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Comparison of Salt Tolerance in Brassicas and Some Related Species

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ABSTRACT

In this paper the salt tolerance in Brassicas and some related species was compared. When seedlings germinated on sand cultures with liquid MS medium were considered, the relative germination rate, root length, shoot length and fresh seedling weight were significantly correlated with each other ($P < 0.01$), and only the relative shoot lengths were significantly different among the tested genotypes ($P < 0.05$); When both seedlings germinated on MS and MS plus 0.4% NaCl were considered, only the relative shoot length of seedlings germinated on MS was significantly different from that germinated on MS + 0.4% NaCl ($P < 0.05$), and also only the relative shoot lengths were significantly different among the tested genotypes ($P < 0.01$). *Raphanus sativa* cv. Changfeng, *B. juncea* cv. JC and *Brassica napus* cv. ZS 10 showed low salt tolerance in terms of relative germination rate, root length, shoot length and fresh seedling weight; *B. oleracea* cv. JF-1, *Sinapis alba* cv. HN-2 showed high salt tolerance in terms of relative germination rate, root length, shoot length and fresh seedling weight. Based on our result we suggest that relative shoot length might be convenient to rank the salt tolerance but cluster analysis based on multiple parameters of relative germination rate, root length, shoot length and fresh seedling weight might be more accurate in screening for salt tolerance in Brassicas and related species.

Keywords: Brassica; Related Species; Salt Tolerance; Multivariate Cluster Analysis

1. Introduction

Soil salinity is an issue that affects an estimated 6% of the world's land surface area or 12,780 million hectares (Mha) and secondary salinization from irrigation impacts an estimated 20% of irrigated land or 1474 Mha [1]. According to the United Nations reports, 20% of agricultural land and 50% of world cropland are salt affected [2]. Furthermore, there is also a dangerous trend of a 10% per year increase in the saline area throughout the world [3]. China has a large area of inland saline-alkali land, approximately 8.1 MHa, equivalent to 40% of the total cultivated land in the country [4,5].

The Brassicaceae family consists of many important field crops such as oilseed rape, the Brassica oleracea vegetable group (cauliflower, broccoli, brussels sprouts, kale) as well as other species such as *Brassica rapa* and *Raphanus sativus* [6]. However, their growth, yield, and oil production are markedly reduced due to salinity [7]. The most common adverse effect of salinity on the crop of *Brassica* is the reduction in plant height, size and yield as well as deterioration of the product quality [8,9].

In previous studies, the Brassicas were usually grown in salt conditions to compare parameters such as relative germination rate, root length, fresh seedling weight or seed yield [10-17]. In the present study the seeds of Brassicas and related species were grown on sand cultures with and without salt to explore an efficient index to compare the salt tolerance in Brassicas and related species and to determine variations in degree of salt tolerance at inter- and intra-specific levels particularly at the germination and seedling stages.

2. Materials and Methods

2.1. Materials

Seeds of *Sinapis alba* (cv.HN1, HN2), *Brassica carinata* cv. EJ and *Isatis indigotica* cv. HN were provided by Pro. Dr. Zaiyun Li of Huazhong Agricultural University,

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China. The *Brassica napus* seeds were from the Oil Crops Institute, CAAS, China. The other seeds were provided by Lab of Plant Genetics and Breeding of Hubei University, China.

2.2. Methods

Sixty healthy seeds for each genotype were inoculated on sand cultures with liquid MS and MS + 0.4% NaCl. Germination rates were scored 4 - 5 days after inoculation. The germinated seeds on MS and MS + 0.4% NaCl were transferred onto sand cultures with MS and MS + 0.4% NaCl for 3 - 4 days and then root length, shoot length and fresh seedling weight were measured. The temperature was set at 25˚C with a 16-hr photo period under 2000 lx. The experiment was arranged in a completely randomized design with three replicates. Salt tolerance indices were calculated according to Zeng *et al*. (2002) [18] and further used to classify genotypes using cluster analysis as described by El. Hendawy *et al*. (2005) [19]. Cluster analysis was carried out based on Euclidean distance of the salt tolerance indices. The cluster group rankings were obtained from the average of means of the multiple parameters in each cluster group. A sum was obtained by adding the number of cluster group rankings in each genotype. The genotypes were finally ranked based on the sums, such that those with the smallest and largest sums were ranked respectively as the most and least tolerant genotypes in terms of relative salt tolerance. Variance analyses, multiple comparisons and cluster analyses were carried out on SPSS.

3. Results

3.1. Correlations among Relative Germination Rate, Root Length, Shoot Length and Fresh Seedling Weight

From **Table 1** it was found that when seeds were germinated on sand cultures with liquid MS medium, the relative germination rate, root length, shoot length and fresh seedling weight were significantly correlated with each other ($P < 0.01$); When seeds were germinated on sand cultures with liquid MS medium plus 0.4% NaCl, the relative germination rate was only significantly correlated with shoot length ($P < 0.05$), the relative root length was significantly correlated with shoot length and fresh seedling weight ($P < 0.01$), and relative shoot length was significantly correlated with fresh seedling weight ($P < 0.01$).

3.2. Effect of Germination Condition on Relative Root Length, Shoot Length and Fresh Seedling Weight

When only seeds germinated on MS medium were considered, variance analysis indicated that the difference of relative shoot lengths among the tested genotypes was significant ($P < 0.05$), while that of relative root length and fresh seedling weight were not significant (data not shown).

When both seeds germinated on MS and MS + 0.4% NaCl were considered, variance analysis indicated that the relative shoot length of seedlings germinated on MS was significantly different from that germinated on MS + 0.4% NaCl ($P < 0.05$), while that of relative root length and fresh seedling weight were not significant; the relative shoot lengths were significantly different among the tested genotypes ($P < 0.01$), while that of relative germination rate, root length and fresh seedling weight were not significant (data not shown).

From **Table 2** it was found that based on relative shoot length *B. carinata* cv. EJ, *B. oleracea* cv. JF-1 and *Eruca sativa* cv. hub12 showed high salt tolerance while *Raphanus sativus* cv. changfeng and *B. napus* cv. ZS 10 were identified as most salt-sensitive.

Table 1. Correlations among relative germination rate, root length, shoot length and fresh seedling weight.

	Relative germination rate	Relative root length	Relative shoot length	Relative fresh weight
Relative germination rate		0.430^{**}	0.416^{**}	0.454^{**}
Relative root length	0.019		0.423^{**}	0.486^{**}
Relative shoot length	0.251^{*}	0.593^{**}		0.804^{**}
Relative fresh weight	−0.075	0.552^{**}	0.500^{**}	

Note: Left Bottom: Correlations of relative germination rate, root length, shoot length and fresh weight of seedlings germinated on MS + 0.4% NaCl; Top Right: Correlations of relative germination rate, root length, shoot length and fresh weight of seedlings germinated on MS; $^{**}P < 0.01$; $^{*}P < 0.05$.

3.3. Cluster Analysis of Salt Tolerance

From **Table 3** it was found that *R. sativa* cv. changfeng, *B. juncea* cv. JC and *B. napus* cv. ZS 10 showed low salt tolerance in terms of relative germination rate, root length, shoot length and fresh weight; *B. oleracea* cv. JF-1, *S. alba* cv. HN-2 showed high salt tolerance in terms of relative germination rate, root length, shoot length and fresh seedling weight; *B. carinata* cv. EJ showed high salt tolerance in terms of root length, shoot length and fresh seedling weight but with very low salt tolerance in terms relative germination rate.

From **Figure 1** it was found that *B. oleracea* cv. JF-1, *B. carinata* cv. EJ and *Sinapis alba* cv. HN-2 were identified as highly salt-tolerant. *S. alba* cv. HN-1, *I. indigotica* cv. HN and *B. rapa* cv. xiaobaicai were identified

Table 2. Multiple comparison of relative shoot length among the tested genotypes when seeds germinated on MS were considered.

	Genotye	1	2	3	4	5
Duncan[a,b]	*R. sativus* cv. changfeng	51.2267				
	B. napus cv. ZS 10	52.8000	52.8000			
	B. oleracea cv. zigan	65.7533	65.7533	65.7533		
	B. napus cv. ZY 821	66.6100	66.6100	66.6100		
	R. sativus cv. red	67.1000	67.1000	67.1000		
	C. abyssinica cv. hubu-1	68.2667	68.2667	68.2667		
	B. oleracea cv. xueyuan	68.4967	68.4967	68.4967		
	B. oleracea cv. qinghua	70.9833	70.9833	70.9833		
	B. juncea cv. JC	71.0333	71.0333	71.0333		
	B. napus cv. ZS 9	72.7133	72.7133	72.7133		
	C. abyssinica cv. hubu-2	74.0267	74.0267	74.0267		
	R. sativus cv. shunyuan	77.6567	77.6567	77.6567	77.6567	
	C. abyssinica cv. hubu-3	78.7367	78.7367	78.7367	78.7367	
	B. rapa cv. dabaicai	78.8000	78.8000	78.8000	78.8000	
	B. napus cv. ZYZ 7	81.7033	81.7033	81.7033	81.7033	
	R. sativus cv. nanpan	82.0167	82.0167	82.0167	82.0167	
	B. napus cv. ZS 11	82.4967	82.4967	82.4967	82.4967	
	R. sativus cv. jiujin	82.5067	82.5067	82.5067	82.5067	
	R. sativus cv. may	83.8400	83.8400	83.8400	83.8400	
	B. juncea cv. yongsheng	84.6500	84.6500	84.6500	84.6500	
	B. oleracea cv. GJ-1	86.2933	86.2933	86.2933	86.2933	
	E. sativa cv. hub11	86.4000	86.4000	86.4000	86.4000	
	S. alba cv. HN-1	86.6667	86.6667	86.6667	86.6667	
	I. indigotica cv. HN	87.1133	87.1133	87.1133	87.1133	
	B. rapa cv. xiaobaicai	89.6400	89.6400	89.6400	89.6400	
	E. sativa cv. hub10		94.9733	94.9733	94.9733	
	S. alba cv. HN-2			95.8467	95.8467	
	B. rapa cv. shiyue			97.8600	97.8600	
	E. sativa cv. hub12			102.1733	102.1733	102.1733
	B. oleracea cv. JF-1				117.0767	117.0767
	B. carinata cv. EJ					135.7000

Note: The genotypes were classified into 5 groups. Genotypes in the same group are not significantly different from each other.

as salt-tolerant, *R. sativa* cv. changfeng and *B. napus* cv. ZS 10 were identified as most salt-sensitive.

4. Discussion

Although salt stress affects all growth stages of a plant, seed germination and seedling growth stages are known to be more sensitive in most plant species [8,20,21]. Huang *et al*. (2010) used 0.37% NaCl to compare the salt tolerance during germination and seedling growth among genotypes of *B. napus, B. juncea* and *B. rapa* [13]. Our preliminary studies also showed that seed germination, root length, shoot length and fresh seedling weight of Brassicas were inhibited by 20% - 60% when the NaCl concentration was 0.4%. Therefore we used 0.4% NaCl for seed inoculation and seedling growth to compare the salt tolerance.

In earlier screening of *Brassica* species for salt tolerance, the superiority of amphidiploid species *B. carinata*, *B. juncea*, and *B. napus* over the diploid species, *B.rapa*, *B. nigra*, and *B. oleracea* was proposed from different studies [8,22-24]. It has been further suggested that the

Table 3. Rankings of genotypes for their salt tolerance in terms of relative germination rate, root length, shoot length and fresh seedling weight.

Genotype	Given No.	Rank of relative germination rate	Rank of relative root length	Rank of relative shoot length	Rank of relative seedling weight	Final genotype ranking
R. sativus cv. jiujin	1	29	12	14	21	20
R. sativus cv. changfeng	2	26	29	31	31	31
R. sativus cv. shunyuan	3	7	7	20	26	13
R. sativus cv. nanpan	4	15	21	16	17	17
R. sativus cv. may	5	25	10	13	19	15
R. sativus cv. red	6	19	14	27	29	27
B. napus ZS 11	7	17	6	15	18	12
B. napus cv. ZS 9	8	20	22	22	7	18
B. napus cv. ZY 821	9	16	27	28	12	21
B. napus cv. ZYZ 7	10	24	9	17	13	14
B. napus cv. ZS 10	11	31	13	30	30	29
C. abyssinica cv. hubu-1	12	27	26	26	14	28
C. abyssinica cv. hubu-2	13	12	28	21	23	22
C. abyssinica cv. hubu-3	14	10	15	19	24	16
E. sativa cv. hub12	15	9	23	3	9	8
E. sativa cv. Hub11	16	21	30	10	25	25
E. sativa cv. Hub10	17	13	24	6	11	11
B. oleracea cv. qinghua	18	4	31	24	28	26
B. oleracea cv. zigan	19	23	16	29	16	22
B. oleracea cv. xueyuan	20	22	18	25	20	24
B. oleracea cv. JF-1	21	1	1	2	2	1
B. oleracea cv. GJ-1	22	11	11	11	4	7
B. juncea cv. yongsheng	23	5	19	12	8	8
B. juncea cv. JC	24	30	25	23	27	30
B. rapa cv. xiaobaicai	25	8	8	7	6	4
B. rapa cv. shiyue	26	18	20	4	5	10
B. rapa cv. dabaicai	27	14	17	18	22	18
S. alba cv. HN-1	28	3	5	9	15	5
S. alba cv. HN-2	29	2	2	5	3	2
B. carinata cv. EJ	30	28	3	1	1	6
I. indigotica cv. HN	31	6	4	8	10	3

salt tolerance of amphidiploids has been acquired from the A (*B. rapa*) and C (*B. oleracea*) genomes [10]. However, there were also different conclusions as significant inter- and intraspecific variation for salt tolerance exists within Brassicas, which can be exploited through selection and breeding for enhancing salt tolerance of the crops [9,12,13,25-27]. For example, of turnip cultivars, Shaljum desi surakh was highest in seed germination, while it was lowest in seedling shoot dry biomass production. However, Neela Shaljum having lower seed germination percentage produced maximum seedling shoot dry biomass. In the same way, cv. Desi of radish with minimum seed germination had highest shoot dry weight under saline conditions [12]. Therefore efficient and accurate indices and criteria for identifying salt-tolerance in Brassicas and related species are needed.

The multivariate cluster analysis method was recommended as the best criteria for the identification of salt tolerance in crop species such as rice [18,28,29], green gram [30], wheat [19], tomato [31], sugarcane [32], peanut [33], cauliflower [34]. As pointed out by Khrais *et al*. [35] and Zeng *et al*. [18], the advantages of using a multivariate analysis in the evaluation of salt tolerance are that it allows: 1) a simultaneous analysis of multiple

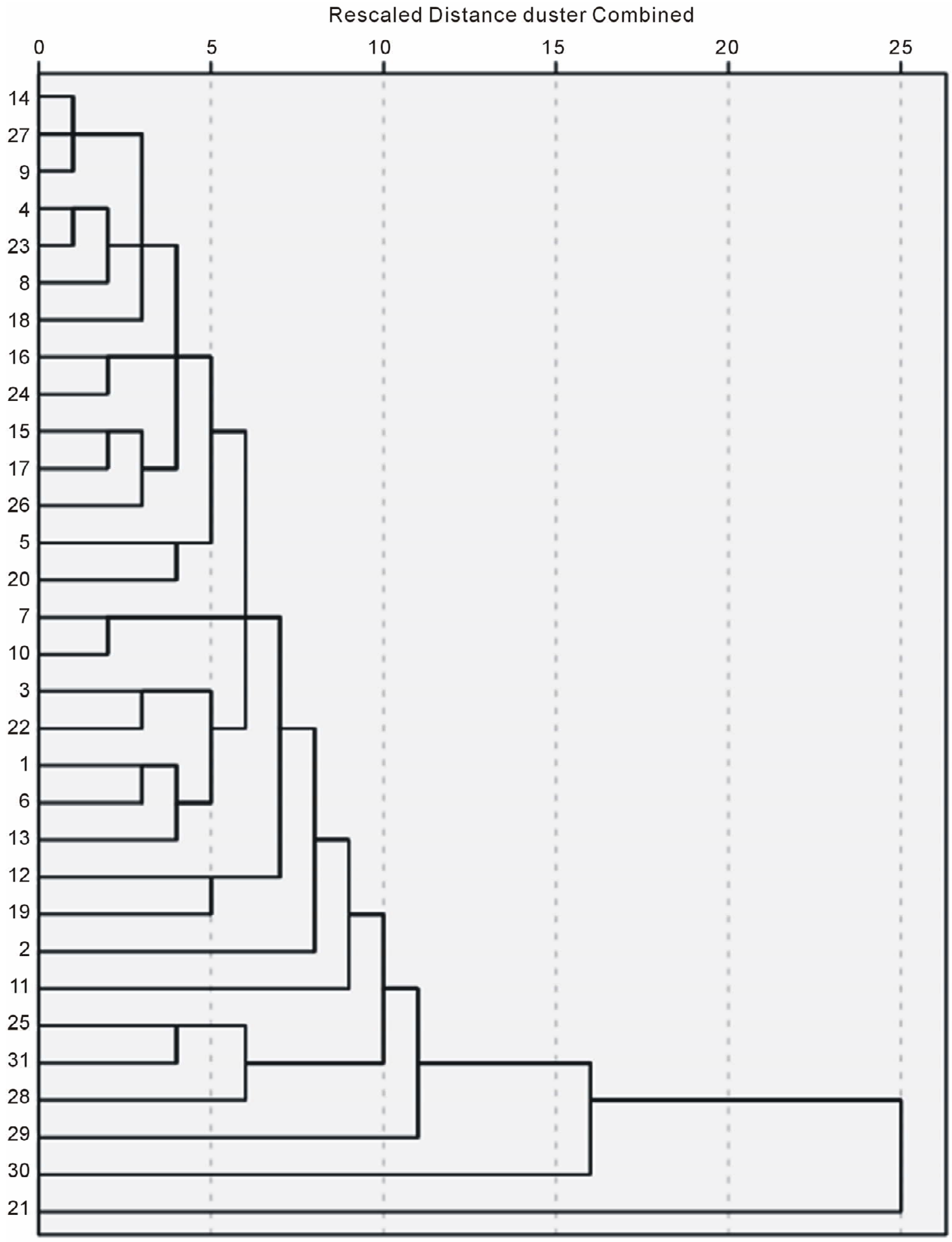

Figure 1. Hierarchical cluster analysis of the genotypes based on multiple salt tolerance indices of seedlings germinated on MS medium (Euclidean distance). The genotypes that the numbers represent were indicated in Table 3.

parameters to increase the accuracy of the genotype ranking; 2) the ranking of genotypes even when plants are evaluated at different salt levels and salt tolerance varies with salinity levels, especially when the salt tolerance indices are averaged across salt levels; and 3) a more convenient and accurate estimation of salt tolerance among genotypes by simply adding the numbers in cluster group ranking at different salt levels.

In the present study, when seedlings germinated on sand cultures with liquid MS medium were considered, the relative germination rate, root length, shoot length and fresh seedling weight were significantly correlated with each other ($P < 0.01$), and the relative shoot lengths were significantly different among the tested genotypes ($P < 0.05$), while the relative root length and relative fresh seedling weight were not significantly different; when seedlings germinated on liquid MS medium plus 0.4% NaCl were considered, the relative germination rate was only significantly correlated with shoot length ($P < 0.05$), the relative root length was significantly correlated with shoot length and fresh seedling weight ($P < 0.01$), and relative shoot length was significantly correlated with fresh seedling weight ($P < 0.01$). When both seedlings germinated on MS and MS plus 0.4% NaCl were considered, the relative shoot length of seedlings germinated on MS was significantly different from that germinated on MS + 0.4% NaCl ($P < 0.05$), suggesting that germination in salt condition had some selection effect for shoot length, while relative root length and fresh seedling weight were not significantly different; the relative shoot lengths were significantly different among the tested genotypes ($P < 0.01$), while the relative germination rate, root length and fresh seedling weight were not significantly different.

Based on relative shoot length *B. carinata* cv. EJ, *B. oleracea* cv. JF-1 and *E. sativa* cv. hub12 were classified as highly salt-tolerant while *R. sativus* cv. Changfeng, and *B. napus* cv. ZS 10 were identified as most salt-sensitive. *R. sativa* cv. changfeng, *B. juncea* cv. JC and *B. napus* cv. ZS 10 showed low salt tolerance in terms of relative germination rate, root length, shoot length and fresh seedling weight; *B. oleracea* cv. JF-1, *S. alba* cv. HN-2 showed high salt tolerance in terms of relative germination rate, root length, shoot length and fresh seedling weight; *B. carinata* cv. EJ showed high salt tolerance in terms of root length, shoot length and fresh seedling weight but very low salt tolerance in terms relative germination rate.

Based on our result we suggest that relative shoot length might be convenient to rank the salt tolerance but cluster analysis based on multiple parameters of relative germination rate, root length, shoot length and fresh seedling weight might be more accurate in screening for salt tolerance in Brassicas and related species.

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