Heritability of Salt Tolerance at Germination Based on Parent-Offspring Correlation and Selection Response in Barley (*Hordeum vulgare* L.)

Yoshiro Mano^{1),2)} and Kazuyoshi Takeda¹⁾

¹⁾ Research Institute for Bioresources, Okayama University, Kurashiki 710, Japan

²⁾ Present address: National Institute of Agrobiological Resources, Tsukuba 305, Japan

Summary

Heritability of salt tolerance in germinating seeds of barley (*Hordeum vulgare* L.) was estimated by parent-offspring correlation and selection response. Germination tests in 250 mM NaCl solution were conducted using the seeds of F₁, F₂, and F₃ plants (F₂, F₃, and F₄ generations, respectively) derived from the crosses between salt tolerant (OUJ417 and OUK666) and sensitive (OUE211) varieties. Salt tolerance of the two crosses showed polygenic segregations in F₂, F₃, and F₄ generations. Correlation coefficients between the parents and offsprings in two crosses were about 0.5 between F₂-F₃ generations and 0.6~0.7 between F₃-F₄ generations. Realized heritabilities estimated from the ratio of (selection response)/(selection differential) were 0.2~0.4 in the F₂-F₃ and 0.8~0.9 in the F₃-F₄. Although dominant genetic variance of salt tolerance at germination is larger than additive genetic variance in the F₂ generation, heritability of this trait is relatively high even between F₃-F₄ generations and higher heritability may be obtained in later generations. We have already selected highly salt tolerant barley varieties from over 6,700 accessions. Using such genetic resources of salt tolerance as the parents, selection for increasing salt tolerance at germination may be carried out.

Key Words: germination, heritability, *Hordeum vulgare*, salt tolerance, selection test.

Introduction

In arid and semi-arid regions in the world, soil salinity due to extensive irrigation is one of the most important environmental stress factors limiting agricultural production (Epstein *et al.* 1980, Boyer 1982, Batie and Healy 1983). One solution to the problem of soil salinity is to apply technological approaches, such as soil amendment and irrigation. They are, however, not always economical or practical. A biological approach, such as development of salinity-tolerant varieties by plant breeding, may in combination with irrigation technology prove an effective way to overcome the limitation of crop production in a salinized area.

Heritability for salt tolerance has been studied in cereal crops such as rice (Moeljopawiro and Ikehishi 1981, Akbar *et al.* 1985, Yeo 1992, Gregorio and

Senadhira 1993), sorghum (Azhar and McNeilly 1988), and barley (Maddur 1977, Jana *et al.* 1980, Tripathi *et al.* 1983). In addition, genetic information on salt tolerance at various growth stages in barley has been accumulated (Mano and Takeda 1996, 1997a, b).

Salt tolerance during germination is important because salinity is usually higher in the soil surface at high groundwater levels (Matsumoto 1989) and the initial plant stand affects the final production. We have previously assessed the salt tolerance in germinating barley using over 6,700 accessions from the world barley collection preserved at the Barley Germplasm Center of Okayama University. Salt tolerance of these varieties measured as growth capacity ranged from only root developed in 171 mM NaCl solution to shoot developed over 25 mm in 428 mM NaCl solution after ten days of incubation (Mano *et al.* 1996). Using a set of 8×8 diallel crosses, salt tolerance at germination was shown to be controlled by recessive genes. Dominance genetic variance was larger than additive genetic variance and narrow sense heritability was as high as 0.75 (Mano and Takeda 1997a). For practical breeding, it is desirable to estimate realized heritability which directly indicate the parent-offspring relationship.

The present study was conducted to visualize the effectiveness of early generation selection for salt tolerance at germination in barley. Two sets of barley populations, derived from the crosses between highly salt tolerant and sensitive varieties, were used to analyze parent-offspring correlation and selection response to salt tolerance at germination.

Materials and Methods

Two sets of F₂, F₃, and F₄ populations, derived from crosses between Kumoi (OUJ417) × Ethiopia 633 (OUE211) and Jeomchon Naked 2 (OUK666) × Ethiopia 633 (OUE211), were used. At germination, both OUJ417 and OUK666 expressed the greatest tolerance for salt, while OUE211 showed the least tolerance for salt among over 6,700 accessions tested (Mano *et al.* 1996).

A total of 155 (OUJ417×OUE211) and 158 (OUK666×OUE211) seeds (F₂ generation) developed on the F₁ plants were germinated on two layers of filter paper moistened with 250 mM NaCl solution in 135 (diameter) × 20 mm Petri dishes as described in Mano *et al.* (1996). One hundred seeds of their parental varieties were also tested. The germination test was carried out at 20 °C

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under a 12 hour light/12 hour dark photoperiod in the growth chamber. After ten days of incubation, salt tolerance was evaluated by measurements of the shoot length (mm). In germination tests, salt sensitive lines developed only roots, while moderately or highly tolerant lines developed both roots and shoots. Unless otherwise stated, the same procedure was applied in all experiments.

After measurement of the shoot length, all the F_2 seeds were planted into the field at the Research Institute for Bioresources, Okayama University, Kurashiki, Japan. Seeds from each F_2 plant were harvested to generate the F_3 lines. One hundred non-dormant seeds of each F_2 plant and their parental varieties were germinated in NaCl solution and the salt tolerance of each line was determined by estimating the average shoot length (mm).

In the next season, the F_4 lines were raised by the single seed descent method. One hundred non-dormant seeds of each F_3 plant (F_4 generation) and their parental varieties were evaluated for salt tolerance.

Parent-offspring correlation or heritability in standard unit (Frey and Horner 1957) was calculated between F_2 plants and F_3 line means, and between the mean of F_3 and F_4 lines. Realized heritabilities were calculated

from the ratio of (selection response)/(selection differential) in the F_2 plants- F_3 line means and F_3 line means- F_4 line means. Selection tests were carried out in both directions for evaluation of the inheritance of this trait. For the F_2 - F_3 selection experiment, seeds developing shoots longer than 50 mm (OUJ417 $\times$ OUE211 cross) and 40 mm (OUK666 $\times$ OUE211), respectively, were selected for tolerance tests, and seeds from which no shoots were developed, were selected for sensitivity tests. In the F_3 - F_4 generation, the tolerant 10 % and the sensitive 10 % of the F_3 lines (16 lines each) were selected.

Results

Salt tolerance in F_2 , F_3 , and F_4 generations

Salt tolerance evaluated by the shoot length of the F_2 population of OUJ417 $\times$ OUE211 cross showed a wide and bimodal distribution and the mean of 155 plants was 25.5 mm (Fig. 1a). In the F_2 population of OUK666 $\times$ OUE211 cross, the distribution of tolerance was shifted to the sensitive range and the mean of 158 plants was

Fig. 1. Frequency distributions for salt tolerance at germination in F_2 (a), F_3 (b) and F_4 populations (c) derived from the cross of OUJ417 (P_1) $\times$ OUE211 (P_2) by the SSD method. The arrows indicate the parental values.

Fig. 2. Frequency distributions for salt tolerance at germination in F_2 (a), F_3 (b) and F_4 populations (c) derived from the cross of OUK666 (P_1) $\times$ OUE211 (P_2) by the SSD method. The arrows indicate the parental values.

15.4 mm (Fig. 2a). In the parental varieties, the average shoot lengths were 45.2 mm (range: 16–66 mm, standard error: 0.9) for OUJ417, 35.9 mm (range: 0–74 mm, standard error: 1.6) for OUK666, and 0 mm for OUE211, respectively. Salt tolerance of the parental varieties was stable for those of the two following seasons. (Figs. 1 and 2).

In both crosses, salt tolerance of the F_3 and F_4 lines showed continuous distribution, and the means of the F_3 (18.7 mm for OUJ417 $\times$ OUE211 and 8.7 mm for OUK666 $\times$ OUE211) and F_4 lines (14.5 mm for OUJ417 $\times$ OUE211 and 13.0 mm for OUK666 $\times$ OUE211) were lower than those of the mid-parent (26.8, 27.7 mm for OUJ417 $\times$ OUE211 and 22.5, 17.5 mm for OUK666 $\times$ OUE211) (Figs. 1 and 2).

Estimation of heritabilities from parent-offspring correlation

Heritabilities of salt tolerance at germination were estimated from the correlation coefficient between the F_2 – F_3 and F_3 – F_4 generations (Table 1). Correlation coefficients of F_2 – F_3 were 0.515 in the OUJ417 $\times$ OUE211 cross and 0.479 in the OUK666 $\times$ OUE211 cross, respectively. Between F_3 – F_4 generations, correlation coefficients were 0.670 in the OUJ417 $\times$ OUE211 cross

and 0.574 in the OUK666 $\times$ OUE211 cross, respectively (all coefficients are significant at the 0.1 % level).

Estimation of heritabilities from selection response

Realized heritabilities of salt tolerance were estimated from the ratio of selection responses of F_3 lines to selection differentials of F_2 populations in the direction of tolerance, sensitivity, and both directions (Figs. 3a and 4a, Table 1). Realized heritabilities in both directions were 0.359 in the OUJ417 $\times$ OUE211 cross and 0.204 in the OUK666 $\times$ OUE211 cross, and those estimated in the direction of tolerance and sensitivity were 0.354 and 0.365 (OUJ417 $\times$ OUE211), and 0.168 and 0.279 (OUK666 $\times$ OUE211), respectively.

Figs. 3b and 4b show the selection response for salt tolerance between F_3 – F_4 generations. In both crosses, moderately tolerant F_4 lines were generated from the tolerant F_3 lines, while no tolerant F_4 lines were generated from the sensitive F_3 lines. Realized heritabilities in both direction were 0.755 in the OUJ417 $\times$ OUE211 cross and 0.951 in the OUK666 $\times$ OUE211 cross, respectively (Table 1). In both crosses, heritabilities for salt sensitivity were higher than those for salt tolerance.

Table 1. Heritability (h^2) estimated from the ratio of selection response (R) to selection differential (S) and parent-offspring correlation (r) for salt tolerance at germination

Cross Generation	Tolerance			Sensitivity			Total			r
	S	R	h ²	S	R	h ²	S	R	h ²	
OUJ 417/OUE 211										
F ₂ – F ₃	28.0 (12) ¹⁾	9.9	0.354	25.5 (37)	9.3	0.365	53.5	19.2	0.359	0.515
F ₃ – F ₄	28.2 (16)	20.5	0.727	17.9 (16)	14.3	0.799	46.1	34.8	0.755	0.670
OUK 666/OUE 211										
F ₂ – F ₃	32.7 (27)	5.5	0.168	15.4 (69)	4.3	0.279	48.1	9.8	0.204	0.479
F ₃ – F ₄	18.0 (16)	13.8	0.767	8.3 (16)	11.2	1.349	26.3	25.0	0.951	0.574

Salt tolerance was evaluated by the shoot length (mm) after ten days of incubation in 250 mM NaCl solution at 20 °C.

¹⁾ : Number of plants selected.

Fig. 3. Selection experiment for salt tolerance at germination in the OUJ417 $\times$ OUE211 cross. (a): F_2 to F_3 generation. (b): F_3 to F_4 generation.

Fig. 4. Selection experiment for salt tolerance at germination in the OUK666 × OUE211 cross. (a): F₂ to F₃ generation. (b): F₃ to F₄ generation.

Fig. 5. Relationships between the mean and the variance of salt tolerance at germination in F₃ (a) and F₄ lines (b) derived from the cross between OUJ417 × OUE211.

Fig. 6. Relationships between the mean and the variance of salt tolerance at germination in F₃ (a) and F₄ lines (b) derived from the cross between OUK666 × OUE211.

Figs. 5 and 6 show the relationships between the mean and the variance of salt tolerance in F₃ and F₄ lines of the two crosses. Salt sensitive F₃ lines of the two crosses showed a smaller variance within lines, while moderately and highly tolerant F₃ lines generally showed a larger variance. Among the F₄ lines, highly tolerant lines with small degree of variance were found in both crosses. Some of these lines may be homozygous for salt tolerance because the within line variance of

these lines were smaller than the within pure line error variance (about 230) estimated by Mano (1996).

Discussion

Several workers have reported on the heritability of salt tolerance at germination. Foolad and Jones (1992)

found that the correlation and regression coefficients between F_2 - F_3 and F_3 - F_4 for tomato were 0.6 to 0.8, indicating that selection in the early generations would be effective for this trait. In addition, heritability for this trait is also high in alfalfa (Allen *et al.* 1985) and forage species (Ashraf *et al.* 1987). Also in barley, narrow sense heritability for salt tolerance at germination, estimated by diallel analysis, has been reported to be quite high (0.75) (Mano and Takeda 1997a).

In this study, fairly high heritabilities (0.6 to 0.9) were estimated from correlation coefficients and selection responses between F_3 - F_4 generations (Table 1). Between F_2 - F_3 generations, however, relatively low heritabilities (0.2 to 0.5) were observed (Table 1). A study of salt tolerance at germination in barley showed that dominant genetic variance was larger than additive genetic variance in the F_2 generation (Mano and Takeda 1997a). Besides, because salt tolerance at germination in the F_2 generation was evaluated on a single seed basis and that of the F_3 lines were estimated from the mean value of 100 seeds, environmental variance in the F_2 generation was 100 times as large as those in the F_3 lines. Thus, lower heritabilities between F_2 - F_3 generation may be due partly to a larger dominant genetic variance and/or larger environmental variance in each F_2 plant.

Frequency distributions for salt tolerance at germination in F_2 , F_3 , and F_4 populations were generally shifted towards the sensitive range (Figs. 1 and 2), indicating that salt sensitivity may be mainly controlled by dominant genes. However, direction of dominance in the selection tests was opposite to that observed in the frequency distributions, i. e., heritabilities for salt sensitivity were generally higher than those for salt tolerance (Table 1), and moderately or highly tolerant F_4 lines were not generated from the sensitive F_3 lines (Figs. 3b and 4b). Therefore, direction of dominance for this trait was not clear in this experiment. In our previous diallel analysis using eight barley varieties including OIJ417, OUK666, and OUE211, salt sensitivity at germination was mainly controlled by dominant genes (Mano and Takeda 1997a).

One reason may be that the salt sensitive parent, OUE211, which did not develop shoots even in 170 mM NaCl solution (Mano *et al.* 1996), harboured a large number of dominant genes controlling salt sensitivity at germination (Mano and Takeda 1997a), and the relatively high (250 mM) NaCl treatment masked the segregation of these sensitive genes. It is possible that salt tolerance at germination is a threshold character, but further studies are required to establish this. Another reason may be that heterosis increased salt tolerance of the F_2 plants and the decreased heterosis effect in the tolerant F_3 lines weakened their tolerance, and hence relatively lower heritabilities were observed in the selection for high tolerance.

Although dominant genetic variance of salt tolerance at germination is larger than additive genetic variance

in the F_2 generation (Mano and Takeda 1997a), heritability of this trait is relatively high even between F_3 - F_4 generations (Table 1) and higher heritability may be obtained in later generations. In addition, the selection technique used in this study should be of value in a practical breeding strategy since it is simple allowing the tests of a very large number of populations or lines. We have already used this method to select highly salt tolerant barley varieties from over 6,700 accessions of the world barley collection (Mano *et al.* 1996). Using salt tolerant parents, selection for increasing salt tolerance at germination may be carried out on a huge number of early segregating generations and used for subsequent line selection.

By combining conventional breeding techniques with information on the molecular genetics of salt tolerance, the development of more reliable selection methods for salt tolerance in segregating populations of barley can be achieved. Mano and Takeda (1997b) reported that the quantitative trait loci controlling salt tolerance at germination of barley were located on 1H, 4H, 5H and 6H, and it will be possible to use marker-assisted selection to combine different genes controlling salt tolerance. In tomato, marker-assisted selection has proven to be very efficient in developing salt tolerant recombinant inbred lines (Monforte *et al.* 1996).

Takeda *et al.* (1995) selected several barley and wheat varieties from over 10,000 accessions in the Heilonggang region of China, which were tolerant to semi-arid and highly saline conditions. Cultivation of these tolerant varieties has started in this region in China. Beside the applied approaches of the program, we are currently generating genetic information on salt tolerance at several growth stages in barley (present study, Mano and Takeda 1996, 1997a,b). In order to combine the genes for salt tolerance at several growth stages with agronomic traits and other stress tolerances, a marker assisted selection system needs to be developed.

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