

Genetical Studies on Salt Tolerance at Germination in Recombinant Inbred, Isogenic, and Doubled Haploid Lines of Barley (*Hordeum vulgare* L.)

Yoshiro MANO and Kazuyoshi TAKEDA

To determine the relationship between morphological markers and salt tolerance at germination in barley (*Hordeum vulgare* L.), a total of 125 recombinant inbred (RI) lines of Russia 6×HES 4, a series of 70 isogenic (IG) lines for *V/v* gene derived from Russia 6×HES 4, and 145 doubled haploid (DH) lines of Leger×CI 9831 were evaluated for their salt tolerance at germination.

Comparison between each set of character pairs revealed that the six-rowed type was significantly more tolerant than the two-rowed type in the RI and IG lines of Russia 6×HES 4, and the two-rowed type and the short haired rachilla type were significantly more tolerant than the six-rowed type and the long haired rachilla type in the DH lines of Leger×CI 9831. On the other hand, no significant difference was observed in salt tolerance such as the smoothness of awn, ear density, and lemma color.

The findings suggested that *v* (kernel row) and *s* (rachilla hair length) genes related with genetic factors controlling salt tolerance at germination, while *r* (awn type), *l* (ear density), and *Re-2* (lemma color) genes inherited independent of gene(s) for salt tolerance at germination.

Key words: Doubled haploid lines, *Hordeum vulgare*, Isogenic lines, Recombinant inbred lines, Salt tolerance,

INTRODUCTION

Salt-affected soils are distributed especially in arid and semi-arid regions in the world. Salinization has been increasing due to the higher level of evaporation and it has decreased crop productivity (Batie and Healy 1983, Boyer 1982, Epstein *et al.* 1980). To solve salinity problems, technological

approaches, for example, reclamation, irrigation, and drainage have been applied, but they are not always economical or practical. In addition, the extensive irrigation often causes severe salinization. Hence, the most effective way to overcome the limitations of crop productivity in a salinized area may be the development of salt tolerant varieties.

To establish an efficient breeding system for salt tolerance, it is important to select and analyze the tolerance at all growth stages, because varietal rankings for salt tolerance could change during different growth stages in cereals (Heenan *et al.* 1988, Mano and Takeda 1995).

In this study, to acquire basic information about the genes controlling salt tolerance at germination, we analyzed the relationship between salt tolerance and morphological marker characters using recombinant inbred (RI), isogenic (IG), and doubled haploid (DH) lines of barley.

MATERIALS AND METHODS

Three sets of barley lines were used. A total of 125 recombinant inbred (RI) lines and a series of 70 isogenic (IG) lines for *V/v* gene of F_8 generation derived from the cross of Russia 6 \times HES 4 were produced by the single seed descent method and the recurrent selfing method, respectively, at the Barley Germplasm Center of Okayama University. A set of 145 doubled haploid (DH) lines derived from the cross of Leger \times CI 9831 were produced by Choo *et al.* (1992) with the *Hordeum bulbosum* method (Kasha and Kao 1970). Table 1 shows the morphological characters of the parental varieties.

Table 1. Salt tolerance at germination and morphological characters of the parental varieties

Variety	Tolerance ¹⁾	Row type	Rachilla hair	Awn type	Lemma color
Russia 6	10.0(%)	Two-rowed	Long-haired	Smooth	Normal
HES 4	62.5	Six-rowed	Long-haired	Rough	Normal
Leger	0.0(%)	Six-rowed	Short-haired	Rough	Normal
CI 9831	70.0	Two-rowed	Long-haired	Rough	Purple

¹⁾: Salt tolerance was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds after ten days of incubation in 1.5% NaCl solution at 20°C.

Twenty seeds of each RI and IG line of Russia 6 \times HES 4 and their parents, and fifty seeds of each DH line of Leger \times CI 9831 and their parents were germinated on two layers of filter paper properly moistened with 1.5% NaCl solution in 90 \times 15 mm and 135 \times 20 mm Petri dishes, respectively, with

two replications. Ten days after incubation, salt tolerance was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds incubated. All experiments were carried out at 20°C under a 12 hours light/ 12 hours dark photoperiod in the growth chamber.

To determine the relationship between genetic factors controlling salt tolerance at germination and morphological markers, RI and DH lines were grouped by several morphological characters and salt tolerance was compared between the allelomorphs. In the IG lines of Russia 6×HES 4, salt tolerance was compared between two-rowed and six-rowed isogenic pairs.

RESULTS

In the experiment of three sets of barley lines, genetic variation due to lines were significant at the 0.1% level (Table 2), and the correlation coefficient for salt tolerance between replication were 0.873 and 0.738 in the RI and IG lines of Russia 6×HES 4, respectively, and 0.786 in the DH lines of Leger×CI 9831 (all correlation coefficients were significant at the 0.1% level).

Table 2. Analysis of variance for salt tolerance at germination in the RI and IG lines of Russia 6×HES 4 and DH lines of Leger×CI9831

Source	RI		IG		DH	
	df	MS	df	MS	df	MS
Lines	124	1431.87***	69	1198.09***	144	939.71***
Replications	1	280.90	1	540.18	1	1.99
Error	124	98.24	69	180.76	144	112.60

*** : Significant at the 0.1 % level.

As shown in Fig. 1, salt tolerance at germination, which was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds were 10.0% for Russia 6 and 62.5% for HES 4. The 125 RI lines of Russia 6×HES 4 showed a continuous distribution for this trait (average 33.0%).

To detect the relationship between genetic factors controlling salt tolerance at germination and morphological markers, 125 RI lines were grouped by several morphological markers. The six-rowed group (average 49.0%) was significantly (at the 0.1% level) more tolerant than the two-rowed group (15.6%) (Fig. 1, Table 3). In addition, the correlation coefficients between salt tolerance at germination and 1,000-kernel weight were

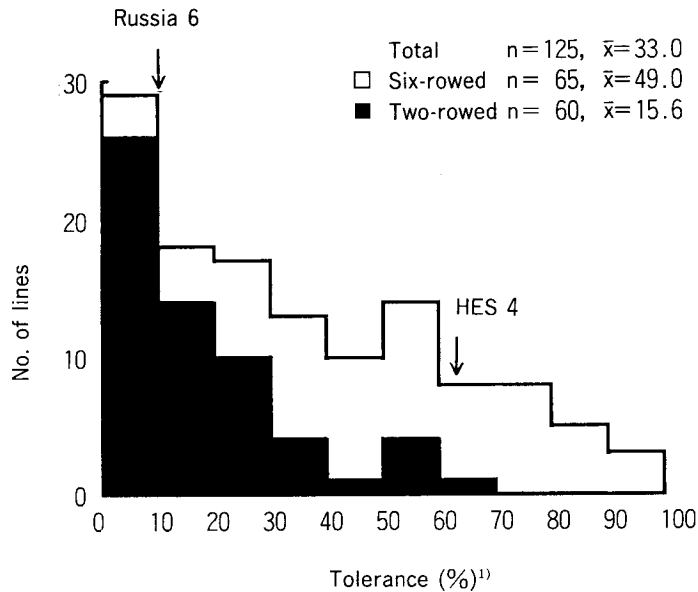


Fig. 1. Frequency distribution for salt tolerance at germination of the RI lines derived from the cross of Russia 6 \times HES 4.

¹⁾ Salt tolerance was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds after ten days of incubation in 1.5% NaCl solution at 20°C.

-0.576 in the whole RI lines, -0.093 in the six-rowed group, and -0.301 in the two-rowed group (Fig. 2). On the other hand, as shown in Table 3, no significant difference was observed in salt tolerance such as the awn type (rough vs. smooth) and the ear type (lax vs. dense).

In the 70 IG lines of Russia 6 \times HES 4, the correlation coefficient for salt tolerance between isogenic pairs was 0.542 (significant at the 0.1% level) suggesting that salt tolerance was controlled by the genetic background of the IG lines. The six-rowed type was more tolerant and it had smaller seed than the two-rowed type (Table 4). Comparing isogenic pairs individually, it was revealed that the six-rowed lines were more tolerant than the two-rowed counterparts without exception or recombinant, indicating that the effect of *V/v* gene on salt tolerance is due to the pleiotropy but not linkage (Fig. 3).

Next, salt tolerance at germination were evaluated in 145 DH lines of Leger \times CI 9831 (Fig. 4). In the parental varieties, the proportion of the number of seeds with germinating shoots longer than 25 mm were 0% for Leger and 70.0% for CI 9831. A total of 145 DH lines showed wide and continuous distribution for this trait (average 17.4%).

Table 3. Comparison of salt tolerance at germination between each set of character pairs in the RI and DH lines

Character		Russia 6 × HES 4 ¹⁾		Leger × CI9831 ¹⁾	
		n	\bar{x}	n	\bar{x}
Row type	Two-rowed	60	15.6(%)	67	20.2*(%)
	Six-rowed	65	49.0***	78	15.1
Rachilla hair	Long	— ²⁾		68	9.0
	Short			77	24.8***
Awn type	Rough	75	32.8	—	
	Smooth	50	33.2		
Lemma color	Purple	—		65	17.7
	Normal			80	17.2
Ear type	Lax	50	33.6	—	
	Dense	75	32.5		

*, ***, : Significant at the 5% and 0.1% levels, respectively.

¹⁾: Salt tolerance was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds after ten days of incubation in 1.5% NaCl solution at 20°C.

²⁾: No difference for the character between the parental varieties.

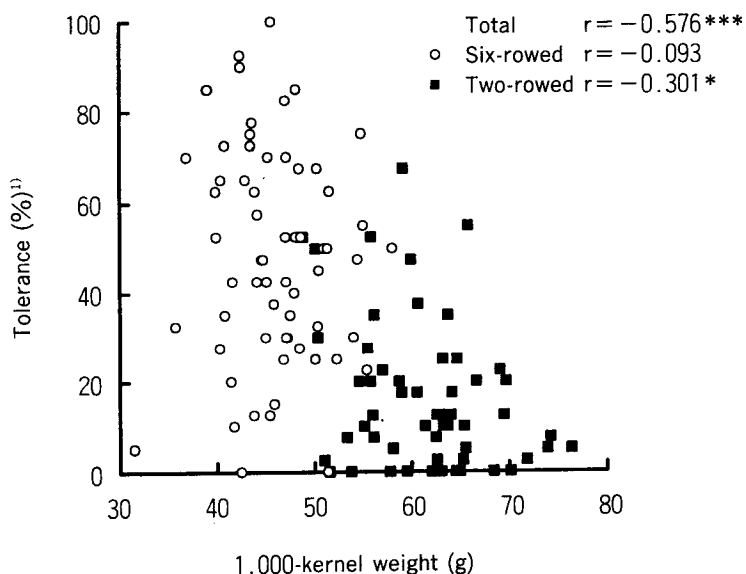


Fig. 2. Correlation between salt tolerance at germination and 1,000-kernel weight in the RI lines derived from the cross of Russia 6 × HES 4.

¹⁾ Salt tolerance was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds after ten days of incubation in 1.5% NaCl solution at 20°C.

Genetical studies on salt tolerance in barley

Table 4. Comparison of salt tolerance at germination between 35 isogenic pairs derived from the cross of Russia 6 × HES 4

Row type	1,000-K.W. (g)	Tolerance ¹⁾ (%)	r ²⁾
Six-rowed	37.8	50.4***	0.542***
Two-rowed	55.6***	17.0	

*** : Significant at the 0.1 % level.

¹⁾: Salt tolerance was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds after ten days of incubation in 1.5 % NaCl solution at 20°C.

²⁾: Correlation coefficient for salt tolerance between isogenic pairs.

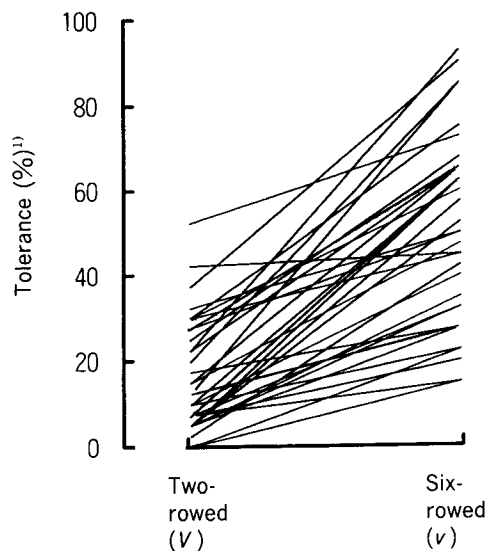


Fig. 3. Comparison of salt tolerance at germination between the 35 isogenic pairs for *V/v* gene derived from the cross of Russia 6 × HES 4.

¹⁾ Salt tolerance was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds after ten days of incubation in 1.5% NaCl solution at 20°C.

In the DH lines of Leger × CI 9831, the two-rowed group (average 20.2%) was significantly (at the 5% level) more tolerant than the six-rowed group (15.1%) (Table 3), and the correlation coefficient between salt tolerance at germination and 1,000-kernel weight was 0.019 (Fig. 5). In addition, as shown in Table 3 and Fig. 4, the group of short haired rachilla (24.8%) was signifi-

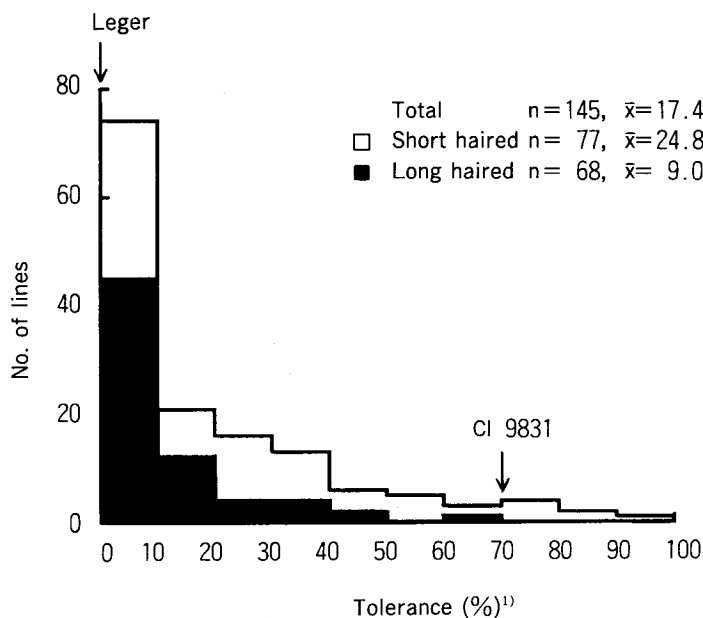


Fig. 4. Frequency distribution for salt tolerance at germination of the DH lines derived from the cross of Leger×CI 9831.

¹⁾ Salt tolerance was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds after ten days of incubation in 1.5% NaCl solution at 20°C.

cantly (at the 0.1% level) more tolerant than that of long haired rachilla (9.0%). On the other hand, no significant difference was observed in salt tolerance as to the lemma color (Table 3).

DISCUSSION

The present findings suggested that *v* (kernel row) and *s* (rachilla hair length) genes related with genetic factors controlling salt tolerance at germination. In the RI and IG lines of Russia 6×HES 4, the six-rowed group was significantly more tolerant than the two-rowed group (Fig. 1, Fig. 3, Table 3). The six-rowed group had a smaller seed than the two-rowed group (Table 4) and the negative correlation was observed between salt tolerance at germination and 1,000-kernel weight in the RI lines (Fig. 2). Jana *et al.* (1980) and Mano *et al.* (1996) also found that the salt tolerant lines generally had smaller seeds using a composite cross population and isogenic lines for *V/v* gene, respectively.

On the contrary, a weak correlation was observed between salt tolerance and 1,000-kernel weight in the two-rowed and six-rowed groups in the

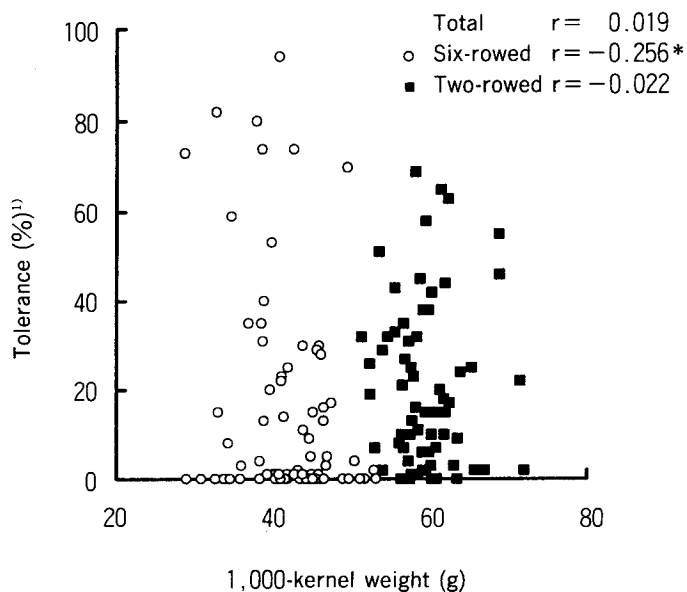


Fig. 5. Correlation between salt tolerance at germination and 1,000-kernel weight in the DH lines derived from the cross of Leger \times CI 9831.

¹⁾ Salt tolerance was evaluated by the proportion of the number of seeds with germinating shoots longer than 25 mm against the number of total seeds after ten days of incubation in 1.5% NaCl solution at 20°C.

RI lines of Russia 6 \times HES 4. Therefore, difference between two-rowed and six-rowed type for salt tolerance in the RI lines of Russia 6 \times HES 4 may be partly due to the pleiotropic effect of v gene for the tolerance and seed weight.

In the DH lines of Leger \times CI 9831, however, no significant correlation was observed between salt tolerance at germination and 1,000-kernel weight, and the two-rowed group (CI 9831 type) was significantly tolerant than the six-rowed group (Leger type). These findings demonstrate that the strong genetic factor(s) for salt tolerance may be linked with V gene in the genetic background of CI 9831.

As to the effect of s gene on salt tolerance, the short haired rachilla group was significantly more tolerant than the long haired rachilla group in the DH lines of Leger \times CI 9831 (Table 3). In addition, Mano *et al.* (unpublished) found out the quantitative trait locus (QTL) for salt tolerance at germination, located about 30cM from the s gene on chromosome 7. These findings indicated that one of the genes controlling salt tolerance may be located near s gene. In the parental varieties, however, Leger (short haired rachilla) was more sensitive than CI 9831 (long haired rachilla), indicating

the existence of gene(s) controlling salt tolerance other than that linked with *s* gene.

The factors affecting salt tolerance at germination detected in this study were ① a pleiotropic effect of kernel row gene (*v*) for the tolerance and seed size, ② genetic factor(s) stronger than the pleiotropic effect of *v* gene, and which may be linked with *V* gene on the long arm of chromosome 2, and ③ the gene(s) controlling salt tolerance linked with short haired rachilla gene (*s*) on the long arm of chromosome 7. On the other hand, the genes controlling awn type (*r*), ear density (*l*), and lemma color (*Re-2*) inherited independent of the gene(s) for salt tolerance at germination.

As well as other crops, many molecular markers have been mapped in barley (Kleinhofs *et al.* 1993, Kasha *et al.* 1994), and detailed linkage analysis for the genes controlling quantitative traits has become possible. We have already mapped the QTLs controlling salt tolerance at the seedling stage using DH population in which mapping data are available (unpublished). Further studies are in progress to detect and to map the genes controlling salt tolerance at other growth stages.

Acknowledgements—The authors would like to thank Dr. Choo, Agr. Canada Res. Sta. for providing the seed samples of DH lines of Leger×CI 9831. We also express thanks for Dr. Sato, Res. Inst. Bioresour. Okayama Univ. for his critical reading of the manuscript and invaluable suggestions.

REFERENCES

- Batie, S.S. and Healy, R.G. 1983. The future of American agriculture. *Sci. Am.* 248: 27-35.
- Boyer, J.S. 1982. Plant productivity and environment. *Science* 218: 443-448.
- Choo, T.M., Ho, K.M., Konishi, T. and Martin, R.A. 1992. Tests for randomness among doubled-haploid lines derived by the bulbosum method in barley (*Hordeum vulgare* L.). *SABRAO J.* 24: 87-92.
- Epstein, E., Norlyn, J.D., Rush, D.W., Kingsbury, R.W., Kelley, D.B., Cunningham, G.A. and Wrona, A. F. 1980. Saline culture of crops: A genetic approach. *Science* 210: 399-404.
- Heenan, D.P., Lewin, L.G. and McCaffery, W.D. 1988. Salt tolerance in rice varieties at different growth stages. *Aust. J. Exp. Agr.* 28: 343-349.
- Jana, M.K., Jana, S. and Acharya, S.N. 1980. Salt stress tolerance in heterogeneous populations of barley. *Euphytica* 29: 409-417.
- Kasha, K.J. and Kao, K.N. 1970. High frequency haploid production in barley (*Hordeum vulgare* L.). *Nature* 225: 874-876.
- Kasha, K.J., Kleinhofs, A. and the North American Barley Genome Mapping Project 1994. Mapping of the barley cross Harrington×TR306. *Barley Genet. Newsl.* 23: 65-69.
- Kleinhofs, A., Kilian, A., Saghai Maroof, M.A., Biyashev, R.M., Hayes, P., Chen, F.Q., Lapitan, N.,

- Fenwick, A., Blake, T.K., Kanazin, V., Ananiev, E., Dahleen, L., Kudrna, D., Bollinger, J., Knapp, S. J., Liu, B., Sorrells, M., Heun, M., Franckowiak, J.D., Hoffman, D., Skadsen, R. and Steffenson, B. J. 1993. A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. *Theor. Appl. Genet.* 86: 705-712.
- Mano, Y., Nakazumi, H. and Takeda, K. 1996. Varietal variation and effects of some major genes on salt tolerance at the germination stage in barley. *Breed. Sci.* (in press)
- Mano, Y. and Takeda, K. 1995. Varietal variation and effects of some major genes on salt tolerance in barley seedlings. *Bull. Res. Inst. Bioresour. Okayama Univ.* 3: 71-81. (in Japanese)

オオムギの組換え型自殖系統，同質遺伝子系統，および倍加半数体系統を用いた発芽時における耐塩性の遺伝解析

間野吉郎・武田和義

オオムギの発芽時における耐塩性と形態マーカーとの連鎖関係を調べるために，Russia 6×HES 4の交雑から養成した組換え型自殖系統125系統と同質遺伝子系統70系統，およびLeger×CI 9831の交雑から養成した倍加半数体145系統の種子を1.5%の塩化ナトリウム溶液を用いて20°C，12時間日長の条件で発芽させ，置床後10日目に調査してシュートを25 mm以上抽出した種子の置床種子に対する割合をもって発芽時の耐塩性とした。

各種主働遺伝子が発芽時の耐塩性に及ぼす影響を解析するために，組換え型自殖系統，同質遺伝子系統，および倍加半数体系統を親品種の間で異なる形質によって群別し，発芽時の耐塩性を比較した。その結果，Russia 6×HES 4の組換え型自殖系統，および同質遺伝子系統においては六条型は二条型よりも耐塩性が有意に強く，また，Leger×CI 9831の倍加半数体系統においては二条型は六条型よりも，底刺の短毛は長毛よりもそれぞれ耐塩性が有意に強く，条性間の耐塩性の強弱は交雑組合せによって逆転した。一方，芒の粗滑，穂密度，および穎色の比較においては有意差が認められなかった。

以上の結果から，条性を支配する *v* 遺伝子，および底刺のタイプを支配する *s* 遺伝子と発芽時の耐塩性を支配する遺伝子との関連性が推察された。また，芒の粗滑を支配する *r* 遺伝子，穂密度を支配する *l* 遺伝子，および穎色を支配する *Re-2* 遺伝子と発芽時の耐塩性は独立であると考えられた。

キーワード：倍加半数体系統，*Hordeum vulgare*，同質遺伝子系統，組換え型自殖系統，耐塩性