

Salinity Tolerance in *Phaseolus* Species during Early Vegetative Growth

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ABSTRACT

The genus *Phaseolus* includes important cultivated species as well as wild species with diverse ecological adaptations. Characterization of the ecological adaptations of the wild species would be useful for improved understanding, conservation, and utilization of these genetic resources. Salinity tolerance during vegetative growth was evaluated for 132 accessions for 14 wild *Phaseolus* species (*P. acutifolius* A. Gray, *P. angustissimus* A. Gray, *P. carteri* Freytag & Debouck, *P. filiformis* Benth., *P. glabellus* Piper, *P. leptostachyus* Benth., *P. lunatus* L., *P. micranthus* Hook & Arnold, *P. microcarpus* Mart., *P. mcvaughii* A. Delgado, *P. oligospermus* Piper, and *P. vulgaris* L.) and 11 accessions representing five cultivated species (*P. acutifolius*, *P. coccineus* L., *P. lunatus* L., *P. polyanthus* Greenman, and *P. vulgaris*) in nutrient solution containing 0 and 180 mM sodium chloride for 21 d. When plants were salinized after the emergence of the first trifoliate leaf, wild accessions of *P. acutifolius*, *P. filiformis*, *P. lunatus*, and *P. vulgaris* showed a wide range of variation in their salinity tolerance as defined by total dry weight reduction (PR) as a percentage of the unsalinized controls, salt susceptibility index (SSI), and root:shoot ratio (RSR). SSI and PR were correlated positively, indicating either trait could be used to select salt-tolerant accessions. Cluster analysis revealed substantial intraspecific and interspecific variation in salinity tolerance. Salinity tolerance was observed in wild *P. micranthus*, *P. mcvaughii*, *P. lunatus*, cultivated *P. coccineus*, and several accessions of wild *P. filiformis*, and *P. vulgaris*. Of these, *P. filiformis* was noteworthy in having 9 of 11 accessions rated as highly tolerant. Wild *P. vulgaris* was more salinity tolerant than the three cultivated *P. vulgaris* accessions included in the study. Many tolerant accessions originated in arid, coastal, or saline areas. We conclude that the genus *Phaseolus* has substantial diversity in salinity tolerance.

EXCESSIVE SOIL SALINITY is an important constraint limiting the distribution of plants in natural habitats, and is an increasingly severe agricultural problem in arid and semiarid regions (Shannon, 1986). Various strategies have been adopted by plant scientists in overcoming salinity (Kingsbury and Epstein, 1984). One important component is the evaluation of genetic variability of the cultivated species or its wild relatives to identify a tolerant genotype that may sustain a reasonable yield on salt affected soils (Kingsbury and Epstein, 1984).

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.), mungbean [*Vigna radiata* (L.) Wilczek] (Läuchli, 1984), pigeonpea [*Cajanus cajan* (L.) Millsp.] (Subbarao et al., 1991), tepary bean (*Phaseolus acutifolius* var. *latifolius*) (Coons and Pratt, 1988), and common bean (*P. vulgaris*) (Mor-

eno-Limón et al., 2000). However, few salt-tolerant genotypes were identified in these studies.

In some crops, wild species have been identified as good genetic resources for biotic and abiotic stress tolerance (Harlan, 1976). For example, there are several wild species of tomato (*Lycopersicon cheesmanii*, *L. peruvianum*, and *Solanum pennellii*) (Tal and Shannon, 1983), wheat (*Triticum* spp.) (McGuire and Dvorak, 1981), barley (*Hordeum spontaneum* K. Koch) (Mano and Takeda, 1998), soybean (*Glycine* spp.) (Pantalone et al., 1997), and cowpea [*Vigna unguiculata* (L.) Walp.] (Gulai and Jaiwal, 1996), which exhibit a wide variation in their salinity tolerance compared to their cultivated species. In addition, some wild relatives of pigeonpea [*Cajanus cajan* (L.) Millse.] including *Atylosia*, *Rhynchosia*, and *Dunbaria* were observed in salt affected, dry land habitats (Subbarao et al., 1991), and these genera could be a useful source of tolerance to salt stress via genetic transformation.

Characters such as yield, survival, vigor, leaf damage, and plant height, have been the most commonly used criteria for identifying salinity tolerance (Maas and Hoffman, 1977; Shannon, 1984). Other indices of tolerance have been proposed that are based on specific physiological characteristics, for instance, accumulation of specific ions in shoots or leaves, or the production of a specific metabolite (Noble and Shannon, 1988). Salinity tolerance, however, is usually assayed in terms of absolute or relative growth or yield (Maas and Hoffman, 1977; Shannon, 1984). This is largely due to ease of measurement and because, in the end, yield (both absolute and relative) under saline conditions is usually the ultimate goal. However, when the developmental pattern of wild populations is so different from that of the cultivars, assessment of the actual salt tolerance may be determined throughout through comparisons of their biomass production over prolonged periods of time (Munns et al., 2000).

Evaluation of salt tolerance in legumes has been attempted by a variety of cultural techniques with plant material ranging from germinating seeds to seedlings to mature plants. Nutrient solution salinized with NaCl + CaCl₂ has been used (Keating and Fisher, 1985), as has artificially salinized soil in containers (Johansen et al., 1990), and sand culture (Ashraf and Waheed, 1990; Subbarao et al., 1991). There have also been attempts to evaluate germplasm in naturally saline areas (Chauhan, 1987) as well as by cell culture techniques and physiological characteristics (Noble and Shannon, 1988).

Evidence collected from various species suggests that salt tolerance is a developmentally regulated, stage-specific phenomenon, so that tolerance at one stage of

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Abbreviations: SII, salt intensity index; SS, salt-stressed; SSI, salt susceptibility index; GM, geometric mean; NS, nonstressed; PR, percent of reduction; RSR, root:shoot ratio.

development may not be correlated with tolerance at other developmental stages (Shannon, 1986). Therefore, specific stages throughout the ontogeny of the plant, such as germination and emergence, seedling survival and growth, should be evaluated separately during the assessment of germplasm for salt tolerance. Such assessments may facilitate development of cultivars with salt-tolerance characteristics throughout the ontogeny of the plant.

Common bean (*P. vulgaris*) is a significant source of dietary protein in many developing countries (Durante and Gius, 1997). Common bean is sensitive to salinity, like many other leguminous crops, and suffers reduced yield even if it is grown at soil salinity less than 2 dS m⁻¹ (Maas and Hoffman, 1977). Therefore, the evaluation of salinity tolerance in wild species may be useful.

Phaseolus is an American genus of approximately 40 species, mainly distributed in the tropics and subtropics (Debouck, 1999). In particular, the wild *Phaseolus* species of the southwestern USA and northwestern Mexico, including *P. angustissimus*, *P. acutifolius*, and *P. filiformis*, are interesting subjects for the study of speciation and environmental adaptation. Many of these species are adapted to arid and semiarid environments and may have genetic potential for improvement of drought and frost tolerance (Goertz and Coons, 1991; Nabhan et al., 1986; Debouck, 1999). Intra- and interspecific variation for salinity tolerance in wild *Phaseolus* species during germination and early seedling growth has been observed (Bayuelo et al., 2002). In particular, *P. angustissimus* and *P. filiformis* are salt-tolerant species during germination and early seedling growth. No reports, however, appear to be available for the salinity tolerance of wild *Phaseolus* species or for comparative responses of these species during the vegetative growth stage. In our study, we investigate the degree of inter- and intra-specific variation for salinity tolerance within the genus *Phaseolus* during early vegetative growth.

MATERIALS AND METHODS

Plant Material

A total of 132 accessions of 14 wild *Phaseolus* species (*P. acutifolius* Gray; *P. angustissimus* A. Gray; *P. carteri* Freytag & Debouck; *P. filiformis* Benth., *P. glabellus* Piper; *P. leptostachyus* Benth.; *P. lunatus* L.; *P. micranthus* Hook & Arnold; *P. microcarpus* Mart; *P. mcvaughii* A. Delgado; and *P. vulgaris* L.) and 11 cultivated accessions representing five species (*P. acutifolius* Gray; *P. coccineus* L.; *P. lunatus* L.; *P. polyanthus* Greenman, and *P. vulgaris* L.) were evaluated for salinity tolerance. The wild *Phaseolus* species were selected on the basis of their geographical distribution and ecological adaptation. *Phaseolus acutifolius*, *P. angustissimus*, *P. carteri*, and *P. filiformis* were chosen from southwestern USA and northwestern Mexico, because of their adaptation to extremely arid conditions and prior reports of the presence of useful genes for tolerance to abiotic stresses. *P. leptostachyus* and *P. microcarpus* were selected because they are widely distributed throughout the Pacific slope of Mexico, where saline soils are common. The wild *P. vulgaris* was selected from the northern and central highlands of Mexico to northwestern Argentina. Useful genetic variability for salinity tolerance could be exhib-

ited in this species because of its adaptation to semi-arid and semi-humid environments.

The selection of cultivated accessions was based on their origin and domestication. Therefore, *P. acutifolius* and *P. coccineus* were selected from northwestern and Central Mexico, whereas accessions of *P. vulgaris*, *P. lunatus*, and *P. polyanthus* were selected from Mexico, Peru, Argentina, and Colombia. Salt-sensitive common bean 'Carioca' (CIAT germplasm accession G4017) (Maas and Hoffman, 1977) was utilized as the control for evaluation of relative tolerance of both wild and cultivated *Phaseolus* accessions during early vegetative growth. Seeds were provided by the United States Department of Agriculture Plant Genetic Resources Unit at Pullman, WA (PI numbers), and the International Center for Tropical Agriculture at Palmira, Colombia (S and G numbers).

Plant Growth

Plants were grown in nutrient solution in a greenhouse at The Pennsylvania State University, PA, (40° 85' N, 77° 83' W) between July and August 1998, 1999, and 2000. Mean growing temperature, relative humidity, and salinity intensity index (SII, based on total dry weight of 143 accessions) were recorded for the three growing seasons (Table 1). The SII for each growing season was calculated as $SII = 1 - X_{ss}/X_{ns}$, where X_{ss} and X_{ns} are the mean of all accessions under salinity-stressed (SS) and non-stressed (NS) environments (Fisher and Maurer, 1978).

Before planting, seeds were surface sterilized with 2.5 g L⁻¹ sodium hypochlorite solution for 5 min and rinsed with sterile distilled water. Seeds were manually scarified and placed in rolled germination paper (Anchor Co., St. Paul, MN) moistened with 0.5 mM CaSO₄ solution and germinated in the dark at 25°C. Seven-day-old seedlings of uniform size were transferred into plastic grids over aerated hydroponic tanks (400-L volume) containing full strength Epstein solution (Epstein, 1972). Roots were slipped through a square 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) between 6 and 6.5. The plants were grown on this control solution until the emergence of the first trifoliate leaf (6–7 d after transplanting), at which time salt stress treatments were initiated. Nutrient solution for plants with salt stress was identical to that for controls except for the addition of NaCl and CaCl₂ to the appropriate concentration. In the salt stress treatment, the first increment of salt, containing 30 mM NaCl and 3 mM CaCl₂, was added 7 d 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 + 18 mM CaCl₂ (electrical conductivity 15.7 dS m⁻¹, 25°C). CaCl₂ was added to retain the root cell membrane

Table 1. Relative humidity, mean growing season temperature, and salt intensity index for the three cropping seasons between 1998 and 2000 used to evaluate 143 accessions of *Phaseolus* species.

Cropping season†	Relative humidity	Mean growing season temperature	Salinity intensity index‡
	%	°C	
1998	62	27	0.647
1999	73	29	0.654
2000	58	25	0.595

† Cropping season = July to August.

‡ Salinity intensity index (SII) = $1 - X_{ss}/X_{ns}$, where X_{ss} and X_{ns} are the mean of all accessions in salt-stressed and non-stressed conditions, respectively.

integrity and maintain the adequate potassium content of the cell (Cramer et al., 1985).

Evaluation of Salinity Tolerance

The 143 accessions were evaluated in three separate experiments. Each experiment was organized in a randomized complete block design with a split plot arrangement of NaCl treatments and four (Experiment 1998) and six (Experiments 1999 and 2000) replications of each treatment (Gomez and Gomez, 1984). The salt-sensitive Carioca was included as a control in all experiments. In the first experiment, 82 wild accessions of *P. vulgaris* and Carioca were evaluated for salinity tolerance. This experiment was conducted from July through August 1998. The second experiment conducted from July through August 1999, consisted of 32 wild accessions of *P. angustissimus*, *P. filiformis*, and *P. leptostachyus*, and Carioca. The remaining 29 accessions of wild (*P. acutifolius*, *P. angustissimus*, *P. carteri*, *P. glabellus*, *P. leptostachyus*, *P. lunatus*, *P. micranthus*, *P. mcvaughii*, *P. oligospermus*, and *P. vulgaris*) and cultivated (*P. acutifolius*, *P. lunatus*, *P. polyanthus*, and *P. vulgaris*) were included in the third experiment, conducted from July through August 2000.

Twenty days after the start of salt treatment, individual plants of both salt stress and nonstress treatments were harvested. Plants were separated into roots and shoots. Plant materials were dried at 65°C for 96 h to determine dry weights. For each accession, plant dry weight under salt stress and as a percentage of dry weight under the nonstress treatment were determined.

Statistical Analysis

Data from the three experiments were combined into a single analysis since there were no significant differences when the performance of salt susceptible Carioca was compared across experiments (seasons) in a one-way analysis of variance. Geometric mean (GM) was determined for total dry weight, and root/shoot ratio as $GM = (NS \times SS)^{1/2}$. Percentage of reduction (PR) due to salinity stress in relation to the NS environment was also determined for the two traits. Salinity susceptibility index for total dry weight for each accession was calculated as follows: $SSI = (1 - Y_s/Y_{ns})/SII$, where Y_s and Y_{ns} are mean total dry weights of a given accession in SS and NS environments, respectively (Fisher and Maurer, 1978). Simple correlation coefficients among different traits were also determined.

On the basis of data collected, the index of salinity tolerance was constructed for total dry weight as percentage of control. The index of salinity tolerance provides data on the relative effects of increasing NaCl concentration on each accession, enabling comparisons to be made in a convenient and standard form (Shannon, 1984).

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 (Gomez and Gomez, 1984). Error variances were homogeneous thus data were not transformed. Statistical differences among species and within a species 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 species ($P = 0.05$).

Quartile calculations for the separation of accessions into categories: tolerant (T), moderately tolerant (MT) or sensitive (S) were analyzed by the Univariate Procedure (SAS Institute, Cary, NC, 1985). The classified data were ranked by the Rank

Procedure (SAS institute, Cary, NC, 1985). Spearman's Coefficient of Rank Correlation was used to analyze the ranked data for the response variables (SAS institute, Cary, NC, 1985).

Ward's minimum variance clustering method was used to classify the *Phaseolus* species and accessions within species into discrete clusters (Romesburg, 1984). The optimum number of clusters was determined by the sum of squares index (E) (Romesburg, 1984).

RESULTS

Salinity Tolerance

Salinity intensity was comparatively moderate in the three growing seasons (Table 1). Salinity stress had significant effects on total dry weight and root: shoot ratio, and differences among and within species for all characters including PR, GM, and SSI were highly significant. Interactions between accessions and salt stress were also significant for root, shoot, and total dry weight and root: shoot ratio. Intraspecific variation in *P. leptostachyus* and *P. microcarpus*, however, was not significant for root, shoot, and total dry weight, although for *P. leptostachyus*, PR and SSI for total dry weight and root: shoot ratio were significant. Because eight of 14 species evaluated only included one accession per species, these accessions were analyzed as a group. Within this group, there was significant variation in root, shoot, and total dry weight (Table 2). The wild accessions of *P. micranthus* (S26184) and *P. mcvaughii* (S31355) and cultivated *P. coccineus* (G35341) had high tolerance to salinity, followed by *P. angustissimus* (S26170), *P. carteri* (S32376), and *P. polyanthus* (G35372), which had an intermediate salt tolerance. *Phaseolus glabellus* (S29186) and *P. oligospermus* (S19150), however, were salt sensitive (Table 3).

Root, shoot, and total dry weight were highly correlated (between $r^2 = 0.74$ and $r^2 = 0.99$). Therefore, only PR for total dry weight due to salinity stress in relation to the NS conditions was reported in Tables 3 and 4. The largest reduction in total dry weight due to salinity stress was in salt-sensitive accessions of *P. microcarpus*, *P. glabellus*, and *P. oligospermus*. In contrast, the most salt-tolerant accessions of *P. micranthus*, *P. coccineus*, and *P. mcvaughii* showed comparatively less total dry weight reduction (Table 3). All other species, on the average, showed comparatively moderate reduction of total dry weight (53 to 67%) (Table 3). Within a species, accessions differed in salinity tolerance. The wild accessions PI535308, PI535309, S26168, PI535303, PI535299, PI535307 (*P. filiformis*), PI535315 (*P. leptostachyus*), PI325687, PI417614, PI201044, PI329247, PI430217, PI417687, PI325678, PI417662, PI533397, G21981 (*P. vulgaris*), and G25704 (*P. lunatus*) had the lowest total dry weight reduction under 180 mM NaCl compared with the other accessions (Table 4).

The SSI for total dry weight was the lowest (signifying greater salinity tolerance) for *P. filiformis* accessions PI535308, PI535309, and S26168 (Table 4). The wild *P. vulgaris* accessions PI325687, PI417614, PI201044, PI329247, PI430217, PI417687, PI325678, and *P. lunatus*

Table 2. Analysis of variance of actual root, shoot and total dry weight, and root: shoot ratio of *Phaseolus* species evaluated in salinity-stressed and non-stressed conditions, in three growing seasons between 1998 and 2000.

Source	df	Mean squares†						
		Root dry weight	Shoot dry weight	Total	Total dry weight		SSI	Root: shoot ratio
					PR	GM		
Replication	5	0.08	4.1	5.7	369.9	1.8	0.08	0.013
Salt stress level	1	29.9**	2353.4**	2857.3**				0.192**
Rep* Salt stress level	5	0.02	3.0	3.7				0.005
Accession	142	0.92**	39.7**	52.1**	1041.8**	19.9**	0.25**	0.062**
Accession* Salt stress level	142	0.18**	11.2**	13.8**				0.024**
Species	6	4.8**	336.6**	433.8**	2446.5**	172.4**	0.62**	0.398**
Accession (Species)	136	0.74**	26.6**	35.4**	979.9**	13.2**	0.23**	0.047**
Acc (<i>P. acutifolius</i>)	5	0.18**	10.5**	12.9*	871.6**	7.7**	0.24**	0.002
Acc (<i>P. filiformis</i>)	17	0.45**	33.6**	39.4**	2551.8**	12.4**	0.59**	0.052**
Acc (<i>P. leptostachyus</i>)	17	0.03	1.2	1.1	1775.6*	0.4	0.36**	0.204**
Acc (<i>P. lunatus</i>)	3	0.70**	32.7**	42.9**	1464.6**	19.1**	0.39**	0.006
Acc (<i>P. microcarpus</i>)	2	0.03	0.9	0.9	102.9	0.4	0.03	0.040
Acc (<i>P. vulgaris</i>)	85	0.72**	26.2**	34.8**	483.3**	12.6**	0.12**	0.021**
All Other Species	7	4.17**	92.9**	136.6**	1404.2**	58.8**	0.38**	0.034**
Salt stress level * Species	6	0.71**	68.3**	86.0**				0.008
Salt stress level (Acc * Species)	136	0.16**	8.6**	10.6**				0.024**
Error	1140	0.0176	0.727	0.772				0.009
	570				164.6	0.26	0.040	

* Indicates significance at $P = 0.05$ ** Indicates significance at $P = 0.01$.† PR = percent reduction in the SS in relation to the NS condition, GM (geometric mean) = $(NS \times SS)^{1/2}$, and SSI (salt susceptibility index) = $(1 - Y_s/Y_{ns})/SSI$, where Y_s and Y_{ns} are the mean total dry weight of a given accession in SS and NS conditions, respectively.

accession G25704 had also relatively low SSI. Among cultivated accessions, only *P. coccineus* (G35341) had a low SSI. As expected, the common bean landrace Carioca had high SSI values, indicating susceptibility to salt stress. The response of the accessions in terms of root: shoot ratio (Table 4) suggests that root growth is less inhibited by salt stress than shoot growth (data not shown; see Bayuelo-Jiménez, 2001 for a complete presentation of the data set).

Correlation

Correlation coefficients between the control and salinized conditions were positive and highly significant ($P < 0.01$) for root, shoot, and total dry weight (Table 5). The geometric mean for total dry weight in salinized and control conditions was positively correlated with root and shoot dry weight. A positive association was found between SSI and PR for root, shoot, and total dry weight and between PR for RSR and root dry weight.

Rating and Ranking

To categorize the accessions as tolerant, moderately tolerant, or sensitive, each was placed in an appropriate quartile on the basis of their percentage of reduction and SSI for total dry weight (Table 4). The distribution pattern of various accessions into various salinity tolerance groups indicates that, in spite of some variations, the overall pattern of behavior of the accessions tested remained fairly constant on the basis of the two different criteria, particularly with respect to the most salt-tolerant and the most salt-sensitive accessions. Because of the similarity of the two criteria, only the pattern of behavior of the best accessions (T and MT) on the basis of PR for total dry weight was reported in Table 4.

Rankings of the 143 accessions according to their percent of reduction of total dry weight at 180 mM NaCl salinity are summarized in Table 4. It is apparent that

although there were some deviations in the rank orders of accessions with intermediate tolerance (MT), particularly when different criteria were used, the most tolerant and the most sensitive occupied the top and the bottom position, respectively, in all cases. This indicates that the criteria for the assessment of salinity tolerance in *Phaseolus* accessions were consistent. Spearman rank correlation analyses between PR and SSI for total dry weight were significant, indicating that the mean values of their responses to salt stress were ranked similarly in all 143 accessions (data not shown).

Cluster Analysis

Ward's clustering technique clearly defined and characterized clusters on the basis of PR and SSI for total

Table 3. Total dry weight of *Phaseolus* species (averaged over all accessions) grown under non-stressed (NS) and salt-stressed conditions (SS), in three seasons between 1998 and 2000. Accessions are ranked by total dry weight as percent of control.

Species		Total dry weight (g plant ⁻¹)		
		NS	SS	PR
<i>P. micranthus</i>	(A = 1)	0.19	0.12	31.06
<i>P. coccineus</i>	(A = 1)	11.24	6.28	40.49
<i>P. macvaughii</i>	(A = 1)	0.73	0.40	42.82
<i>P. lunatus</i>	(A = 4)	7.22	3.14	53.28
<i>P. filiformis</i>	(A = 18)	4.13	1.46	54.25
<i>P. carteri</i>	(A = 1)	0.93	0.39	54.29
<i>P. angustissimus</i>	(A = 1)	1.30	0.60	56.30
<i>P. acutifolius</i>	(A = 6)	7.79	3.22	58.26
<i>P. polyanthus</i>	(A = 1)	9.65	3.45	60.13
<i>P. vulgaris</i>	(A = 86)	5.41	1.89	62.41
<i>P. leptostachyus</i>	(A = 18)	0.64	0.19	67.20
<i>P. glabellus</i>	(A = 1)	0.63	0.18	69.66
<i>P. microcarpus</i>	(A = 3)	2.33	0.66	70.74
<i>P. oligospermus</i>	(A = 1)	0.84	0.17	78.31
LSD (0.05)¶		0.70	0.70	10.26

PR = percent reduction in the SS in relation to the NS conditions.

¶ To compare means among species. Data are shown as mean of six replicates.

§ A = Number of accessions per species.

Table 5. Correlation coefficients among non-stressed (NS), salt-stressed (SS), and percent reduction (PR) for root, shoot, and total dry weight, and root: shoot ratio, and salinity susceptibility index (SSI) for total dry weight for 143 accessions of *Phaseolus* evaluated in three cropping seasons between 1998 and 2000.

	Root dry weight			Shoot dry weight			Total dry weight				Root/shoot ratio			
		SS	PR	NS	SS	PR	NS	SS	PR	GM	SSI	NS	SS	PR
Root dry weight	NS	0.82**	0.17	0.86**	0.77**	0.10	0.89**	0.79**	0.09	0.87**	0.12	-0.04	-0.08	0.09
	SS	-	-0.32	0.76**	0.85**	-0.13	0.79**	0.89**	-0.19	0.86*	-0.17	-0.12	0.05	-0.21
	PR	-	-	0.03	-0.19	0.48**	0.064	-0.22	0.57**	-0.08	0.56**	0.22*	-0.25*	0.62**
Shoot dry weight	NS	-	-	-	0.86**	0.16	0.99**	0.87**	0.15	0.96**	0.18	-0.37*	-0.24*	-0.07
	SS	-	-	-	-	-0.20	0.87**	0.99**	-0.24	0.95**	-0.22	-0.33*	-0.23*	-0.02
	PR	-	-	-	-	-	0.16	-0.19	0.98**	-0.02	0.97**	-0.17	0.11	-0.22
Total dry weight	NS	-	-	-	-	-	-	-0.88**	0.13	0.96**	0.15	-0.34*	-0.21*	-0.05
	SS	-	-	-	-	-	-	-	-0.24	0.97**	-0.21	-0.30*	-0.19*	-0.05
	PR	-	-	-	-	-	-	-	-0.06	0.99**	-0.21	-0.11	0.05	-0.09
	GM	-	-	-	-	-	-	-	-	-	-0.03	-0.37*	-0.20*	-0.06
	SSI	-	-	-	-	-	-	-	-	-	-	-0.14	-0.00	-0.11
Root: shoot ratio	NS	-	-	-	-	-	-	-	-	-	-	-	0.46**	0.30*
	SS	-	-	-	-	-	-	-	-	-	-	-	-	-0.59**
	PR	-	-	-	-	-	-	-	-	-	-	-	-	-

* Indicates significance at $P = 0.05$.** Indicates significance at $P = 0.01$.† PR = percent reduction in the SS in relation to the NS condition and SSI, GM (geometric mean) = $(NS \times SS)^{1/2}$, and (salt susceptibility index) = $(1 - Y_{ss}/Y_{ns})/SSI$, where Y_{ss} and Y_{ns} are the mean total dry weight of a given accession in SS and NS conditions, respectively.

dry weight (salinity tolerance), root: shoot ratio, and the actual mean of root, shoot, and total dry weight at 180 mM NaCl. *Phaseolus* accessions were grouped into three clusters on the basis of distance ranges for the tree. To determine where to cut the tree, we used the sum of squares index (E) (Romesburg, 1984).

Cluster I, II, and III includes 38, 32, and 73 accessions, respectively. Cluster I included accessions of eight species, *P. acutifolius*, *P. coccineus*, *P. filiformis*, *P. leptostachyus*, *P. lunatus*, *P. mcvaughii*, *P. micranthus*, and *P. vulgaris*. These accessions were characterized by the lowest root, shoot, and total dry weight reduction (33, 38, and 38%, respectively) as well as a low SSI (0.63) and an intermediate increase in root: shoot ratio (11%).

Cluster II comprises accessions of seven species, *P. acutifolius*, *P. angustissimus*, *P. carteri*, *P. leptostachyus*, *P. lunatus*, *P. polyanthus*, and *P. vulgaris*, which belong to the moderately tolerant group. These accessions had moderate reduction of root, shoot, and total dry weight (42, 58, and 57%, respectively), as well as an intermediate SSI value (0.92) and root: shoot ratio (14%).

Cluster III comprises accessions of seven species, *P. acutifolius*, *P. filiformis*, *P. glabellus*, *P. microcarpus*, *P. leptostachyus*, *P. oligospermus*, and *P. vulgaris*. These accessions were highly sensitive to salinity stress in all their growth attributes and PR and SSI values for total dry weight. It was characterized by accessions with the largest reduction in root, shoot, and total dry weight (60, 73, and 72%) because of salinity stress. SSI values were also the highest compared with the other species >1.16, indicating an above-average susceptibility to salt stress. Root: shoot ratio increased 16% under salt stress.

In an attempt to examine the genetic variability within species, we extracted, for detailed consideration, accessions of four species (*P. acutifolius*, *P. filiformis*, *P. lunatus*, and *P. vulgaris*) and classified them by using the Ward's clustering technique. The classification was based on the same growth attributes stated earlier.

Accessions of *P. filiformis* grouped into two clusters. The first cluster was divided in two subgroups: subgroup a includes three accessions (PI535308, PI535309, and

S26168) that decreased more than 23% root, shoot, and total dry weight and had the lowest SSI value = 0.35. However, in the subgroup b, there were six accessions that decreased by a similar percent root, shoot, and total dry weight (38–44%) but had intermediate SSI values = 0.66. Both subgroups had a high relative tolerance in root, shoot, and total dry weight as a percentage of control. These accessions, therefore, were tolerant to salinity stress. However, in the second cluster, there were nine accessions that produced very low percentage of total dry weight (30%) and high SSI values (1.1). These accessions species were classified as salt sensitive. Neither Cluster II nor I showed an increased response on root: shoot ratio.

Phaseolus vulgaris accessions grouped into three clusters. The first cluster includes 18 accessions that produced greater than 66% root dry weight but intermediate relative shoot and total dry weight (52–55%). These accessions had also moderately low SSI values = 0.70 and a low increased root: shoot ratio (8%). These accessions, therefore, were tolerant to salinity stress. Cluster II of *P. vulgaris* includes 23 moderately tolerant accessions, which had an intermediate reduction of root, shoot, and total dry biomass (45–60%) as well as an intermediate performance in their SSI values = 0.92 and moderately high root: shoot ratio (28%), whereas the Cluster III comprises 45 salt-sensitive accessions, included Carioca and other cultivated accessions.

The accessions of *P. acutifolius* and *P. lunatus* grouped into two clusters. The first cluster includes five salt-sensitive accessions, which produced very low percentage of root, shoot, and total dry weight and had a very high SSI value (1.1). In the second cluster, there were five salt-tolerant accessions representing four cultivated accessions of *P. acutifolius* (G40022, G40142, and G40148) and *P. lunatus* (G25798) as well as one wild accession of *P. lunatus* (G25704). All of these accessions were distinguished by a high tolerance to salinity on the basis of the two criteria.

DISCUSSION

Data based on two different growth criteria at the early vegetative stage, PR, and SSI for total dry weight clearly demonstrate genetic variation in vegetative growth responses to salinity within and among *Phaseolus* species. This study indicated that two wild accessions, *P. micranthus* S26184 and *P. mcvaughii* S31355, and the cultivated *P. coccineus* G35341, had superior vegetative growth and low salt susceptibility index under intense salt stress. These species, however, only included one accession per species and thus did not reveal the intra-specific variation that was observed in *P. acutifolius*, *P. filiformis*, *P. lunatus*, and *P. vulgaris*. Although these latter species were moderately tolerant, their actual reduction of total biomass was lower for several of their accessions than the former group of species (Table 4). Seventeen accessions including *P. filiformis* (PI535309, PI535308, S26168, PI535303, PI535299, PI535307), *P. vulgaris* (PI325687, PI201014, PI417644, PI329247, PI43-0217, PI417687, PI325678, PI417662, PI533397, PI417770), and *P. lunatus* (G25704) had the lowest total dry weight reduction and highest levels of salinity tolerance (with SSI < 0.9, indicating below-average susceptibility to salinity). These accessions ranked well in total dry weight reduction and SSI values and together with *P. micranthus* (S26184) and *P. mcvaughii* (S31355), and cultivated *P. coccineus* (G35341) had the lowest reduction of total biomass and SSI values under stress. Therefore, these 20 accessions were categorized as the most NaCl tolerant among the 143 accessions evaluated in this study. These accessions were highly tolerant to salinity (180 mM NaCl = EC 15.7 dS m⁻¹) during the early vegetative growth stage, contrasting clearly with an earlier report of the salt sensitivity of *P. vulgaris* (2.1 dS m⁻¹) (Maas and Hoffman, 1977). These results are highly comparable with the range of tolerance observed in tropical grain legumes to salinity (Keating and Fisher, 1985).

Salinity tolerance of these accessions, as a group, significantly out-produced cultivars from *P. vulgaris* under salinity stress. Positive correlation between total dry weight with and without salt stress supported similar findings by Foolad (1996). Thus, accessions with good vegetative growth under stress also had good vegetative growth without stress. Because of a positive correlation between SSI and PR for total dry weight either trait could be used, in combination with the GM and/or arithmetic mean for total dry weight, to select salt-tolerant accessions.

Genetic variation in salinity tolerance probably represents adaptation to diverse environments in the regions of origin of these taxa. These species are distributed in dry habits of southwestern USA and northwestern Mexico, and semihumid habits of the Mexican highlands (Buhrow, 1983; Debouck, 1999). The climatic conditions of these regions, ranging from hot to hot semiarid are a major factor in the creation of saline soils and the establishment of vegetation. Salinity of these regions is mostly associated with coastal areas, inland salinity, and crop irrigation (Fuentes and Saucedo, 1999).

Phaseolus filiformis emerged as extremely salt tolerant in this study and in an earlier report (Bayuelo et al., 2002). This species is widely distributed in southwestern Arizona, western-northwestern Sonora, and Baja California. Plants occur sprawling on beach vegetation at elevations from 0 to 1600 m in hot and semihot arid climates (Buhrow, 1983). These species are highly adapted to extremely arid conditions and able to withstand drought, extreme heat, frost, and salt stress (Buhrow, 1983). The adaptation of this species to truly arid zones seems to be associated with its rapid seed germination, rapid and vigorous growth, continual flowering, and maturity during favorable periods. As is evident from this study, *P. filiformis* (with SSI = 0.74, indicating an above-average tolerance to salinity) was one of the most highly salt-tolerant species during vegetative growth. The tolerance of this species was more associated with a lower reduction of total dry weight than with a higher root: shoot ratio as was observed in other species.

Wild ancestors of the common bean, *P. vulgaris*, are found in Mexico from the state of Chihuahua in the north to the state of Chiapas in the south, under a wide range of climatic conditions ranging from hot, semihot or temperate subhumid to hot semidry in moderately disturbed natural vegetation (Delgado-Salinas, 1985). This wide distribution of wild common bean with their adaptation to different environments contributes to their genotypic and phenotypic variability. We demonstrated that the salt-tolerant accessions of this species were mostly distinguished by a moderate salt susceptibility index (0.8) and an intermediate vegetative growth, but the available range of variability for salinity tolerance in these accessions could come largely from root: shoot ratio. This species maintained relatively high root growth at 180 mM NaCl. The consequent increase in root to shoot growth seems to be associated with increased salinity tolerance in this species. It is possible that under salt stress the plant expends more photosynthetic energy on root production in search of water and/or reducing water loss and thus maintains relatively high plant water relations (Kafkai, 1991).

The level of salt tolerance that we found in wild *P. vulgaris* was relatively high, as would perhaps be expected in natural environments, and is at least comparable to those that have been reported in a wild relative of pigeonpea, *Atylosia albicans* (W. & A) Benth. during vegetative growth at 100 mM NaCl (Subbarao et al., 1991). It appears that the generalization that *P. vulgaris* is a salt-sensitive species (Maas and Hoffman, 1977) is based on studies of a small number of cultivars that happen to be salt-sensitive.

Phaseolus micranthus and *P. mcvaughii* are also salt-tolerant species that commonly grow on the Pacific coast of Mexico. *Phaseolus mcvaughii* generally grows in semidry regions, under deciduous forest at 500 m elevations, whereas *P. micranthus* grows under habitats of pine-oak forest at elevations from 500 to 1800 m (Debouck, 1999). By contrast, *P. coccineus* cultivars are cold-temperate crops, but some genotypes in Mexico have been reported growing in hot and subhumid cli-

mates, or even in dry climates (Delgado-Salinas, 1985). Although the adaptation of these species to salt stress seems to be associated with their low SSI values = 0.6 and a high vegetative growth, their available range of variability for salinity tolerance could not be evaluated. Further studies to determine the extent of variability for salt tolerance within these species are greatly needed.

The tepary bean, *P. acutifolius*, is cultivated successfully in the deserts, grasslands, and subtropical woodlands of northern Mexico and Arizona and New Mexico, where saline soils, low soil moisture, and high temperatures frequently are present (Nabhan, 1985). It is not surprising that some cultivated accessions of *P. acutifolius* have relatively high levels of tolerance to heat, drought, and salinity (Thomas et al., 1983; Parsons and Howe, 1984; Goertz and Coons, 1991). Drought-tolerant plants can use several mechanisms that tend to postpone or tolerate desiccation. These include reduction of water loss by increased stomatal resistance, reduction of absorbed radiation by changes in leaf orientation, or reduction in leaf area (Parsons and Howe, 1984). Mechanisms that tend to promote drought tolerance by maintaining turgor include osmotic adjustment, an increase in cell wall elasticity, or a decrease in cell size (Markhart, 1985). Osmotic adjustment also occurs in plants that have been exposed to saline conditions (Bayuelo-Jiménez, 2001) and might be a mechanism that promote tolerance to salt stress throughout maintenance of relatively high plant water potentials and thus, whole plant growth. Within *P. acutifolius*, we found cultivated accessions that were better than wild accessions. These accessions could be used as a source of genetic variability for low SSI and could be directly exploited in breeding.

Besides *P. acutifolius*, cultivated accessions of *P. lunatus*, on the average, exhibited relatively higher levels of salinity tolerance (i.e., had a relatively lower SSI values). It is likely that both cultivated species possess complementary genetic variability for salinity tolerance because of their distinct evolutionary origins (Delgado-Salinas, 1985). Use of salt-tolerant germplasm from *P. acutifolius* and *P. lunatus* reported in this study should therefore be maximized in cultivar development programs aimed at increasing plant growth in arid and temperate regions under irrigation and return for bean growers in a sustainable farming system.

The highly salt-tolerant species in the diverse germplasm of *Phaseolus*, examined in our study, could be of considerable economic value in increasing bean yield in saline areas with moderate salinity. Wild *P. vulgaris*, which has no barrier to hybridization with cultivated common bean, can provide salinity tolerance gene(s) to cultivated bean through breeding (Singh, 2001). The other wild *Phaseolus* species could also be a valuable source of tolerance to salinity during germination and as young seedlings (*P. angustissimus* and *P. filiformis*) (Bayuelo-Jiménez, 2001), and during vegetative growth (*P. acutifolius*, *P. filiformis*, and *P. lunatus*). Crosses of common bean with *P. filiformis* and *P. angustissimus* (Petzold and Dickson, 1987) and *P. lunatus* (Kuboyama et al., 1991), however, have been attempted without producing fertile viable hybrids progenies. Identifica-

tion and cloning useful genes from these species, and successful regeneration and transformation of common bean, may facilitate gene introgression in the future (Singh, 2001).

Salinity tolerance is known to differ with ontogeny (Shannon, 1984, 1986). Thus, particular species may be differentially affected at various physiological stages of development. In a previous study, we demonstrated that the cultivated accession Carioca (*P. vulgaris*) and wild accessions PI535272 (*P. angustissimus*), PI535315 (*P. leptostachyus*), and PI430196 (*P. microcarpus*) grown under 180 mM NaCl were among the most salt-tolerant species during germination but they were moderately sensitive or sensitive during early seedling growth (Bayuelo-Jiménez, 2001). These results are consistent with reports for wheat (Ashraf et al., 1986), lentil (*Lens culinaris* Medic.) (Ashraf and Waheed, 1990), and wild *Hordeum* (Mano and Takeda, 1998) that also demonstrated that salinity tolerance varies with plant ontogeny.

It has been argued that selection for salinity tolerance at germination, as seedlings and/or early vegetative growth may not produce tolerant adult plants (Kinsbury and Epstein, 1984). In contrast, the performance of seedlings under saline conditions has been considered highly predictive of the response of adult plants to salinity (Azhar and McNeilly, 1987). Ashraf et al. (1986) evaluated seedlings of barley, wheat, and seven grass species (*Trifolium*), and demonstrated considerable tolerance to salinity at the adult stage. In our studies, five accessions of *P. filiformis* (PI535303, PI535304, PI535305, PI535307, PI535309) previously identified as the most tolerant at germination and early seedling growth, were also tolerant during the vegetative growth stage at 180 mM NaCl. The tolerance observed in this species, however, may or may not be expressed during reproduction. Nevertheless, tolerance observed at germination, early seedling, and the vegetative growth stage is of great importance because salinity tolerance at every stage of growth is of considerable value in determining the ultimate tolerance of the species (Shannon, 1984).

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REFERENCES

- Abdel Ghaffar, A.S., H.A. El-Attar, M.H. El-Halfaw, and A.A. Abdel-Salam. 1982. Effects of inoculation, nitrogen fertilizer, salinity, and water stress on symbiotic N₂ fixation by *Vicia faba* and *Phaseolus vulgaris*. p. 72-82. In P.H. Graham and S.C. Harris (ed.) Biological nitrogen fixation technology for tropical agriculture. CIAT, Cali, Colombia.
- Ashraf, M., and A. Waheed. 1990. Screening of local/exotic accessions of lentil (*Lens culinaris* Medic.) for salt tolerance at two growth stages. *Plant Soil* 128:167-176.
- Ashraf, M., T. McNeilly, and A.D. Bradshaw. 1986. The potential for evolution of salt (NaCl) tolerance in seven grass species. *New Phytol.* 103:299-309.
- Azhar, F.M., and T. McNeilly. 1987. Variability for salt tolerance in

- Sorghum bicolor* (L.) Moench. under hydroponic conditions. *J. Agron. Crop Sci.* 195:269–277.
- Bayuelo-Jiménez, J.S. 2001. Salinity tolerance of wild *Phaseolus* species. Ph.D. diss. (Diss. Abstr. AAT3020421). The Pennsylvania State University, University Park, PA.
- Bayuelo-Jiménez, J.S., R. Craig, and J.P. Lynch. 2002. Salinity tolerance of *Phaseolus* species during germination and early seedling growth. *Crop Sci.* 42:2184–2192.
- Buhrow, R. 1983. The wild beans of southwestern North America. *Desert Plants* 5:67–88.
- Chauhan, Y.S. 1987. Screening for tolerance to salinity and water logging: Case studies with pigeonpea and chickpea. p. 19–21. In *Adaptation of chickpea and pigeonpea to abiotic stresses. Proceedings of the consultants. ICRISAT, Patancheru, India.*
- Coons, J.M., and R.C. Pratt. 1988. Physiological and growth responses of *P. vulgaris* and *P. acutifolius* when grown in fields at two levels of salinity. *Annu. Rep. Bean Improv. Coop.* 31:88–89.
- Cramer, G.R., A. Luchli, and V.S. Spolito. 1985. Displacement of Ca by Na from the plasmalemma of root cells: a primary response to salt stress? *Plant Physiol.* 79:207–211.
- Debouck, D. 1999. Diversity in *Phaseolus* species in relation to the common bean. p. 25–52. In S.P. Singh (ed.) *Developments in plant breeding. Common bean improvement in the twenty-first century.* Kluwer Academic Publishers, Dordrecht, the Netherlands.
- Delgado-Salinas, A.O. 1985. Systematics of the genus *Phaseolus* (Leguminosae) in North and Central America. Ph.D. diss. (Diss. abstr. AAT8527553). The University of Texas at Austin.
- Durante, M., and C. Gius. 1997. Legume seeds: protein content and nutritional value. *Field Crop Res.* 53:31–45.
- Epstein, E. 1972. Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, New York.
- Fisher, R.A., and R. Maurer. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res.* 29:897–912.
- Foolad, M.R. 1996. Genetic analysis of salt tolerance during vegetative growth in tomato, *Lycopersicon esculentum* Mill. *Plant Breed.* 115: 245–250.
- Fuentes, C., and H. Saucedo. 1999. Reclamation of soils with deficient drainage and salt problems in irrigated areas of Mexico. p. 113–127. In *International Commission on Irrigation and Drainage, 17th Granada, Spain. 24 Sept. 1999. ICID, New Delhi, India.*
- Goertz, S.H., and J.M. Coons. 1991. Tolerance of tepary and navy beans to NaCl during germination and emergence. *HortScience* 26:246–249.
- Gomez, A.K., and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons, New York.
- Gulai, A., and P.K. Jaiwal. 1996. *In vitro* evaluation of NaCl tolerance in wild and cultivated species of *Vigna*. *Natl. Acad. Sci. Lett.* 19: 101–106.
- Harlan, J.R. 1976. Genetic resources in wild relatives of crops. *Crop Sci.* 16:329–333.
- Johansen, C., N.P. Sexena, Y.S. Chauhan, G.V. Subbarao, R.P.S. Pundir, J.V.D.K. Kumar Rao, and M.K. Jana. 1990. p. 973–978. In S.K. Sinha et al. (ed.) *Genotypic variation in salinity response of chickpea and pigeonpea. Proc. International Congress of Plant Physiology, New Delhi, India, February 1988 (Vol. 2).* Society of Plant Physiology and Biochemistry, New Delhi, India.
- Kafkai, U. 1991. Root growth under stress. p. 375–391. In Y. Waisel et al. (ed.) *Plant roots. The hidden half.* Marcel Dekker, New York.
- Keating, B.A., and M.J. Fisher. 1985. Comparative tolerance of tropical grain legumes to salinity. *Aust. J. Agric. Res.* 36:373–383.
- Kingsbury, R.W., and E. Epstein. 1984. Selection for salt resistant spring wheat. *Crop Sci.* 24:310–315.
- Kuboyama, T., Y. Shintaku, and G. Takeda. 1991. Hybrid plant of *Phaseolus vulgaris* L. and *P. lunatus* L. obtained by means of embryo rescue and confirmed by restriction endonuclease analysis of rDNA. *Euphytica* 54:177–182.
- Läuchli, A. 1984. Salt exclusion: An adaptation of legumes for crops and pastures under saline conditions. p. 171–187. In R.C. Staples and G.H. Toenniessen (ed.) *Salinity tolerance in plants: Strategies for crop improvement.* Wiley, New York.
- Maas, E.V., and G.J. Hoffman. 1977. Crop salt tolerance-current assessment. *J. Irrig. Drain. Eng.* 103:115–134.
- Markhart, A.H. 1985. Comparative water relations of *Phaseolus vulgaris* L. and *Phaseolus acutifolius* Gray. *Plant Physiol.* 77:113–117.
- Mano, Y., and K. Takeda. 1998. Genetic resources in wild *Hordeum* species. *Euphytica* 103:137–141.
- McGuire, P.E., and J. Dvorak. 1981. High salt tolerance in wheat grasses. *Crop Sci.* 21:702–705.
- Moreno-Limón, S., R.K. Maiti, and R. Foroughbakhch. 2000. Genotypic variability in *Phaseolus* bean cultivars exposed to salinity at the germination stage. *Crop Res.* 19:487–492.
- Munns, R., R.A. Hare, R.A. James, and G.J. Rebetzke. 2000. Genetic variation for improving the salt tolerance of durum wheat. *Aust. J. Agric. Res.* 51:69–74.
- Nabhan, G.O. 1985. Native crop diversity in Aridoamerica: Conservation of regional gene pools. *Econ. Bot.* 39:387–399.
- Nabhan, G.P., J.S. Muruaga, F. Cardenas, and B.T. Burns. 1986. Wild bean exploration in northwest Mexico and southwest USA. *Plant Gen. Res. Newslett.* 65:23–25.
- Noble, C.L., and M.C. Shannon. 1988. Salt tolerance selection of forage legumes using physiological criteria. p. 989–994. In S.K. Sinha et al. (ed.) *Proc. International Congress of Plant Physiology, New Delhi, India, February 1988 (Vol. 2).* Society of Plant Physiology and Biochemistry, New Delhi, India.
- Pantalone, V.R., W.J. Kenworthy, and L.H. James. 1997. Chloride tolerance in soybean and perennial Glycine accessions. *Euphytica* 97:235–239.
- Parsons, L.R., and T.K. Howe. 1984. Effect of water stress on the water relations of *Phaseolus vulgaris* and the drought resistance *Phaseolus acutifolius*. *Physiol. Plant* 60:197–202.
- Petzoldt, R., and M.H. Dickson. 1987. Interspecific hybridization of *Phaseolus vulgaris* with *P. angustissimus*, *P. filiformis* and *P. ritensis*. *Annu. Rpt. Bean Improv. Coop.* 30:94–95.
- Romesburg, H.C. 1984. *Cluster Analysis for Researchers.* Lifetime Learning Publications, Belmont, CA.
- SAS Institute. 1985. *SAS user's guide: Statistics.* 5th ed. SAS Inst., Cary, NC.
- Shannon, M.C. 1984. Breeding, selection, and the genetics of salt tolerance. p. 231–283. In R.C. Staples and G.H. Toenniessen (ed.) *Strategies for crop improvement.* John Wiley & Sons, New York.
- Shannon, M.C. 1986. New insights in plant breeding efforts for improved salt tolerance. *Hort. Technol.* 6:96–99.
- Singh, S.P. 2001. Broadening the genetic base of common bean cultivars: A review. *Crop Sci.* 41:1659–1675.
- Subbarao, G.V., C. Johansen, M.K. Jana, and J.V.D.K. Kumar Rao. 1991. Comparative salinity responses among pigeonpea genotypes and their relatives. *Crop Sci.* 31:415–418.
- Tal, M., and M.C. Shannon. 1983. Salt tolerance in the wild relatives of the cultivated tomato: Responses of *Lycopersicon esculentum*, *L. cheesmanii*, *L. peruvianum*, *Solanum pennellii* and F₁ hybrids to high salinity. *Aust. J. Plant Physiol.* 10:109–117.
- Thomas, C.V., R.M. Manshardt, and J.G. Waines. 1983. Teparies as a source of useful traits for improving common beans. *Desert Plants* 5:43–48.