Shoot Biomass Production among Accessions of *Medicago truncatula* Exposed to NaCl

Maren E. Veatch,* Steven E. Smith, and George Vandemark

ABSTRACT

Increased salt tolerance would improve utilization of salt-sensitive crop plants such as alfalfa (*Medicago sativa* L.). In order for selection for salt tolerance to be more efficient, it is useful to know whether improved productivity under saline conditions is due to unique physiological responses to salinity or merely the carry over of increased yield that was selected for in a nonsaline environment. *Medicago truncatula* Gaertn., a self-pollinated relative of alfalfa, was used to examine the response of specific genotypes across a range of salinities. This was done by evaluating the change in fresh shoot biomass production of greenhouse-grown mature plants and seedlings of different accessions of *M. truncatula* in response to four levels of salinity imposed as NaCl. Those accessions with the highest fresh shoot biomass production under nonsaline irrigation also had the highest fresh shoot biomass production under all salinity levels. The high correlation between an accession's fresh shoot biomass under nonsaline and saline irrigation indicate no unique physiological adaptation to salinity in the accessions of *M. truncatula* evaluated.

CROP PRODUCTION IS affected by salinity on approximately one third of the world's nearly 3×10^8 ha of irrigated agricultural land (Apse et al., 1999; Burns et al., 1990; Schachtman and Lui, 1999). The amount of land affected by salinity is increasing (Qadir et al., 1998). A major focus of plant breeding efforts in many areas has been to maintain or improve crop productivity in salt-affected environments, typically by selecting for increased "salt tolerance" (Ashraf, 1994; Epstein, 1985; Nuccio et al., 1999). Maas (1987) defined salt tolerance as either increased (i) plant survival, (ii) yield under saline growth conditions, or (iii) reduced depression in yield under saline conditions relative to that under nonsaline conditions (i.e., increased "relative yield"). Given the spatial and temporal variability in salinity within most agricultural systems, a cultivar with high relative yield would seem to be an ideal short-term solution to permit maintenance of crop production under at least moderate salinity (Igartua, 1995). However, in cases where there is little genetic variation for relative yield within populations, selection has often focused on genotypes that have the highest possible yield over a range of saline environments (Flowers and Yeo, 1995; Igartua, 1995).

Developing selection strategies for improving salt tolerance has not always been straightforward because of the variability typically observed in salinity stress. Rosielle and Hamblin (1981) proposed that the most effective way to increase yield over a range of stressful conditions would be to select for increased yield under nonsaline conditions. They assumed that high crop yield under stress does not require unique stress-specific physiological or developmental processes. Rather, they suggested that high yield is due to improved manifestation of plant traits expressed under essentially all conditions of plant growth. Selection for improved salt tolerance has generally focused on performance under salt stress conditions, and selection for increased yield under saline irrigation has been successful in a variety of crops (Al-Doss and Smith, 1998; Ashraf and Ahmad, 2000; Aslam et al., 1993; Igartua and Garcia, 1998; Kapulnik et al., 1989; Koval, 2000). It is generally not known whether any improvements in salt tolerance were due to improved expression of unique physiological or developmental responses that are specifically triggered by salinity (Borsani et al., 2003), which are expressed as high relative yield. These improvements could also be due to improved overall yield per se of the sort proposed by Rosielle and Hamblin (1981) which is expressed in both stress and nonstress environments. Differentiating these two routes toward stress response is greatly facilitated if individual genotypes can be evaluated across a range of stresses (Falconer and Mackay, 1996). In cases where significant variation in stress tolerance exists, the lack of a positive correlation between performance of a given genotype in stress and nonstress environments could indicate that stress tolerance may be due to unique stress-associated responses. Conversely, consistent performance of a given genotype in comparison to other genotypes across environments would suggest that such stress-associated tolerance mechanisms might not be operating and that selection should focus on improving overall yield in nonstress conditions.

Alfalfa is moderately sensitive to salinity stress, which is typically imposed experimentally as NaCl (Ashraf, 1994; Djilianov et al., 1997; Noble et al., 1984; Zhu et al., 1996). However, the heterozygous, outcrossing nature of alfalfa (Smith, 1993) and the inbreeding depression it displays (Holland and Bingham, 1994) make it difficult to study the response of individual genotypes across a range of salinities. *Medicago truncatula*, an annual relative of alfalfa, has an outcrossing rate of less than 3% (Bonin et al., 1996) allowing the generation of highly homozygous genotypes and lines (Cook, 1999). Because of its rapid generation time and diploid genome (Cook, 1999), *M. truncatula* has been used as a model for understanding growth and development in legumes (Jimenez-Zurdo et al., 2000; Oldroyd, 2001; Schoenbeck et al., 1999). *Medicago truncatula* may also be a good model for understanding salt response in alfalfa and other legumes. The objective of this research was to evaluate forage yield in *M. truncatula* under salt stress

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Abbreviations: DAP, days after planting; NPGS, National Plant Germplasm System.

and determine if the resulting yield is due to a response triggered by salt stress or merely the result of increased yield potential per se. Understanding more about the basis for salt tolerance would permit the use of more appropriate selection environments and therefore improve selection efficiency. This research involved separate evaluations of shoot biomass of greenhouse-grown mature plants and seedlings of several *M. truncatula* genotypes irrigated with saline solutions ranging from 0 to 115 m*M* NaCl.

MATERIALS AND METHODS

All *M. truncatula* seed used were obtained from the United States Department of Agriculture National Plant Germplasm System (NPGS), Western Regional Plant Introduction Center, Pullman, WA. Individual accessions were treated as pure lines because of the high level of self pollination in *M. truncatula* (Bonin et al., 1996). Seeds were scarified by rubbing between medium grain and fine-grain sandpaper, and were stored at 20°C for approximately 1 to 7 d before planting. The growth medium was an artificial soil consisting of Sunshine Mix (Sun Gro Inc.) and sand (3:1 V: V ratio). Soil was put in 3.8- by 21-cm cylindrical containers ("conetainers," SC-10 Super Cell, Stuewe and Sons, Inc., Corvallis, OR), which were filled and compacted to a volume of 120 mL leaving 25 mm of headspace (Johnson, 1990).

All plants were grown in a greenhouse at the Campus Agricultural Center at the University of Arizona, Tucson, AZ, without supplemental lighting. Two studies were conducted in a randomized complete block design arranged as a split plot with salinity level as the main plot factor and accession (genotype) as the subplot factor. The saline irrigation solutions were a $0.25 \times$ Hoagland's solution (Hoagland and Arnon, 1950) with concentrations of NaCl of 0, 50, 75, and 115 m*M*. Twenty *M. truncatula* accessions and one alfalfa population were evaluated. Eight of the *M. truncatula* accessions were from the core collection for annual *Medicago*s of the NPGS. The other 12 accessions were chosen on the basis of their fresh shoot biomass after 38 d of growth in a preliminary study that involved 91 randomly chosen accessions that were exposed to salinity of 0 and 75 m*M* NaCl for 24 d (M.E. Veatch, unpublished results). The accessions included from this preliminary study were selected in the following way: the top three highest biomass producing accessions under nonsaline irrigation (PI 577602, W6 6079, W6 6021), the three lowest biomass producing accessions under nonsaline irrigation (PI 464816, W6 6103, W6 6102), three of the highest biomass producing accessions under 75 m*M* NaCl irrigation (PI 190082, PI 577643, W6 6078), and three accessions with some of the highest relative yields with moderate biomass production (PI 493295, PI 577639, PI 577614). Selection was done this way to cover a range of yield potential in *M. truncatula*. The alfalfa population used was AZ-97 MEC-ST, which was derived from two cycles of selection for high forage yield under saline irrigation conditions (Al-Doss and Smith, 1998). Alfalfa, unlike *M. truncatula* accessions, is outcrossing and this population was presumably more genetically and phenotypically variable. There were 10 replications with two plants of each accession per replication in both studies.

Mature Plant Study

This study was conducted from November 2000 to January 2001. The mean high and low greenhouse temperatures were 28.9 and 6.0°C. Five seeds were sown in each conetainer and covered with 7 mL of dry unwashed sand. All seeds were irrigated with tap water (0 m*M* NaCl) until 15 d after planting

Table 1. Spearman's rank correlation between shoot biomass produced under 0 m*M* NaCl by 20 *M. truncatula* accessions and one alfalfa population and biomass produced under 50, 75, and 115 m*M* NaCl, and the rank correlation between mature plants and seedlings at each salinity level.

	Irrigation treatment (m*M* NaCl)			
Age of plants (date)	0	50	75	115
Mature (summer 1999) 0 m*M* NaCl	–	0.80	0.89	–
Mature (winter 1999) 0 m*M* NaCl	–	0.90	0.91	0.91
Seedling (spring 2000) 0 m*M* NaCl	–	0.89	0.68	0.84
Mature vs. seedling (winter 1999 and spring 2000)	0.75	0.74	0.74	0.77

(DAP). Plants were thinned to one per conetainer 14 DAP and the number of true leaves for the remaining plant recorded. At 15 DAP those plants not assigned to irrigation with the 0 m*M* NaCl treatment were irrigated with the 50 m*M* NaCl solution followed by irrigation with the assigned saline solution (50, 75, or 115 m*M* NaCl) 2 d later. A one-conetainer border of *M. truncatula* 'Jemalong' was placed on the outer edge of the experimental plants and was irrigated with the assigned irrigation solution of the adjacent plants. Irrigation occurred every 3 d and involved wetting the soil to beyond field capacity. Fifty-six DAP the aboveground shoot biomass was cut at the soil line and its fresh weight recorded. Fresh weight was used as a measure of shoot biomass since shoot dry weight was previously shown to be significantly correlated with shoot fresh weight in mature plants (52 DAP) of *M. truncatula* ($R = 0.93$) (M.E. Veatch, unpublished results).

The original study of effect of salinity on the selected accessions was conducted June through August 2000 minus the 115 m*M* NaCl treatment. The rank correlation data were similar to that obtained in the winter study (Table 1); however, the effect of salinity on biomass was not as pronounced or clear as was expected (M.E. Veatch, unpublished data). The November 2000 to January 2001 study was conducted with the additional salinity level (115 m*M* NaCl) in an attempt to obtain a more definitive picture of how increasing NaCl affects biomass production and to increase the stress response of the selected accessions.

Seedling Study

The study was conducted in April 2001, with mean high and low outside temperatures at the Campus Agricultural Center being 26.7 and 8.9°C., with the greenhouse temperatures being typically 1 to 2°C higher. Before sowing, seed mass was estimated by weighing four random samples of 20 seeds from each accession. Seeds of different accessions of *M. truncatula* germinate at very different speeds. Since the goal was to evaluate differences in biomass accumulation in seedlings under NaCl, and not to evaluate differences in germination under NaCl, dry seeds were not directly planted into the wetted growth medium. Rather, seeds were germinated on filter paper wetted with 4 mL of a 0.02% (w/v) solution of captan [*N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide] and tap water in Petri plates placed in selfsealing clear plastic bags with wetted paper towels for 2 d at 25°C in the dark. On the basis of differences in germination speed, seeds of different accessions were placed on filter paper on different days such that all the seedlings were at the same developmental stage on the day when they were to be placed in the soil. All seedlings were transplanted into soil that had either been wetted with the 0 m*M* NaCl solution or the 50 m*M* NaCl solution. Seedlings had radicles 5 to 8 mm long at this time and were placed radicle down with the cotyledons at the soil surface with a flat metal spatula. The soil was then gently compacted around the radicle. All plants were irrigated with the assigned saline solution 2 d following transplanting. At the third trifoliate leaf

stage in alfalfa axillary meristems often become active, which is considered the initiation of mature plant growth (Meyer, 1999). All seedlings within a replication were harvested when the most rapidly developing accession in that replication reached the third trifoliate leaf stage, which occurred 15 to 17 d after sowing. At harvest the number of leaves and the fresh shoot biomass of each plant were recorded. As there were no previous data on the correlation between fresh and dry biomass in seedlings, each plant was then placed in a manila envelope and dried at 85°C for 3 d and its dry weight recorded.

Data Analyses

Data for the alfalfa population were included with the *M. truncatula* accessions during statistical analysis. Shoot biomass values for both studies were analyzed by multifactor analysis of variance in JMP (Fit Model Platform, Sall et al., 2001). Relative yield values for each study were calculated by dividing the mean shoot biomass of both plants of an accession within a replication under 50, 75, or 115 m*M* NaCl irrigation by the overall mean shoot biomass of that accession under 0 m*M*

NaCl irrigation. In analyzing shoot biomass of mature plants, the leaf number on the day before saline irrigation began was used as a covariate to normalize for differences in size at the start of treatment. For seedling data, covariates were leaf number at the time of harvest and mean seed mass of the accession. The relationships between an individual accession's mean shoot biomass under different salinity levels, and between an accession's mean shoot biomass as a mature plant and as a seedling were analyzed by Spearman's Rank Correlation (Multivariate Platform in JMP, Sall et al., 2001). *P* values ≤ 0.05 were considered significant throughout.

RESULTS

Mature Plant Study

As the concentration of NaCl in the irrigation solution increased, mean shoot biomass decreased significantly. The overall trend was that irrigation with a 115 m*M* NaCl solution decreased mean shoot biomass by over 46% relative to that with nonsaline irrigation (Table 2).

Table 2. Mean shoot fresh weight (±SE) (g plant⁻¹) and relative yield of mature plants of 20 *M. truncatula* accessions and an *M. sativa* irrigated with four nutrient solutions (0, 50, 75, and 115 m*M* NaCl) sorted by biomass under nonsaline irrigation (0 m*M* NaCl).

Accession		Irrigation treatment (m*M* NaCl)			
		0	50	75	115
W6 6079	Fresh weight (g)	4.28 ± 0.25 a†	3.82 ± 0.12 a	3.09 ± 0.13 a	2.83 ± 0.14 a
	Relative yield‡		0.89 ± 0.06 a	0.72 ± 0.05 a	0.66 ± 0.03 ab
W6 6078	Fresh weight (g)	4.03 ± 0.21 ab	3.44 ± 0.18 ab	2.87 ± 0.19 ab	2.56 ± 0.12 a
	Relative yield		0.85 ± 0.06 a	0.71 ± 0.05 a	0.64 ± 0.03 ab
PI 190082	Fresh weight (g)	3.90 ± 0.40 a–d	3.16 ± 0.16 bc	2.83 ± 0.22 ab	2.39 ± 0.19 abc
	Relative yield		0.80 ± 0.05 a	0.75 ± 0.06 a	0.59 ± 0.06 ab
PI 292436	Fresh weight (g)	3.86 ± 0.28 a–d	3.07 ± 0.26 bc	2.38 ± 0.23 bcd	2.69 ± 0.16 a
	Relative yield		0.82 ± 0.08 a	0.62 ± 0.06 a	0.69 ± 0.04 ab
PI 384648	Fresh weight (g)	3.80 ± 0.31 abc	2.84 ± 0.23 bcd	2.77 ± 0.21 abc	2.43 ± 0.14 ab
	Relative yield		0.75 ± 0.07 a	0.74 ± 0.06 a	0.63 ± 0.06 ab
PI 577602	Fresh weight (g)	3.52 ± 0.25 a–e	2.54 ± 0.24 cde	2.23 ± 0.21 cde	2.08 ± 0.13 b–e
	Relative yield		0.72 ± 0.08 a	0.62 ± 0.07 a	0.59 ± 0.04 ab
PI 566887	Fresh weight (g)	3.45 ± 0.23 a–g	2.90 ± 0.23 b–e	2.46 ± 0.17 bc	1.89 ± 0.16 b–e
	Relative yield		0.82 ± 0.07 a	0.73 ± 0.06 a	0.54 ± 0.04 ab
PI 537168	Fresh weight (g)	3.17 ± 0.26 c–f	2.61 ± 0.23 cde	2.06 ± 0.20 de	1.93 ± 0.16 bcd
	Relative yield		0.82 ± 0.09 a	0.79 ± 0.08 a	0.53 ± 0.06 ab
PI 566890	Fresh weight (g)	3.38 ± 0.39 a–f	2.88 ± 0.22 b–e	2.53 ± 0.13 ab	2.31 ± 0.18 a–d
	Relative yield		0.82 ± 0.07 a	0.75 ± 0.05 a	0.66 ± 0.07 ab
PI 566889	Fresh weight (g)	3.30 ± 0.18 a–f	1.97 ± 0.14 e	1.77 ± 0.21 def	1.21 ± 0.16 fg
	Relative yield		0.59 ± 0.05 a	0.56 ± 0.18 a	0.39 ± 0.06 b
PI 577643	Fresh weight (g)	3.20 ± 0.21 b–f	2.32 ± 0.15 b–f	1.96 ± 0.19 cde	1.67 ± 0.14 def
	Relative yield		0.74 ± 0.05 a	0.61 ± 0.07 a	0.52 ± 0.03 ab
PI 577639	Fresh weight (g)	3.09 ± 0.22 b–f	2.53 ± 0.14 b–e	2.34 ± 0.13 b	1.70 ± 0.10 cde
	Relative yield		0.81 ± 0.05 a	0.75 ± 0.05 a	0.55 ± 0.04 ab
PI 566888	Fresh weight (g)	3.00 ± 0.23 d–g	2.40 ± 0.16 de	1.93 ± 0.13 def	1.61 ± 0.08 efg
	Relative yield		0.80 ± 0.06 a	0.65 ± 0.05 a	0.53 ± 0.04 ab
W6 6021	Fresh weight (g)	2.91 ± 0.16 d–g	2.34 ± 0.17 d	1.88 ± 0.15 def	1.68 ± 0.14 def
	Relative yield		0.80 ± 0.06 a	0.64 ± 0.05 a	0.58 ± 0.06 ab
W6 6103	Fresh weight (g)	2.90 ± 0.30 d–h	2.48 ± 0.27 c	2.10 ± 0.26 cde	1.80 ± 0.24 cde
	Relative yield		0.86 ± 0.08 a	0.70 ± 0.09 a	0.60 ± 0.09 ab
PI 493295	Fresh weight (g)	2.74 ± 0.22 e–h	2.50 ± 0.20 cde	2.10 ± 0.11 de	1.72 ± 0.17 cd
	Relative yield		0.91 ± 0.09 a	0.75 ± 0.06 a	0.63 ± 0.06 ab
W6 6102	Fresh weight (g)	2.30 ± 0.26 hg	1.51 ± 0.12 gh	1.23 ± 0.11 f	1.13 ± 0.12 fg
	Relative yield		0.67 ± 0.07 a	0.54 ± 0.05 a	0.48 ± 0.05 ab
PI 577614	Fresh weight (g)	2.12 ± 0.22 f–i	1.57 ± 0.10 fg	1.35 ± 0.09 f	0.92 ± 0.10 g
	Relative yield		0.73 ± 0.04 a	0.63 ± 0.05 a	0.44 ± 0.05 ab
PI 566886	Fresh weight (g)	2.03 ± 0.24 hi	1.71 ± 0.16 g	1.72 ± 0.16 ef	1.16 ± 0.06 f
	Relative yield		0.82 ± 0.08 a	0.79 ± 0.07 a	0.53 ± 0.04 ab
Az 97 Mec St§	Fresh weight (g)	1.58 ± 0.18 ij	1.58 ± 0.10 h	1.31 ± 0.06 f	1.16 ± 0.06 fg
	Relative yield		1.00 ± 0.06 a	0.83 ± 0.05 a	0.73 ± 0.05 a
PI 464816	Fresh weight (g)	0.84 ± 0.07 j	0.72 ± 0.08 i	0.73 ± 0.10 g	0.44 ± 0.06 h
	Relative yield		0.89 ± 0.10 a	0.81 ± 0.14 a	0.51 ± 0.08 ab
Treatment mean¶	Fresh weight (g)	3.0 ± 0.07 a	2.43 ± 0.05 b	2.09 ± 0.04 c	1.77 ± 0.04 d

† Means in a column with the same letter are not significantly different according to Tukey's HSD.
‡ Relative yields were calculated by dividing the mean biomass in a replication of an accession under saline irrigation by the overall mean biomass of that accession under nonsaline irrigation.
§ = *M. sativa*. All other accessions are *M. truncatula*
¶ Means within row with the same letter are not significantly different according to Tukey's HSD.

In addition to the overall differences in shoot biomass among treatments, there were significant differences in shoot biomass between *M. truncatula* accessions within each treatment. Accessions 6079 and 464816 consistently had the highest and lowest fresh shoot biomass, respectively, over all treatments (Table 2). With few exceptions the relative rank of the mean fresh shoot biomass of the accessions remained consistent across salt treatments (Table 2). Between the rank of an accession under nonsaline irrigation and its rank under each of the three levels of saline irrigation for both plants grown in the summer and the winter there was a positive correlation (Table 1).

Analysis of variance indicated a significant interaction ($p < 0.001$) between salinity level and accession for relative yield. However, there were no significant differences between mean relative yields among the *M. truncatula* accessions (Table 2). The only significant difference in mean relative yield was between AZ-97 MEC-ST, and

M. truncatula accession 566889, under irrigation with 115 mM NaCl (Table 2).

Seedling Study

Fresh shoot biomass was highly correlated with dry weight ($r = 0.96$); therefore, only the fresh shoot biomass and relative yield data is shown (Table 3). Increasing salinity in the nutrient solution decreased mean fresh shoot biomass by an average of 38% under 75 mM NaCl treatment relative to that under 0 mM NaCl. An increased NaCl concentration in the irrigation solution (115 mM NaCl) caused no further significant decrease in shoot biomass (Table 3).

Within salt treatments there were significant differences among accessions for fresh shoot biomass (Table 3). As seen in mature plants, accession 6079 had the highest fresh shoot biomass under all treatments and accession 464816 the lowest (Table 3). There was also a positive rank correlation between mean fresh shoot

Table 3. Mean shoot biomass (±SE) (mg plant^{-1}) and relative yield of seedlings of 20 *M. truncatula* accessions and an *M. sativa* irrigated with four nutrient solutions (0, 50, 75, and 115 mM NaCl) sorted by biomass under nonsaline irrigation (0 mM NaCl).

Accession		Irrigation treatment (mM NaCl)			
		0	50	75	115
W6 6079	**Fresh weight (mg)**	410 ± 20 a†	290 ± 30 a	290 ± 30 a	250 ± 20 a
	Relative yield‡		0.70 ± 0.07 a	0.70 ± 0.09 ab	0.61 ± 0.05 ab
W6 6078	**Fresh weight (mg)**	390 ± 20 a	270 ± 20 a	260 ± 20 ab	230 ± 20 ab
	Relative yield		0.69 ± 0.06 a	0.66 ± 0.06 ab	0.58 ± 0.04 ab
PI 190082	**Fresh weight (mg)**	270 ± 30 b	240 ± 20 b	200 ± 10 b	160 ± 20 cde
	Relative yield		0.89 ± 0.09 a	0.73 ± 0.05 ab	0.59 ± 0.07 ab
PI 292436	**Fresh weight (mg)**	250 ± 20 bc	190 ± 20 b–d	120 ± 20 b–e	160 ± 20 cd
	Relative yield		0.75 ± 0.10 a	0.48 ± 0.13 ab	0.63 ± 0.07 ab
PI 566890	**Fresh weight (mg)**	250 ± 20 bcd	200 ± 20 bc	160 ± 10 bcd	130 ± 20 bc
	Relative yield		0.81 ± 0.08 a	0.62 ± 0.07 ab	0.51 ± 0.07 ab
PI 577643	**Fresh weight (mg)**	220 ± 10 b–e	170 ± 20 c–h	160 ± 10 bcd	140 ± 20 cde
	Relative yield		0.76 ± 0.06 a	0.72 ± 0.06 ab	0.62 ± 0.06 ab
W6 6021	**Fresh weight (mg)**	210 ± 10 b–e	160 ± 10 b–f	120 ± 10 b–e	80 ± 20 c–f
	Relative yield		0.74 ± 0.04 a	0.57 ± 0.06 ab	0.42 ± 0.06 ab
PI 577639	**Fresh weight (mg)**	210 ± 20 b–e	150 ± 20 b–e	90 ± 20 b–e	120 ± 10 e–f
	Relative yield		0.70 ± 0.10 a	0.43 ± 0.06 ab	0.58 ± 0.06 ab
PI 384648	**Fresh weight (mg)**	200 ± 20 b–e	200 ± 20 bc	170 ± 20 bc	160 ± 20 cd
	Relative yield		1.01 ± 0.12 a	0.86 ± 0.12 a	0.79 ± 0.08 a
PI 566889	**Fresh weight (mg)**	200 ± 20 c–g	140 ± 20 d–h	60 ± 20 c–f	70 ± 20 c–f
	Relative yield		0.63 ± 0.13 a	0.20 ± 0.08 b	0.38 ± 0.11 ab
PI 493295	**Fresh weight (mg)**	190 ± 10 c–g	130 ± 20 c–h	120 ± 10 cde	100 ± 10 c–f
	Relative yield		0.67 ± 0.06 a	0.66 ± 0.07 ab	0.55 ± 0.09 ab
PI 566887	**Fresh weight (mg)**	190 ± 20 c–g	170 ± 20 b–f	150 ± 20 cde	100 ± 20 c–f
	Relative yield (fw)		0.86 ± 0.08 a	0.80 ± 0.08 a	0.55 ± 0.09 ab
Az 97 Mec S§	**Fresh weight (mg)**	190 ± 10 c–e	140 ± 10 b–h	110 ± 10 c–f	100 ± 10 c–f
	Relative yield		0.76 ± 0.08 a	0.58 ± 0.10 ab	0.54 ± 0.04 ab
PI 577602	**Fresh weight (mg)**	180 ± 10 d–h	130 ± 20 c–h	130 ± 20 c–f	110 ± 10 c–f
	Relative yield		0.72 ± 0.11 a	0.72 ± 0.12 ab	0.60 ± 0.08 ab
W6 6102	**Fresh weight (mg)**	180 ± 20 e–h	120 ± 20 e–h	90 ± 20 def	100 ± 20 ef
	Relative yield		0.67 ± 0.11 a	0.52 ± 0.06 ab	0.57 ± 0.08 ab
PI 566888	**Fresh weight (mg)**	160 ± 20 c–g	130 ± 20 c–h	130 ± 20 def	100 ± 10 ef
	Relative yield		0.83 ± 0.13 a	0.84 ± 0.13 a	0.63 ± 0.07 ab
W6 6103	**Fresh weight (mg)**	160 ± 30 c–g	90 ± 20 fgh	80 ± 30 c–f	80 ± 20 c–f
	Relative yield		0.55 ± 0.15 a	0.49 ± 0.19 ab	0.52 ± 0.15 ab
PI 537168	**Fresh weight (mg)**	150 ± 10 gh	80 ± 20 d–h	60 ± 20 def	40 ± 10 c–f
	Relative yield		0.56 ± 0.17 a	0.40 ± 0.15 ab	0.30 ± 0.09 ab
PI 566886	**Fresh weight (mg)**	150 ± 10 f–h	120 ± 10 gh	90 ± 10 f	80 ± 10 f
	Relative yield		0.77 ± 0.07 a	0.60 ± 0.09 ab	0.52 ± 0.09 b
PI 577614	**Fresh weight (mg)**	130 ± 10 d–h	100 ± 10 e–h	80 ± 10 def	70 ± 10 c–f
	Relative yield		0.74 ± 0.09 a	0.65 ± 0.09 ab	0.53 ± 0.08 ab
PI 464816	**Fresh weight (mg)**	110 ± 20 h	40 ± 10 h	40 ± 10 ef	30 ± 10 def
	Relative yield		0.40 ± 0.11 a	0.40 ± 0.15 ab	0.24 ± 0.11 b
Treatment mean¶	**Fresh weight (mg)**	210 ± 5.00 a	160 ± 5.00 b	130 ± 5.00 c	120 ± 5.00 c

† Means in a column with the same letter are not significantly different according to Tukey's HSD.
‡ Relative yields were calculated by dividing the mean biomass in a replication of an accession under saline irrigation by the overall mean biomass of that accession under nonsaline irrigation.
§ = *M. sativa.* All other accessions are *M. truncatula*
¶ Means within row with the same letter are not significantly different according to Tukey's HSD.

biomass of the accessions under nonsaline and saline irrigation (Table 1).

The only significant differences in relative yields among accessions were between the accession with the highest relative yield (384648) and the *M. truncatula* accessions with the lowest relative yield values at the 75 and 115 mM NaCl levels, which were 566889 and 464816 respectively (Table 3). In addition to the significant rank correlations between treatments, there was a positive correlation between mature plants and seedlings at each salinity level (Table 1).

DISCUSSION

Salt tolerance can be expressed as increased overall yield or increased relative yield (Maas, 1987). Even though there was significant variation among accessions' shoot biomass under saline irrigation, we observed little evidence of differences in relative yield among *M. truncatula* accessions (Tables 2 and 3). Any differences in relative yield were between *M. truncatula* accessions with the lowest relative yield and the genotype with the highest relative yield, which was alfalfa in the mature plants (Table 2) or the *M. truncatula* accession with the highest relative yield in the seedlings (Table 3).

The lack of differences in relative yield among *M. truncatula* accessions examined provides evidence against the existence of a specific and unique physiological response to salinity in this species. This is also supported by the positive correlations between an accession's biomass production under nonsaline irrigation and under the three levels of saline irrigation (Table 1). This is further supported by the positive rank correlations in shoot biomass between seedlings and mature plants (Table 1). Although we examined *M. truncatula* genotypes displaying a range of yield potential, there is a possibility that there are unique adaptations in *M. truncatula* that are in one of the accessions not evaluated. Both *M. truncatula* and alfalfa are native to areas where water stress is encountered but salt stress is uncommon (Clarkson and Russell, 1976; Heyn, 1963; Lesins and Lesins, 1979; Little et al., 1992). The few differences in relative yield and the high rank correlations of accessions across environments may simply be the result of little history of natural selection for growth under saline conditions. These results suggest that advances in the improvement of *M. truncatula* yields could be simplified by selecting for the highest possible yields in a nonsaline environment.

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