Measurements included shoot length, root length, shoot fresh & dry weight and root fresh & dry weights. After forty days of growth, the seedlings were uprooted and washed with distilled water to remove sand particles. Shoot and root lengths of five randomly selected seedlings from each replicate were measured in centimeters (cm) from the base of hypocotyls to the tip of the shoot. The average of each replication was calculated. After the measurement of root and shoot lengths, the shoots were separated from the roots and wrapped with filter paper to remove any drop of water present on their leaves and shoots. Then these were placed on the digital balance for the calculation of fresh weights. The same procedure was used for the calculation of root fresh weights. After measuring the fresh weights shoots and roots were placed in paper bags and dried in an oven at 70 ^{o}C.

Determination of ionic attributes

Before the estimation of ionic attributes (Na^+ and K) 0.5 g of leaf material was digested with concentrated sulfuric acid (5 mL) in digestion tubes as described by Wolf (1990). The digested leaf samples of pea genotypes were analyzed for Na^+ and K^+ by Flame photometer (Jenway PFP-7, UK). A standard curve (SC) was drawn based on a graded series of standards (ranging from 10 to 100 mg L^{-1}) of Na^+ and K^+.

Experimental design and statistical analysis

The experiment was laid out in two factor factorial arrangement under Completely Randomize Design (CRD). All the values indicated in this investigation are the mean of five replicates. The data was analyzed with the help of statistical software, Statistix 8.1, USA. A two way analysis of variance (ANOVA) was carried out and Tukey's honestly significant difference (HSD) was used to compare means.

Conclusion

On the basis of the above findings, it is concluded that germination, emergence, plant fresh/dry biomass and leaf inorganic osmolytes are significant screening criteria for salt tolerance in pea genotypes. On the other hand, we also determined that salt tolerance potential in pea genotypes is highly linked with accumulation of inorganic osmolytes (Na^+, K^+, Ca^{2+}, K^+/Na^+, Ca^{2+}/Na^+) in their leaves. Since, samarina zard, climax, 9800-5, 9800-10 and 2001-55 well maintained the above mentioned attributes as compared to the remaining

genotypes submitted to salt stress, therefore classified as the most highly salt tolerant genotypes.

Acknowledgments

The authors acknowledge the Higher Education Commission of Pakistan and Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Pakistan for providing financial support and lab facilities, respectively.

References

Abbas W, Ashraf M, Akram NA (2010) Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by glycinebetaine and sugar beet extracts. Sci Hortic 125:188-195.

Ahmad MZ, Khan MA (2010) Tolerance and recovery responses of playa halophytes to light, salinity and temperature stresses during seed germination. Flora 205:764-771.

Akram M, Ashraf MY, Ahmad R, Waraich EA, Iqbal J, Mohsan M (2010) Screening for salt tolerance in maize (*Zea mays* l.) hybrids at an early seedling stage. Pak J Bot 42:141-154.

Ali Z, Khan AS, Asad MA (2002) Salt tolerance in bread wheat: genetic variation and heritability for growth and ion relation. Asia J Plant Sci 1:420-422.

Alian A, Altman A, Heuer B (2000) Gynotypic difference in salinity and water stress tolerance of fresh market tomato cultivars. Plant Sci 152:59-65.

Al-Karaki GN (2000) Growth, water use efficiency and sodium and potassium acquisition by tomato cultivars grown under salt stress. J Plant Nutr 23:1-8.

Al-Niemi TS, Campbell WF, Rumbaugh MD (1992) Response of alfalfa cultivars to salinity during germination and post- growth. Crop Sci 32:976-980.

Apse MP, Blumwald E (2007) Na^+ transport in plants. FEBS Letters 581:2247-2254.

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206-216.

Ashraf M, Oleary JW (1996) Responses of some newly evolved salt-tolerant genotypes of spring wheat to salt stress. Acta Bot 45:29-39.

Ashraf M, Athar HR, Haris PJC, Kwon TR (2008) Some prospective strategies for improving crop salt tolerance. Adv Agron 97:45-110.

Ashraf MY, Akhtar K, Hussain F, Iqbal J (2006) Screening of different accession of three potential grass species from Cholistan desert for salt tolerance. Pak J Bot 38:1589-1597.

Azhar FM, Ahmad R (2000) Variation and heritability of salinity tolerance in upland cotton at early stage of plant development. Pak J Bio Sci 3:1991-1993.

Balal RM (2010) Morpho-physiological and biochemical characterization and *in vitro* propagation of salt-tolerant citrus rootstocks. Discussion Ph.D thesis. University of Agriculture Faisalabad, Pakistan.

Beltran JM, Manzur CL (2005) Overview of salinity problems in the world and FAO strategies to address the problem. Proceedings of the International Salinity Forum; Riverside, California, p. 311-313.

Bhantana P, Lazarovitch N (2010) Evapotranspiration, Crop coefficient and growth of two young pomegranate (*Punica granatum* L.) varieties under salt stress. Agri Water Manage 97:715-722.

Cambrolle J, Redondo-Gomez S, Mateos-Naranjo E, Luque T, Figueroa ME (2011) Physiological responses to salinity in the yellow-horned poppy, *Glaucium flavum*. Plant Physiol Biochem 49:186-194.

Carpici EB, Celik N, Bayram G (2009) Effects of salt stress on germination of some maize (*Zea mays* L.) cultivars. Afr J Biotech 8:4918-4922.

Dasgan HY, Aktas H, Abak K, Cakmak I (2002) Determination of screening techniques to salinity tolerance in tomatoes and investigation of genotype responses. Plant Sci 163:695-703.

Dubey RS, Rani M (1990) Influence of NaCl salinity on the behaviour of protease, amino peptidase and carboxyl-peptidase in rice seedlings in relation to salt tolerance. Aus J Plant Physiol 17:215-224.

FAO (2008) Land and plant nutrition management service. Available online at: http://www.fao.org/ag/agl/agll/spush/. Accessed 25 April 2008.

Gama PBS, Inanana S, Tanaka K, Nakazawa R (2007) Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. Afr J Biotechnol 6:79-88.

Garg BK, Vyas SP, Kathju S, Lahiri AN, Mali PC, Sharma PC (1993) Salinity–fertility interaction on growth, mineral composition and nitrogen metabolism of Indian Mustard J Plant Nutr 16:1637-1650.

Goodman, AM, Ganf GG, Maier HR, Dandy GC (2011) The effect of inundation and salinity on the germination of seed banks from wetlands in South Australia. Aquatic Bot 94:102- 106.

Grewal HS (2010) Water uptake, water use efficiency, plant growth and ionic balance of wheat, barley, canola and chickpea plants on a sodic vertosol with variable subsoil NaCl salinity. Agri Water Manage 97:148-156.

Grieve AM, Prior DL, Bevington BK (2007) Long-term effect of saline irrigation water on growth, yield, and fruit quality of Valencia orange trees. Plant and Soil 45:313-317.

Guan YJ, Hu J, Wang XJ, Shao CX (2009) Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. J Zhejiang Uni Sci 10:427-433.

Hasaneen MNA, Younis ME, Tourky SMN (2009) Plant growth metabolism and adaptation in relation to stress conditions XXIII. Salinity-biofertility interactive effects on growth, carbohydrate and photosynthetic efficiency *lactuca sativa*. Plant Omics J 2:60-69.

Hayat S, Hasan SA, Yusuf M, Hayat Q, Ahmad A (2010) Effect of 28- homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in *Vigna radiata*. Environ Exp Bot 69:105-112.

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

IAEA (1995) Management strategies to utilize salt affected soils: Isotope and conventional research method. IAEA-TECDOC-8th, Vienna, Austria.

Ibraheem O, Dealtry G, Roux S, Bradley G (2011) The effect of drought and salinity on the expressional level of sucrose transporters in rice (*Oryza sativa* Nipponbare) cultivar plants. Plant Omics J 4:68-74.

Johnson DW, Smith SE, Dobrenz AK (1992) Genetics and phenotypic relationships in response to NaCl at different developmental stages in alfalfa. Theor Appl Genet 83:833-838.

Kamal A, Qureshi MS, Ashraf MY, Hussain M (2003) Salinity induced changes in growth and some physio-chemical aspects of two soybean (*Glycine max* L.) genotypes. Pak J Bot 35:93-97.

Khan AA, Rao SA, McNilly TM (2003a) Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.). Euphytica 131:81-89.

Khan AS, Asad MA, Ali Z (2003b) Assessment of genetic variability for NaCl tolerance in wheat. Pak J Agri Sci 40:33-36.

Khan MA., Shirazi MU, Ali M, Mumtaz S, Sherin A, Ashraf MY (2006) Comparative performance of some wheat genotypes growing under saline water. Pak J Bot 38:1633-1639.

Khayat PN, Jamaati-e-Somarin S, Zabihi-e-Mahmoodabad R, Yari A, Khayatnezhad M, Gholamin R (2010) Screening of salt tolerance canola cultivars (*Brassica napus* L.) using physiological markers. World Appl Sci J 10:817-820.

Kramer PJ (1983) Water Relations of Plants. New York: Academic Press, Inc. p. 489.

Lauchli A, Epstein E (1990) Plant responses to saline and sodic conditions, in: Tanji KK (Ed.), agricultural salinity assessment and management, American Society of Civil Engineering, New York, p. 113-137.

Levy Y, Syvertsen JP (2004) Irrigation water quality and salinity effects in citrus trees. Hortic Rev 30:37-82.

Li R, Shi F, Fukuda K (2010) Interactive effects of salt and alkali stresses on seed germination, germination recovery, and seedling growth of a halophyte *Spartina alterniflora* (Poaceae). S Afr J Bot 76:380-387.

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: An overview. Arch Biochem Biophys 444:139-153.

Malcolm CV, Lindley VA, O'Leary JW, Barrett-Lennard EG (2003) Halophyte and glycophyte salt tolerance at germination and the establishment of halophyte shrubs in saline environments. Plant and Soil 253:171-185.

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

Munns R (2005) Gene and salt tolerance: bringing them together. New Phytol, p. 167.

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 BiolSci 10:2752-2755.

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.

Patel AD, Pandey AN (2007) Effect of soil salinity on growth, water status and nutrient accumulation in seedlings of *Cassia montana* (Fabaceae). J Arid Environ 70:174-182.

Patel AD, Bhensdadia H, Pandey AN (2009) Effect of salinisation of soil on growth, water status and general nutrient accumulation in seedlings of *Delonix regia* (Fabaceae). Acta Ecol Sinica 29:109-115.

Patel NT, Gupta A, Pandey AN (2010) Salinity tolerance of *Avicennia marina* (Forssk.) Vierh from Gujarat coasts of India. Aquat Bot 93:9-16.

Perez-Perez JG, Robles JM, Tovar JC, Botia P (2009) Response to drought and salt stress of lemon 'Fino 49' under field conditions: Water relations, osmotic adjustment and gas exchange. Sci Hortic 122:83-90.

Pessarakli M, Tucker TC (1988) Nitrogen-15 uptake by eggplant under sodium chloride stress. Soil Sci Soc Am J 52:1673-1676.

Qu XX, Huang ZY, Baskin JM, Baskin CC (2008) Effect of temperature, light and salinity on seed germination and radicle growth of the geographically widespread halophyte shrub *Halocnemum strobilaceum*. Ann Bot 101:293-299.

Raveh E (2005) Methods to assess potential chloride stress in citrus: analysis of leaves, fruit, stem-xylem sap, and roots. HortTechnology 15:104-108.

Rubio-Casal AE, Castillo JM, Luque CJ, Figueroa ME (2003) Influence of salinity on germination and seeds viability of two primary colonizers of Mediterranean salt pans. J Arid Environ 53:145-154.

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.

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.

Song J, Fan H, Zhao Y, Jia Y, Du X, Wang B (2008) Effect of salinity on germination, seedling emergence, seedling growth and ion accumulation of a euhalophyte *Suaeda salsa* in an intertidal zone and on saline inland. Aquat Bot 88:331-337.

Tester M, Davenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Ann Bot 91: 503-527.

Tlig T, Gorai M, Neffati M (2008) Germination responses of *Diplotaxis harra* to temperature and salinity. Flora 203:421-428.

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity, and extreme temperatures: toward genetic engineering for stress tolerance. Planta 218:1-14.

Wolf B (1990) A comparative system of leaf analysis and its use for diagnosing nutrient status. Commun Soil Sci Plant Anal 13:1053-1059.

Zekri M (2004) Strategies to manage salinity problem in citrus. Proc Int Soc Citri, p. 639-643.