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Tolerance of Tepary and Navy Beans to NaCl During Germination and Emergence

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Abstract. **Seeds of two tepary bean lines *(Phaseolus acutifolius* Gray var. *latifolius)* and one navy bean cultivar *(P. vulgaris* L. 'Fleetwood') were tested with 0.0-, – 0.3-, –0.6-, -0.9-, -1.2-, or – 1.5-MPa NaCl solutions to determine their relative salt tolerance during germination and emergence. Developmental stage was not affected at – 0.3 MPa, but with salinities more negative than -0.9 MPa, 'Fleetwood' developed more slowly than the tepary lines; no plants emerged at – 1.5 MPa. Teparies tended to maintain higher water and osmotic potentials than navy over the range of NaCl concentrations used, although turgor was similar for all three genotypes. Leaf area was reduced more in navy than in white tepary at – 0.6 and – 0.9 MPa. Dry weights of navy were higher than those of either tepary bean at all NaCl concentrations, although decreases at higher salinities relative to 0.0 MPa were greater for navy than for teparies. Root : shoot ratios were higher at – 0.3 MPa than at 0.0 MPa, but were lower at the higher NaCl concentrations for all three genotypes. Overall, tepary beans tolerated NaCl better than navy. The characteristic that best indicated differences in salt tolerance was developmental stage.**

Hendry (1918) observed that the tepary bean tolerated salinity better at vegetative and reproductive stages than other *Phaseolus* species tested. Marcarian (1981) also suggested a greater salt tolerance of tepary compared to common bean in field plantings, although levels of salinity were not part of the experimental design. Perez and Minguez (1985) observed a greater salt tolerance in plant vegetative growth and seed yield of tepary, as compared to that previously reported for common bean (Ayers and Westcot, 1976; Bernstein and Hayward, 1957; Gelburd, 1985; Lorenz and Maynard, 1988; Maas, 1986). However, none of these studies focused on salt tolerance of tepary relative to navy during germination and emergence, a time when the plant may be exposed to extremely high salt in the soil because salts often concentrate in the upper soil surface. For this reason, use of growth characteristics during early development as first indicators of salt tolerance is necessary (Norlyn and Epstein, 1984). In an earlier study (Goertz and Coons, 1989), tepary had higher germination percentages, rates, and seedling fresh weights than *Phaseolus vulgaris* at 0.0 to – 2.5 MPa NaCl. Whether tepary would exhibit greater vigor than navy in establishing a seedling in a saline environment was not tested. Salt tolerance from imbibition through seedling emergence does not necessarily coincide with tolerance at seed germination, as shown for carrot, chili pepper, tomato, and guayule (Miyamoto et al., 1985). Seedling vigor at stand establishment is important in any environment, but especially in semi-arid saline areas where soils often crust at the surface and where high temperatures compound salt problems.

The objectives of this study were to: 1) compare the NaCl tolerance during germination and emergence of two *Phaseolus* species, one extremely salt sensitive (common bean) and the other reportedly salt tolerant (tepary), and 2) determine some developmental and physiological characteristics that distinguish differences in salt tolerance of the two species.

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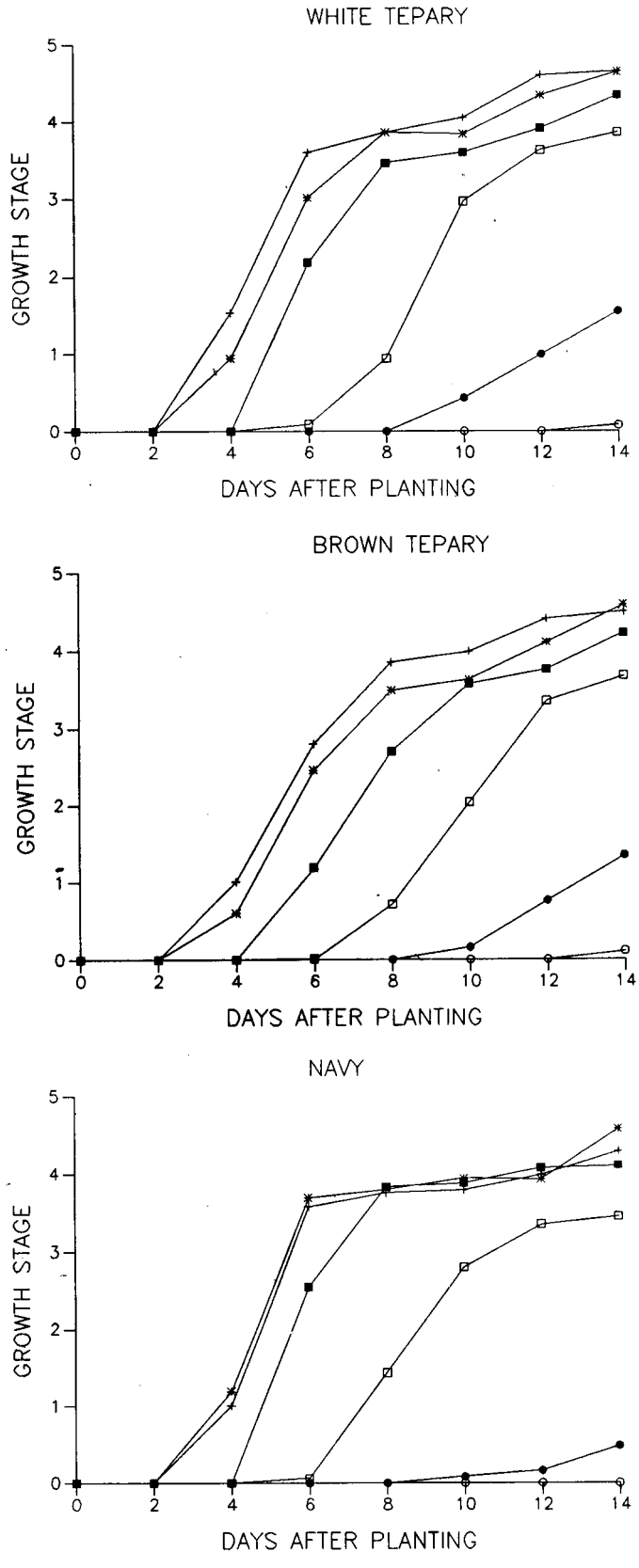

Fig. 1. Developmental growth stages of white and brown tepary bean lines and of one navy bean cultivar over 14 days for six NaCl solutions. 0 = no emergence, 1 = hypocotyl hook visible, 2 = cotyledonary leaves above surface of vermiculite, 3 = stem straight with primary leaves beginning to unfold, 4 = full expansion of primary leaves, and 5 = expansion of first true leaves, generally trifoliolate. (+ – +) 0.0 MPa, (* – *) –0.3 MPa, (■ – ■) –0.6 MPa, (❑ – ❑) –0.9 MPa, (● – ●)-l.2MPa, (❍ – ❍) -1.5 MPa.

Brown (UN ACC2) and white (UN ACC1) bean were used. Tepary beans were sized for tepary bean accessions and 'Fleetwood' navy uniformity, although some variation remained. Seeds of navy beans were uniform. Seed weights averaged 116, 133, and 198 mg/seed for white tepary, brown tepary, and navy, respectively. Seeds were dusted with tetramethylthiuram disulfide (thiram) fungicide before planting.

Aqueous solutions of NaCl were prepared with osmotic potentials of 0.0, – 0.3, –0.6, –0.9, – 1.2, or – 1.5 MPa (0.0, 3.5, 7.1, 10.6, 14.2, or 17.7 $g \cdot liter^{-1}$, respectively) established with a vapor pressure osmometer.

Fine vermiculite (3 liters of Terra-lite; Grace Horticultural Products, Cambridge, Mass.) was added to 1.5 liters of NaCl solution in 27 × 19 × 6-cm plastic trays (Carolina Scientific, Burlington, N.C.). Seeds were planted in six rows with 10 holes/row and 2.5 cm between seeds. Seeds of each genotype were placed 2.5 cm deep in two of the six rows, with all three genotypes in each tray, and covered with vermiculite. Trays then were sealed in plastic bags and placed in a growth chamber at 25 ± 2C and relative humidity of 46% ± 6%. A pan of water in the bottom of the chamber was kept filled to help stabilize humidity when seedlings began to emerge. Chamber irradiance averaged 320 ± 40 $\mu mol \cdot s^{-1} \cdot m^{-2}$ during the 16-h photoperiod.

The experiment was a randomized complete-block design, with each block containing 20 seedlings of each genotype-salinity combination. Each tray contained all three genotypes sown in two adjacent rows whose location was randomized, as was the placement of trays in the growth chamber. Four replications of the 14-day experiment were conducted over 2 months. Data from all four replicates were combined for analysis of variance. Least significant differences at the 0.05 level were calculated to compare genotypes. Regression equations were calculated for each genotype using all NaCl concentrations.

Trays were weighed every other day, and the weight loss was replaced with distilled water that was sprinkled lightly over the entire surface to minimize local dilution of salt. The amount of replacement water varied for different salt concentrations and with time. The six trays, one for each salt level, were rotated every 2 days in the chamber.

Developmental stages of seedlings were recorded every 2 days using the following scale: 0 = no emergence, 1 = hypocotyl hook visible, 2 = cotyledonary leaves above surface of vermiculite, 3 = stem straight with primary leaves beginning to unfold, 4 = full expansion of primary leaves, and 5 = expansion of first trifoliolate leaves. Seedlings were harvested after 14 days.

Measurements of water potential and osmotic potential, as well as calculation of turgor, were made on three plants per treatment using two of the four replications. Two disks per plant from the middle of expanding primary leaves were punched at 0900 HR 14 days after planting and 2 days after the last watering. Disks were placed immediately into Merrill 75-13 psychrometer chambers (Merrill Specialty Equipment, Logan, Utah) that were placed in a 25C water bath to equili-

Table 1. Developmental stage[z] of two tepary bean lines and of one navy bean cultivar at 14 days after growth in NaCl solutions of increasing salinity, expressed as osmotic potential.

Genotype	Osmotic potential (– MPa) 0.0	0.3	0.6	0.9	1.2	1.5	Regression equation[y]	Correlation coefficient (r)
				Stage[x]				
White tepary	4.6	4.6	4.3	3.9	1.6	0.1	$y = 4.5 + 1.6x - 3.1x^2$	0.98
Brown tepary	4.5	4.6	4.2	3.7	1.4	0.1	$y = 4.5 + 1.3x - 2.9x^2$	0.98
Navy	4.3	4.6	4.1	3.4	0.5	0.0	$y = 4.5 + 0.8x - 2.7x^2$	0.93

[z]0 = no emergence, 1 = hypocotyl hook visible, 2 = cotyledonary leaves above surface of vermiculite,3 = stem straight with primary leaves beginning to unfold, 4 = full expansion of primary leaves, and 5 = expansion of first true leaves, generally trifoliolate.
[y]y = Developmental stage, x = NaCl osmotic potential.
[x]Interaction between NaCl and genotype was nonsignificant at $P = 0.05$; LSD (0.05) = 0.20 to compare genotypes.

Table 2. Relative leaf area (percent of 0.0 MPa area for each genotype) of two tepary bean lines and of one navy bean cultivar at 14 days after growth in NaCl solutions of increasing salinity, expressed as osmotic potential.

Genotype	Osmotic potential (– MPa) 0.0	0.3	0.6	0.9	Regression equation[z]	Correlation coefficient *(r)*
			Relative leaf area (%)[y,x]			
White tepary	100	97	*73*	31	$y = 100.2 + 20.5x - 108.3x^2$	0.99
Brown tepary	100	100	60	24	$Y = 102.2 + 0.7X - 100x^2$	0.98
Navy	100	100	57	17	$y = 102.3 + 2.7x - 111.1x^2$	0.98

[z]y = Leaf area, x = NaCl osmotic potential
[y]leaf areas (cm^2/plant) at 0.0 MPa were 14.3, 16.0, and 25.2 for white tepary, brown tepary, and navy, respectively.
[x]Interaction between NaCl and genotype was not significant at $P = 0.05$; LSD (0.05) = 8.2 to compare genotypes.

Table 3. Dry weight (mg/plant) of shoots plus roots of two tepary bean lines and of one navy bean cultivar at 14 days after growth in NaCl solutions of increasing salinity, expressed as osmotic potential.

Genotype	Osmotic potential (– MPa) 0.0	0.3	0.6	0.9	1.2	1.5	Regression equation[z]	Correlation coefficient (r)
				Dry wt (mg)[y]				
White tepary	123	97	83	68	83	75	$y = 121.6 - 89.6x + 40.9x^2$	0.92
Brown tepary	131	112	77	65	89	86	$y = 134 - 123.2x + 62.7x^2$	0.87
Navy	178	162	129	95	102	129	$y = 187.6 - 155.3x + 74.4x^2$	0.88

[z]y = Dry weight, x = NaCl osmotic potential.
[y]Interaction between NaCl and genotype was not significant at $P = 0.05$; LSD (0.05) = 9.2 to compare genotypes.

Table 4. Root : shoot dry weight ratios of two tepary bean lines and of one navy bean cultivar at 14 days aftergrowth in NaCl solutions of increasing salinity, expressed as osmotic potential.

Genotype	Osmotic potential (– MPa) 0.0	0.3	0.6	0.9	Regression equation[z]	Correlation coefficient *(r)*
			Ratio[y]			
White tepary	0.40	0.43	0.40	0.19	$y = 0.4 + 0.4X - 0.7X^2$	0.98
Brown tepary	0.45	0.54	0.35	0.16	$y = 0.5 + 0.4x - 0.8x^2$	0.95
Navy	0.26	0.28	0.20	0.21	$y = 0.3 - 0.08x$	0.59

[z]y = Root : shoot dry weight ratio, x = NaCl osmotic potential.
[y]Interaction between NaCl and genotype was not significant at $P = 0.05$; LSD (0.05) = 0.06 to compare genotypes.

brate for *3* h. Water potential was calculated from microvolt readings (MJ55; Wescor, Logan, Utah). Subsequently, the chambers were frozen in liquid nitrogen for 20 see, allowed to equilibrate to room temperature for 30 rein, and placed in a water bath at 25C for 1 h. Using the same meter and psychrometers, readings then were made of osmotic potential. Turgor potential was calculated as the difference between osmotic and water potentials.

Emerged seedlings were weighed after cleaning vermiculite from the roots in running water and blotting plants to remove excess water. For seedlings stressed with – 1.2 and – 1.5 MPa, weights included nonemerged plant material, as well as emerged seedlings. The plants were separated into leaf blades, roots, and stems plus petioles. Leaf area was determined with a leaf area meter (Model 3100; LI-COR, Lincoln, Neb.). Dry weights were determined after 48 h at 68C. Root : shoot dry weight ratios were calculated.

Development of all three genotypes was retarded as NaCl concentration increased (Table 1). At 0.0, –0.3, and –0.6 MPa NaCl, seedlings of all genotypes had cotyledons and expanding first true leaves (stage 4) by day 14. At –0.6 MPa, some delay in development was noted in all genotypes, but at salinities more negative than – 0.9 MPa, emergence was delayed more in navy bean than in white tepary bean (WT) or brown tepary bean (BT). At – 1.2 MPa, the hypocotyl hook was straightening in all seedlings of both tepary lines, while hypocotyls were visible in only half of the navy bean seedlings. At – 1.5 MPa, only a few tepary seedlings and none of the navy beans emerged. For all genotypes, NaCl also delayed seedling emergence from the medium surface and the speed at which they emerged (Fig. 1).

All genotypes maintained leaf turgor potential due to parallel decreases in water and osmotic potentials with higher NaCl concentrations (Fig. 2). These findings differ from those of Neumann et al. (1988) who reported a decrease in turgor when 10-day-old *P. vulgaris* seedlings were placed in NaCl for 24

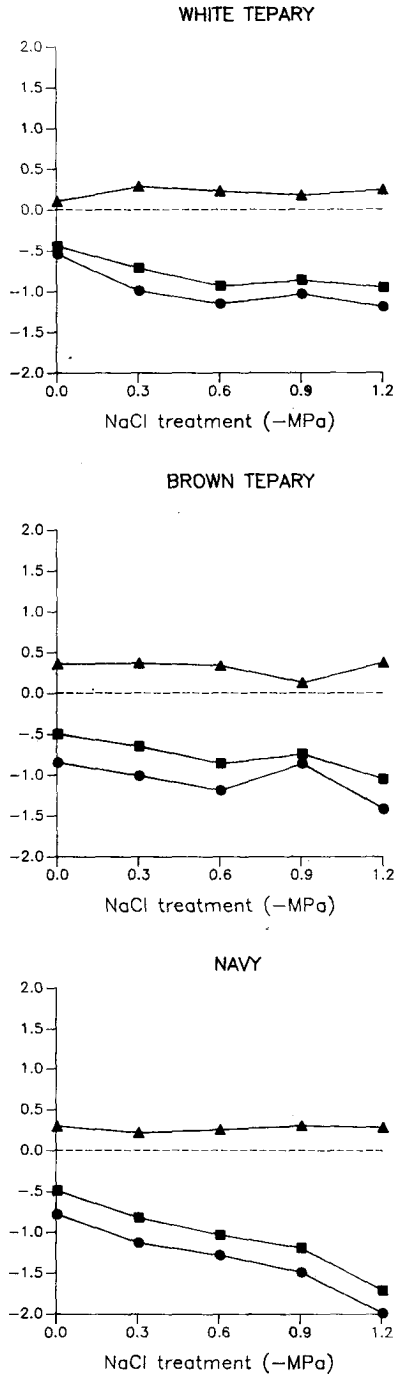

Fig. 2. Turgor, osmotic, and total water potentials in leaves of white and brown tepary bean lines and of one navy bean cultivar after plants were in one of five NaCl solutions for 14 days. (▲ – ▲) turgor potential (■ - ■) water potential, (● - ●) osmotic potential.

h. This discrepancy may be attributable to the holding of their beans with NaCl for only 24 h; thus, they still may have been adapting, whereas our beans had been in NaCl since the start of imbibition. With higher NaCl concentrations, tepary beans tended to maintain less negative water and osmotic potentials than navy, although turgor was similar in all three genotypes (Fig. 2). The larger decline in water and osmotic potentials of navy suggests that it was stressed more than either WT or BT, as was also reflected in delayed development.

NaCl inhibited leaf area expansion during the 14 days of the tests (Table 2). Leaf area is expressed as a percentage of that of 0.0 MPa area for each genotype, because large differences in leaf area were noted among genotypes, with navy having the largest. At -0.6 and – 0.9 MPa, the percentage leaf area maintained was highest with WT, intermediate with BT, and lowest with navy. Leaf area of both tepary genotypes was affected less at – 0.9 MPa salinity than that of navy because navy started at a higher absolute value at 0.0 MPa. Leaf area was not measured on stressed plants at – 1.2 and – 1.5 MPa, as leaves expanded only minimally at these salinities.

Dry weight of navy bean was significantly higher than those of tepary beans with all NaCl treatments (Table 3). All genotypes had lower dry weight with higher NaCl from 0.0 to – 0.9 MPa, and then levelled between – 1.2 and – 1.5 MPa. The decreases in dry weight with higher NaCl were greater for navy than for the teparies, relative to weights at 0.0 MPa.

Root : shoot ratios of genotypes were lower at higher NaCl levels (Table 4). Since plants stressed with – 1.2 and – 1.5 MPa NaCl were not sufficiently developed to make a good comparison among genotypes, they were not used for ratio determinations. Tepary beans had higher root : shoot ratios than navy at 0.0 MPa. The root: shoot ratio was higher for WT and BT than for navy at 0.0 through – 0.6 MPa. The ratios remained high for WT and BT through – 0.6 MPa, then dropped to less than half of their values at 0.0 MPa; those for navy were nearly the same at all salinities.

In conclusion, tepary beans have greater salt tolerance than navy beans during germination and emergence, just as during later vegetative and reproductive stages (Hendry, 1918; Perez and Minguez, 1985). The tepary beans had reached a more advanced developmental stage at – 1.2 MPa and had more rapid establishment, less percentage reduction in leaf area, and higher water potentials than navy bean following 2 weeks of growth with NaCl. Dry weights and root : shoot ratios were not good indicators of salt tolerance, due in part to large initial differences between navy and tepary seedlings. The characteristic that most easily distinguished salt tolerance of seedlings was developmental stage at 14 days. This technique required only initial preparation of growth trays followed by developmental stage determination at harvest and gave a consistent indication of the greater salt tolerance in tepary vs. navy bean.

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