SEED PHYSIOLOGY, PRODUCTION & TECHNOLOGY

Salinity Tolerance of *Phaseolus* Species during Germination and Early Seedling Growth

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ABSTRACT

Salinity tolerance during germination and early seedling growth was evaluated for 24 accessions representing four wild *Phaseolus* species (*P. angustissimus* A. Gray, *P. filiformis* Bentham, *P. leptostachyus* Bentham, and *P. microcarpus* Mart.) and four accessions of cultivated common bean (*P. vulgaris* L.) at 0, 60, 120, and 180 m *M* NaCl. Salinity stress delayed germination in all accessions to varying degrees. Eight accessions of *P. filiformis* germinated fastest under high salinity (120 m*M* NaCl). Additional wild accessions exhibiting rapid germination at 120 m *M* NaCl were *P. angustissimus*, PI535272, *P. leptostachyus*, PI535336, and *P. microcarpus*, PI430196. Among accessions, median germination time (days to 50% germination, T50) at 120 m*M* NaCl was correlated positively ($r^2 = 0.55$, $P \leq 0.01$) with germination in the control treatments. 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 d, demonstrating high genetic potential within *Phaseolus* 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 (ratio of median germination time at 120 m *M* NaCl versus control), and reduced seedling growth. Group II included salt tolerant accessions with rapid germination, high sensitivity index, and enhanced seedling growth. Group III included cultivated accessions corresponding to the Mesoamerican and Andean gene pool with rapid germination, low sensitivity index, and intermediate seedling growth. The results confirm that wild *Phaseolus* species, and in particular *P. filiformis*, represent a genetic resource for improvement of salinity tolerance in common bean.

Soil salinity is an important constraint to crop production, affecting about 95 million hectares worldwide (Szabolcs, 1994). Although some crops are moderately tolerant of saline conditions, many crops are adversely affected by even low levels of salt (Greenway and Munns, 1980). The common bean is an example of a salt sensitive species (Maas and Hoffman, 1977). In Eastern Africa and Latin America, common bean is cultivated on 14 million hectares with an annual production of 11.6 million Mg (FAO, 1998). The common bean is primarily grown in semiarid tropical environments but it is also grown in irrigated soils of these regions. About 20 to 30% of the bean-production area in the Middle East and 5 to 10% in Latin America are affected by soil salinity (CIAT, 1992). These soils are subject to high salt concentrations in the topsoil because of capillary rise and evaporation of soil water during the dry season or from salinity of irrigation waters.

Salinity impairs seed germination, reduces nodule formation, retards plant development and reduces crop yield (Greenway and Munns, 1980). 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 intraspecific 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 (Austin, 1993). Legumes are considered a relatively salt sensitive family (Maas and Hoffman, 1977) within which limited variability for salinity tolerance has been detected (Garg and Gupta, 1997; Johansen et al., 1990). In contrast to the cultivated legumes, the genetic diversity of wild relatives may provide useful genes for improving tolerance. For example, there are several wild relatives of pigeonpea [*Cajanus cajan* (L.) Millse.] belonging to the genera *Atylosia*, *Dunbaria*, and *Rhynchosia* (Subbarao et al., 1991), which exhibit wide variation in their salinity tolerance and may represent genetic resources for improvement of salinity tolerance in cultivated pigeonpea. Similarly, salinity tolerance is found in wild species of tomato (*Lycopersicon*) like *Lycopersicon cheesmanii* Riley, *Solanum pennellii* Corr. (Tal and Shannon, 1983), and *Lycopersicon pimpinellifolium* (Jusl.) Mill. (Foolad and Lin, 1997).

Phaseolus is an American genus of approximately 40 species, mainly found in tropical and subtropical zones (Debouck, 1999). In Mexico, most *Phaseolus* species are distributed from sea level to 2900 m altitude under a wide range of climatic conditions ranging from hot, semi-hot or temperate sub-humid to hot semi-dry in moderately disturbed natural vegetation (Delgado-Salinas, 1985). The wide distribution of wild populations and their adaptation to diverse environments contributes to their genotypic and phenotypic variability (Delgado-Salinas, 1985). In particular, the *Phaseolus* of the southwestern USA and northwestern Mexico, including *P. angustissimus* and *P. filiformis*, are interesting from the perspective of speciation and environmental adaptation. Other interesting *Phaseolus* species, which are widely distributed in lowlands of the Pacific slope of Mexico, include *P. leptostachyus* and *P. microcarpus* (Delgado-Salinas, 1985). These species are adapted to diverse climates and soils and might represent a potential genetic resource for tolerance to salinity, drought, heat, and frost.

Wild *Phaseolus* species represent a genetic resource

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for improvement of characters related to disease and pest resistance (Cardona et al., 1989), stress tolerance (Debouck, 1999; Goertz and Coons, 1991), improved nutritional quality and agronomic traits (González et al., 1995; Lynch et al., 1992; Shellie-Dessert and Bliss, 1991). The extent of genetic diversity of wild *Phaseolus* species for salinity tolerance, however, has not been thoroughly investigated.

The present study was undertaken to characterize inter- and intraspecific variability for NaCl salinity tolerance in wild *Phaseolus* species, during seed germination and early seedling growth.

MATERIALS AND METHODS

Plant Material

Two experiments were conducted to examine a range of genetic variability for salinity tolerance among and within *Phaseolus* species, and to confirm the reproducibility of the germination and seedling growth performance. In the first experiment, twenty-four wild *Phaseolus* accessions representing four species (*P. angustissimus* A. Gray, *P. filiformis* Bentham, *P. leptostachyus* Bentham, and *P. microcarpus* Mart) and four cultivated accessions of common bean (*P. vulgaris* L.) were used (Table 1). The second experiment consisted of four wild *Phaseolus* species (*P. angustissimus*, PI535372, *P. filiformis*, PI535310, *P. leptostachyus*, PI535315 and *P. microcarpus*, PI430196) and one cultivated accession of *P. vulgaris* (G4017). The wild *Phaseolus* species were selected on the basis of their geographical distribution and ecological adaptation (Table 1). *P. angustissimus* 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. *Phaseolus leptostachyus* and *P. microcarpus* were selected because they are widely distributed throughout the Pacific slope of Mexico, where saline soils are common.

The selection of cultivated accessions was based on contrasting seed size, origin, and growth habit. The Brazilian landrace 'Carioca' (CIAT germplasm accession G4017) is of the Mesoamerican gene pool. It has an indeterminate prostrate growth habit (Type III), shallow root system and small seeds ($\leq$300 mg seed^{-1}). The Mesoamerican genotype BAT 271 has an indeterminate growth habit (Type III) and small seeds ($\leq$300 mg seed^{-1}). The Peruvian landrace G19833 is of the Andean gene pool and has an indeterminate prostrate growth habit (Type III), and medium seeds (>300 mg seed^{-1} $\leq$ 400 mg seed^{-1}), and the Andean genotype 'Calima' (G4494) has a determinate bush growth habit (Type I) and large seeds (>400 mg seed^{-1}) (Singh, 1982).

Seeds of all the wild accessions were provided by the United States Department of Agriculture Plant Genetic Resources Unit at Pullman, WA, and the cultivated accessions by the International Center for Tropical Agriculture (CIAT). The seeds of the wild *Phaseolus* accessions used in this study were produced in a common environment in 1995 in Hawaii, USA. All wild seeds were stored under identical conditions at Pullman from harvest until use. To add a comparison with a few representative cultivated genotypes, we used seeds recently produced at CIAT, Colombia. Seeds from CIAT were produced and stored in uniform conditions. The four cultivated accessions were examined to provide reference points for the evaluation of wild *Phaseolus* accessions during germination and early seedling growth.

Table 1. Geographical origin of wild (W) and cultivated (C) accessions included in this study.

Species/Accession	Type	Origin†	State	Latitude	Longitude	Altitude (masl)
P. angustissimus						
PI535272‡	W	USA	New Mexico	32°26′N	106°34′W	1700
PI535273	W	USA	New Mexico	32°20′N	106°33′W	1700
P. filiformis						
PI535310‡	W	USA	Texas	31°46′N	106°29′W	NA§
PI535290	W	USA	Arizona	32°27′N	111°29′W	914
PI535309	W	USA	Arizona	33°36′N	111°12′W	518
PI535305	W	USA	Arizona	33°22′N	114°06′W	NA
PI535303	W	USA	Arizona	33°22′N	114°06′W	NA
PI535295	W	USA	Arizona	33°36′N	111°12′W	550
PI535306	W	USA	Arizona	33°27′N	111°29′W	792
PI535304	W	USA	Arizona	32°22′N	114°06′W	NA
PI535300	W	USA	Arizona	33°36′N	111°12′W	518
PI535507	W	MEX	Sonora	31°08′N	113°06′W	150
P. leptostachyus						
PI535336	W	MEX	Sonora	27°30′N	108°40′W	1460
PI535315‡	W	MEX	Jalisco	19°44′N	103°29′W	2000
PI535322	W	MEX	Zacatecas	23°29′N	103°36′W	2250
PI535317	W	MEX	Jalisco	19°43′N	104°13′W	1040
PI535334	W	MEX	S. L. Potosi	22°31′N	101°30′W	2088
PI535327	W	MEX	Oaxaca	16°49′N	96°20′W	1680
PI535330	W	GUA	Jalapa	14°40′N	89°51′W	1040
PI535331	W	GUA	Jalapa	14°40′N	89°56′W	1180
PI535324	W	MEX	Nayarit	21°25′N	104°56′W	1220
PI535328	W	MEX	Oaxaca	16°45′N	96°18′W	1480
P. microcarpus						
PI430196‡	W	MEX	Durango	NA	NA	NA
PI430197	W	MEX	Durango	NA	NA	NA
P. vulgaris						
G4017‡	C	BRA	Sao Paulo	NA	NA	NA
G19833	C	PERU	Amazonas	6°26′S	77°45′W	1860
G4494	C	COL	Valle del Cauca	3°30′N	76°21′W	965
BAT271	C	COL	Valle del Cauca	3°30′N	76°21′W	965

† MEX: Mexico, GUA: Guatemala, BRA: Brasil, COL: Colombia.
‡ Accessions included in both Exp. I and II.
§ NA, Information not available.

Germination Assay

Preliminary greenhouse experiments were conducted to establish techniques and to identify appropriate NaCl treatment levels (Bayuelo-Jiménez and Lynch, 1998). In this study, the *Phaseolus* accessions were evaluated for salt tolerance during germination and early seedling growth at 0, 60, 120, and 180 m*M* NaCl concentration (with electrical conductivity values of 0.2, 8.0, 15.0, and 19.0 dS m^{-1} and a water potential of the salt solution of −0.06, −0.31, −0.57, and −0.81 MPa, respectively). NaCl was used since it is a common salt that adversely affects plant growth under natural conditions, although single salt solutions are not found in nature (Bernstein, 1962). High levels of NaCl were used in the study to provide a range of germination responses from the control treatment and of selected accessions. Seeds were manually scarified by removing approximately 1 mm of the testa at the cotyledon with a scalpel. Before scarification, seeds were surface sterilized with 2500 mg L^{-1} sodium hypochlorite solution for 5 min, rinsed with sterile distilled water several times, and briefly blotted onto sterile paper towels. Seeds were germinated in covered, sterilized disposable Petri dishes 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) (0_2 permeable) to prevent evaporation of water, thus minimizing changes in concentration of solutions. In the first experiment, there were seven seeds in a Petri dish and four replications of each treatment (28 seeds/accession). A randomized complete block design with a split plot arrangement of treatments and four replications was used with NaCl levels as the main plots and accessions (as a group of seven seeds per dish) randomized within each main plot. In the second experiment, there were 14 seeds for each species in a Petri dish and eight replications of each treatment (112 seeds/accession). The experimental design was a factorial set of five accessions and four salinity levels arranged in a randomized complete block design. Experiments were conducted in a dark growth chamber. The mean temperature was 30 ± 0.5°C and relative humidity was 80% for both experiments. 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 d. On the 7th day, fresh weights of radicles and hypocotyls were measured. Subsequently the radicles and hypocotyls were dried at 60°C for 48 h, and weighed. Cotyledons were not included in fresh and dry weight comparisons, since they reflect imbibition rather than growth. When calculating the time of germination (i.e., 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.

Statistical Analysis

The effects of NaCl concentration on germination were compared by means of four response parameters: time to 25% germination (T25), time to 50% germination (T50), time to 75% germination (T75), and final percent germination. Analysis of variance (ANOVA) as well as rank correlation using Spearman's Coefficient of Rank Correlation were used to analyze the data for the response variables (Zar, 1996). Analysis of variance assumes normal distribution and common error variance. However, seed germination data are frequently skewed to the right and censored. Therefore, the data were analyzed by means of non-parametric methods by (i) assigning ranks to the entire set of observations within each block separately and (ii) analyzing the ranks as if they were the observed responses using the classical analysis of variance (Conover, 1999). When the differences were significant, least significant differences (LSD) were calculated on the ranked data. The fresh weight and dry weight of seedlings in the various treatments for each species and among species were tested for significant differences at 5% probability by a two-way analysis of variance (SAS, 1995). Treatment means were compared by the least significant difference (LSD) test at $P \leq 0.05$. Ward's minimum variance clustering method was used to classify accessions into discrete clusters (Romersburg, 1988). The optimum number of clusters was determined by the sum of squares index (E) (Romersburg, 1988).

RESULTS

Germination

Germination responses averaged over all accessions and species on the basis of T25, T50, T75, and final germination are summarized in Table 2. Mean days to 25, 50, and 75% germination were significantly delayed at 120 and 180 m*M* but not at 60 m*M* NaCl. Comparisons of mean average final germination at different salt concentrations indicated that the highest germination percentage was achieved at 60 (both experiments) and 120 m*M* NaCl (first experiment), which was significantly higher ($P < 0.01$) that the final germination under 180 m*M* salt stress (Table 2).

Germination responses measured as mean days to 50% germination (median germination, T50) and final germination percentage at 120 and 180 m*M* NaCl are presented in Tables 3 and 4. Germination responses to 60 m*M* NaCl were not significantly different than controls and so are not shown. In almost all accessions, the germination rate was delayed in response to salt stress (Tables 3 and 4). However, there were differential responses to salt stress within species (Table 3) and among

Table 2. Germination responses to NaCl levels averaged over all accessions (Exp. I) and across all species (Exp. II).

	Time to germination							
	T25†		T50†		T75†		Final Germination	
NaCl level (mM)	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II
	days						%	
0	0.82	0.61	0.95	0.74	0.99	0.84	93.8	93.9
60	0.92	0.77	1.08	0.78	1.15	1.05	97.3	92.1
120	1.17	0.84	1.32	0.97	1.40	1.75	95.9	89.5
180	1.82	1.11	1.89	1.18	2.25	1.76	82.1	84.4
$LSD_{0.05}$	0.12	0.08	0.18	0.16	0.21	0.21	7.40	3.97

† T25, T50, and T75 days to 25, 50, and 75% germination, respectively.

Table 3. Germination responses and Sensitivity Index (SI) for *Phaseolus* species at 0, 120, and 180 m*M* NaCl stress Exp. I.

		Salt concentration							
		0 m*M*		120 m*M*			180 m*M*		
Rank	Species	T50‡	Final Germ¶	T50	Final Germ	SI§	T50	Final Germ	SI
		d	%	d	%		d	%	
	P. angustissimus								
11	PI535272	0.67 ± 0.17	95	1.00 ± 0.20	96	1.6	1.55 ± 0.21	93	2.3
17	PI535273	0.75 ± 0.14	96	1.33 ± 0.17	100	1.7	>6# ± –	19	–
	P. filiformis								
1	PI535310	0.50 ± 0.00	96	0.50 ± 0.00	96	1.0	0.88 ± 0.17	96	1.8
2	PI535290	0.50 ± 0.00	96	0.63 ± 0.13	100	1.3	1.50 ± 0.35	89	3.0
3	PI535309	0.50 ± 0.00	100	0.63 ± 0.13	100	1.3	1.63 ± 0.54	82	3.4
4	PI535305	0.50 ± 0.00	100	0.75 ± 0.14	96	1.5	1.38 ± 0.13	93	2.8
5	PI535303	0.50 ± 0.00	100	0.75 ± 0.25	93	1.5	1.50 ± 0.11	96	3.0
6	PI535295	0.50 ± 0.00	100	0.88 ± 0.24	100	1.8	1.50 ± 0.41	93	3.0
8	PI535306	0.50 ± 0.00	93	0.88 ± 0.24	100	1.8	1.75 ± 0.22	100	3.5
9	PI535304	0.50 ± 0.00	100	1.00 ± 0.20	86	2.0	1.75 ± 0.25	80	3.5
10	PI535300	0.50 ± 0.00	100	1.00 ± 0.29	89	2.0	2.18 ± 0.45	82	4.4
20	PI535307	0.50 ± 0.00	100	1.88 ± 0.38	96	3.8	3.63 ± 0.84	17	7.4
	P. leptostachyus								
15	PI535336	1.00 ± 0.00	100	1.25 ± 0.25	96	1.3	2.75 ± 0.43	79	2.8
18	PI535315	1.25 ± 0.14	79	1.63 ± 0.13	71	1.4	2.63 ± 0.23	72	2.1
19	PI535322	1.38 ± 0.13	96	1.75 ± 0.25	79	1.3	3.08 ± 0.52	71	2.5
21	PI535317	1.50 ± 0.05	89	1.67 ± 0.17	75	1.1	4.13 ± 0.63	20	2.9
22	PI535334	1.25 ± 0.14	89	1.75 ± 0.14	89	1.5	3.18 ± 0.73	71	2.6
23	PI535327	1.25 ± 0.14	96	1.75 ± 0.14	93	1.5	3.01 ± 0.68	68	2.5
24	PI535330	1.63 ± 0.13	93	1.83 ± 0.44	89	1.1	3.38 ± 0.97	55	2.2
25	PI535331	1.50 ± 0.20	93	2.13 ± 0.31	82	1.4	4.00 ± 0.80	68	2.8
26	PI535324	1.25 ± 0.14	86	2.00 ± 0.20	82	1.7	4.64 ± 0.94	19	3.8
27	PI535328	1.25 ± 0.14	86	2.50 ± 0.20	86	2.0	4.83 ± 1.19	17	3.9
	P. microcarpus								
13	PI430196	0.50 ± 0.00	96	1.25 ± 0.25	100	2.5	1.50 ± 0.25	91	3.0
28	PI430197	0.83 ± 0.17	86	>6#	72	–	>6# –	53	–
	P. vulgaris								
7	G4017	1.13 ± 0.13	90	1.00 ± 0.20	92	0.9	1.88 ± 0.13	88	1.7
12	G19833	1.13 ± 0.13	100	1.00 ± 0.20	100	0.9	2.33 ± 0.13	86	2.2
14	G04494	1.75 ± 0.25	93	1.25 ± 0.14	100	0.8	3.63 ± 0.38	77	2.1
16	BAT271	1.13 ± 0.13	100	1.38 ± 0.13	100	1.3	2.88 ± 0.27	89	2.6

‡ Days to 50% germination averaged over four replications ± SE.
¶ Percent germination from a total of 28 seeds.
# Seeds did not reach 50% germination at 6 d.
§ Ratio of median response at 120 or 180 m*M* NaCl to median response at 0 m*M*.

species (Table 4). For example, seven accessions of *P. filiformis* were the most salt tolerant at 120 m*M*, reaching 50% germination in less than 1 d, whereas accessions of *P. leptostachyus* were the most salt sensitive, requiring more than 1.5 d to reach the same germination percentage. Seeds of *P. microcarpus* accession PI430197 did not reach 50% germination at 120 m*M* NaCl (Table 3), whereas three cultivated accessions of *P. vulgaris* reached 50% germination in the same or less time than control treatment.

The mean time to germination of almost all *Phaseolus* species increased with the addition of NaCl (Fig. 1 and 2). The increase in median germination time was greater at 180 m*M* than 120 m*M* NaCl, and higher in *P. leptostachyus* than *P. vulgaris*, *P. angustissimus*, and *P. micocarpus*. In the second experiment, median germination response of *P. filiformis* was not significantly affected at any of the NaCl levels (Fig. 2).

With regard to final germination percentage, *P. angustissimus*, *P. filiformis* accessions and four cultivated

Table 4. Germination responses and Sensitivity Index (SI) of *Phaseolus* species at 0, 120, and 180 m*M* NaCl. Exp. II.

		Salt concentration							
		0 m*M*		120 m*M*			180 m*M*		
Rank	Species	T50‡	Final Germ¶	T50	Final Germ	SI§	T50	Final Germ	SI
		d	%	d	%		d	%	
2	*P. angustissimus* PI535272	0.57 ± 0.12	96	0.75 ± 0.13	93	1.3	1.16 ± 0.29	93	2.0
1	*P. filiformis* PI535310	0.50 ± 0.00	98	0.50 ± 0.00	99	1.0	0.50 ± 0.00	95	1.0
5	*P. leptostachyus* PI535315	1.28 ± 0.31	89	1.78 ± 0.33	84	1.5	2.10 ± 1.36	64	1.8
3	*P. microcarpus* PI430196	0.53 ± 0.09	93	0.86 ± 0.13	86	1.6	0.89 ± 0.13	69	1.7
4	*P. vulgaris* G4017	0.78 ± 0.08	100	0.98 ± 0.08	95	1.3	1.22 ± 0.31	84	1.6

‡ Days to 50% germination (T50) averaged over eight replications ±SE.
¶ Percent germination from a total of 112 seeds.
§ Ratio of median response at 120 or 180 m*M* NaCl to median response at 0 m*M*.

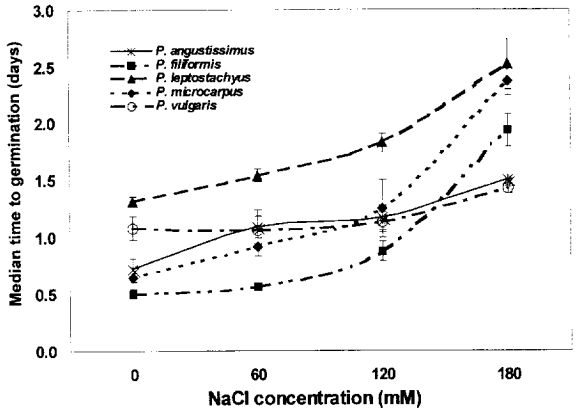

Fig. 1. The effect of increasing NaCl concentration on median germination time (T50) of *Phaseolus* species (averaged over all accessions). Data shown as mean ± standard error of the mean of four replicates, each replicate is the average of seven seeds. ANOVA indicated that median germination response was influenced by species at 0 m*M* ($F = 70$, $P \leq 0.0001$), 60 m*M* ($F = 44.1$, $P \leq 0.0001$), 120 m*M* ($F = 18$, $P \leq 0.0001$), and 180 m*M* ($F = 3.5$, $P \leq 0.0179$) NaCl concentration. Exp. I.

accessions were relatively salt tolerant, exceeding 90% germination at 120 m*M* NaCl (Table 3). In contrast, some accessions of *P. leptostachyus* and one accession of *P. microcarpus* had <90% germination at 120 m*M* NaCl (Table 3).

Percent germination within *Phaseolus* species varied at the highest level of salt stress (Table 3). The 180 m*M* NaCl treatment markedly reduced final germination percentage in *P. angustissimus* accession PI535273, *P. filiformis* accession PI535307, and *P. leptostachyus* accessions PI535317, PI535324, and PI535328 (Table 3). Final germination for *P. angustissimus* and *P. filiformis* averaged 94 and 97% over all salinity levels (Table 4), and the germination of *P. vulgaris* was reduced from 100 to 84% when salinity was increased from 0 to 180 m*M* salt stress. The final germination of *P. leptostachyus* and *P. microcarpus* decreased from 89 and 93% in a salt-free medium to 64 and 69% at 180 m*M* salt stress,

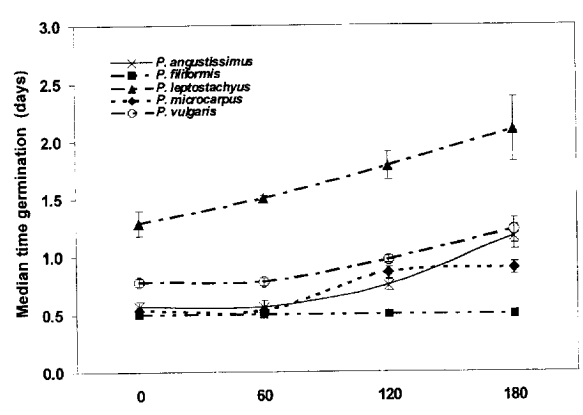

Fig. 2. The effect of increasing NaCl concentration on median response time (T50) of *Phaseolus* species. Data shown as mean ± standard error of the mean of eight replicates, each replicate is the average of 14 seeds. ANOVA indicated that median germination response was influenced by species at 0 m*M* ($F = 17.2$, $P \leq 0.0001$), 60 m*M* ($F = 20.5$, $P \leq 0.0001$), 120 m*M* ($F = 9.2$, $P \leq 0.0018$), and 180 m*M* ($F = 23$, $P \leq 0.0003$) NaCl concentration. Exp. II.

respectively. Germination responses to salinity were similar in both experiments.

Ranking

Overall comparisons of 28 accessions indicated significant differences at 0 and 120 m*M* NaCl. At 120 m*M* NaCl, 6 *P. filiformis* (PI535310, PI535290, PI535309, PI535305, PI535303, and PI535295) germinated most rapidly, followed by G4017 (*P. vulgaris*), and PI535272 (*P. angustissimus*) (Table 3). All remaining accessions ranked significantly lower in performance, with median responses greater than 1.5 d. In the absence of NaCl, the fastest germination times were exhibited by all accessions of *P. filiformis* and PI430196 (*P. microcarpus*) (Table 3).

Estimates of the relative stability of germination (sensitivity index) of individual accessions were made by examining the relative increase in median response time with the addition of NaCl (Table 3). The cultivated accessions G4017 and G19833 showed the smallest increase in median response time under 120 m*M* NaCl salt stress (12% sensitivity index) (Table 3). These were closely followed by *P. filiformis* accessions PI535310, PI535390, and PI535309 (0–26%), and *P. leptostachyus* accession PI535336 (25%) (Table 3). These accessions germinated most rapidly and showed the greatest stability in response.

The second experiment was consistent with the first (Table 4). In the absence of NaCl, the fastest germination times were exhibited by *P. filiformis*, *P. angustissimus*, and *P. microcarpus*. At 120 m*M* NaCl, *P. filiformis* germinated significantly more rapidly than any other species (Table 4), followed by *P. angustissimus*, *P. microcarpus*, *P. vulgaris*, and *P. leptostachyus*. At 180 m*M* NaCl, *P. filiformis* germinated first (Table 4). Ranked germination times for *P. microcarpus*, *P. angustissimus*, and *P. vulgaris* were not significant, but they were significantly less than *P. leptostachyus* at 180 m*M* salt stress. *Phaseolus filiformis* consistently ranked as the top species in the absence or presence of salt.

Phaseolus filiformis germinated most rapidly and had the greatest stability in response (0% sensitivity index) (Table 4). The cultivated *P. vulgaris* and the wild accessions *P. angustissimus* and *P. leptostachyus* (26–39%), showed smaller increase in response time than *P. microcarpus* (63%) (Table 4). Ranking in this fashion was similar to median germination response, to the extent that both indices ranked *P. vulgaris* and *P. angustissimus* as good performers (2nd and 3rd). However, when ranked by the stability measure, the relative ordering of accessions changed.

Correlation

Correlation analyses indicated that in all salt treatments, the time to 25, 50, and 75% germination were highly correlated (Table 5). In general, accessions that germinated rapidly at lower salt concentrations also germinated rapidly at higher salt concentrations (Table 5). In both experiments, Spearman rank correlations between median germination times were significant be-

Table 5. Spearman rank correlation between the time to 25, 50, and 75% germination quartiles for bean seed at four salt stress levels. Exp. I and II.

		Germination quartile			
		50%		75%	
Germination quartile	NaCl level mM	Exp. I	Exp. II	Exp. I	Exp. II
25%	0	0.81†	0.68	0.77	0.65
	60	0.81	0.94	0.69	0.72
	120	0.77	0.77	0.80	0.45
	180	0.81	0.47	0.77	0.77
50%	0			0.91	0.87
	60			0.68	0.74
	120			0.83	0.48
	180			0.87	0.42

† All correlation coefficients were significant at $P \leq 0.01$.

tween 60 and 120 m*M* (r^2 = 0.66–0.79) and between 60 and 180 m*M* (r^2 = 0.58–0.30). Similarly, there was a significant correlation occurred in both experiments between median response time rank at 60 and 120 m*M* NaCl and the rank observed in the absence of NaCl (data not shown).

Seedling Growth

Salt stress inhibited hypocotyl growth more than radicle growth (Table 6). The magnitude of reduction was highly dependent upon the species and NaCl concentrations. Hypocotyl fresh weight was significantly reduced in all accessions at all salinity levels (60–180 m*M*), whereas radicle fresh weight was reduced only at higher salt levels (Table 6). At either salt concentration, analysis of variance of seedling fresh weights indicated highly significant differences among and within species. Among species, total fresh weight of *P. microcarpus* (second experiment) (data not shown) and *P. leptostachyus* (both experiments) accessions were significantly lower at 60 m*M* NaCl, whereas considerably less inhibition was observed in other species (Table 6). Within species, there were significant differences in tolerance to salinity. Seedlings of PI535273 (*P. angustissimus*), PI535334, PI535336 (*P. leptostachyus*), PI535304 and PI535305, PI535310 (*P. filiformis*), and PI430196 (*P. microcarpus*) had the highest total fresh weight under 120 m*M* salt stress compared to other accessions (data not shown). These results are in agreement with those results obtained during germination. A faster rate of germination allowed the emerging seedlings to accumulate more biomass relative to the control (Table 3). Conversely, total fresh weight of cultivated accessions was significantly reduced with increased salt stress. Therefore, even though some cultivated seeds germinated rapidly (i.e., G4017 and G19833) in high NaCl concentrations, vigorous seedlings did not develop. Even at the lowest concentration of salt (60 m*M* NaCl), all four cultivated accessions had severely reduced seedling growth relative to the control.

Table 6. Early seedling growth of *Phaseolus* species after 7 d of growth under NaCl stress. Exp. I.

		Fresh weight			Dry weight		
Species‡	NaCl	Radicle	Hypocotyl	Total	Radicle	Hypocotyl	Total
	m*M*	mg seedling^{-1}					
P. angustissimus (2 accessions)	0	208	725	933	8.4	26.5	34.5
	60	245	639	884	8.1	18.6	27.1
	120	231	325	556	6.7	10.7	16.1
	180	118	139	257	4.7	9.4	15.4
	$LSD_{0.05}$	119	84	279	4.3	8.3	12.4
P. filiformis (10 accessions)	0	153	797	950	5.0	26.3	31.4
	60	167	650	818	5.2	23.8	29.1
	120	151	449	600	5.1	15.9	21.0
	180	69	119	189	3.0	6.7	9.8
	$LSD_{0.05}$	21.2	112	130	0.5	2.4	2.8
P. leptostachyus (10 accessions)	0	68	338	407	2.6	11.1	13.7
	60	70	251	301	2.5	9.4	11.9
	120	58	199	187	1.9	5.0	7.0
	180	23	34	57	0.8	1.8	2.6
	$LSD_{0.05}$	11	58	64	0.26	0.91	0.11
P. microcarpus (2 accessions)	0	135	649	785	3.4	18.9	22.4
	60	138	576	714	4.2	23.2	27.4
	120	105	355	459	3.9	14.8	18.7
	180	39	114	153	1.6	3.6	5.3
	$LSD_{0.05}$	30	259	259	0.8	2.2	2.5
P. vulgaris (4 accessions)	0	2040	7181	9225	78.5	305.7	384.3
	60	1794	4363	6156	78.8	240.3	316.1
	120	1434	2752	4183	62.6	140.5	207.9
	180	500	1138	1638	26.1	86.9	113.0
	$LSD_{0.05}$	473	1592	2028	7.6	33.1	38.7

LSD: Least Significant Difference at $P \leq 0.05$.

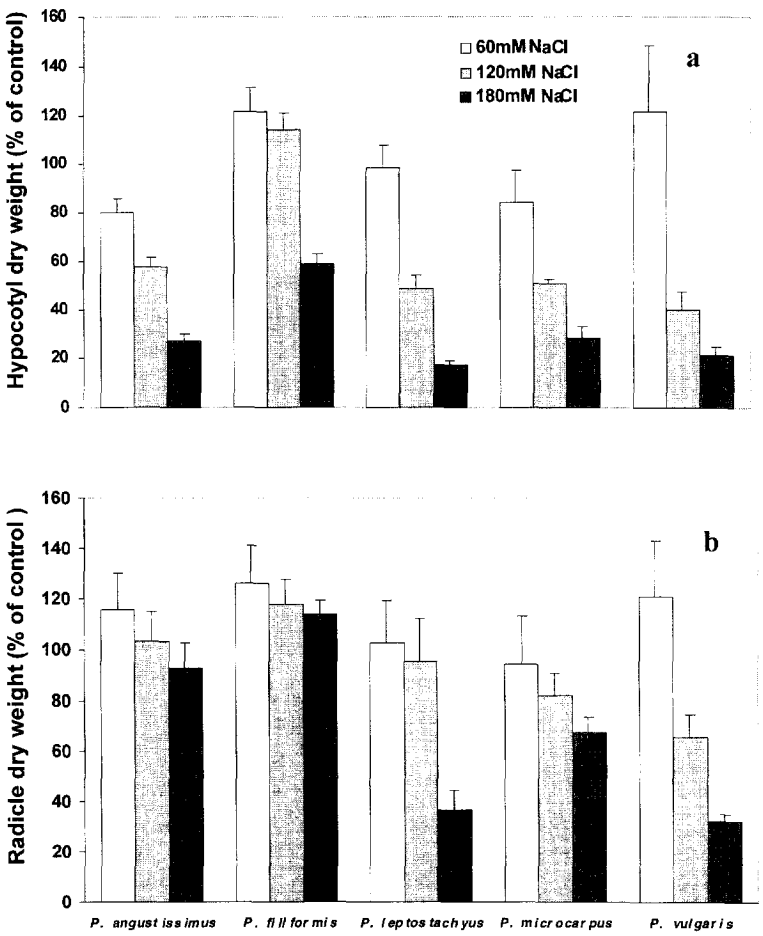

Fig. 3. Effects of salinity on (a) hypocotyl and (b) radicle dry weight of *Phaseolus* species. Data shown as mean ± standard error of the mean of eight replicates. Experiment II.

NaCl stress significantly reduced seedling dry weight. A significant species–salinity interaction indicated that species responded differentially to salinity stress (Table 6). The accessions PI535303 and PI535310 (*P. filiformis*), PI535336 (*P. leptostachyus*), and PI430196 (*P. microcarpus*) showed the lowest reduction of hypocotyl dry weight, less than 20% relative to the control, and no reduction of radicle dry weight under 120 m*M* salt stress level. At 180 m*M* salt stress, however, seedling dry weight was reduced 50% or more in all accessions. Differences in salt tolerance at 180 m*M* NaCl were found only in PI535303, PI535304, and PI535310 (*P. filiformis*), which exhibited a reduction of about 20% in radicle dry weight and 60% in hypocotyl dry weight (data not shown). Surprisingly, in the second experiment, *P. filiformis* accession PI535310 exhibited a significant increase in the radicle dry weight (13% of the control value) and a decrease of about 40% in the hypocotyl dry growth at 180 m*M* salt stress (Fig. 3a–b). These accessions were the most tolerant, as indicated by significantly faster germination, relative stability and greater seedling growth.

The difference in seedling growth among wild and cultivated accessions, as shown by the absolute seedling growth (Table 6), could be related species differences in seed size. The correlation of seedling growth and seed size was significant ($r^2 = 0.98$, $P < 0.01$) at 120 m*M* NaCl, with cultivated accessions having the largest seeds and the highest seedling growth under salt stress. Conversely, seedling growth and salt tolerance of small-seeded species was not correlated with seed size ($r^2 = 0.17$, $P < 0.01$).

Cluster Analysis

Ward's clustering technique clearly defined clusters on the basis of final germination, median germination response (T50), sensitivity index, and seedling biomass at 120 m*M* NaCl. The accessions grouped into three clusters on the basis of distance ranges for the tree shown in Fig. 4. To determine the optimum number of clusters, we used the sum of square index, E (Romersburg, 1988) and had a choice of six clusters for classification considering the width of the range E (Fig. 4).

Cluster I and II included 11 and 13 accessions, respectively, whereas Cluster III had two accessions in each of two subgroups. Cluster means of different quantitative variables of individual clusters are presented in Table 7. Cluster I was characterized by accessions with intermediate final germination (86%), late germination response,

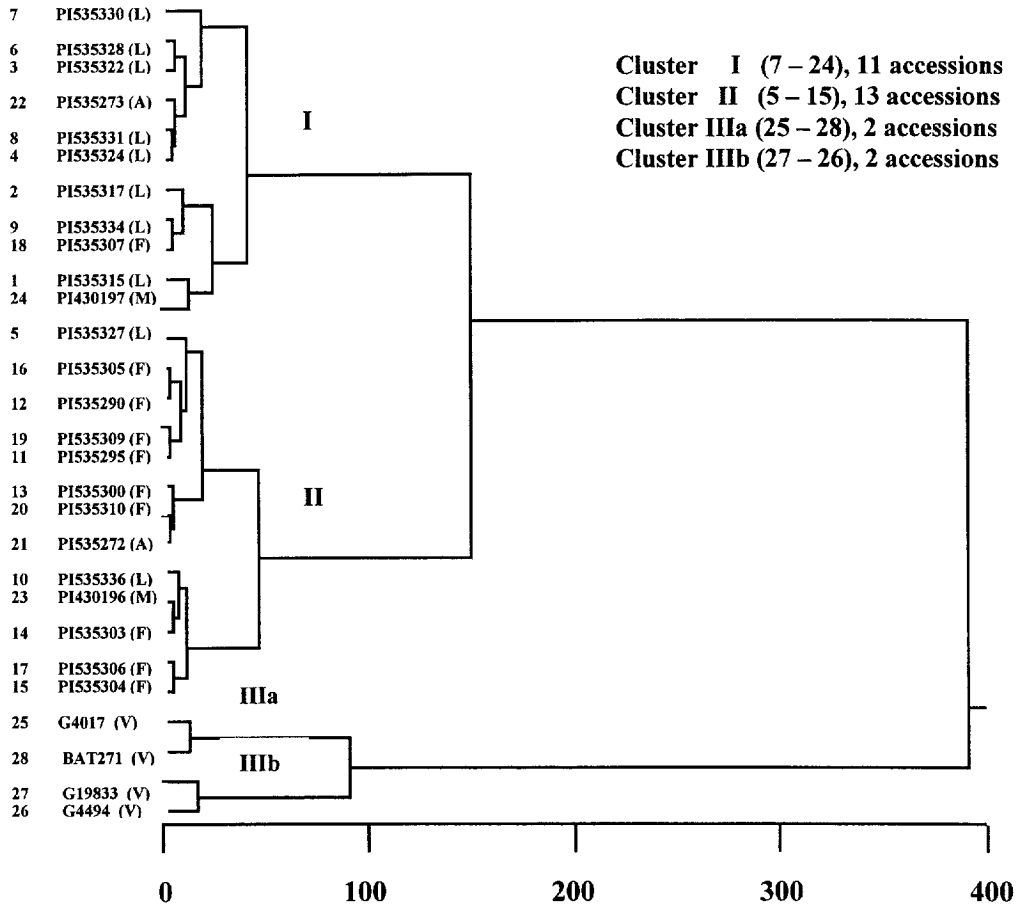

Fig. 4. Ward's Minimum Variance Dendrogram of 24 wild *Phaseolus* accessions and four cultivated accessions of common bean. A = *P. angustissimus*, F = *P. filiformis*, L = *P. leptostachyus*, M = *P. microcarpus*, and V = *P. vulgaris*. Exp. I. Optimum number of clusters was determined with the sum of square index, E.

high sensitivity index, and reduced biomass of radicle and hypocotyl (47%). These species were the most sensitive to salinity stress. In contrast, accessions of *P. filiformis*, one accession of *P. microcarpus* (PI430196), and one accession of *P. angustissimus* (PI535272) in Cluster II were characterized by high final germination (97%), rapid germination, high sensitivity index, and high biomass accumulation in radicle and hypocotyl. These species, therefore, were the most tolerant to salinity stress. Cluster III comprises four cultivars of common bean, *P. vulgaris*. This group was divided in two subgroups. Subgroup *a* includes accessions G4017 and BAT271, which belong to the Mesoamerican gene pool. These cultivated accessions had a high final germination, small increase in median time to germination, and low sensitivity index. However, they had reduced growth of radi-

Table 7. Quantitative variables used in the identification of the different clusters Exp. I. Data are mean ± SE of four replicates.

	Cluster			
			III	
Variable	I	II	Subgroup a	Subgroup b
Final germination (%)§	85.5 ± 4.37	96.9 ± 0.96	100 ± 2.78	100 ± 2.38
Median germination (T50)	1.8 ± 0.14	0.97 ± 0.11	1.1 ± 0.27	1.1 ± 0.13
Sensitivity Index‡	1.7 ± 0.20	1.8 ± 0.21	1.1 ± 0.19	0.8 ± 0.06
Radicle dry weight (mg)	2.6 ± 0.52	4.8 ± 0.35	37.3 ± 2.11	87.8 ± 12.8
Radicle dry weight (%)†	64.2 ± 6.11	114.3 ± 4.37	69.9 ± 10.5	88.8 ± 16.9
Hypocotyl dry weight (mg)	6.1 ± 1.18	15.5 ± 0.97	103.0 ± 10.0	177.1 ± 1.45
Hypocotyl dry weight (%)†	41.0 ± 4.37	68.6 ± 3.19	41.3 ± 0.41	49.2 ± 0.47
Total dry weight (mg)	8.8 ± 1.52	20.3 ± 1.23	140.4 ± 12.1	264.9 ± 11.3
Total dry weight (%)†	47.1 ± 4.12	75.6 ± 3.01	46.3 ± 1.90	57.6 ± 2.92

§ Percent germination at 102 m*M* NaCl.
‡ Ratio of median response at 120 m*M* NaCl to median response at 0 m*M*.
† Radicle, hypocotyl, and total dry weight at 120 m*M* NaCl relative to growth under non-stress conditions.

cle and hypocotyl under stress. Subgroup *b* includes accessions G19833 and G4494, both of the Andean gene pool. These cultivated accessions had high final germination, small increase in median time to germination, the lowest sensitivity index, and a high accumulation of biomass of radicle compared with hypocotyl. Total biomass accumulation, however, was moderately affected at 120 m*M* NaCl.

DISCUSSION

Salinity stress can affect seed germination through osmotic effects (Welbaum et al., 1990) or by ion toxicity (Bliss et al., 1986; Huang and Reddman, 1995). Physiological studies to distinguish between the two effects are limited (Bliss et al., 1986), but evidence suggests that low water potential of the germination medium is a major limiting factor (Bradford, 1995). 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 nonstress conditions. On the basis of these two criteria, our results demonstrated genetic variation in seed germination and early seedling growth responses to salinity among and within wild *Phaseolus* species. This study indicated that *P. angustissimus* and *P. filiformis* had superior germination performance at low and moderate levels of salt stress. The performance of *P. filiformis* accessions PI535310, PI535290, PI535209, PI535303, and PI535205 is favorable. These accessions were identified as the most tolerant, being able to germinate rapidly under both control (no stress) and salt stress conditions. These accessions ranked well in germination response time and together with PI535272 had the smallest relative increase in median germination time under stress (Tables 3 and 4). A high correlation ($r^2 = 0.55$) between median germination time at 120 and 0 m*M* indicated that germination processes that facilitate rapid germination under salt and nonstress conditions possibly were controlled by similar genetic and physiological mechanisms (Foolad, 1996). Conversely, several species such as *P. filiformis* accession PI535307 and *P. microcarpus* accession PI430196 germinated rapidly under control conditions but germinated poorly at the highest salt stress levels, thus exhibiting high sensitivity indices. Consequently, in 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 Lin, 1997). Evaluation of selected accessions at the three salt-stress levels demonstrated that accessions that germinated rapidly at lower salt concentrations also germinated rapidly at higher salt concentrations (Tables 3 and 4). This result was consistent with the high correlation ($r^2 = 0.79$) found between germination at 60 and 120 m*M* NaCl, suggesting similarity of genes contributing to rapid germination at different salt stress levels.

At the lowest NaCl concentration (60 m*M*), all accessions reached 50% germination before the end of the experiments (6 d); however, at 180 m*M* NaCl, two accessions did not reach 50% germination by 6 d (Table 3). Although many accessions were not able to germinate rapidly under the highest salt-stress level (180 m*M*), this concentration is higher than what is observed in most saline agricultural soils (75-150 m*M*; Bernstein, 1962).

Wild *P. filiformis* accessions were the most tolerant to salinity stress, as indicated by rapid germination, relative stability, and greater seedling growth. In contrast, accessions PI535336 (*P. leptostachyus*) and PI535272 (*P. angustissimus*) and cultivars G4017 and G19833 (*P. vulgaris*), in which germination response was very stable, were less salt tolerant in terms of early seedling growth. These results demonstrate that tolerance to salinity in *Phaseolus* species might also vary with developmental stages. Mano et al. (1996) reported that salt tolerance at germination was independent of salt tolerance at the seedling stage in 6646 barley genotypes. This phenomenon has also been reported for rice (*Oryza sativa* L.) (Heenan et al., 1988), wheat (*Triticum aestivum* L.) (Maas and Poss, 1989), and tomato (*Lycopersicon esculentum* Mill.) (Foolad and Lin, 1992). 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 barley (*Hordeum vulgare* L.) (Huang and Reddman, 1995), tomato (*Lycopersicon*) (Foolad, 1996), pigeon pea (*Cajanus 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.

It is noteworthy that the tolerance range of *P. filiformis* to salinity is high considering the fact that legumes are rated as salt sensitive (Maas and Hoffman, 1977). The common bean (*P. vulgaris*) and mung bean (*Vigna radiata*), for example, suffer yield losses at soil salinity less than 2 dS m^{-1}. Fifty millimolar NaCl reduced bean growth nearly 50% (Imamul and Larher, 1983), whereas 100 m*M* NaCl reduced seed germination 50% (Singh and Singh, 1971). In our study, several accessions of *P. filiformis* tolerated salinity levels higher than 120 m*M* NaCl during germination and early seedling growth. These results are comparable with the tolerance range found in a wild relative of pigeonpea. *Atylosia albicans* (W. & A) Benth was able to maintain growth at $\leq$ 12 dS m^{-1} equivalent to 100 m*M* NaCl without salt toxicity symptoms (Subbarao et al., 1991). Paliwal and Maliwal (1980) reported the seed of mung bean (*Vigna radiata* Wilczek.) could tolerate 6 dS m^{-1} salinity equivalent to 53 m*M* NaCl comparable to sorghum [*Sorghum bicolor* (L.) Moench.] (6 dS m^{-1}) and pearl millet [*Pennisetum americanum* (L.) Leeke] (9 dS m^{-1}).

Our results suggest a relationship between salinity

tolerance and the geographic origin of accessions. In the cluster analysis, *P. filiformis* and accession PI535272 (*P. angustissimus*) (group II), which have similar geographical origin, clustered together. These species are distributed in dry habitats of southwestern USA and northwestern Mexico (Buhrow, 1983). 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 salt-tolerant vegetation. Salinity of these regions is mostly associated with soils derived from soft shale, siltstones, lake-bed deposits and valley alluvium formed from ancient saline seas (Schmidt, 1989).

Phaseolus filiformis occurs sprawling on beach vegetation at elevations from 0 to 1600 m. (Delgado-Salinas, 1985). This species is characterized by its indeterminate annual habit, although many plants grow in the winter, become semidormant, and regrow in the summer before dying. *Phaseolus filiformis* is adapted to extremely arid conditions and is able to grow in environments receiving less than 100 mm rainfall each year, with air temperatures reaching 38°C, and soils with high pH (Buhrow, 1983). The adaptation of this species to truly arid zones seems to be associated with its rapid seed germination, rapid growth, continual flowering, and maturity during favorable periods (Buhrow, 1983).

Phaseolus angustissimus is another salt tolerant species, which generally grows in semidesert regions along dry rivers or creek beds, or on igneous or calcareous rocky hillsides, under pine-oak forest at elevations from 1130 to 2500 m (Buhrow, 1983). This species is distinguished by its indeterminate perennial habit, waxy leaves, woody tap root, explosive dehiscent pods, and rugose seed testa. These characteristics together with its rapid germination and high seedling growth are probably specializations to survive in xeric environments.

It is interesting to note that small seeds and a rugose seed testa distinguish both species. According to Debouck (1999), the rugose testa of these species is an adaptation to increase the contact between the seed and the soil, and thereby promote quick germination. In general, wild *Phaseolus* species are characterized by an irregular seed surface, testa thickness and small seed size, as well as a high quantity of protein and few starch granules (Murray, 1984). The hard and thick testa and few starch granules may be related to the mechanisms of adaptation to wild conditions. Under natural conditions, erratic precipitation causes rapid imbibition of cultivated species with thin testa, thereby initiating rapid hydrolysis of starch granules and emergence. On the contrary, this situation does not arise in the wild species with high protein content and late metabolism of protein, leading to delayed germination. Therefore, the thickness and high protein content of rugose seed testa are possible adaptive mechanisms to the natural habitat (Moreno et al., 1993).

The accessions which make up group I in the cluster analysis correspond to the salt-sensitive species of *P. leptostachyus* and *P. microcarpus*. These species grow in tropical and temperate subhumid climates, on rocky or sandy soils associated with tropical deciduous, evergreen seasonal, and oak-pine forests of the Pacific slope of western Mexico (Delgado-Salinas, 1985). Although the climatic and environmental range of both species is wide, the geographical distribution of the selected accessions seems not to be associated with the pattern of incidence of hot semiarid climates and saline soils.

The accessions, which make up groups IIIa and IIIb correspond to the Mesoamerican and Andean gene pool, respectively. These cultivated accessions are mostly distinguished by a rapid germination, moderate sensitivity index, and an intermediate seedling growth. The available range of variability for salinity tolerance in these accessions could come largely from seed size. Larger seeded species have more seed reserves to support seedling growth during stress periods. A high correlation coefficient ($r^2 = 0.98$) between seedling growth and seed size confirm that cultivated accessions having the largest seeds, exhibiting the greatest seedling growth under salt stress. 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 during germination and early seedling were not tolerant during vegetative growth (Bayuelo-Jiménez, 2001). Thus, particular species may be differentially affected at various physiological stages of development and may not produce tolerant adult plants. The establishment of patterns and classification of genetic diversity within the wild germplasm surveyed through the employment of cluster analysis has enabled clear groupings to be identified. The resulting information will be useful in improving the understanding of the diversity of wild *Phaseolus* species. The morphological characters underlying these 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 *Phaseolus* species. Wild *Phaseolus* species, and in particular *P. filiformis*, represent a genetic resource for improvement of salt tolerance of common bean.

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