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Morpho-biochemical responses to salinity tolerance in common bean (*Phaseolus vulgaris* L.).

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The effect of salinity stress during germination, early seedling and vegetative growth on morphological and biochemical traits was evaluated for 18 genotypes of common bean *(Phaseolus vulgaris* L.) at 0, 60, 120, and 180 m*M* NaCl. Analysis of variance showed that the salinity stress had significant effect on all traits except shoot to root length and dry weight ratios. Though salinity stress delayed germination in all accessions, three local landraces, 'Naein', 'Lordegan' and 'Talash' germinated fastest under high salinity (120 *mM* NaCl). The Na uptake among the cultivars studied suggested that 'COS-16' (1.12 mg/g) and 'Naein' (1.07 mg/g) were most tolerant to salinity. Conversely, 'Cardinal' (1.89 mg/g) and 'Talash' (1.89 mg/g) that had the highest Na uptake were considered as the most susceptible cultivars. Seeds that germinated rapidly at 60 m*M* NaCl also germinated rapidly at 120 m*M* NaCl. At 180 m*M* NaCl, several accessions reached 50% germination by 6 days, demonstrating high genetic potential within *P. vulgaris* for salinity tolerance during germination. The biomass of radicles plus hypocotyls decreased with increasing salinity. Cluster analysis separated the accessions into three groups. Group I included salt sensitive accessions with late germination, high sensitivity index, and reduced seedling growth. Group II included salt tolerant accessions with rapid germination, high sensitivity index, and enhanced seedling growth. Group III only included cultivated accessions corresponding to the CIAT gene pool with rapid germination, low sensitivity index, and intermediate seedling growth.

Key words: *Phaseolus vulgaris* L., salinity stress, Na^+ ions, morphological and biochemical traits.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important source of dietary protein in many developing countries. Common bean like many other leguminous crops is sensitive to salinity, and suffers reduced yield even if it is grown at soil salinity less than 2 dS m^{-1} (Maas and Hoffman, 1977). Plants growing under saline conditions are stressed basically in three ways; (1) Reduced water potential in the root zone causing water deficit; (2) Phytotoxicity of ions such as Na^+ and Cl^-, and (3) Nutrient imbalance by depression in uptake and/or shoot transport (Lauchli, 1984; Munns and Termatt, 1986; Gama et al., 2007). This is attributed to the fact that Na^+ competes with K^+ for binding sites essential for cellular function. This role makes K^+ an important element as more than 50

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enzymes are activated by K^+, and Na^+ can not substitute in this role. On one hand, the latter implication of these two macronutrients in salinity is thought to be one of the factors responsible for reduction in the biomass and yield components. Several studies such as genetic variability of cultivated *Phaseolus* bean cultivars exposed to salinity at germination stage, seedling stage (Bayuelo-Jimenes et al., 2002a) and early vegetative growth (Bayuelo-Jimenes et al., 2002b) have been conducted.

One approach to reducing the deleterious effects of soil salinity on crop production is the development of salt-tolerant cultivars (Epstein et al., 1980). In certain species, this may be achieved by exploiting intra specific variability. However, when such variability is limited, as occurs in many crop species, genes may be transferred from closely related wild species adapted to high salinity. A large number of accessions of cultivated species of Leguminosae have been evaluated for salt tolerance. These include faba bean *(Vicia faba* L.) (Abdel-Ghaffar et al., 1982), chickpea *(Cicer arietinum* L.), mung bean *[Vigna radiata* (L.) Wilczek] (Lauchi, 1984), pigeon pea *[Cajanus cajan*(L.) Millsp.] (Subbarao et al., 1991), and common bean *(P. vulgaris)* (Moreno-Limon et al., 2000). However, few salt tolerant genotypes were identified in these studies.

Evidence collected from various species suggests that salt tolerance is dependent on the stage of development; such that tolerance at one stage of development may not be correlated with tolerance at other developmental stages. The objective of this study was to evaluate morphological and biochemical characteristics responses of eighteen common bean varieties to salinity stress and was undertaken to characterize variability for NaCl salinity tolerance in, during seed germination, early seedling and vegetative growth.

MATERIALS AND METHODS

Two independent split plot experiments were conducted in the form of complete randomized block design in growth chamber and greenhouse. State the arrangement and number of replications.

Germination assay and early seedling growth

In this study, 18 *Phaseolus* accessions were evaluated for salt tolerance during germination and early seedling growth at 0, 60, 120, and 180 *mM* NaCl concentration (with electrical conductivity values of < 0.1, 5.2 , 11.1, and 17.0 dS m^{-1} and a water potential of the salt solution of -0.05, -0.28, -0.57, and -0.85 MPa, respectively). Seeds were manually scarified by removing approximately 1 mm of the testa with a scalpel. Before scarification, seeds were surface sterilized with 10% sodium hypochlorite solution for 5 min, rinsed with sterile distilled water several times, and briefly blotted onto sterile paper towels. Ten seeds were used for germination in covered, sterilized disposable Petri dishes (110×110×10) containing germination paper (Anchor Paper Co., St. Paul, MN) moistened once with 10 mL of distilled water or NaCl solution. The Petri dishes were tightly sealed with Parafilm (American Can Co., Greenwich, CT) (O_2 permeable) to prevent evaporation of water, thus minimizing changes in concentration of solutions. A randomized complete block design with a split plot arrangement of treatments and three replications was used with NaCl levels as the main plots and accessions (as a group of ten seeds per dish) randomized within each main plot. The Petri dishes were placed in a dark growth chamber. The mean temperature was 30°C and relative humidity was 80%. Temperature and relative humidity were measured and controlled automatically in a computerized growth chamber.

Seeds were considered germinated when the emergent radicle reached 2 mm in length. Percentage germination was recorded each 12 h for 6 day. On the 7th day, fresh weights of radicles and hypocotyls were measured. Subsequently the radicles and hypocotyls were dried at 65°C for 72 h, and weighed. Mean radicle dry weight was calculated based on total radicle dry weight related to each Petri dish in the number of germinated seeds in each Petri dish to evaluate speed of germination (Cotyledons were not included in fresh and dry weight comparisons, since they reflect imbibition rather than growth). In calculating the time of germination (that is, time from imbibition to radicle emergence), seeds that germinated within an interval were presumed to have germinated at the midpoint of that interval. The control treatment was used to estimate potential germination of seeds within each accession.

Establishment and vegetative growth

Seeds were sown in bed for germination and seven days old seedlings of uniform size from each genotype were transferred to rectangular containers of 90×60×25 cm size which were filled with half strength Hoagland's solution. Spacing was 10 cm between and within rows. Roots were slipped through a hole in the grid and the plants were held in place with a wrapping of Dacron batting around bases. The internal surface of the grid was covered with foil to prevent algal growth in the solution. The pH of the solution was periodically adjusted (usually once a day) to pH 5.8 ± 0.2. The plants were grown on this control solution until the emergence of the first trifoliate leaf (6-7 days after transplanting), and then salt stress treatments were initiated. Nutrient solution for plants with salt stress was identical to that for controls except for the addition of NaCl to the appropriate concentration. In the salt stress treatment, the first increment of salt, containing 60 *mM* NaCl was added 7 days after transplanting and additional increments of the same composition were added daily until the salt concentration reached the final treatment level of 180 *mM* NaCl. Treatments were replicated 3 times and arranged into spilt plot in the form of randomized complete block design. Finally, 3 weeks after conduction of salinity treatment, data were collected on plant height, root length, shoot dray weight, root dray weight, shoot to root biomass ratio, plant height to root length ratio, uptake and K^+/Na^+ and Na^+/Ca^{2+} ratio.

Ion analysis

In order for ion analysis, 0.5 g of finely grounded shoot samples were burned at 550 for 5 h, then cooled and 5 ml HCL 6 N added, heated for few minutes. The resulting filtrates were stored at a temperature of 4°C until measurement. Sodium ions were determined using flame emission spectrophotometer AA6700 (Shimadzu Corporation, Kyoto, Japan). Ca^{2+} concentration was estimated based on titration approach.

Statistical analysis

Data from two experiments were independently analyzed. Before analysis of variance, data of mean values of tolerance for each accession for each variable were subjected to tests for heterogeneous error variances by the Bartlett's test. Error variances were homogeneous thus data were not transformed. Statistical differences were ascertained from the SAS Generalized Linear Models Procedure. A protected least significant difference (PLSD) was constructed when the *F-tests* indicated statistically significant differences among genotypes *(P*< 0.05). Ward's minimum variance clustering method was used to classify the accessions into discrete clusters. The optimum number of clusters was determined by MANOVA procedure (Sorkhe et al., 2007).

RESULTS

Morphological characteristics related to salinity tolerance

Comparison of mean values of genotypes showed high variability, ranging from minimum (0.619 g) to maximum (1.729 g) for 'Aligodarz' and 'Khomain-2' landraces, respectively. Maximum RaFW was obtained for 'G-14088' (2.965 g), 'Khomain-2' (2.202 g) and 'MCD-4024' (0.621 g) genotypes at salinity levels of 60, 120 and 180 mM NaCl, respectively (Table 1). In addition, 'Khomain-2' had maximum RaDW at 0 (0.381 g) and 60 (0.289 g) m*M* NaCl. However, differences of genotypes for RaFw and RaDW at 180 m*M* NaCl were not significant (Table 1).

Comparison of mean values showed that genotypes for germination speed (Table 1) were significantly different for GP in different salinity levels, but the difference between genotypes was only significant at 120 and 180 m*M* NaCl for GS. 'Kohdasht' local landrace showed minimum GS (0.01) at 120 and 180 m*M* NaCl salinity level. However, cultivars 'CRAN75' with 0.74 and 'Talash' with 0.34 were maximum for this trait at 120 and 180 m*M* NaCl salinity level, respectively (Table 1). Root growth was reduced in all genotypes as the salinity level increased. The local landrace 'Naein' produced maximum root dry weight (RDW) of 1.05, 0.88, 0.87 and 0.83 g in 0, 60, 120 and 180 mM NaCl salinity level, respectively (Table 1). Root length (RL) was also reduced as salinity level increased. Mean per all genotypes was ranged from 7.26 to 12.87 cm at 180 mM NaCl and control, respectively. 'Naein' landrace produced longer roots relative to other genotypes at all salinity levels with maximum 20.6 cm in control, while minimum RL (4.3 cm) was produced by 'Aligodarz' at 180 mM NaCl (Table 1). Genotypes were significantly different for plant height. The landrace 'Naein' (41.76 cm) and 'Mich Map' (16.93 cm) showed maximum and minimum PH in control. In addition, 'Naein' landrace showed maximum PH in all salinity levels (Table 1). The SDW reduced as result of increasing salt concentration, ranging from 4.99 g for 'Talash' in control to 0.65 g for 'Daneshju' in 180 mM NaCl , respectively (Table 1). The values obtained for SDW/RDW ranged from 2.61 to 8.43 (Table 1). Higher and lower values for this ratio were observed in 'G-14088' and 'COS-16', respectively (Table 1).

Biochemical characteristics related to salinity tolerance

Sodium analysis showed that when used overall salinity levels are, 'COS-16' with 1.12 mg/g and 'Naein' with 1.07 mg Na^+ per gram dry leaf accumulated less amount of Na^+ than other genotypes. Conversely, 'Cardinal' and 'Talash' with 1.89 mg/g had the highest amount of Na^+ uptake. Non significant differences were observed among genotypes at control, however, genotypes showed significant differences in the salinity level (Table 2). The 'Naein' accumulated the least amount of Na^+ at 60 mM (0.43 mg/g) and 120 mM (1.23 mg/g) salinity. The amount of Na^+ accumulation of 'Naein' landrace at 180 mM salinity was also low (Table 2). Among genotypes, 'CRAN75' and 'Cardinal' were the most and the least K^+ accumulating at all salinity levels, respectively (Table 2). Assessment of genotypes in different salinity levels showed that in control condition 'Aligodarz' had the least amount of Ca (0.33 mg/g) and 'Talash' had the highest amount (3.04 mg/g) of Ca uptake. The amount of Ca accumulation of 'Naein' in 60 and 120 m*M* NaCl was also high (Table 2). Evaluation of genotypes for Na^+/Ca^{2+} ratio showed that 'Khomain-5'with mean 0.57 had the least and 'G-14088' with mean 1.97 had the highest amount. Differences of genotypes for this ratio were non-significant at control level, but were highly significant in other salinity levels (Table 2). The results obtained for K^+/Na^+ ratio indicated highly significant differences among genotypes, salinity levels and interaction of genotypes into salinity (Table 2). Genotypes 'CRAN75' 220.97 and 'Kohdasht' 63.13 had the highest and the least values of this ratio in control. However, difference of genotypes in other salinity level was not significant (Table 2).

Correlation

Correlation coefficients were positive and highly significant *(P* < 0.01) for root dry weight (RDW) and shoot dry weight (SDW) with plant height (PH), root length (RL), germination percent (GP) and speed of germination (GS). Correlation coefficients between radicle fresh weight (RaFW) and radicle dry weight (RaDW) with germination percentage and speed of germination were positive and highly significant *(P* < 0.01). A positive association was found between plant height, root length and shoot dry weight with Ca content. A negative association was found between amount of K and RDW, SDW, PH and RL (Table 3).

Cluster analysis

The MANOVA method was used in this study to cluster

Table 1. Mean comparison for radicle fresh weight (RaFW), radicle dry weight (RaDW), root length (RL) and root dry weight (RDW) of 18 common bean genotypes evaluated at 0, 60, 120 and 180 mM NaCl salinity levels.

Genotype	Salinity levels							
	0	60	120	180	0	60	120	180
Naien	§1.520^{abc} 20.60^{a}	0.985^{defg} 13.30^{a}	0.843^{bcde} 12.03^{a}	0.415^{a} 12.43^{a}	ᵑ0.248^{cdef} 1.05^{b}	0.155^{ef} 0.88^{b}	0.105^{defgh} 0.87^{a}	0.043^{a} 0.83^{b}
Mich map	1.575^{bcd} 18.50^{ab}	1.575^{cde} 12.60^{abc}	0.963^{bcd} 9.90^{ab}	0.244^{a} 6.90^{cde}	0.355^{ab} 0.62^{a}	0.253^{abc} 0.35^{e}	0.155^{cd} 0.26^{ef}	0.053^{a} 0.23^{def}
COS-16	2.44^{ab} 17.90^{abc}	1.323^{cdef} 11.6^{abc}	0.399^{de} 9.10^{abcd}	0.356^{a} 6.23^{cde}	0.257^{cde} 0.36^{fgh}	0.138^{f} 0.26^{fghi}	0.049^{ghi} 0.21^{efghi}	0.032^{a} 0.23^{def}
CRAN75	1.504^{bcd} 12.80e^{fg}	1.693^{fg} 9.13^{bcde}	1.183^{bc} 7.70^{bcdef}	0.552^{a} 7.56^{cde}	0.256^{cde} 0.28^{i}jk	0.261^{ab} 0.21^{i}j	0.142^{cde} 0.21^{efg}	0.064^{a} 0.19^{efg}
Sharekord	1.328^{cd} 8.1jk	0.885^{fg} 7.03^{de}	0.405^{de} 5.30^{ef}	0.248^{a} 4.73^{e}	0.197^{efg} 0.22kl	0.061^{g} 0.21^{i}j	0.039^{hi} 0.17^{gh}	0.027^{a} 0.16^{fg}
MCD-4024	1.660^{bcd} 9.06^{hi}jk	1.676^{cde} 7.33^{de}	1.463^{b} 6.30^{bcdef}	0.621^{a} 5.22^{de}	0.288^{bcd} 0.35^{ghi}	0.200^{bcdef} 0.26^{fghi}	0.166^{cd} 0.22^{efg}	0.074^{a} 0.2^{efg}
Cardinal	1.940^{abc} 12.03e^{fgh}	0.559^{g} 11.13^{abc}	0.437^{de} 9.35^{abc}	0.258^{a} 5.43^{de}	0.293^{bc} 0.32^{ghi}j	0.134^{f} 0.30^{efg}	0.191^{bc} 0.22^{efg}	0.028^{a} 0.19^{efg}
Khomain-5	1.801^{bc} 16.60^{bcd}	0.201^{defg} 11.40^{abc}	0.276^{cde} 9.16^{abcd}	0.081^{a} 8.50^{bcd}	0.217^{defg} 0.77^{c}	0.144^{f} 0.50^{d}	0.304^{hi} 0.47^{c}	0.007^{a} 0.40^{c}
Tylor	2.076^{ab} 16.43^{bcd}	1.048^{defg} 10.50^{abcd}	0.716^{cde} 9.85^{ab}	0.294^{a} 8.16^{bcde}	0.218^{defg} 0.54^{e}	0.145^{f} 0.50^{d}	0.118^{defg} 0.46^{cd}	0.038^{a} 0.41^{c}
Aligodarz	1.085^{d} 6.40k	1.059^{defg} 6.25^{e}	0.185^{e} 4.36^{f}	0.149^{a} 5.13^{cde}	0.174^{fg} 0.19l	0.166^{def} 0.16^{i}j	0.318^{hi} 0.14^{h}	0.007^{a} 0.12^{g}
MCD-4017	2.060^{ab} 8.35^{i}jk	2.383^{ab} 7.50^{de}	0.470^{de} 6.00^{cdef}	0.389^{a} 5.68^{de}	0.309^{bc} 0.28^{hi}jk	0.239^{abcd} 0.28^{fghi}	0.041^{ghi} 0.22^{efg}	0.033^{a} 0.22^{def}
Daneshju	1.253^{cd} 11.6e^{fgh}	1.090^{defg} 10.40^{abcd}	0.848^{bcde} 5.60^{def}	0.351^{a} 6.72^{cde}	0.145^{g} 0.43^{f}	0.140^{f} 0.23^{hi}j	0.073^{efghi} 0.18^{fgh}	0.042^{a} 0.19^{efg}
Khomain-2	2.214^{ab} 10.90^{fghi}	1.894^{bc} 9.00^{cde}	2.202^{a} 7.06^{bcdef}	0.572^{a} 6.76^{cde}	0.381^{a} 0.29^{hi}j	0.289^{a} 0.24^{ghi}	0.273^{b} 0.23^{efg}	0.068^{a} 0.24^{de}
G-O1437	1.534^{bcd} 12.03e^{fgh}	0.912^{fg} 10.60^{abcd}	0.722^{cde} 6.00^{cdef}	0.292^{a} 7.20^{cde}	0.260^{cde} 0.39^{fg}	0.181^{cdef} 0.29^{efgh}	0.308^{a} 0.22^{efg}	0.062^{a} 0.16^{efg}
Talash	2.092^{ab} 14.63^{cde}	0.977^{efg} 12.70^{ab}	0.873^{bcde} 12.30^{a}	0.057^{a} 11.03^{ab}	0.319^{bc} 0.79^{c}	0.162^{def} 0.59^{c}	0.128^{cdef} 0.4^{d}	0.005^{a} 0.35^{c}
G-14088	2.516^{a} 9.88^{ghi}j	2.965^{a} 9.06^{bcde}	0.210^{e} 8.36^{bcde}	0.083^{a} 8.00^{bcde}	0.298^{bc} 0.36^{gh}	0.233^{abcde} 0.33^{ef}	0.021^{i} 0.28^{e}	0.001^{a} 0.273^{d}
Kohdasht	1.273^{cd} 11.91e^{fgh}	1.253^{cdefg} 9.30^{bcde}	0.328^{de} 7.23^{bcdef}	0.063^{a} 5.86^{cde}	0.067^{h} 0.24jkl	0.126^{fg} 0.24^{ghi}	0.057^{fghi} 0.15^{gh}	0.005^{a} 0.14^{g}
Lordegan	1.088^{d} 14.00^{d}e^{f}	1.347^{cdef} 12.10^{abc}	0.857^{bcde} 9.47^{ab}	0.350^{a} 9.10^{bc}	0.167^{g} 1.12^{a}	0.156^{ef} 0.96^{a}	0.119^{defg} 0.66^{b}	0.045^{a} 0.50^{b}
Naien	†0.96^{a} 41.76^{a} 4.94^{ab}	0.60^{bc} 37.00^{a} 4.36^{a}	0.36^{ef} 32.36^{a} 3.41^{a}	0.30^{de} 30.60^{a} 3.28^{ab}	‡0.72^{a} 4.58^{b} 2.10^{def}	0.74^{a} 3.68^{a} 2.86^{b}	0.41^{def} 2.97^{a} 2.73^{abc}	0.34^{a} 2.68^{a} 2.52^{ab}
Mich map	0.83^{c}	0.60^{bc}	0.46^{cd}	0.60^{a}	0.67^{a}	0.44^{a}	0.41^{def}	0.13^{d}

Table 1. Contd.

	16.93^{g}	14.90^{gh}	17.78^{de}	15.6^{de}	2.81^{cd}	2.33^{c}	1.46^{cde}	1.31^{cd}
	4.55^{ab}	6.79^{a}	5.91^{a}	6.082^{ab}	1.89^{defg}	1.74^{cde}	1.79^{defg}	2.35^{abc}
	063^{f}	0.53^{d}	0.36^{ef}	0.20^{fg}	0.69^{a}	0.90^{a}	0.26^{ghi}	0.22^{abcd}
COS-16	19.80^{g}	16.30^{fgh}	12.08^{f}	11.56^{efg}	3.02^{c}	1.19^{fg}	1.00^{efghi}	0.88^{def}
	3.64^{ab}	4.49^{a}	4.87^{a}	3.94^{ab}	1.46^{fg}	1.46^{e}	1.38^{g}	2.004^{abc}
	0.56^{g}	0.50^{d}	0.53^{b}	0.56^{a}	0.24^{a}	0.33^{a}	0.74^{a}	0.33^{ad}
CRAN75	20.06^{g}	16.46^{fgh}	14.85^{def}	12.33^{efg}	1.62^{f}	1.42^{ef}	1.17^{efgh}	0.98^{cdef}
	5.73^{ab}	6.90^{a}	5.65^{a}	5.45^{ab}	1.10^{g}	1.82^{cde}	1.97^{cdefgi}	1.672^{bc}
	0.63^{f}	0.56^{cd}	0.40^{de}	0.20^{fg}	0.30^{d}	0.34^{a}	0.52^{cd}	0.16^{cd}
Sharekord	21.43e^{fg}	14.76^{gh}	12.63^{ef}	9.63^{fg}	1.60^{f}	1.28^{fg}	1.06^{efghi}	1.01^{cdef}
	7.56^{ab}	6.28^{a}	6.28^{a}	8.43^{ab}	1.612^{fg}	2.13^{bcde}	2.45^{abcde}	2.06^{abc}
	0.93^{a}	0.63^{b}	0.53^{b}	0.30^{de}	0.51^{a}	0.42^{a}	0.52^{cd}	0.22^{bcd}
MCD-4024	17.00^{g}	13.50^{h}	10.70^{f}	10.18^{efg}	1.56^{f}	1.32^{fg}	1.22^{defg}	1.01^{cdef}
	4.54^{ab}	5.29^{a}	5.63^{a}	4.74^{ab}	2.63^{bcd}	1.85^{cde}	1.703^{efg}	1.95^{abc}
	0.73^{d}	0.33^{f}	0.56^{b}	0.20^{fg}	0.50^{a}	0.51^{a}	0.36^{efg}	0.23^{abcd}
Cardinal	26.30^{d}e	24.20^{dc}	18.51^{cd}	14.58^{efg}	1.81^{f}	1.58^{ef}	1.10^{efghi}	0.74^{ef}
	5.82^{ab}	6.24^{a}	4.95^{a}	3.8^{ab}	2.24^{cdef}	2.25^{bcde}	2.014^{bcdefg}	2.73^{a}
	0.5^{g}	0.40^{e}	0.266^{h}	0.23^{f}	0.27^{a}	0.55^{a}	0.25^{ghi}	0.22^{abcde}
Khomain-5	34.70^{bc}	18.93^{fgh}	23.13^{bc}	21.06^{bc}	3.15^{c}	2.31^{c}	1.73^{c}	1.20^{cde}
	4.15^{ab}	4.63^{a}	3.69^{a}	2.9^{ab}	2.10^{def}	2.53^{bcd}	2.48^{abcde}	2.5^{abc}
	0.86^{bc}	0.66^{b}	0.50^{bc}	0.23^{f}	0.62^{a}	0.52^{a}	0.47^{cde}	0.15^{cd}
Tylor	27.60^{d}	19.10^{efg}	13.42^{def}	12.10^{efg}	3.16^{c}	2.75^{b}	1.31^{cdef}	1.00^{cdef}
	5.87^{ab}	5.63^{a}	2.89^{a}	2.61^{ab}	1.68^{efg}	1.99^{bcde}	1.54^{fg}	1.603^{c}
	0.66e^{f}	0.50^{d}	0.46^{cd}	0.30^{de}	0.40^{a}	0.45^{a}	0.33^{fgh}	0.12^{d}
Aligodarz	18.73^{g}	15.25^{gh}	12.613^{ef}	12.14^{efg}	1.42^{f}	1.4^{ef}	0.78^{ghi}	0.68^{f}
	7.69^{ab}	8.19^{a}	5.62^{a}	5.88^{ab}	3.09^{abc}	2.48^{bcd}	2.903^{ab}	2.49^{abc}
	0.66e^{f}	0.53^{d}	0.53^{b}	0.26^{ef}	0.46^{a}	0.64^{a}	0.33^{fgh}	0.26^{abc}
MCD-4017	25.50^{d}e^{f}	19.53^{efg}	14.68^{def}	12.9^{efg}	1.83^{f}	1.26^{fg}	0.97^{fghi}	1.04^{cdef}
	8.02^{ab}	4.84^{a}	4.88^{a}	5.12^{ab}	3.11^{ab}	2.62^{bc}	2.468^{abcde}	2.58^{ab}
	0.66e^{f}	0.50^{d}	0.30^{fg}	0.20^{fg}	0.03^{a}	0.75^{a}	0.54^{bc}	0.13^{d}
Daneshju	26.80^{d}	17.60^{fgh}	11.06^{f}	11.13^{efg}	2.53^{de}	1.46^{ef}	0.66^{i}	0.65^{f}
	5.74^{ab}	6.63^{a}	3.67^{a}	5.20^{ab}	2.54^{bcde}	1.67^{de}	1.96^{cdefg}	1.75^{bc}
	0.90^{ab}	0.83^{a}	0.76^{a}	0.46^{b}	0.32^{a}	0.66^{a}	0.51^{cd}	0.26^{abc}
Khomain-2	26.60^{d}e	25.70^{cd}	16.1^{def}	15.00^{defg}	2.43^{de}	1.76^{de}	0.76^{ghi}	0.77^{ef}
	8.25^{a}	7.44^{a}	3.42^{a}	4.35^{ab}	2.76^{bcd}	2.85^{b}	2.309^{abcdef}	2.312^{abc}
	0.83^{c}	0.66^{b}	0.46^{cd}	0.36^{cd}	0.55^{a}	0.53^{a}	0.31^{fgh}	0.23^{abcd}
G-O1437	25.43^{d}e^{f}	21.60^{def}	14.08^{def}	15.16^{ef}	2.54^{de}	2.08^{cd}	0.72^{hi}	0.81^{ef}
	7.4^{ab}	7.99^{a}	3.33^{a}	5.44^{ab}	2.17^{def}	2.09^{bcde}	2.437^{abcde}	2.177^{abc}
	0.70^{d}e	0.33^{f}	0.40^{cdef}	0.13^{h}	0.52^{a}	0.50^{a}	0.64^{ab}	0.34^{ab}
Talash	37.86^{ab}	34.10^{ab}	26.30^{b}	25.83^{b}	4.99^{a}	3.91^{a}	2.75^{ab}	2.56^{ab}
	6.37^{ab}	7.07^{a}	7.52^{a}	7.94^{ab}	2.76^{bcd}	2.79^{b}	2.165^{abcdefg}	2.386^{abc}
	0.46^{h}	0.43^{e}	0.26^{i}	0.16^{gh}	0.24^{a}	0.25^{a}	0.23^{hi}	0.26^{abcd}
G-14088	37.78^{ab}	33.2^{ab}	25.26^{b}	21.13^{c}	2.32^{e}	2.24^{c}	1.62^{cd}	1.42^{c}
	6.62^{ab}	6.93^{a}	6.43^{a}	5.28^{ab}	3.83^{a}	3.65^{a}	3.47^{a}	2.672^{a}
Kohdasht	0.50^{gh}	0.40^{e}	0.20^{fghi}	0.10^{h}	0.16^{a}	0.16^{a}	0.01j	0.01^{e}

Table 1. Contd.

	20.63^{fg}	15.83^{g}	10.66^{f}	9.46^{g}	0.89^{g}	0.91^{g}	0.69^{i}	0.67^{f}
	4.08^{ab}	3.53^{a}	4.51^{a}	4.94^{ab}	1.74^{efg}	1.71^{de}	1.494^{fg}	1.61^{c}
	$0.66e^{f}$	0.60^{bc}	0.36^{defg}	0.40^{bc}	0.63^{a}	0.44^{a}	0.16^{i}	0.32^{ab}
Lordegan	32.30^{c}	29.60^{bc}	24.24^{b}	19.95^{cd}	2.99^{c}	2.17^{c}	2.44^{b}	2.21^{b}
	2.62^{b}	2.96^{a}	3.78^{a}	4.93^{ab}	2.13^{def}	2.52^{bcd}	2.67^{abcd}	2.19^{abc}

[§]The number including RaFW and RL, respectively; [n] The number including RaDW and RDW, respectively; Values with different letters showed statistically significant differences (α=5% Duncan Test); [†] The number, indicating GP, PH and SDW/RDW ratio, respectively; [‡] The number indicating GS, SDW and PH/RL ratio, respectively.

Table 2. Evaluation of biochemical traits including Na^+, K^+, Ca^{2+}, K^+/Na^+ and Na^+/Ca^{2+} in the 18 common bean genotypes separated for 0, 60, 120 and 180 mM NaCl salinity levels.

Genotype	Salinity levels							
	0	**60**	**120**	**180**	**0**	**60**	**120**	**180**
Naien	$^{†}0.07^{a}$ 1.22^{e}	0.42^{g} 1.90^{abc}	1.23^{de} 2.193^{a}	2.56^{efg} 1.95^{a}	$^{‡}7.50^{efg}$ 102.74^{cdef} 0.06^{a}	6.20^{efg} 14.67^{a} 0.22^{e}	5.46^{de} 4.42^{a} 1.03^{def}	5.52^{cd} 2.12^{a} 1.31^{h}
Mich map	0.07^{a} 1.46^{de}	0.56^{efg} 1.84^{abc}	1.74^{abcd} 1.23^{abcd}	2.35^{fgh} 1.54^{abcd}	10.46^{c} 150.72^{b} 0.047^{a}	10.62^{b} 18.93^{a} 0.31^{e}	8.43^{b} 4.84^{a} 1.40^{cde}	8.48^{b} 3.61^{a} 1.52^{gh}
COS-16	0.11^{a} 2.24^{bc}	0.54^{fg} 2.20^{ab}	1.54^{cde} 1.16^{def}	2.31^{gh} 0.94^{def}	11.72^{b} 108.34^{cde} 0.04^{a}	8.36^{c} 15.45^{a} 0.24^{e}	8.37^{b} 5.41^{a} 1.32^{cde}	8.37^{b} 3.61^{a} 2.46^{de}
CRAN75	0.06^{a} 1.14^{e}	0.48^{fg} 1.63^{bcd}	1.51^{cde} 1.25^{bcde}	3.01^{de} 1.30^{bcde}	13.7^{a} 220.97^{a} 0.05^{a}	13.05^{a} 27.14^{a} 0.29^{e}	11.79^{a} 7.63^{a} 1.19^{cde}	10.67^{a} 3.54^{a} 2.31^{ef}
Sharekord	0.07^{a} 1.33^{de}	0.48^{fg} 1.06^{defgh}	1.620^{bcde} 0.93^{def}	3.56^{abc} 0.99^{def}	7.66^{defg} 111.01^{cd} 0.05^{a}	6.74^{e} 13.87^{a} 0.45^{de}	5.31^{de} 3.28^{a} 1.74^{bcd}	5.24^{def} 3.65^{a} 3.60^{c}
MCD-4024	0.11^{a} 1.52^{de}	1.06^{cde} 0.62^{h}	1.63^{bcde} 0.93^{ab}	3.09^{cde} 1.89^{ab}	8.56^{de} 74.05^{gh} 0.08^{a}	6.51^{ef} 6.14^{a} 1.71^{b}	5.23^{de} 3.21^{a} 1.73^{bcd}	5.00^{def} 1.61^{a} 1.63^{fgh}
Cardinal	0.06^{a} 1.25^{e}	0.73^{defg} 1.02^{defgh}	1.49^{cde} 0.80^{cdef}	2.44^{fg} 1.21^{cdef}	$4.8i$ 80.00^{fgh} 0.05^{a}	$3.11i$ 4.26^{a} 0.71^{cde}	2.83^{f} 1.89^{a} 1.86^{bc}	2.04^{g} 1.13^{a} 2.02^{cd}
Khomain-5	0.01^{a} 2.96^{a}	0.960^{def} 2.42^{a}	1.20^{e} 2.41^{a}	2.84^{ef} 1.31^{abcde}	6.84^{fg} 71.25^{gh} 0.03^{a}	5.17^{gh} 5.38^{a} 0.39^{de}	4.73^{de} 1.67^{a} 0.49^{f}	4.05^{ef} 1.43^{a} 2.11^{efg}
Tylor	0.06^{a} 2.17^{bc}	1.08^{cd} 1.27^{cdefg}	1.370^{de} 1.45^{bcde}	3.48^{abc} 1.35^{bcde}	6.51^{gh} 109.78^{cd} 0.03^{a}	5.37^{fgh} 4.96^{a} 0.85^{cde}	5.64^{de} 4.10^{a} 0.94^{ef}	5.30^{cd} 1.52^{a} 2.58^{de}
Aligodarz	0.07^{a} 0.33^{f}	0.43^{g} 1.37^{cdef}	2.23^{a} 1.55^{abcde}	2.94^{e} 1.35^{bcde}	8.81^{d} 120.68^{c} 0.22^{a}	6.47^{ef} 15.02^{a} 0.31^{e}	5.78^{de} 2.59^{a} 1.43^{cde}	4.77^{def} 1.62^{a} 2.18^{efg}
					5.50^{he}	5.30^{gh}	5.78^{de}	4.09^{f}

Table 2. Contd.

MCD-4017	0.06^{a} 2.23^{bc}	0.60^{defg} 1.06^{defgh}	1.95^{abc} 1.16^{def}	2.70^{efg} 1.16^{def}	84.66^{cdgh} 0.03^{a}	8.79^{a} 0.56^{de}	2.92^{a} 1.68^{bcd}	1.51^{a} 2.31^{ef}
Daneshju	0.06^{a} 2.27^{bc}	0.65^{defg} 2.18^{ab}	2.05^{ab} 0.99^{def}	2.68^{efg} 1.09^{def}	4.70i 78.38^{gh} 0.03^{a}	4.93^{h} 7.51^{a} 0.30^{e}	3.27^{f} 1.59^{a} 0.48^{f}	2.21^{g} 0.83^{a} 2.45^{de}
Khomain-2	0.08^{a} 1.40^{de}	0.88^{defg} 1.03^{defgh}	1.68^{bcde} 1.05^{ef}	3.44^{bcd} 0.76^{ef}	6.77^{fg} 89.08^{defg} 0.05^{a}	4.36^{h} 4.95^{a} 0.85^{cde}	2.52^{f} 1.50^{a} 1.6^{bcde}	2.14^{g} 0.62^{a} 4.51^{b}
G-O1437	0.09^{a} 1.94^{cd}	1.45^{bc} 1.35^{cdef}	1.35^{de} 0.93^{cdef}	2.88^{efg} 1.19^{cdef}	6.90^{fg} 72.66^{gh} 0.04^{a}	6.34^{efg} 4.35^{a} 1.07^{bcd}	5.06^{de} 3.73^{a} 1.45^{bcde}	5.88^{cd} 2.04^{a} 4.42^{de}
Talash	0.01^{a} 3.04^{a}	1.49^{bc} 0.93^{efgh}	2.02^{abc} 0.94^{f}	3.97^{a} 0.60^{f}	7.38^{efg} 76.87^{gh} 0.03^{a}	6.43^{ef} 4.31^{a} 1.60^{b}	4.49^{e} 2.22^{a} 2.15^{ab}	4.05^{f} 1.02^{a} 6.59^{a}
G-14088	0.08^{a} 1.03^{e}	0.64^{defg} 0.72^{gh}	1.90^{abc} 0.72^{ef}	3.58^{abc} 0.83^{ef}	8.20^{de} 107.89^{cde} 0.07^{a}	7.16^{de} 11.20^{a} 0.89^{cde}	7.13^{c} 3.75^{a} 2.64^{a}	6.44^{c} 1.79^{a} 4.31^{b}
Kohdasht	0.10^{a} 1.39^{ab}	1.82^{ab} 1.40^{cde}	1.52^{cde} 1.11^{abc}	3.61^{ab} 1.81^{abc}	6.50^{gh} 63.13^{h} 0.07^{a}	5.35^{gh} 2.93^{a} 1.30^{bc}	5.39^{de} 3.55^{a} 1.37^{cde}	5.99^{cd} 1.63^{a} 2.00^{efg}
Lordegan	0.10^{a} 2.76^{bc}	2.04^{a} 0.76^{fgh}	1.54^{bcde} 0.90^{ef}	1.96^{h} 0.86^{ef}	7.91^{def} 75.12^{gh} 0.04^{a}	7.93^{cd} 3.88^{a} 2.69^{a}	5.21^{de} 3.36^{a} 1.71^{bcd}	5.55^{cd} 2.82^{a} 2.28^{ef}

†The numbers indicating Na^{+} and Ca^{2+}, respectively. ‡ The numbers indicating K^{+}, K^{+}/Na^{+} and Na^{+}/Ca^{2+}, respectively.

analysis and the cutting point was 0.45. The stability of nodes on the dendrogram was estimated with a bootstrap procedure. The distance coefficients for genotypes of common bean varied from a maximum of 27.201 (between 'Naein' landrace and 'Aligodarz') to a minimum of 3.133 (between 'COS-16' and 'Taylor'), with average of 13.859. The dendrogram distance coefficient of 0.90, consisted of three clusters, that is, three groups of genotypes; 9, 3, 11, 12, 13, 7, 17, 14, 4, 6, 5 and 10 (cluster I); and 18, 15, 8, 2, and 1 (cluster II); and cluster III only consisted of genotype 16. Cluster I divided into two subgroups in distance of 0.72, for which subgroup Ia contained 3, 14, 4, 6, 5, 1, 9, 17 and subgroup IIb contains genotypes 7, 10, 11, 12, and 13. In addition, cluster II also divided into two subgroups in distance of 0.66, for which subgroup Ic contained genotypes 2, 8, 15, 18 and subgroup IId only contained genotype 1 (Figure 1).

DISCUSSION

In the context of this discussion, the term salt tolerance during seed germination is used only to refer to situations where the seed germinates rapidly under salt stress conditions. No distinction is made between osmotic and ionic effects of the salinity stress. Likewise, salt tolerance during early seedling growth is assessed on the absolute growth at a given salt concentration as well as the percentage of growth under salt stress relative to growth under non-stress conditions. On the basis of these two criteria, our results demonstrated genetic variation in seed germination and early seedling growth responses to salinity among *P. vulgaris* genotypes. This study indicated that 'CRAN75', 'Naein' and 'Talash' had superior germination performance at 120 and 180 m*M* NaCl levels of salt stress. A high correlation between mid germination time at 120 and 0 m*M* indicated that germination processes that facilitate rapid germination under salt and non-stress conditions possibly were controlled by similar genetic and physiological mechanisms (Foolad, 1996). Conversely, several accessions germinated rapidly under control conditions but germinated poorly at the highest salt stress levels, thus exhibiting high sensitivity indices. Consequently, in

Table 3. Correlation analysis of different traits.

Traits	RaFW	RaDW	GP	GS	RDW	SDW	PH	RL	PH/RL	SDW/RDW	Ca^{2+}	K^{+}	Na^{+}	K^{+}/Na+	Na^{+}/Ca^{2+}
RaFW	1.00														
RaDW	0.87**	1.00													
GP	0.53**	0.64**	1.00												
GS	0.41**	0.47**	0.36**	1.00											
RDW	0.13**	0.20**	0.52**	0.29**	1.00										
SDW	0.28**	0.41**	0.74**	0.34**	0.81**	1.00									
PH	0.39**	0.44**	0.68**	0.29**	0.72**	0.85**	1.00								
RL	0.29**	0.43**	0.63**	0.37**	0.69**	0.73**	0.69**	1.00							
PH/RL	-0.60**	-0.66**	0.79**	0.03**	-0.22**	-0.38**	-0.36**	-0.50**	1.00						
SDW/RDW	-0.28**	-0.33**	-0.38**	-0.009	-0.29**	-0.19**	-0.19**	-0.33**	0.46**	1.00					
Ca^{2+}	-0.36**	0.31**	-0.28**	0.09**	-0.09**	0.108	0.24**	0.36**	-0.37**	-0.21**	1.00				
K^{+}	-0.55	0.61**	0.71**	-0.087	-0.27**	-0.42**	-0.44**	-0.44**	0.81**	0.38**	0.35**	1.00			
Na^{+}	-0.40**	-0.46**	0.48**	0.41**	-0.02	-0.21**	-0.25**	-0.06	0.06	0.27**	0.30**	-0.59**	1.00		
K^{+}/Na^{+}	0.42**	0.49	0.63**	0.059	0.18**	0.35**	0.34**	0.38**	0.73**	0.32**	0.38**	-0.85**	-0.55**	1.00	
Na^{+}/Ca^{2+}	-0.53**	-0.59**	0.45**	0.36**	-0.22**	-0.13**	-0.39**	-0.43**	0.02	0.07	0.48**	0.36**	0.82**	0.50**	1.00

*, ** indicates significance at $P < 0.05$ and $P < 0.01$ respectively.

these accessions, the physiological processes required for germination were sensitive to salt. Thus, these accessions might be deficient in genetic elements required for coping with salinity (Foolad and Jones, 1993).

'Naein' landrace was the most tolerant to salinity stress, as indicated by rapid germination, relative stability, and greater seedling growth. In contrast, 'Kohdasht' landrace was less salt tolerant in terms of early seedling growth. These results demonstrate that tolerance to salinity in *P. vulgaris* genotypes might also vary with developmental stages. Salt tolerance at germination and at the seedling stage appears to be controlled by more than one gene and is highly influenced by salt concentration (Foolad and Jones, 1993). Salt stress inhibited the growth of hypocotyls more than radicles in all *Phaseolus* taxa. Similar observations have been reported in pigeon pea, *C. cajan* (Subbarao et al., 1991), and tepary bean, *Phaseolus acutifolius* A. Gray (Goertz and Coons, 1991). The consequent increase in root to shoot ratio may be helpful for salinized seedlings by improving water relations.

Reductions in the biomass of *P. vulgaris* under saline condition were indicative of severe growth limitations. Salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio. In several legumes, such as faba bean (Zahran and Sprent, 1986) and P*. vulgaris* (Wignarajah, 1992), salinity was reportedly found to reduce shoot and root weights.

Our results showed that landrace of 'Naein' exhibited lower Na uptake than the others, while 'Cardinal' and 'Talash' had comparatively, the highest Na uptake. This suggests that 'Naein' is more resistant genotype because common bean is known to exclude Na^{+} from the shoot by re-absorption of Na^{+} from the xylem, but takes up Cl^{-} in proportion to external NaCl concentrations (Jacobi and Ratner, 1984). The genotypes 'Cardinal' and 'Talash' with the highest Na uptake had a low survival rate with distinct visual symptoms of salinity damage. This observation tends to confirm the report which identified correlations of high shoot Na^{+} concentrations with shoot damage as a physiological marker during screening for salinity tolerance (Gama et al., 2007). The low survival rates noticed for other cultivars could be explained by the fact that high concentrations of sodium ions in the protoplasmic constituents not only effectively inhibit metabolic functions (Gama et al., 2007), but also result to high viscosity in the cell, therefore increasing the chances of molecular interactions that cause

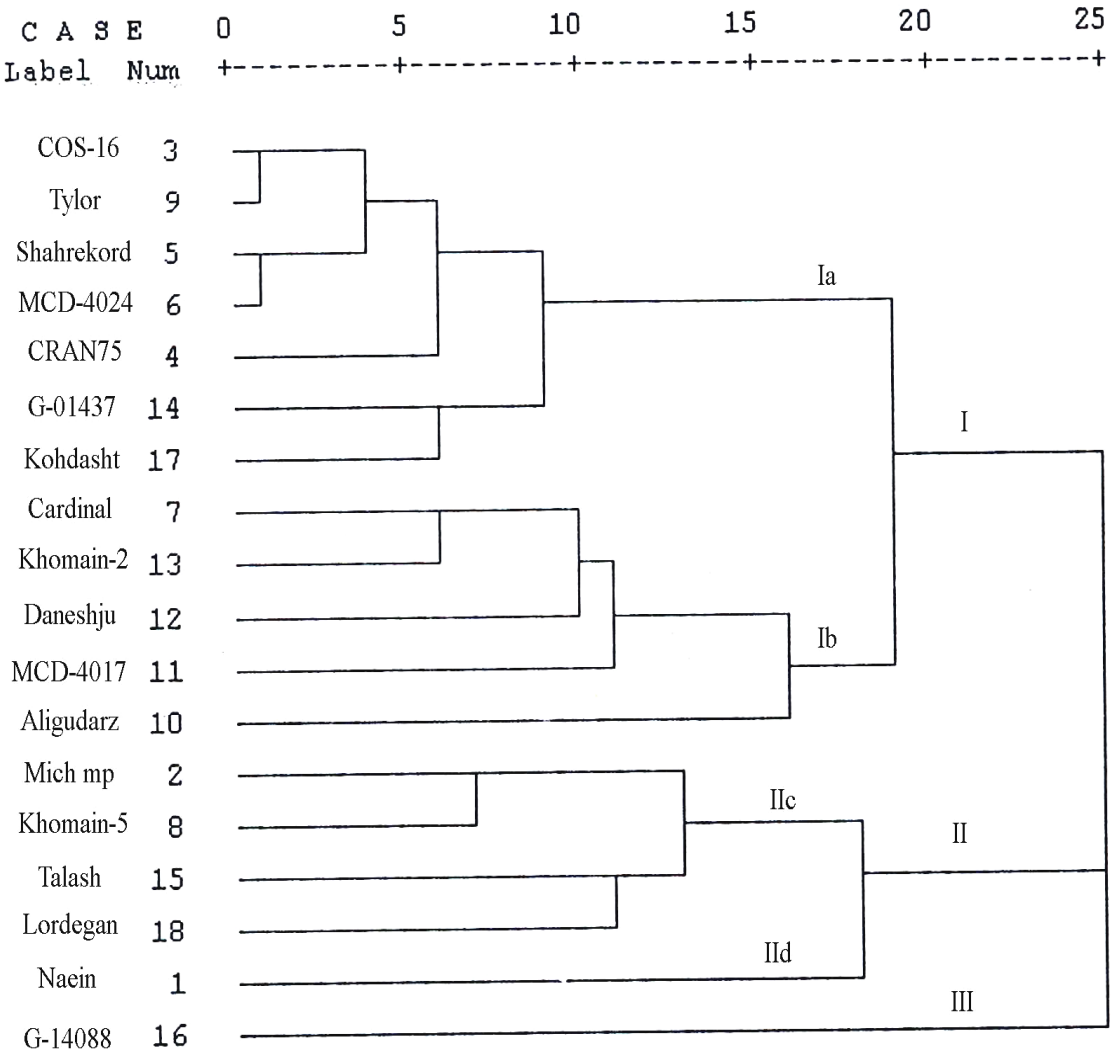

Figure 1. Ward's Minimum Variance Dendrogram of 18 *Phaseolus Vulgaris* L. genotypes. Optimum number of cluster was determined by MANOVA procedure (Sorkheh et al., 2007).

protein denaturation and membrane fusion. One interesting phenomenon about *Phaseolus* is that it tends to show signs of salinity shock at the 5th day of salt exposure and recovery in case of the salt tolerant landrace of 'Naein' that is in agreement with results that had obtained by Gama et al. (2007). The results here are unlikely in favor of findings of Bayuelo-Jiménez (2002a) and others that most of the cultivars of *P. vulgaris* compared to their wild relatives were sensitive to salinity stress because the response of 'Naein' to salinity by maintaining high dry weight and a low Na^+ concentration in shoot tissues is a unique characteristic in cultivated beans. Thus, this provides more evidence that some of the cultivated cultivars of common bean in Iran have substantially higher degree of tolerance to salinity. This is probably due to wide crosses with wild relatives for disease resistance. These retrogressed disease resistant traits, therefore, might also be of multiple or diverse importance to other environmental stresses such as salinity.

However, to evaluate biochemical, physiological and morphological responses of locally adapted common bean varieties to salinity stress, we suggest more robust methodologies, in terms of time and resources, for screening common bean for salinity tolerance. These include physiological markers such as survival rates, ion concentrations, SDW and RDW, SDW/RDW ratio and relative growth rate as essential parameters for screening for salinity. However, other morphological characters like plant height, number of leaves, leaf area, and root length and density are difficult to correlate to salinity tolerance where cultivars have different growth pattern (Gama et al., 2007).

The accessions which make up group Ia and Ib in the cluster analysis correspond to the salt sensitive genotypes. These accessions grow in tropical and

temperate subhumid climates, on rocky or sandy soils associated with tropical deciduous, although the climatic and environmental range of accessions seem not to be associated with the pattern of incidence of hot semiarid climates and saline soils (Bayuelo-Jimenez et al., 2002a).

The accessions which makes up groups IIc and IId correspond to the Iran and CIAT gene pool, respectively. These cultivated genotypes mostly distinguished by the highest RDW, RL, PH and SDW. The available range of variability for salinity tolerance in these accessions could come largely from seed size. Large seeded genotypes have more seed reserves to support seedling growth during stress periods. A high correlation coefficient between seedling growth and seed size conform that cultivated accessions having the largest seeds, exhibit the greatest seedling growth under salt stress. These results according with Bayuelo-Jimenez et al. (2002a) but in our study the correlation coefficient was relatively moderate to high. Although increased seedling growth was positively related to seed size under salt stress, such tolerance may vary with plant ontogeny. Cultivated accessions identified in this study as the most tolerant, despite results had obtained by Bayuelo-Jimenez et al. (2002a), during germination and early seedling and vegetative growth, specifically the local landrace of 'Naein' is most tolerant during different stages. Thus particular species may be differentially affected at various physiological stages of development and may not produce tolerant adult plants, for example in this study, landrace of 'Aligudarz' and 'Daneshju' from Iran gene pool clustered in subgroup Ib. The resulting information will be useful in improving the understanding of the diversity of cultivated and wild common bean. The morphological characters and ionic analysis such as Na^+ underlying theses groups provide a useful aid to target the search for new germplasm needed for future crop improvement.

In conclusion, the results of this study demonstrate that salt tolerance during germination and early seedling growth exists within *P. vulgaris* genotypes. The local landrace 'Naein' represents a genetic resource for improvement of salt tolerance in common bean.

Conflict of Interests

The authors have not declared any conflict of interest.

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