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Genetic analysis of salt tolerance in early growth stages of rapeseed (*Brassica napus* L.) genotypes

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Abstract

Genetic parameters for different characteristics related to salinity tolerance were estimated using 8 × 8 diallel crosses in rapeseed (*B. napus*). Salt stress was induced by adding incremental levels of NaCl to Hoaglands solution in a sand culture until electrical conductivity of 12.9dSm^{-1} was attained. Analysis of variance revealed significant general and specific combining ability mean squares for all studied characteristics, indicating the involvement of both additive and non-additive gene actions in the inheritance of characters. High ratios of $\frac{2\,\sigma^2_{gca}}{\left(2\,\sigma^2_{gca}+\sigma^2_{sca}\right)}$ and high narrow-sense heritability estimates were observed for Ca^{2+}, K^{+}, Na^{+}, K^{+}/Na^{+}, Ca^{2+}/Na^{+}, and stress tolerance index (TOL), indicating the prime importance of additive effects in their genetic control. Significant positive correlations were observed between shoot dry weight and Ca^{2+}/Na^{+} (0.531), and K^{+}/Na^{+} (0.562**). The results revealed that the parents BL2, Ceres, and Shiralee and the crosses Falcon × BL2, BL2 × Ceres and Ceres × Shiralee were superior to perform effective selection for salinity tolerance in rapeseed.**

Key words: Rapeseed, *Brassica napus*, combining ability, salinity tolerance.

Introduction

Salinity of agricultural lands and irrigation water is a major environmental factor which limits the growth and yield of *Brassica* species and also other crops in many arid and semiarid regions of the world. The stresses imposed by salinity are mainly due to ion compositions and concentrations in rhizosphere and also in plant tissues [1]. Information on the mechanisms involved in salt tolerance and their genetic control is essential to facilitate selection for characteristics and to design an efficient breeding programme for genetic improvement of salinity tolerance [2]. However little is known about the genetic control of salinity tolerance in *Brassica* species [3].

Identifying proper selection criteria for salinity tolerance is also a major problem. Rapid screening techniques based on heritable characteristics for selecting salt tolerant plants are needed. Besides measuring ions and metabolites that act on osmotic adjustment, comparison of yield potentials of genotypes in stress and non-stress condition is a suitable way to identify stress tolerance [4]. In this respect, some selection criteria including stress tolerance index, STI, [5], and tolerance index, TOL, [6] have been defined.

Although, the nature of variation for salt tolerance in *Brassica* species has been the subject of different studies [3, 7-10], the gentic control of salt tolerance has not been fully investigated. The present study was designed to estimate the genetic parameters for some mineral contents and two selection indices related to salinity tolerance in eight rapeseed breeding lines and cultivars crossed in a half diallel fashion.

Materials and methods

Eight diverse rapeseed (*Brassica napus* L.) genotypes were crossed in a half diallel fashion,then 28 F$_1$ progenies along with their parents were evaluated in sand culture under normal and saline environments in greenhouse. Experimental design for each condition was a randomized complete block design with three replications. Sterilized seeds were germinated in petridishes at 25° ± 2°C for 4 days. Ten uniform seedlings were transplanted into 4-liter pots filled with washed and sterilized river sand, covered with polythene beads, and after establishment, five plants were maintained for evaluation. Temperatures during the experiment were averaged 25/20°C (day/night) and relative humidity was 50-60%. The photoperiod was 14 h and the light source was fluoresent-incandescent lamps with a PAR of 414 μmol m^{-2}s^{-1}. Plants were given deionized water up to 10 days after transplanting and saline and non-saline (control) grown plants were irrigated thereafter every 2 days with half-strength Hoaglands nutrient solution [11] with and without NaCl, (EC = 12.9 and EC = 1.05 dS^{-1}m, respectively and pH = 7). Electrical conductivity of the saline treatment was increased to the desired level by incremental addition of the salt over 10-day period to avoid osmotic shock to the seedlings.

Plants in both environments were harvested 40 days after planting at 4-5 leaf stage. The characteristics were shoot leaf and stem dry weight (SDW) in g/pot, root length (cm), shoot dry weight Ca^{2+}, K^+, and Na^+ contents (mg g^{-1}), Ca^{2+}/Na^+ and K^+/Na^+. Sodium and K^+ levels of each sample were measured by flame photometery and Ca^{2+} was measured by atomic absorption spectrophotometry [12].

The stress tolerance index (TOL) as differences in SDW between the stress (y_s) and non-stress (y_p) environments [5], and stress tolerance index (STI) as $[(y_p).(y_s)/(y_{\bar{p}})^2]$, where $y_{\bar{p}}$ is the mean of SDW in non-stress environment [6] were calculated for each replicate of a genotype.

Data were subjected to analysis of variance and means were compared using the least significant differences (LSD). Variations in general combining ability (*gca*) of the parental lines and specific combining ability (*sca*) of crosses for the measured characteristics were partitioned from the total genetic variance using Griffings [13], Method II, Model I. The components of variance ratio as $\left[\frac{2\sigma^2_{gca}}{\left(2\sigma^2_{gca}+\sigma^2_{sca}\right)}\right]$ was computed for each characteristic to determine the relative importance of additive and non-additive gene effects [14]. High-parent heterosis was calculated as mean deviation of a cross performance from the mean of its superior parent [15]. Finally, narrow-sense heritability estimates for each characteristic were determined as the ratio of additive genetic variance to phenotypic variance [14].

Results and discussion

Significant variations in general combining ability and specific combining ability estimates were observed for shoot dry weight, root length, Na^+, K^+, Ca^{2+}, K^+/Na^+, Ca^{2+}/Na^+, TOL and STI (Table 1), indicating the importance of both additive and non-additive genetic effects for these characteristics. High narrow-sense heritability estimates and high ratios of $2\sigma^2_{gca}/(2\sigma^2_{gca}+\sigma^2_{sca})$ for Na^+, K^+, Ca^{2+}, K^+/Na^+, Ca^{2+}/Na^+ and TOL, indicated the importance of additive genetic effects in their genetic control. Therefore, the efficiency of selection based on these characters is expected to be high but, shoot dry weight and STI were controlled predominantly by non-additive genetic effects (Table 1). When *sca* effects are predominant in self-pollinated crops, the major portion of the variability then, is due to additive × additive genetic effects or divergence among progenies in the same parental array, therefore, selection should be delayed to later generations.

Significant positive and very high correlations were observed between *gca* effects of all the characteristics investigated with their means (ranging from 0.81 for root length to 0.96 for K^+/Na^+ and Ca^{2+}/Na^+). Therefore, for those characters with high narrow-sense heritability estimates, selection based on the means would improve their *gca* effects.

For ion compositions, the parents with superior means were BL2 and Falcon with 32.41 and 35.96 mg g^{-1} respectively for Na^+; Ceres, Shiralee, and Falcon with 12.05, 11.85 and 12.25 mg g^{-1}, respectively for K^+; Ceres and Shiralee with 23.80 and 22.06 mg g^{-1} respectively for Ca^{2+}; Falcon, Ceres, and Shiralee with 0.341, 0.313 and 0.313 respectively for K^+/Na^+; and finally BL2, Ceres, and Shiralee with 0.684, 0.618 and 0.617 respectively for Ca^{2+}/Na^+ ratio. These parents also had superior *gca* effects (Table 2).

The parent BL2, followed by Falcon, and Shiralee were good general combiners for shoot dry weight. These parents also exhibited low amounts of (negative) *gca* effects for TOL (Table 2). Although, significant differences ($P < 0.05$) were observed among the parental lines for SDW, the range of variability for this characteristic was low. However, F_1 progeny differences were higher and the F-test for parent vs. progeny mean square was significant, indicating average heterotic response under salt stress and different gene combinations in parental lines. For STI, only the parent Regent had significant positive *gca* effect, so it had

Table 1. Analysis of variance for shoot dry weight, root length, shoot Na^+, K^+, Ca^{2+}, Ca^{2+}/Na^+ and K^+/Na^+ in saline environment and TOL[a] and STI[b] indices based on Griffings Method II, Model I for diallel crosses

		Mean squares								
Sources of variation	df	Shoot dry weight	Root length	Na^+	K^+	Ca^{2+}	K^+/Na^+	Ca^{2+}/Na^+	TOL	STI
Crosses	35	0.047**	4.181**	33.256**	7.226**	14.720**	0.009**	0.024**	0.170**	0.030**
GCA	7	0.072**	11.143**	125.811**	23.729**	44.778**	0.031**	0.086**	0.625**	0.042**
SCA	28	0.041**	0.440**	10.117**	3.100**	6.705**	0.004**	0.009**	0.057**	0.027**
Error	70	0.009	0.449	3.533	1.069	3.045	0.0004	0.001	0.014	0.009
$2\sigma^2_{gca}/(2\sigma^2_{gca}+\sigma^2_{sca})$		0.28	0.52	0.78	0.69	0.70	0.62	0.68	0.74	0.27
Narrow-sense heritability		0.23	0.46	0.71	0.59	0.55	0.60	0.65	0.68	0.20
C.V (%)		13.39	11.34	4.89	9.06	8.11	6.66	5.64	13.60	21.04

**Significant at P = 0.01; a and b as defined in the text refer to tolerance index and stress tolerance index, respectively.

Table 2. Three top *gca* and *sca* effects of dry weight, root length, shoot Na^+, K^+, Ca^{2+}, K^+/Na^+ and Ca^{2+}/Na^+ in saline environment and TOL[a] and STI[b] indices for eight rapeseed genotypes and their diallel crosses

Characters	Three top *gca* effects		Three top *sca* effects	
	Parents	*gca*	Crosses	*sca*
Shoot dry weight	Falcon	0.044*	PF7045/91 × Regent	0.142**
	BL2	0.045*	PF7045/91 × Darmor	0.242**
	Shiralee	0.043*	Ceres × Shiralee	0.169**
Root length (cm)	BL2	1.328**	BL2 × Shiralee	1.186**
	Shiralee	0.319*	Ceres × Regent	1.142**
	BL1	–0.021	Regent × Darmor	2.690**
Na^+ (mg g^{-1})	BL2	–3.587**	Ceres × Regent	–5.453
	Falcon	–1.675**	BL1 × Falcon	–2.261
	Shiralee	–0.244	PF7045/91 × Shiralee	–1.567
K^+ (mg g^{-1})	Ceres	1.127**	PF7045/91 × Darmor	1.999**
	Shiralee	0.879**	BL1 × Shiralee	1.785**
	Falcon	0.802**	Falcon × BL2	1.161*
Ca^{2+} (mg g^{-1})	Ceres	1.645**	PF7045/91 × Darmor	3.313**
	Shiralee	1.185**	PF7045/91 × Ceres	2.795**
	BL2	0.518	Ceres × Regent	1.793
K^+/Na^+	Falcon	0.034**	Ceres × Regent	0.071**
	Ceres	0.030**	BL1 × Shiralee	0.056**
	Shiralee	0.022**	PF7045/91 × Darmor	0.050**
Ca^{2+}/Na^+	BL2	0.069**	PF7045/91 × Darmor	0.084**
	Ceres	0.045**	PF7045/91 × Ceres	0.073**
	Shiralee	0.030**	Falcon × Shiralee	0.060**
TOL	BL2	–0.175**	BL2 × Ceres	–0.282**
	Shiralee	–0.125**	Regent × Shiralee	–0.175**
	Ceres	–0.085**	BL1 × Falcon	–0.165*
STI	Regent	0.067**	Falcon × Shiralee	0.141*
	Falcon	0.025	PF7045/91 × Shiralee	0.121*
	Shiralee	0.008	BL2 × Ceres	0.120*

*,** Significant at P = 0.05 and P = 0.01, respectively; a and b as defined in the text refer to tolerance index and stress tolerance index, respectively.

high salt stress tolerance and high biomass potential in early growth stages. The parent BL2 had the highest mean and *gca* effect for root length.

Mean performance of the crosses (Table 3) and the estimates of their *sca* effects (Table 2) indicated that no cross had both high *sca* effect and high mean value (low values for TOL and Na^+) for all the characters, although a few crosses revealed to be the best combinations for more than one character. Highly significant and positive correlations were found between *sca* effects and mean performances of the crosses for all the characteristics, ranging from 0.62 for Na^+ concentration to 0.88 for STI. The cross PF 7045/19 × Darmor was one of the best combinations based on its significant positive *sca* effect for SDW, Ca^{2+} content and K^+/Na^+ and Ca^{2+}/Na^+ ratios. However, none of its parents exhibited high positive *gca* effects for these characteristics (Table 2). On the other hand, the best crosses based on shoot dry weight and TOL value *per se* was BL2 × Ceres, with at least one parent with high *gca* effect for these characteristics. Most of the crosses with high *sca* effects for SDW had at least one parent with positive or significant positive high-parent heterosis (Table 3). In general, 39% and 50% of the crosses had significant high-parent heterosis for SDW and TOL, respertively. Most of the crosses involving BL2 and Ceres had negative and significant high-parent heterosis for TOL.

For STI, the cross Falcon × Shiralee was the best combination, however, Regent × Shiralee had the highest mean value for STI. The parents of these crosses had average *gca* effects, except Regent which exhibited the highest *gca* effect and high mean performance. With respect to *gca* effects the superiority of average × average combination may be due to the presence of genetic diversity among the parents and there could be some complementation indicating importance of non-additive effects. As mentioned earlier, STI seemed to be predominantly under the control of non-additive gene effects. These crosses had salt stress tolerance and high shoot dry weight in non-stress environment and also gave positive and significant

Table 3. Range, mean and three top high parent heterosis for dry weight, root length, shoot Na^+, K^+, Ca^{2+}, K^+/Na^+ and Ca^{2+}/Na^+ in saline environment and TOL[a] and STI[b] indices

Characters	Range	Mean ± S.E	Three top high parent heterosis	
			Crosses	Heterosis
Shoot dry weight	0.89	0.71±0.003	BL2 × Ceres	0.327**
			Regent × Shiralee	0.250**
			Falcon × Shiralee	0.220**
Root length (cm)	7.87	5.95±0.23	Regent × Darmor	3.523**
			Ceres × Shiralee	1.630**
			Ceres × Regent	1.520**
Na^+ ($mg\ g^{-1}$)	42.49	38.39±1.85	PF7045/91 × BL2	–9.378**
			Ceres × Regent	–8.185**
			Falcon × PF7045/91	–6.473**
K^+ ($mg\ g^{-1}$)	12.70	11.41±0.40	BL1 × Shiralee	2.027**
			Falcon × Ceres	1.863*
			Ceres × Shiralee	1.77*
Ca^{2+} ($mg\ g^{-1}$)	23.04	21.46±0.80	PF7045/91 × Darmor	2.017
			Ceres × Regent	1.610
			Falcon × Shiralee	0.817
K^+/Na^+	0.01	0.301±0.001	Ceres × Regent	0.088**
			BL2 × Ceres	0.067**
			BL1 × Shiralee	0.053**
Ca^{2+}/Na^+	0.730	0.566±0.001	Ceres × Regent	0.158**
			Falcon × Regent	0.082**
			Falcon × Shiralee	0.055*
TOL	1.490	0.874±0.009	Regent × Shiralee	–0.410**
			PF7045/91 × Ceres	–0.367**
			Falcon × BL2	–0.360**
STI	0.520	0.451±0.002	Falcon × Shiralee	0.267**
			PF7045/91 × Shiralee	0.247**
			BL2 × Ceres	0.180*

*,** Significant at $P = 0.05$ and $P = 0.01$, respectively; a and b as defined in the text refer to tolerance index and stress tolerance index, respectively.

high-parent hoterosis (Table 3). The crosses Regent × Darmor, with significant positive *sca* effect and the highest heterosis, and BL2 × Shiralee with the highest mean *per se* were good specific combiations for root length. The cross Ceres × Regent exhibited the highest *sca* effects, mean performance and high parent heterosis for Ca^{2+}/Na^+ and K^+/Na^+. Also, the lowest *sca* effect and mean for Na^+, the highest mean for Ca^{2+} and the second highest mean for STI were observed for this cross. Nearly, 54% of the crosses exhibited significant negative high-parent heterosis for Na^+ concentration. The cross BL1 × Falcon had significant negative and positive *sca* effects for Na^+ and SDW, respectively. So it can be used for improving these characteristics, simultaneously.

Shoot dry matter is an important selection criterion which combines various responses to salinity into one measure of tolerance. However, this is a destructive method which can not be used for early generation selection. To overcome this difficulty, selection for tolerance could be performed on correlated characteristics. Significant positive correlations were estimated between SDW and other characteristics including Ca^{2+} (0.36*), K^+(0.41*), Ca^+/Na^+(0.53**) and K^+/Na^+ (0.57**). The high narrow-sense heritability estimates for K^+, Ca^{2+}/Na^+ and K^+/Na^+ were also high (Table 1), hence these characteristics can be used as good selection criteria for improving dry weight in saline environment. In previous studies [1, 2, 7, 10 & 16], also the importance of Ca^{2+}, K^+, Ca^{2+}/Na^+ and K^+/Na^+ for salinity tolerance have been emphasised. Significan positive correlations were observed between K^+ and Ca^{2+} (0.60**) and between Ca^{2+}/Na^+ and K^+/Na^+ (0.80**). As it was expected, negative and significant correlations were observed between Na^+ and K^+ (–0.41*), Ca^{2+} (–0.42*), Ca^{2+}/Na^+ (–0.82**) and K^+/Na^+ (–0.75**).

Pronounced results regarding selection criteria for salt tolerance were obtained using stress indices (TOL and STI). The parent BL2 exhibited the lowest (negative) *gca* effects and mean values for TOL and Na^+ and had the highest significant positive *gca* effects for SDW, root length, and Ca^{2+}/Na^+ ratio. The parent Ceres had the highest *gca* effects and means for Ca^{2+} and K^+. The cross of these parental lines including BL2 and Ceres or the crosses involving at least one of these cultivars gave the best combinations for most of the characteristics. According to Fernandez [6] a small value of TOL represent relatively more tolerance to stress. Selection based on TOL favours genotypes with low yield potential under non-stress environment and high yield under stress conditions. One of the difficulties with the use of plant dry matter to screen tolerant genotypes is that they may differ in their potential growth which under salinity stress it may present their potential capacity rather than specific tolerance [4]. Fernandez [6], reported that STI can be used to identify genotypes that produce high yields under both stress and non-stress environments. In other words, STI is an overall index of yield potential and stress tolerance. For example, Regent with average tolerance based on SDW, was considered as a susceptible cultivar based on TOL, but it had the highest mean value and *gca* effect for STI. However, this parental line was also classified as moderate tolerant genotype based on Na^+, K^+ and Ca^{2+} concentrations and K^+/Na^+ and Ca^{2+}/Na^+ ratios.

The genotypes had different mechanisms of salt tolerance, such as Na^+ regulation for osmotic adjustment, K^+ absorption for turgor maintenance and Ca^{2+} ion homeostasis and growth maintainance. For example, Falcon, a moderately salt sensitive parent based on TOL and STI indices, had a low Na^+ content, the highest K^+/Na^+ ratio and high K^+ and Ca^{2+} concentrations. Similarly, BL2 and Regent were the most salt sensitive parental lines based on TOL, but they were classified as superior parents based on STI. Also, Ceres, the moderately tolerant parent based on TOL value and Na^+ content, had the highest values and *gca* effects for Ca^{2+} and K^+ concentrations. However, as it is evident from the results of this study, the parental lines BL2, Shiralee and Ceres were tolerant and good general combiners based on most of the studied characteristics. These parents which were classified as tolerant based on higher SDWs in saline condition, had low TOL values, relatively higher K^+ and lower Na^+ concentrations and therefore, greater K^+/Na^+ ratios.

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