

# Genetic Diversity in the Batini Barley Landrace from Oman:

## II. Response to Salinity Stress

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### ABSTRACT

Understanding the diversity for salt tolerance in barley (*Hordeum vulgare* L.) landraces will facilitate their use in genetic improvement. Our objectives were to screen a collection of 2308 accessions in seven subpopulations of the Omani Batini barley landrace under salinity stress, quantify genetic variation in germination and early seedling growth attributes, establish the forage yield-salinity response functions for 10 families within each subpopulation, and select genotypes with high yield potential under salinity. Subpopulations displayed high levels of genetic diversity and differed significantly for seed germination and seedling growth attributes at 0.0 and 20.0 dS m<sup>-1</sup>, and at tillering stage for forage yield at 0.85, 10.0, and 20.0 dS m<sup>-1</sup>. A multivariate-based selection criterion for high forage yield at tillering stage under salinity stress, based on simultaneous selection for low temporal variation in germination and high shoot dry weight under 20.0 dS m<sup>-1</sup>, identified highly salt tolerant accessions. Twenty-five out of 70 families representing seven subpopulations were classified as salt tolerant on the basis of their salinity susceptibility indices at 20.0 dS m<sup>-1</sup>. Accessions with short rachilla or with high root number and root length under salinity stress ranked highest in salt tolerance. Forage yield at 10.0 and 20.0 dS m<sup>-1</sup> can be predicted with high ( $R^2 = 0.67$ ,  $P < 0.0001$ ) and moderate ( $R^2 = 0.23$ ,  $P < 0.01$ ) accuracy by forage yield under 0.85 dS m<sup>-1</sup>. On average, forage yield was reduced by 2.4 and 7.9% per dS m<sup>-1</sup> at 10.0 and 20.0 dS m<sup>-1</sup>, respectively. Although genetically not improved, the landrace, in general, and subpopulation Batini 4, in particular, contain diversity that remains to be exploited.

SALINITY STRESS negatively affects agricultural yield throughout the world, whether production is for subsistence or economic gain (Epstein et al., 1980; Flowers and Yeo, 1995). The United Nations Environment Program estimates that 20% of the agricultural land and 50% of the cropland in the world is salt-stressed (Flowers and Yeo, 1995). For example, intensive flood and basin irrigation with water pumped from alluvial aquifers adjacent to the Arabian Sea in the Batinah region of Oman during the last 20 to 30 yr resulted in seawater intrusion and the salinization of large parts of the intensively cultivated lands in this region (El-Kharbotly et al., 2003).

Salinity and overgrazing impose serious environmental problems that affect grassland cover and the availability of animal feed in Oman (El-Kharbotly et al.,

2003). Few native plant species other than barley (*Hordeum vulgare* L.) are currently available as animal feed. Introduced forage species not fully adapted to the climatic and edaphic conditions in Oman may not achieve their full production potential. The use of salt tolerant forage barley could be a solution for feed shortages in Oman and other regions with saline water and soil in the Arabian Peninsula. Further enhancement of barley's relatively high tolerance to salinity stress could improve the profitability of some of the salt-affected soils around the world (Flowers and Yeo, 1995), including the Batinah region in Oman.

Barley is one of the most salt-tolerant crop species among the glycophytes (Maas, 1986), with genotypes that can germinate in seawater (i.e., about 47.0 dS m<sup>-1</sup>) (Mano and Takeda, 1997). However, the literature is replete with conflicting reports as to whether a positive phenotypic or genetic correlation exists between its tolerance at the germination and seedling growth stages under stress and nonstress conditions (von Well and Fossey, 1998), or whether salt tolerance at the germination and seedling growth stages is expressed at subsequent growth stages (Mano and Takeda, 1995). One reason for this is the difficulty of measuring the tolerance of salinity as distinct from the tolerance of water or osmotic stress (Munns et al., 2002) and the difficulty of screening large numbers of germplasm accessions for small, repeatable, and quantifiable differences in relevant seed, seedling, and plant attributes (Foolad et al., 1998).

Screening for salt-tolerant barley germplasm is important to determine whether there is a genetic basis for selection and breeding purposes (Alonso et al., 1999). Salt tolerance has been identified as a developmentally regulated, stage-specific phenomenon with tolerance at one stage of plant development being poorly correlated with tolerance at other stages (Foolad et al., 1998). In addition, at each stage of plant development, salt tolerance appears to be controlled by more than one gene and to be highly influenced by environmental factors (Mano and Takeda, 1995). Although field screening for salt tolerance has the advantage of testing germplasm under natural conditions, it is less efficient and more expensive than screening under controlled conditions (Shannon and Noble, 1990).

Numerous traits related to salt tolerance that have been used to screen germplasm include germination percentage, seedling root and shoot attributes, degree of leaf injury, rates of Na<sup>+</sup> or Cl<sup>-</sup> accumulation in leaves (Munns et al., 1995), ion concentration in root cells (Flowers and Hajibagheri, 2001), carbon isotope discrimination, and midday differential canopy temperature (Isla et al., 1998). Salt tolerance at germination is easy to measure, but the reports on the relationship

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between salt tolerance at germination and that of the seedling or the adult plant stages in many plant species, including barley, are conflicting (Mano and Takeda, 1997). Barley has been found to be tolerant to salinity at germination, sensitive at the seedling and early vegetative growth stages, then tolerant again at maturity (Epstein et al., 1980; Maas, 1986). Therefore, barley selection for salt tolerance requires selection throughout the growing season (Munns, 2002).

A germplasm collection of the barley landrace Batini, which originated in a region of increasing salinity and decreasing fresh water resources for irrigation (ICBA, 2000), was found to be highly diverse for 26 quantitative and qualitative traits (Jaradat et al., 2004). The objectives of this study were to: (i) screen a germplasm collection of seven subpopulations of the Batini landrace for germination and early seedling growth under increasing salinity stress levels and select salt tolerant genotypes, (ii) quantify the level of variation available in these subpopulations for forage yield, and (iii) establish the forage yield-salinity response functions for each subpopulation at the growth stage most appropriate for forage production.

## MATERIALS AND METHODS

### Seed Germination Experiment

A total of 2308 accessions of seven subpopulations of the Omani barley landrace Batini (Jaradat et al., 2004) were screened for tolerance to salinity stress at the germination and early seedling growth stages (Table 1). Two replicates, each of 10 seeds of uniform size and 120 g kg<sup>-1</sup> moisture content, were weighed and surface sterilized with 5% (w/v) calcium hypochlorate for 10 min and thoroughly washed with sterile deionized water. The seeds were transferred to sterile Petri dishes (100 mm in diameter) containing two Whatman No. 1 filter paper moistened with 15 mL half-strength control solution, i.e., no NaCl added (Timm et al., 1991), or the control solution with NaCl added to produce a salinity level of 20 dS m<sup>-1</sup>. This salinity level was chosen to represent the highest salinity level of saline water aquifers in the Arabian Peninsula (Hussain et al., 1997; ICBA, 2000). Batches of 400 Petri dishes were placed in the dark in a precision incubator (RU MED Type 3001-3601, Rubarth Apparate GmbH, Germany). Temperature was maintained during the 10-d duration of the ger-

mination tests at 25°C (±0.5). Germination response to salinity stress was monitored visually at 8-h intervals for the duration of the germination test. A seed was considered to have germinated if the radicle exceeded 2 mm in length (ISTA, 1993).

At the end of the germination test, the total number of germinated seeds was counted. For each seedling, the number of roots was counted and root length and shoot length were measured. Seedlings from each subpopulation and salinity treatment were dried in a forced-air oven for 24 h at 70°C. The following derived variables were calculated: ratio of root-to-shoot length, milligrams root dry weight per milligrams seed dry weight, and milligrams shoot dry weight per milligrams seed dry weight. The last two variables were combined to estimate the fraction of seed dry weight recovered in the seedling dry weight as milligrams seedling dry weight per milligrams seed dry weight.

### Sand Culture Experiment

From the Omani barley landrace Batini, seeds were collected from each of 10 randomly selected individual plants of each of seven subpopulations in the summer of 2000 (Jaradat et al., 2004). Progenies grown from seed of each plant were considered to belong to one family. A sand culture was prepared according to Yoshida et al. (1971) with a bulk density of approximately 1.4 g cm<sup>-3</sup> in a controlled-environment growth chamber at a light intensity of approximately 500 μmol m<sup>-2</sup> s<sup>-1</sup> with a 16-/8-h (light/dark) photoperiod. Relative humidity was maintained at about 70 (±5)%, and the day/night temperature was maintained at 24/16 (±2)°C. An experiment was designed as a split-plot with three replicates. Three salinity levels (0.85, 10.0, and 20.0 dS m<sup>-1</sup>) were the main plots and genotypes (subpopulations) were the subplots. The 0.85 dS m<sup>-1</sup> saline solution was used in the sand culture to simulate natural field conditions (Kader and Jutzi, 2002). The 10.0 dS m<sup>-1</sup> salinity level was chosen to represent the predominant salinity level of saline water aquifers in the Arabian Peninsula (Hussain et al., 1997; ICBA, 2000). A nutrient solution (Timm et al., 1991) was prepared separately for each treatment. Irrigation solutions were prepared in 250-L reservoirs. A separate preprogrammed pump provided irrigation three times daily to plants in each treatment. Overflow irrigation was returned to the reservoirs by gravity.

A total of 6300 seeds (i.e., 10 seeds per subpopulation, family, treatment, and replicate) were planted in the sand culture at a distance of 20 cm between rows and 10 cm between seeds within rows. NaCl and CaCl<sub>2</sub> (5:1 molar concentration) were added to the nutrient solutions on the first day after

**Table 1.** Germination index (GI), thermal time to 50% germination (d50), mg seedling dry weight mg-seed-dry-weight<sup>-1</sup> (delta), and salinity susceptibility indices (SSI) based on arcsine-transformed percent reduction in seedling weight (SSI<sub>sw</sub>), root length (SSI<sub>r</sub>), shoot length (SSI<sub>s</sub>), number of roots (SSI<sub>n</sub>), and percent tolerant accessions of 2308 accessions in seven subpopulations of the Batini barley landrace under 0.0 and 20.0 dS m<sup>-1</sup> salinity stress.

Subpopulation	Number of accessions	Salinity stress level, dS m <sup>-1</sup>										Percent tolerant accessions
		0.0		20.0		0.0		20.0		0.0		
Batini 1	327	1.72a†	8.25a	36a	74ab	0.382c	0.364d	0.97ab	0.83a	0.82bc	1.19a	45.8
Batini 2	363	1.31c	5.98b	24cd	64c	0.467b	0.416b	0.84b	0.54b	0.62d	0.87c	78.8
Batini 3	383	1.25d	9.12a	24cd	82a	0.470b	0.409b	1.05a	0.63b	0.76c	0.82c	74.1
Batini 4	492	1.41b	5.23b	28bc	62c	0.514a	0.468a	0.71b	0.48b	0.67d	0.72d	83.3
Batini 5	247	1.30c	7.88a	28bc	76a	0.439b	0.370cd	0.95b	0.69a	0.82ab	1.02b	54.6
Batini 6	184	1.12e	7.09a	22d	72b	0.470b	0.390c	1.18a	0.86a	1.04a	1.09a	44.6
Batini 7	312	1.38b	4.38b	30b	60c	0.450b	0.415b	1.15a	0.92a	0.99a	0.84d	30.4
Mean		1.36	6.84	27.4	70.0	0.456	0.405	0.98	0.71	0.82	0.94	66.5
Total	2308											

† Values followed by the same letter, for each measured variable within each column, do not differ significantly at the 0.05 level of probability (Tukey HSD for unequal N).

planting. Salinity levels were maintained throughout the eight week-duration of the experiment. Electrical conductivity ( $EC_w$ ) of the nutrient solutions was measured, and if necessary adjusted, three times a week. Seedling emergence was monitored and recorded at 8-h intervals until no further germination was observed. Data on germination percentage and seedling emergence as well as cumulative thermal time (in degrees Celsius per day,  $^{\circ}Cd^{-1}$ , above a base temperature of  $0.0^{\circ}C$ ) and fresh forage yield of first and second cuts were collected on the basis of single replicates, families, and treatments. Fresh forage of first and second cuts was dried in a forced-air oven for 24 h at  $70^{\circ}C$ , and the dry forage weight was recorded for each replicate, subpopulation, treatment, and family. A salinity intensity index, analogous to the drought intensity index of Fisher and Maurer (1978), was calculated for variables measured under stress and nonstress conditions as  $SII = 1 - (X_{ss}/X_{ns})$ , where  $X_{ss}$  and  $X_{ns}$  are means of all subpopulations, or families under salinity, and nonsalinity stress, respectively.

### Statistical Analysis

Data for each variable were plotted to test for normality, and the homogeneity of variances among subpopulations was verified by a Bartlett's test (Zar, 1996). Since no significant differences were found among replicates within each salinity treatment and experiment (data not presented), data for each variable from all replicates within a salinity treatment and experiment were combined for statistical analyses. The positive and significant correlation coefficients ( $r > 0.92$ ;  $P < 0.01$ ) found among replicates of a certain treatment were considered as indicators of repeatability of the experiment (Zar, 1996).

A germination index (GI) was calculated for each subpopulation as  $GI = (\sum T_i N_i)/S$ , where  $T_i$  is the thermal time ( $^{\circ}Cd^{-1}$ ) between seed imbibition and germination,  $N_i$  is the number of seed germinated on the  $i$ th day, and  $S$  is the total number of seeds germinated by the end of the experiment (Foolad et al., 1999).

Temporal patterns of seed germination in the seed germination experiment or seedling emergence in the sand culture experiment (González-Astroga and Núñez-Farfán, 2000) were statistically analyzed by calculating a coefficient of aggregation (CA) as  $CA = \sigma^2/\bar{x}$ , where  $\sigma^2$  and  $\bar{x}$  are the variance and mean values, respectively, of germination or seedling emergence data (i.e., based on number of germinating seed or emerging seedlings per unit thermal time). If  $CA = 1.0$ ,  $<1.0$ , or  $>1.0$ , then the distribution of these variables is random, more uniform than random or contagious (over-dispersed), respectively. Deviations of CA from unity were tested by a  $t$  test (Zar, 1996).

Statistical analyses were performed on arcsine-transformed data expressed as percentages (Table 1), whereas number of germinated seed and number of roots, two discontinuous variables, were transformed by a modified square root formula [ $Y = (X + 3/8)^{0.5}$ ] (Alonso et al., 1999). Salinity susceptibility indices (SSI) were calculated for seedling weight, root length, shoot length, and number of roots in the seed germination experiment, and for the dry forage yield in the sand culture experiment as  $SSI = (1 - Y_{ss}/Y_{ns})/SII$ , where  $Y_{ss}$  and  $Y_{ns}$  are subpopulation, or family means for a particular trait under salinity stress and nonstress, respectively (Fisher and Maurer, 1978). Dry forage yield at the 0.85 and  $10.0 dS m^{-1}$  salinity stress levels was used as  $Y_{ns}$  to calculate SSI estimates for the 10.0 and  $20.0 dS m^{-1}$  treatments, respectively (Fig. 1).

A mixed effects model was used to perform the analysis of variance of all data recorded on seedling and plant growth attributes. The variance components due to subpopulations

( $\sigma_g^2$  or genetic variance), genetic  $\times$  salinity variance ( $\sigma_{g,sl}^2$ ), and error variance ( $\sigma_e^2$ ) were estimated according to Comstock and Moll (1963). The phenotypic variance was calculated as  $\sigma_p^2 = \sigma_g^2 + (\sigma_{g,sl}^2/s) + (\sigma_{e/rs}^2)$ , where "r" is the number of replicates and "s" is the number of salinity levels. An estimate of the broad-sense heritability (Falconer, 1981) was calculated as the ratio of the genetic ( $\sigma_g^2$ ) and the phenotypic ( $\sigma_p^2$ ) variances.

Thermal time ( $^{\circ}Cd^{-1}$ ) for 50% germination ( $d_{50}$ ) in the seed germination experiment was calculated according to Flowers and Hajibagheri (2001) assuming a base temperature of  $0.0^{\circ}C$  (Alvarado and Bradford, 2002). Seedling survival rate in the sand culture experiment was measured at  $100^{\circ}Cd^{-1}$  and was calculated as the percentage of live seedlings from germinated seed. All plants, in each replicate and treatment in the sand culture experiment, were clipped (5 cm above ground) at the end of the tillering stage, and just before the start of stem elongation (i.e., most appropriate stage for forage harvest), and their  $^{\circ}Cd^{-1}$  was recorded. All above-ground biomass was harvested 4 wk after the first cut (i.e., at  $525^{\circ}Cd^{-1}$  after the first cut), and the fresh weight for both cuts was recorded. Thermal time was used in both experiments to provide a more precise assessment of seed and seedling response to salinity (Flowers and Hajibagheri, 2001).

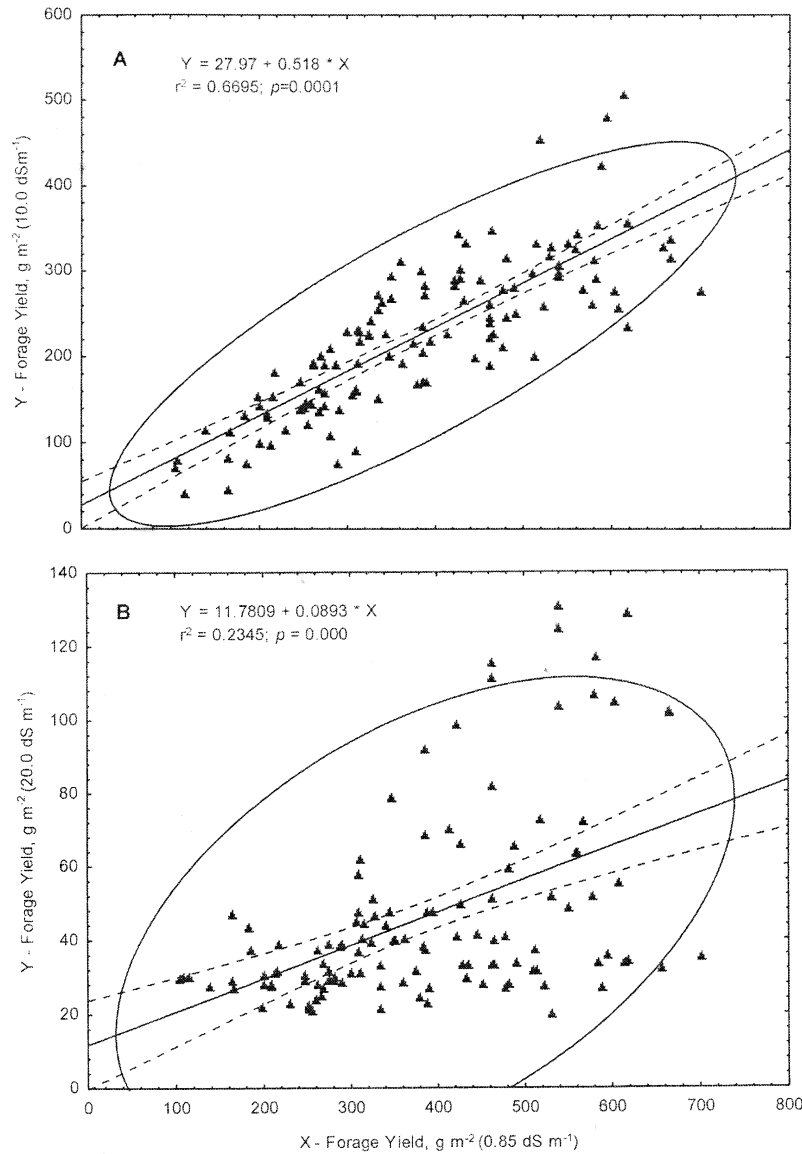
The principal components analysis (PCA) was used to reduce the dimensionality of, and extract maximum variance in, 26 indices and derived-variables calculated for the seven subpopulations in both experiments (Fig. 2a). Additionally, PCA was used to plot the seven subpopulations on the basis of their factor coordinates (Fig. 2b).

For the continuous data (i.e., percent germination at 0.0 and  $20.0 dS m^{-1}$ ,  $d_{50}$ , seedling dry weight at 0.0 and  $20.0 dS m^{-1}$ , shoot length, and number and length of roots at 0.0 and  $20.0 dS m^{-1}$ , cumulative  $^{\circ}Cd^{-1}$  to first cut, and forage yield for first and second cuts at 0.85, 10.0, and  $20.0 dS m^{-1}$ ), a mean and standard deviation (s.d.) were calculated for each variable. These statistics were used to classify accessions into three categories (i.e., low [ $\leq \bar{x} - s.d.$ ], medium [ $\geq \bar{x} - s.d.$  to  $\leq \bar{x} + s.d.$ ], and high [ $\geq \bar{x} + s.d.$ ]), according to Zar (1996). For discrete multivariate analyses (Agregi, 1990), this method of grouping was considered more appropriate than the classification based on the 33rd and 67th centiles. A polymorphic diversity index was calculated from frequency data of the low, medium, and high categories for each subpopulation, and was used to estimate the average distance among all pairs of subpopulations (Yeh et al., 2000). Tukey's HSD mean separation test for unequal number of observations per mean ( $P = 0.05$ ) was used for pairwise comparisons among subpopulation phenotypic distances. Finally, families within each subpopulation were ranked according to their performance (based on mean and standard deviation) in the germination and sand culture experiments as tolerant, medium tolerant, or susceptible. All statistical analyses were conducted with several modules in the statistical packages STATISTICA 6.0 (StatSoft Inc., 2001), SYSTAT 10.2 (SYSTAT Software Inc., 2002, p. 665), and POPGENE (Yeh et al., 2000).

## RESULTS

### Germination Test

All subpopulations displayed a continuous distribution for all variables under the 0.0 and  $20.0 dS m^{-1}$  salinity levels; however, variances were 2 to 3 times higher under  $20.0 dS m^{-1}$  (data not presented). Seed germination of all subpopulations was delayed by salinity stress. Germination index, thermal time to 50% germination ( $d_{50}$ ),



**Fig. 1.** Forage yield ( $\text{g m}^{-2}$ ) at 10.0 (a) and 20.0  $\text{dS m}^{-1}$  (b) as a function of forage yield at 0.85  $\text{dS m}^{-1}$  salinity stress for seven subpopulations of the Batini barley landrace.

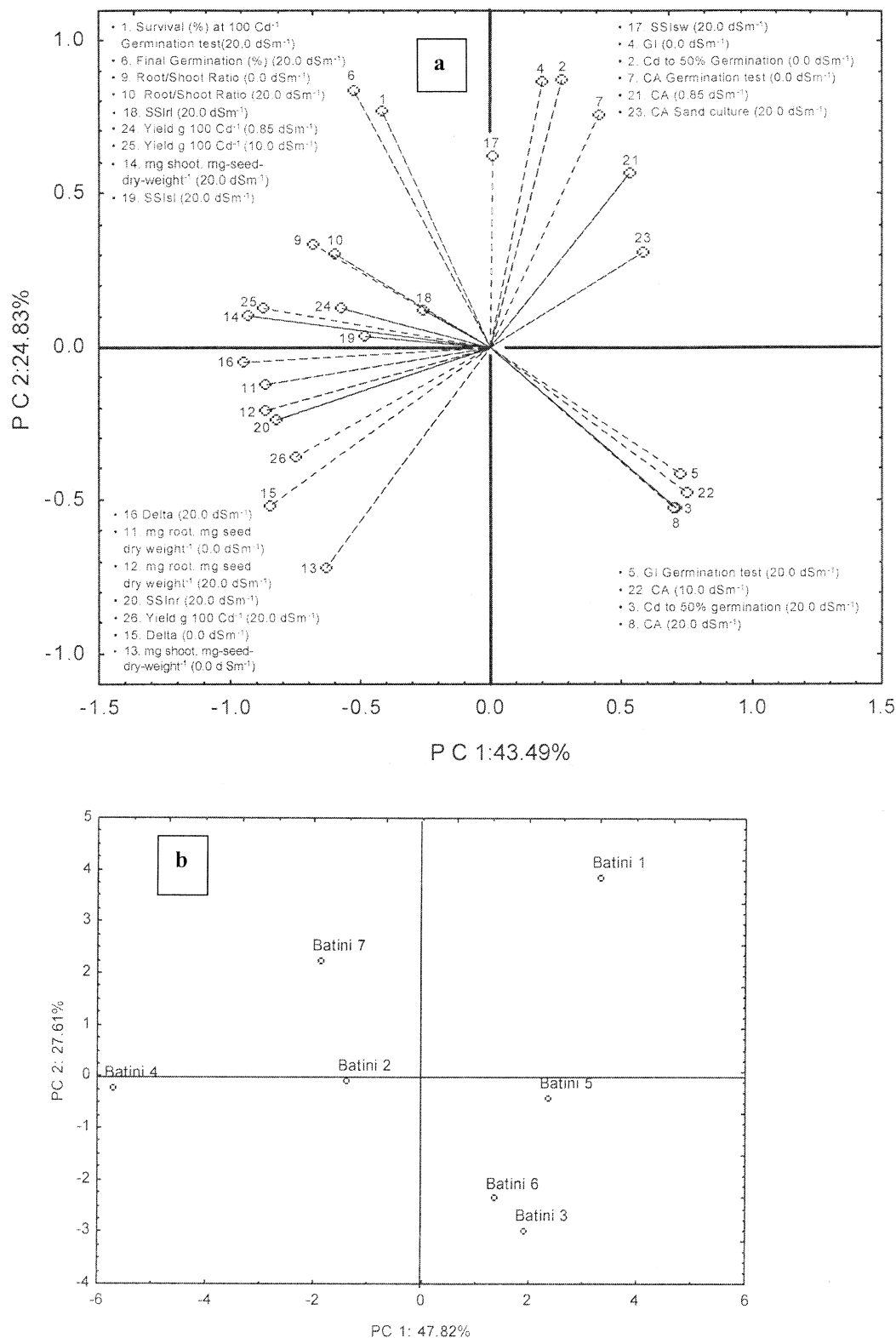
milligrams seedling dry weight per milligrams seed dry weight ( $\Delta$ ), and salinity susceptibility indices based on four seedling attributes, varied widely among subpopulations in response to the 20.0  $\text{dS m}^{-1}$  salinity treatment (Table 1). Germination index averaged 1.36 (range 1.12–1.72) under no-salinity stress; however, salinity stress delayed germination 5-fold (mean GI = 6.84), and GI of the slowest germinating subpopulation (Batini 3) was twice as high as GI of the fastest (Batini 7).

Mean thermal time to 50% germination more than doubled (27.43–70.0 $^{\circ}\text{Cd}^{-1}$ ) under salinity stress. Subpopulations Batini 7, Batini 4, and Batini 2 germinated faster (60–64 $^{\circ}\text{Cd}^{-1}$ ) than the remaining subpopulations that germinated either at an average (Batini 6 and Batini 1) or slow (Batini 5 and Batini 3) rate. Germination indices (GI) at 0.0 and 20.0  $\text{dS m}^{-1}$  were not significantly correlated; however, GI and final germination percentage, under 20.0  $\text{dS m}^{-1}$  salinity stress, were negatively and significantly correlated ( $r = -0.71$ ;  $P < 0.05$ ).

The averaged value of  $\Delta$  was 0.456 and 0.405 for the 0.0 and 20.0  $\text{dS m}^{-1}$  salinity treatments (Table 1). Variation among subpopulations (Tukey HSD,  $P = 0.05$ ) was higher at the 20.0 than at the 0.0  $\text{dS m}^{-1}$  salinity level. Batini 4 produced significantly higher dry seedling weight per unit seed weight at both salinity stress levels, as compared with the remaining subpopulations.

Four salinity susceptibility indices (Table 1) separated the seven subpopulations into different groupings (Tukey HSD,  $P = 0.05$ ). On average, seedling dry weight (SSI = 0.98) and number of roots per seedling (SSI = 0.94) were drastically reduced in response to salinity stress. The least affected seedling attribute was root length (SSI = 0.71), whereas shoot length was moderately reduced (SSI = 0.82) in response to salinity stress.

The least discriminating index was the one based on seedling dry weight, followed by the one based on root length. Salinity susceptibility indices based on shoot



**Fig. 2.** Plot of the first two principal components of 26 indices and derived-variables (a) and seven subpopulations (b) of the Batini barley landrace.

length and number of roots were most discriminating among subpopulations and were the only positively and significantly correlated indices ( $r = 0.76$ ;  $P < 0.05$ ).

A large portion (66.5% or 1535 of the original 2308 accessions) of the total germplasm collection was classi-

fied as tolerant to salinity (Table 1). However, subpopulations differed considerably in this regard. For example, Batini 7 and Batini 4 exhibited the lowest (30.4%) and the highest (83.3%) salt tolerance, respectively. Salt tolerant accessions in Batini 4 and Batini 2 were associated

**Table 2. Genetic ( $\sigma_g^2$ ), genetic  $\times$  salinity level interaction ( $\sigma_{g \times sl}^2$ ), error ( $\sigma_e^2$ ), and phenotypic ( $\sigma_p^2$ ) variances, along with estimates of broad-sense heritability ( $H$ ) for five germination and seedling attributes of 2308 accessions in seven subpopulations of the Batini barley landrace under 0.0 and 20 dS m<sup>-1</sup> salinity stress levels.**

Variable	$\sigma_g^2$	$\sigma_{g \times sl}^2$	$\sigma_e^2$	$\sigma_p^2$	$H$
Germination index	128.32***	23.68*	8.019	164.25	0.78
Seedling dry weight	1.0643***	0.0034**	0.0002	1.56	0.68
Number of roots	725.5***	2.59*	0.98	933.8	0.78
Root length	22 164***	10.46*	4.82	27 232	0.81
Shoot length	3 937.5***	11.19ns	6.502	4 958.8	0.79

\* Significant at the 0.05 level of probability.

\*\* Significant at the 0.01 level of probability.

\*\*\* Significant at the 0.001 level of probability.

ns, not significant.

with longer seedling roots than average ( $SSI_{sw} = 0.71$  and  $0.84$ , respectively), whereas Batini 3 produced an above average number of roots per seedling ( $SSI_{nr} = 0.82$ ).

### Genetic Variance and Temporal Variation

Variance structures for GI and for four seedling attributes (seedling dry weight, number of roots, root length, and shoot length) were dominated by among-subpopulation effects (Table 2). The broad-sense heritability ( $H$ ) estimates ranged from 0.68 for seedling dry weight to 0.79 for shoot length. The interaction components were all significant, except for shoot length.

Temporal variation in the seed germination experiment averaged 0.40 (range 0.18–0.82) and 1.02 (range 0.57–1.68) for the 0.0 and 20.0 dS m<sup>-1</sup> salinity stress treatments, respectively (Table 3). A  $t$  test indicated that temporal variation for four subpopulations (Batini 1, Batini 2, Batini 4, and Batini 7) did not increase as a result of salinity stress. Only three temporal variation estimates (Batini 1, Batini 5, and Batini 3 in increasing order) were significantly higher than 1.0 under salinity stress. Temporal variation in seedling emergence, at three salinity levels in the sand culture experiment, increased progressively from 0.48 to 5.39 (Table 3) as salinity stress level increased from 0.85 to 20.0 dS m<sup>-1</sup>.

Differential subpopulation responses to increasing salinity levels are evident when results of mean separation under each salinity stress level (Tukey HSD,  $P = 0.05$ ) are compared. Batini 1 was significantly different from the remaining subpopulations at 0.85 dS m<sup>-1</sup>, whereas the 10.0 and 20.0 dS m<sup>-1</sup> salinity treatments separated the

seven subpopulations into three and two significantly different groupings, respectively.

Variance components derived from analyses of variance for the arcsine-transformed seedling emergence percentage at each of the three salinity levels (Table 4) are dominated by the among-subpopulations effect. The among-families (within subpopulations) components were also significant and slightly decreased in magnitude at the 20.0 dS m<sup>-1</sup> salinity stress level. The contribution of the within-families component was uniformly low (1% or less of the total variance in each case). By contrast, the contributions of among-subpopulations variation, and among-families variation to the overall ANOVAs, when tested by the Satterthwait's method of denominator synthesis (StatSoft Inc., 2001), were highly significant at all salinity levels.

### Thermal Time and Forage Yield

Mean estimates of pretiltering thermal time (first cut) and fresh forage yield (g m<sup>-2</sup>) at tillering, and at 525°Cd<sup>-1</sup> after the first cut (Table 5), were highly influenced by salinity stress level and subpopulation. Total forage yield, whether expressed on unit-area (g m<sup>-2</sup>) or thermal-time (g 100°Cd<sup>-1</sup>) basis, was significantly reduced, especially for the second cut at the medium (10.0 dS m<sup>-1</sup>) and high (20.0 dS m<sup>-1</sup>) salinity stress levels. Cumulative °Cd<sup>-1</sup> to first cut at 0.85, 10.0, and 20.0 dSm<sup>-1</sup> averaged 523.0, 523.4, and 579.4, respectively; the last mean estimate was significantly different from the first two.

Total fresh forage yield (first and second cuts combined) was reduced by 22 and 82% at 10.0 and 20.0 dS m<sup>-1</sup>, respectively. Differences among subpopulations

**Table 3. Temporal variation in germination of 2308 accessions at two (0.0 and 20.0 dS m<sup>-1</sup>) salinity levels, and in seedling emergence of 10 families from each of seven subpopulations of the Batini barley landrace at three salinity (0.85, 10.0, and 20.0 dS m<sup>-1</sup>) levels, as measured by the variance-to-mean ratio ( $\sigma^2/\bar{x}$ , coefficient of aggregation).**

Subpopulation	Seed germination experiment				Sand culture experiment		
	Salinity level, dS m <sup>-1</sup>		$t$	$P$	Salinity level, dS m <sup>-1</sup>		
	0.0	20.0			0.85	10.0	20.0
	$\sigma^2/\bar{x}$				$\sigma^2/\bar{x}$		
Batini 1	0.82	1.18	-0.114	0.913	0.95a†	1.21b	8.65a
Batini 2	0.33	0.62	-0.374	0.219	0.42b	0.73c	4.09b
Batini 3	0.30	1.68	-3.172	0.019	0.38b	1.53a	8.15a
Batini 4	0.37	0.57	-0.524	0.619	0.42b	0.64c	2.76b
Batini 5	0.38	1.45	-2.956	0.025	0.38b	1.05b	3.14b
Batini 6	0.18	1.07	-2.735	0.034	0.48b	1.19b	3.81b
Batini 7	0.34	0.54	-0.313	0.765	0.34b	0.75c	7.12a
Mean	0.40	1.02			0.48	1.014	5.39

† Values followed by the same letter, for each measured variable within each salinity level, do not differ significantly at the 0.05 level of probability (Tukey HSD for unequal N).

**Table 4.** Variance components derived from analyses of variance for the arcsine-transformed emergence percentage at each of three salinity levels for 10 families from each of seven subpopulations of the Batini barley landrace.

Variance component	Salinity stress level, dS m <sup>-1</sup>		
	0.85	10.0	20.0
Among subpopulations	0.787**	0.663**	0.34**
Among families within subpopulations	0.065**	0.068**	0.045**
Within families	0.0057	0.0062	0.0059

\*\* Significant at the 0.01 level of probability.

for forage yield within each salinity level were highly significant. In addition, the highest yielding subpopulations at the low or medium salinity levels were not the highest yielding at the high salinity level. Batini 3 ranked first, second, and second at the 0.85, 10.0, and 20.0 dS m<sup>-1</sup> salinity levels, respectively. On the other hand, Batini 4 ranked third, first, and first at the 0.85, 10.0 and 20.0 dS m<sup>-1</sup> salinity levels, respectively. On average, fresh forage yield of Batini 4, at the 10.0 dS m<sup>-1</sup> salinity level, exceeded that of the lowest yielding subpopulation (Batini 5) and the overall mean by 44 and 22%, respectively. Additionally, its fresh forage yield at the 20.0 dS m<sup>-1</sup> salinity level exceeded that of the lowest yielding subpopulation (Batini 1) and the overall mean by 132 and 45%, respectively.

Mean fresh forage yield of the first cut decreased at a rate of 2.07% when salinity stress increased from 0.85 to 10.0 dS m<sup>-1</sup> and at a rate of 7.5% when salinity stress increased from 10.0 to 20.0 dS m<sup>-1</sup>. The respective values for the second cut were 2.4 and 7.9%. Batini 4 experienced the least (6.9 g m<sup>-2</sup> dS m<sup>-1</sup>) and Batini 2 the most (36.5 g m<sup>-2</sup> dS m<sup>-1</sup>) decrease at the 10.0 dS m<sup>-1</sup> salinity stress level. At the highest salinity stress

**Table 5.** Salinity susceptibility index (SSI), mean thermal time (°Cd<sup>-1</sup>) to reach end of the tillering stage, forage yield at end of the tillering stage (first cut) and four weeks after the first cut (at 525°Cd<sup>-1</sup>) for 10 families from each of seven subpopulations of the Batini barley landrace grown under 0.85, 10.0, and 20.0 dS m<sup>-1</sup> salinity stress levels in a sand culture.

Salinity	Variable	Subpopulation							Mean
		Batini 1	Batini 2	Batini 3	Batini 4	Batini 5	Batini 6	Batini 7	
<b>dS m<sup>-1</sup></b>									
0.85	Cumulative °Cd <sup>-1</sup> to 1st cut	456e†	520d	528d	437f	550c	576b	592a	523.0
	First cut, g m <sup>2</sup>	1051a	989a	998a	925b	764c	889b	923b	934.0
	Yield, g 100°Cd <sup>-1</sup>	230.6	190.2	188.9	214.3	138.4	154.3	155.9	181.8
	Second cut, g m <sup>2</sup>	546b	627a	633a	665a	486c	429c	548b	562.0
	Yield, g 100°Cd <sup>-1</sup>	104	119.4	120.5	126.7	92.6	81.7	104.4	107.0
	Forage yield, g m <sup>2</sup>	1597a	1616a	1631a	1590a	1318c	1471b	1496b	1496.0
10.0	Cumulative °Cd <sup>-1</sup> to 1st cut	448d	520c	528c	440d	560b	568b	600a	523.0
	First cut, g m <sup>2</sup>	867a	624d	807b	857a	654d	635d	737c	740.1
	Yield, g 100°Cd <sup>-1</sup>	193.5	120.0	152.9	194.7	116.8	111.8	122.8	144.6
	Second cut, g m <sup>2</sup>	351c	438b	456b	568a	336c	358c	488b	427.9
	Yield, g 100°Cd <sup>-1</sup>	66.8	83.5	84.6	108.2	64.0	68.2	92.9	81.2
	Forage yield, g m <sup>2</sup>	1218b	1062c	1263b	1424a	990c	993c	1225b	1168
20.0	Salinity susceptibility index§	1.09b	1.59a	1.04b	0.50d	0.97bc	1.50a	0.86c	
	Cumulative °Cd <sup>-1</sup> to 1st cut	576b	584b	576b	472c	616a	608a	624a	579.4
	First cut, g m <sup>2</sup>	104d	201b	225b	269a	172c	162c	159c	184.6
	Yield, g 100°Cd <sup>-1</sup>	17.9	34.4	38.3	56.9	27.8	26.6	25.5	32.49
	Second cut, g m <sup>2</sup>	65d	87c	103b	124a	78c	75c	70cd	86.0
	Yield, g 100°Cd <sup>-1</sup>	12.3	16.6	19.6	23.6	14.9	14.3	13.2	16.36
	Forage yield, g m <sup>2</sup>	169f	288c	328b	393a	250de	237e	229e	270.6
	Salinity susceptibility index‡	1.12a	0.94b	0.96b	0.94b	1.13a	1.00b	1.05ab	
	LSD for cumulative °Cd <sup>-1</sup> to 1st cut	P = .05							35.0
	LSD for 1st cut, g m <sup>2</sup>	P = 0.5							124.8
	LSD for second cut, g m <sup>2</sup>	P = 0.05							76.5
	LSD for mean forage yield, g m <sup>2</sup>	P = 0.05							174.8

† Values followed by the same letter, for each measured variable within each salinity level, do not differ significantly at the 5% level of probability (Tukey HSD for unequal N).

§ Based on forage dry weight under 0.085 and 10.0 dS m<sup>-1</sup>.

‡ Based on forage dry weight under 0.85 and 20.0 dS m<sup>-1</sup>.

**Table 6.** Genetic ( $\sigma_g^2$ ), genetic  $\times$  salinity level interaction ( $\sigma_{g \times sl}^2$ ), error ( $\sigma_e^2$ ), phenotypic ( $\sigma_p^2$ ) variance estimates for germination index and forage yield, and estimates of the broad-sense heritability (H) of 10 families from each of seven subpopulations of the Batini barley landrace under 0.85, 10.0 and 20 dS m<sup>-1</sup> salinity stress levels.

Variable	$\sigma_g^2$	$\sigma_{g \times sl}^2$	$\sigma_e^2$	$\sigma_p^2$	H
Germination index	153.8**	46.14**	12.08	198.7	0.77
Forage yield					
First cut	201 572**	375 948**	5 922	327 380	0.62
Second cut	96 754.5**	176 696**	4 308	147 329	0.65

\*\* Significant at the 0.01 level of probability.

level (20.0 dS m<sup>-1</sup>), however, Batini 2 experienced the highest (76.3 g m<sup>-2</sup> dS m<sup>-1</sup>) and Batini 4 the lowest (42.3 g m<sup>-2</sup> dS m<sup>-1</sup>) decrease in forage yield.

Forage yields (expressed as g 100°Cd<sup>-1</sup>) at 0.85, 10.0, and 20.0 dS m<sup>-1</sup> salinity levels were positively correlated with root dry weight (at 0.0 dS m<sup>-1</sup> in the germination test) in an increasing magnitude ( $r = 0.42$ , ns;  $0.46$ , ns; and  $0.79$ ,  $P = 0.05$ , respectively). However, forage yield at 20.0 dS m<sup>-1</sup> was only positively and significantly correlated ( $r = 0.80$ ,  $P = 0.01$ ) with the salinity susceptibility index based on number of roots (SSI<sub>nr</sub>) in the germination test.

Genetic, genetic  $\times$  salinity level, phenotypic variances for GI, and forage yield at the first and second cuts (Table 6) were dominated by among-subpopulation effects. Nevertheless, all genetic  $\times$  salinity interaction components were significant ( $P < 0.01$ ). Broad-sense heritability estimates were 0.77, 0.62, and 0.65 for the germination index and forage yield for the first cut and forage yield for the second cut, respectively.

The relationships between forage yield at the 0.85,

**Table 7. Linear regression equations describing forage yield under 10.0 and 20.0 dS m<sup>-1</sup> salinity stress (FY<sub>10</sub>, and FY<sub>20</sub>, respectively) as a function of forage yield under no stress (FY<sub>Max</sub>) of 10 families from each of seven subpopulations of the Batini barley landrace.**

Subpopulation	Variable		Intercept, $\alpha$	Regression coefficient, $\beta$	Adjusted $R^2$	$P$
	Dependent	Independent				
Batini 1	FY <sub>10</sub>	FY <sub>Max</sub>	-18.23	0.42	0.94	0.000
Batini 2			3.409	0.45	0.96	0.000
Batini 3			6.799	0.49	0.94	0.000
Batini 4			-2.1458	0.59	0.94	0.000
Batini 5			5.0211	0.66	0.96	0.000
Batini 6			3.3851	0.74	0.99	0.000
Batini 7			0.1249	0.83	0.96	0.000
Batini 1	FY <sub>20</sub>	FY <sub>Max</sub>	30.464	0.008	0.203	0.191
Batini 2			-2.505	0.119	0.471	0.001
Batini 3			17.642	0.093	0.181	0.100
Batini 4			-15.579	0.169	0.328	0.001
Batini 5			24.518	0.048	0.107	0.148
Batini 6			33.144	0.006	0.004	0.800
Batini 7			25.390	0.034	0.129	0.307

10.0, and 20.0 dS m<sup>-1</sup> salinity levels are presented in Fig. 1 for all subpopulations combined and in Table 7 for individual subpopulations. Forage yield at the moderate (10.0 dS m<sup>-1</sup>) salinity level can be predicted with reasonable accuracy (adjusted  $R^2 = 0.67$ ) as a function of forage yield at the 0.85 dS m<sup>-1</sup> salinity level (Fig. 1a). The separate regression equations (Table 7) clearly point to the large variable responses among subpopulations to salinity stress, but with high  $R^2$  values. Forage yield under moderate salinity can be predicted with high accuracy with forage yield under no salinity stress conditions as a predictor (adjusted  $R^2$  values ranged from 0.94–0.99). The accuracy with which forage yield at the highest salinity level (20.0 dS m<sup>-1</sup>) can be predicted decreased dramatically, however, with a low adjusted  $R^2$  of 0.23 (Fig. 1b). Separate regression equations (Table 7, lower part) suggest that forage yield under 20.0 dS m<sup>-1</sup> salinity stress can be predicted with some accuracy (adjusted  $R^2 = 0.48$ , and 0.33;  $P = 0.001$ ) for at least two (Batini 2 and Batini 4) subpopulations.

### Multivariate Data Analysis

Principal components analysis was utilized to group all 26 measured and derived variables (Fig. 2a) into the minimum number of components that can account for the maximum variance available in the multivariate data set. Seventeen variables loaded heavily on PC1, whereas the remaining nine were associated with PC2. The first and second PCs accounted for 43.49 and 24.83% of the total variance, respectively. PC1 was dominated by variables that describe responses to high salinity stress, whereas variables describing germination and survival at low and medium salinity stress dominated PC2. Figure 2b shows subpopulation loadings on the first two PCs.

Variables that explained maximum variation in PC1, in decreasing magnitude, were  $\Delta$  (20.0 dS m<sup>-1</sup>), milligrams shoot dry weight per milligrams seed dry weight, forage yield g 100°Cd<sup>-1</sup>, milligrams root dry weight per milligrams seed dry weight (at 0.0 and 20.0 dS m<sup>-1</sup>),  $\Delta$  (0.0 dS m<sup>-1</sup>), and salinity susceptibility index based on number of roots per seedling at 20.0 dS m<sup>-1</sup>. On the other hand, the variables °Cd<sup>-1</sup> to 50% germination at 0.0 dS m<sup>-1</sup>, germination index at 0.0 dS m<sup>-1</sup>, final germination (%) at 20.0 dS m<sup>-1</sup>, survival rate (%) at 100°Cd<sup>-1</sup>,

CA at 0.0 dS m<sup>-1</sup>, and milligrams shoot per milligrams seed dry weight (0.0 dS m<sup>-1</sup>), loaded high on, and explained maximum variation in, PC2.

Four subpopulations (Batini 1, Batini 3, Batini 5, and Batini 6) exhibited five variables with positive loadings on PC1 and five variables with positive loadings on PC2. The remaining three subpopulations (Batini 2, Batini 4, and Batini 7) exhibited one, and 13 variables with positive and negative loadings on PC1, respectively; and one and two variables with negative and positive loadings on PC2, respectively.

A few variables (variables 18, 19, and 24, in increasing order; Fig. 2a) had relatively small and negative, and small and positive loadings on PC1, and PC2, respectively, as compared with other variables. However, these variables were positively correlated with yield at low and medium salinity stress, and negatively correlated with temporal variation at medium and high salinity stress.

The relationships between two variables measured at the seedling growth stage (CA at 20.0 dS m<sup>-1</sup> [variable 8] and milligrams shoot per milligrams seed dry weight at 20.0 dS m<sup>-1</sup> [variable 14]), and two other variables measured during plant growth in sand culture (yield g 100°Cd<sup>-1</sup> at 10.0 and at 20.0 dS m<sup>-1</sup> [variables 25, and 26, respectively]) are of particular interest. The first two variables were negatively and significantly correlated ( $r = -0.70$ ,  $P = 0.05$ ), whereas the second two were positively and significantly correlated ( $r = 0.73$ ,  $P = 0.5$ ). However, variable 8 was negatively correlated with variable 24 ( $r = -0.61$ ,  $P = 0.06$ ) and with variable 25 ( $r = -0.10$ ,  $P = 0.36$ ). On the other hand, variable 14 is positively and significantly correlated with both variables 25 and 26 ( $r = 0.92$ ,  $P = 0.01$ ; and  $r = 0.72$ ,  $P = 0.05$ , respectively). Consequently, accessions with high yield at medium and high salinity stress levels could be identified at the germination stage by simultaneously selecting for low variance-to-mean ratio and high shoot dry weight at 20.0 dS m<sup>-1</sup>.

### Salinity Susceptibility Index and Ranking

Average SSI, based on dry forage yield in the sand culture experiments, separated the seven subpopulations into three groups: Batini 4, with the lowest SSI (0.72) was the most salt tolerant; Batini 7, Batini 3, and



**Table 8. Salinity susceptibility index, mean phenotypic distance among, and number of families classified as salt tolerant, medium tolerant or susceptible in seven subpopulations of the Batini barley landrace.**

Subpopulation	Batini 1	Batini 2	Batini 3	Batini 4	Batini 5	Batini 6	Batini 7
Salinity susceptibility index	1.1b†	1.27a	1.00b	0.72c	1.05b	1.25a	0.96b
Mean phenotypic distance	0.65c	0.86b	0.97a	0.95a	0.44d	0.52d	0.57cd
Number of families rated as:							
Tolerant	3	2	4	6	4	2	4
Medium tolerant	3	5	3	2	3	2	2
Susceptible	4	3	3	2	3	6	4

† Values, within each row, followed by the same letter do not differ significantly at  $P = 0.05$ .

Batini 5 were intermediate; and Batini 2 and Batini 6 were the most susceptible (Table 8). There were 13 (or 62%) pairwise significant differences out of the 21 possible pairwise comparisons for mean phenotypic distances among all seven subpopulations (Table 8). Results of mean comparisons among subpopulations indicate that Batini 3, Batini 4, and Batini 2, in decreasing order, were the most distant from the others. The remaining 4 subpopulations were less distant, and differed significantly from the first three.

Categorical classification of families within subpopulations, based on their SSI estimates, grouped them into one of three categories: salt tolerant, moderately tolerant, or sensitive. Twenty-six, 19, and 25 families were classified as tolerant, medium tolerant, and susceptible, respectively. Out of the 10 families in each subpopulation tested in this study, Batini 4 has most (six) of its families classified as salt tolerant, followed by Batini 3 (four), Batini 5 (four), Batini 7 (four), Batini 1 (three), Batini 2 (two), and Batini 6 (two).

## DISCUSSION

Crops more often are confronted with higher salinity at the germination and early seedling growth stages than at later stages when plants are vigorously growing because germination and early seedling growth occur in surface soils where there is higher salt accumulation due to evaporation and capillary rise of water (Almansouri et al., 2001). Screening a wide barley germplasm base (Flowers and Hajibagheri, 2001) helped establish differences in salt tolerance as evidenced by a number of salinity susceptibility indices in two different experimental environments and at germination, seedling and tillering stages.

Genetic variation in salinity tolerance of a large number of accessions of the Batini barley landrace may represent adaptation to diverse local environments in the Batinah region. The climatic, edaphic, and cultural factors in the hot, semiarid Batinah are major factors in the creation of saline soils that are associated with coastal areas and flood irrigation (El-Kharbotly et al., 2003). Many salt tolerant accessions of barley (Munns et al., 2002) and other species such as *Phaseolus vulgaris* L. (Bayuelo-Jimenez et al., 2002), *Lycopersicon esculentum* Mill. (Foolad et al., 1998), and *Oryza sativa* L. (Zeng et al., 2002) originated in arid, coastal, or saline areas.

Results of the uni-, and multivariate analyses demonstrated genetic variation, within and among subpopulations of the landrace, in germination, seedling growth, and forage yield in response to salinity stress. Although

the study covered approximately 60% of the barley plant ontogeny under the Batinah environmental and edaphic conditions, salinity tolerance demonstrated in this study is of considerable value in determining the ultimate tolerance of the species (Zeng et al., 2002). For plants that are grown at high temperature, 10 to 15 d in salinity is sufficient to generate differences in biomass between genotypes that correlate well with differences in yield (Munns, 2002). Moreover, a 14-d screening period was sufficient to differentiate between sensitive and tolerant genotypes of *Triticum durum* Desf., a relatively salt sensitive species (Sadat Noori and McNeilly, 2000).

Temporal variation in, and thermal time to, 50% germination, combined with differences among subpopulations in relative seedling dry weight under salinity stress, support earlier screening results of barley (Flowers and Hajibagheri, 2001) and clearly identified highly salt-tolerant subpopulations at the germination stage. Temporal variation in germination can be attributed (González-Astroga and Núñez-Farfán, 2000) to variation among subpopulations in the threshold level of salinity beyond which germination is significantly reduced.

We identified genotypes with short rachilla hair in Batini 4 (Jaradat et al., 2004), a trait that was found to be linked to high salt tolerance (Mano and Takeda, 1997). These genotypes fully germinated, survived, and produced 22 and 45% above-average forage yield at 10.0 and 20.0 dS m<sup>-1</sup>, respectively. Additionally, we identified genotypes in Batini 2 and Batini 3 with increased root-to-shoot ratio under salinity, a trait found to be associated with increased salinity tolerance in durum wheat (Sadat Noori and McNeilly, 2000) and common bean landraces (Bayuelo-Jimenez et al., 2002).

Considering the physiological complexities of seed germination under salinity stress (Alvarado and Bradford, 2002), the positive phenotypic correlations between germination attributes under stress and nonstress conditions (Fig. 2a), can be exploited through phenotypic selection to improve germination (Foolad et al., 1999) and to increase salt tolerance at the adult plant level (Ashraf et al., 1986). Moreover, if salt tolerance at germination and seedling growth stages are controlled by independent genes, it will be possible, upon genetic analysis, and by marker-assisted selection, to develop more resistant germplasm by combining genes for salt tolerance (Mano et al., 1996; Mano and Takeda, 1997). Batini 4 and, to some extent Batini 2 and Batini 3, harbor the variability needed to pursue these objectives.

Evaluation of yield potential under salinity stress may be a critical component of selection in breeding pro-

grams, since improving yield is the main target in plant breeding (Zeng et al., 2002). A favorable combination of salt tolerance at germination, seedling, and tillering stages is critical in selecting for forage production under saline conditions (Mano and Takeda, 1997; Munns et al., 2002). Multivariate analyses procedures allowed simultaneous analysis of multiple indices and derived variables (Fig. 1a) and resulted in improved evaluation of salt tolerance and genotypic ranking of subpopulation and families (Zeng et al., 2002).

Yield reduction due to increased salinity, as estimated by two salinity indices (Fig. 1a,b), and regression coefficients for each subpopulation (Table 7), confirmed the variable responses of the seven subpopulations to increasing salinity stress levels. Genotypic differences in reaction to similar salinity stresses were reported for barley varieties differing in salt tolerance (Munns et al., 1995); tolerant and sensitive varieties experienced 38 to 56% and 64 to 68% reduction in biomass, respectively, after a 30-d exposure to 17.5 dS m<sup>-1</sup>. On the other hand, and under comparable salinity stress levels, reductions in barley grain and straw yield of up to 80.0 and 46.5%, respectively, were reported (Royo and Aragues, 1999). High forage yields under salinity were attributed to either longer crop growth duration before clipping (Royo, 1999), higher leaf and tiller numbers per plant, or higher plant density (Hussain et al., 1997).

Forage yield reductions of 41 and 85% were reported for barley (Hussain et al., 1997) in response to increased salinity level to medium (9.26) and high (16.28 dS m<sup>-1</sup>) levels, respectively, as compared to mean reductions of 22 and 82% in response to increased salinity to 10.0 and 20.0 dS m<sup>-1</sup>, respectively, in this study. Respective minimum reductions of 10.5 and 72.5% were estimated for Batini 4, a highly salt tolerant subpopulation. In comparison to a study by Slavich et al. (1990), forage yield reduction per unit increase in salinity in this study was lower (2.4 as compared with 4.18%) at medium salinity levels (9–10 dS m<sup>-1</sup>) and comparable to the reduced rate (7.9 as compared with 6.9%) at higher (17.0–20.0 dS m<sup>-1</sup>) salinity levels.

Rate of recovery after first cut, as estimated by forage yield of the second cut, confirmed genotypic differences, and can be attributed to differences in tolerance to salinity, or to differences in leaf area expansion and photosynthetic rates (Bonachela et al., 1995). Genotypic differences in cumulative °Cd<sup>-1</sup> to first cut and the delay in °Cd<sup>-1</sup> to reach the end of tillering stage in response to increased salinity stress indicated large variation among subpopulations. Moreover, the significant interaction component between genotypes and salinity levels in this study confirms the findings of Zeng et al. (2002).

Screening for salt tolerance under controlled conditions is more efficient and less expensive than screening under field conditions (Shannon and Noble, 1990). However, unless more reliable and inexpensive field-screening procedures are developed (Royo and Aragues, 1999), the high broad-sense heritability of tolerance to salinity at the germination ( $H > 0.68$ ) and tillering ( $H > 0.62$ ) stages, demonstrated in this and other (Ashraf et al.,

1986) studies may have to be exploited for further genetic gain.

The consistently high genetic variance component for germination index for four seedling attributes (Table 2) and for the emergence data (Table 4) in the germination and sand culture experiments indicate a strong genetic control over seed germination under salinity stress (Flowers and Yeo, 1995). The relatively high  $H$  estimates (0.62–0.81) for the same attributes at the germination stage may reflect high heritability for tolerance to salinity (Ashraf et al., 1986) and is expected to result in high genetic gains in a selection and breeding program. Genetic differentiation among barley populations is a predicted consequence of its breeding system (Shannon and Nobel, 1990). Consequently, there were significant differences among subpopulations in the amount of among-family variance for all traits, especially at the highest salinity level. Within-family variances were generally quite uniform across subpopulations. Multivariate assessment was instrumental in grouping a large number of indices and derived-variables into a small number of principal components, thus allowing for targeted multitrait selection, a procedure much needed to select for tolerance to salinity throughout the life cycle of the plant (Munns, 2002).

## CONCLUSIONS

A high level of diversity for salt tolerance, within and among subpopulations of the Batini landrace, was demonstrated at the germination, seedling, and tillering growth stages. We measured tolerance to salinity and screened a large number of individual seedlings and plants at the tillering stage for small, repeatable, and quantifiable differences in several seedling and biomass attributes.

A large proportion of variance in seed germination attributes was accounted for by genetic differences among subpopulations. Positive associations were identified between germination attributes under stress and nonstress conditions. Targeted multitrait selection was instrumental in identifying genotypes in the Batini landrace with improved salt tolerance, high biomass production, and high rate of recovery after the first cut under medium and high salinity stress levels. These genotypes were characterized as having either short rachilla hairs or high root-to-shoot ratios under salinity stress. The salt-tolerant barley germplasm evaluated in this study should contribute to increasing barley production in arid regions under saline irrigation.

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