Genetic Diversity and Selection for Salt Tolerant accessions of *Brassica rapa*populations

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Abstract: The present study was carried out to evaluate genetic diversity and genomic relationships of 14 populations of *B. rapa* with different centers of originsbased on polymorphism of four isozyme systems. The selectionof salt tolerant accessions was carried out by treatment with 150 mMNaCland the molecular characterization was achieved using RAPD analysis. The studied populations were classified into three groups based on allele frequency confirming genetic divergence of the studied populations as a result of different breeding traditions in different parts of the world. The highest values of genetic diversity measures were observed for the populations of *B. rapa*ssp. *chinensis* with Chinese and unknown origin followed by the Chinese *B. rapa* ssp. *pekinensis*. The genetic relationships among the studied populations based on Nei's genetic distance derived from isozyme data coincide with their breeding history. The variation between the salt tolerant and salt sensitive accessions as observed from RAPD pattern was attributed to the genetic divergence among salt tolerant and sensitive accessions. The salt tolerant accessions can be selected as resources to improve the salt stress tolerance in *B. napus*by sequencing of characteristic RAPD bands in marker assisted selection.
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1. Introduction

Brassica rapa L. is a diploid multipurpose crop that is used as condiments, vegetables, vegetable oils, biodiesel and fodder crops (Reiner *et al.,* 1995; Tahir *et al.,*2012). The continuing breeding of *B. rapa* crop (A genome, n=10) in different parts of the world has increased variation within the species (Zhao *et al.,*2005).This variation has led to the divergence of the species into major cultivar groups referred to as subspecies (Qian *et al.,* 2006; Snowdon, 2007; Zhao *et al.,*2009). In southern China, three cultivars have been developed depending on the part used (Zhao*et al.,*2009):Chinese turnip rape (ssp. *rapa*), the leafy vegetables Pak choi (ssp. *chinensis*) as well as the Chinese cabbage (ssp. *pekinensis*).The Sarson types (ssp*trilocularis*) was developed in India (Snowdon, 2007) while the oil seed types (ssp. *oleifera*) are the dominating European forms (Reiner *et al.,*1995; Zhao *et al.,* 2009).

Interspecific hybridizations between *B. rapa*and *B. oleracea*L. (C genome, n=9) contribute to derive the amphidiploid (AACC, *n* = 19) oil crop *B. napus*(Marhold and Lihova, 2006; Snowdon, 2007; Koch and Al-Shehbaz, 2009; Maoteng *et al.,* 2010; Zou *et al.,* 2010; Schmidt *et al.,*2011). Several studies indicated that the amphidiploid *B. napus* is more salt tolerant than its diploid parents (Ashraf *et al.,* 2001; Ashraf and McNeilly, 2004) therefore it was suggested that the combination of A and C genomes are responsible for the salt tolerance advantage of *B. napus*(Ashraf *et al.,* 2001; Zou *et al.,* 2010; Girke *et al.,*2012).

Due to successive breeding for desired phenotypes, some crop types are subjected to progressive genetic erosion and reduction in genetic diversity within the crop gene pool. *B. napus*has been subjected to progressive selection for varieties with low glucosinolate and low erucic acid content (Ofori *et al.,* 2008; Kim *et al.,* 2013) giving rise to a narrow genetic base of this crop.This initiates approaches to extend genetic variation through breeding programs including *B. rapa* cultivarsas genetic resources (Ren *et al.,*2000; Zou *et al.,* 2010; Li *et al.,*2013; Roy and Tester, 2013). The selection of genetic resources has to be based on sufficient information about its genetic diversity as well as genomicrelationships among the selected breeding lines. Several attempts were performed to revise the genomic relationships among selected accessions of *B. rapa*using different markers. These markers includes AFLP (Takuno *et al.,* 2007), simple sequence repeats (Ofori *et al.,* 2008), Single nucleotide polymorphisms and insertion/deletions (Park *et al.,* 2010).

The present study was devoted to evaluate genetic diversity and genomic relationships among *B. rapa*populations using isozyme polymorphism. Besides, the yield of *Brassica* crops is greatly affected by soil salinity and the need for salt tolerant crops is of prime importance.Therefore, selection of salt tolerant and molecular characterization using RAPD of

accessions of *B. rapa*were also investigated to be used as *superior* parents included in breeding programs for the improvement of oilseed rape *B. napus*

2. Material and methods

Plant Material

A total of 56 accessions of *B. rapa*were obtained representing 14 populations of different centers of origins (Table 1). Out of these accessions, 49 were supplied by IPK gene bank Gatersleben Germany and the other 7 were purchased from seed markets in Egypt. The distribution of populations was (5) populations of *B. rapa*ssp. *chinensis*, (4) of *B. rapa*ssp. *pekinensis*,(3) of *B. rapa* ssp. *rapa*, (1) of *B. rapa* ssp. *oleifera ruvo-gruppe* and (1) of*B. rapa* ssp. *trilocularis.*

Seed germination

Seeds were surface sterilized by soaking in 70% (v/v) ethanol for 1 min, then rinsed several times with sterile distilled water. The seeds were then germinated for 3 days at 25^{o}C in sterilized Petri dishes with three moist filter papers.

Isozyme extraction

Seedlings (3-day-old) were macerated in 5 ml saline solution containing 0.8% NaCl and 0.2% $NaNO_3$, and then centrifuged at 12000 rpm for 15 minutes. Supernatants were collected in pre-chilled tubes and stored at -20^{o}C until use for electrophoretic separation of isozyme.

Isozyme electrophoresis

Mini vertical slabs of 7.5% acrylamide concentration were prepared according to Laemmli (1970). Aliquots (15 µl) of extracts were mixed with equal volumes of loading buffer (50% glycerol containing 1% bromophenol blue) and loaded onto the gels. Electrophoresis was carried out at 15 mA/gel for 60 min.

The gels were stained for four isozymes according to Eduardo Valejos (1983). The isozymes are acid phosphatase (ACP), catalase (CAT), esterase (EST), and peroxidase (PER).

Isozyme phenotypes were interpreted genetically according to standard principles of Wendel and Weeden (1989) and were scored collectively for all studied accessions. The genotypes were used in assessing genetic variability by the computation of allele frequency (A_f), mean alleles per locus (A), mean effective allele number per locus (A_e), number of polymorphic alleles per locus (A_p), percentage of polymorphic loci (P_p) and average of both observed and theoretical heterozygosity (H_{obs} *and* H_{exp}respectively) according to Hedrick (1984).To analyze the population homozygosity level, the Wright's fixation index (F) as well as number of loci with significant excess (H_E) or deficiency (H_D) heterozygosity were calculated (Hedrick, 1984). Genetic divergence among *B. rapa*populations were quantified by computingpairwise values for Nei'sGenetic distance (D) according to Nei (1978). The Nei's genetic distance was used to construct the dendrogram using UPGMA (Sneath and Sokal, 1973).

Selection for salt tolerant accessions

Twenty seeds from each accession were spread on a Petri dish (150mm diameter, 15mm height) lined with two filter papers (Whatman No 1) and watered using 12 ml of a gradient of NaCl concentrations of 0.0 mM (control), 50ml mole (EC = 3.9), 100 ml mole (EC = 8) and 150 ml mole (EC = 11.9). The dishes were incubated at 24°C for three days then the percentage of germination was calculated. Accessions within populations that maintained the highest germination percentage under the highest NaCl concentration of 150 mM were selected as salt tolerant while those with the lowest percentage of germination were considered salt sensitive. The genome of the selected salt tolerant and sensitive accessions in each population was characterized using Random amplified polymorphic DNA (RAPD) as genetic marker.

RAPD analysis

Genomic DNA samples were extracted from 5 mg of germinated seed of each accession according to Porebski *etal*. (1997). Five random primers termed A10 (5'GTGATCGCAG3'), B16 (5'TTTGCCCGGA3'), C10 (5'TGTCTGGGTG3'), Z10 (5' CCGACAAACC 3'), and Z12 (5'TCAACGGGAC3') were used for generation of RAPD fragments. The mix for a 50 µl reaction comprised 50 µg genomic DNA, 1 µM primer, 1.0 µMdNTP's (supplied as a 50X deoxynucleoside triphosphate Mastermix; Bioline USA, Inc.), 5 mM $MgCl_2$ (supplied as a 50 mM stock; Bioline), 10 mM 10X Taq reaction buffer (Bioline) and 1 U BIOTAQ DNA polymerase (Bioline). Amplifications were performed in the DNA Thermal Cycler (Hybaid Ltd.), programmed for an initial 6 min denaturation at 94°C followed by 35 cycles of 1 min at 94°C,1 min at 46°C and 1 min at 72°C. PCR products were separated in a 1.5% agarose gel containing 0.2 pg/ml of ethidium bromide. The molecular weight of the PCR products was estimated by using a 100-bp ladder (Amersham-Pharmacia-Biotech, Uppsala, Sweden) as a molecular size standard.

A data matrix was created from photographs of gels by using gel documentation system (Advanced American Biotechnology 1166 E, Valencia Dr. Unit 6 C, Fullerton CA 92631). RAPD bands were scored as present (1) or absent (0), and a pair-wise correlation distance matrix was obtained. To compare RAPD fragments of salt tolerant and salt sensitive accessions, sequential agglomerative hierarchical non-overlapping (SAHN) clustering using the unweighed pair group method with arithmetic mean (UPGMA)method was then performed, and a phenogram was generated as

described by Sneath and Sokal (1973) using NTSYS-pc version 2.02 (Rohlf 2000).

Table 1 Accession origin, code and percentage germination under 150 mMNaCl of the studied *B. rapa*populations

Origin	Accession code	% germination	Origin	Accession code	% germination
B. rapa ssp. chinensis			***B. rapa ssp. rapa***		
UNG	BRA 1321/91	85	UNG	BRA 337/79	90**
UNG	BRA 1320/91	100**	U.S.A	BRA 1116/99	20**
UNG	BRA 332/80	55	Egypt	..	80
UNG	BRA 1828/00"K7649"	50	Egypt	..	65
Taiwan	BRA 1308/ 88	80	Egypt	..	85
Taiwan	BRA 1634/94"8894"	60	Egypt	..	85
China	BRA 116/ 80	45	Egypt	..	80
China	BRA 226/ 84	50	Egypt	..	80
China	BRA 447/ 78	60	Egypt	..	35
China	BRA1621/94"K8866"	50	***B. rapa ssp. oleifera. ruvo-gruppe***		
China	BRA	60	UNG	CR 2596/87	100**
China	BRA 1597/92	95	UNG	CR 3315/87	30
China	BRA 218/81	70	UNG	CR 3323/00	90
China	BRA 446/85	70	UNG	CR 3322/98	80
China	BRA 219/84	40	UNG	CR 2592/96	25**
China	BRA 217/81	80	***B. rapa ssp. trilocularis***		
China	BRA 77/84	90	IND	CR 2232/88	100**
China	BRA 119/80	60	IND	CR 2233/88	90
U.S.A	K 9420/95	25**	IND	CR 2217/01	10**
Japan	BRA 1638/95	90	IND	CR 2215/88	35
Japan	BRA 1609/93	90	IND	CR 2244/88	95
B. rapa ssp. pekinensis					
UNG	BRA 1620/97	45			
UNG	BRA 78/84	100**			
UNG	BRA 430/00	85			
UNG	BRA 80/88	90			
Japan	BRA 1125/77	85			
Japan	BRA 1307/91	95			
Japan	BRA 1596/99	30			
Japan	BRA 1612/93	90			
Japan	BRA 1124/77	35			
China	BRA 122/81	20**			
China	BRA 1628/94"K8873"	90			
China	K65R	90			
Korea	BRA 201/76	40			
Korea	BRA 988/85	60			
Korea	BRA 1586/95	95			

3. Results

Allele frequency

A total of 15 isozyme loci were observed all over the studied populations (Table 2). Populations varied according to presence or absence of a specific locus. The highest number of loci (11 loci) was recorded in *B. rapa* ssp. *rapa* of unknown origin followed by the population of *B. rapa* ssp. *chinensis* from Taiwan and China, *B. rapa* ssp. *pekinensis* from Korea as well as *B. rapa* ssp. *rapa* from Egypt (10 loci). A total of 30 alleles were exhibited allover the recorded 15 loci and their frequencies were computed for each population.

Based on allele frequency at a specific locus, populations varied into populations with rare alleles (a or b, $A_f \leq 0.20$), others with monomorphic loci (having a predominant allele with frequency $A_f = 1$), while the remaining population showed balanced alleles ($A_f = 0.50$). For example, the population of *B. rapa* ssp. *chinensis* with unknown origin exhibited Cat1a as the rare allele while Cat3a was observed as the rare allele in the populations of *B. rapa* ssp. *chinensis* from unknown origin and China as well as *B. rapa* ssp. *pekinensis* from Korea and *B. rapa* ssp. *rapa* from unknown origin. Populations having the allele (b) as the rare one were Acp1b in the population of *B. rapa* ssp. *oleifera ruvo-gruppe*with unknown origin; Acp2b in the population of *B. rapa* ssp. *rapa* from USA; Est1b in the population of *B. rapa* ssp. *chinensis* from China; as well as Per1b in the population of *B. rapa* ssp. *oleifera ruvo-gruppe* with unknown origin (Table 2). At the monomorphic loci, some populations share the same predominant allele at a specific locus (Acp1b, Acp2b, Acp3a, Cat1b, Cat2a, Est1a, Est4a, Per2b and Per3b) while at other populations, the predominant allele varied from population to the other.

The genetic diversity measures (Table 3) revealed that the highest values of number of alleles per locus (A) were observed for *B. rapa* ssp. *chinensis* from China, *B. rapa* ssp. *chinensis* with unknown origin followed by that of *B. rapa* ssp. *rapa* from Egypt and *B. rapa* ssp. *trilocularis* (India). The population of *B. rapa* ssp. *rapa* from Egypt and *B. rapa* ssp. *chinensis* from China showed the highest values of effective allele number (A_e). *B. rapa* ssp. *chinensis* from China was distinguished by the highest percentage of polymorphic loci (P_p). *B. rapa* ssp. *trilocularis* (India) showed the highest value of observed heterozygosity (H_{obs}) followed by *B. rapa* ssp. *rapa* from Egypt then *B. rapa* ssp. *pekinensis* from China and unknown origin. It was also observed that *B. rapa*ssp. *chinensis* as well as *B. rapa* ssp. *pekinensis* from Japan were distinguished by having the highest values of Wright's index per population (F). Most of the studied populations showed significant deficiency of heterozygosity (H_D; Table 3) with the highest values observed in the Japanese populations of *B. rapa* ssp. *chinensis* (11 loci 73%) and*B. rapa* ssp. *pekinensis* (12 loci 80%).

The UPGMA clustering based on genetic distance of isozyme data resulted in the separation of *B. rapa* ssp. *trilocularis* from India and *B. rapa* ssp. *oleifera ruvo-gruppe* with unknown origin in two separate clusters at D=0.6 (Fig 1). Besides, four additional groups can be recognized in which the populations representing ssp. *chinensis* comprisedtwo groups while those of ssp. *pekinensis* and ssp. *rapa*were included in the other two groups (Fig 1).

The five RAPD 10-mer primers revealed a total of 244 polymorphic fragments that were utilized to compare the tolerant and sensitive accessions (Fig 2). The salt tolerant accession of *B. rapa*ssp. *chinensis* (BRA 1320/91 with unknown origin) was characterized by 20 RAPD fragments ranged from 131bp (using primer B-16) to 2318bp (using primer Z-12). The salt sensitive accession of this subspecies (K 420/95 form U.S.A) revealed 47 RAPD fragments varied from 129bp to 1200bp in case of primer Z-12.

The salt tolerant accession of *B. rapa*ssp. *pekinensis* (78/84 with unknown origin) was characterized by 32 RAPD fragments ranged from 205bp (primer B-16) to 1400 bp (primer Z-10). On the other hand, the salt sensitive accession (122/81, China) was distinguished by 38 RAPD fragments varied from 227bp to 664bp (primer C-10).Considering *B. rapa*ssp. *rapa*, the salt tolerant accession (BRA 337/79 with unknown origin) revealed 43 RAPD fragments varied from 209bp (primer B-16) to 7606bp (primer Z-10). The salt sensitive accession (BRA 337/79 with unknown origin) revealed 42 fragments ranging from 132bp to 7606bp with primer Z-12. In case of *B. rapa* ssp. *oleifera ruvo-gruppe*, salt tolerant accession (CR 2596/87 with unknown origin) was characterized by 43 fragments their varied from 122bp with primer B-16 to 1403 with primer Z-12 while the salt sensitive accession (CR 2592/96 with unknown origin) revealed 44 fragments ranged from 169bp to 814bp with primer Z-12. *B. rapa* ssp. *trilocularis* salt tolerant accession (CR 2232/88, India) showed 34 fragments ranged from 140bp with primer B-16 to 1606 with primer Z-12 while the salt sensitive accession (2217/01, India) revealed 15 fragments varied from 207bp with primer B-16 to 1219bp with primer C-10.

Each of the studied tolerant accessions was characterized by the occurrence of specific RAPD fragments (Fig 2). These fragments were 274bp (primer A10) observed in *B. rapa*ssp. *chinensis*; 261bp and 349bp (primer A10)as well as 299bp (primer B16) characteristic for *B. rapa* ssp. *rapa*; 503bp (primer A10) in *B. rapa* ssp. *oleifera ruvo-gruppe*; 951bp (primer Z12) in *B. rapa* ssp. *pekinensis* and 1088bp (primer Z12) in *B. rapa* ssp. *trilocularis.*

At D = 0.95, the dendrogram based on RAPD data of both salt tolerant and sensitive accessions grouped the accessions of *B. rapa* ssp. *chinensis* and *B. rapa* ssp. *pekinensis* in one cluster; while those of *B. rapa* ssp. *rapa*,*B. rapa* ssp. *trilocularis*,*B. rapa* ssp. *oleifera ruvo-gruppe*occupied separate clusters. At D = 0.9, both tolerant and sensitive accessions representing each subspecies were observed in a distinctive cluster.

Table 2. Allele frequency of the studied *Brassica rapa*populations

Populations		*B. rapa* ssp. *chinensis*					*B. rapa* ssp. *pekinensis*				*B. rapa* ssp. *rapa*			*B. rapa* ssp. *oleifera ruvo gruppe*	*B. rapa* ssp. *trilocularis*
Loci	alleles	Unknown	Taiwan	China	USA	Japan	Unknown	Japan	China	Korea	Unknown	USA	Egypt	Unknown	India
Acp1	a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.50	0.90	0.50
	b	0.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.50	0.00	0.50	0.10	0.50
Acp2	a	0.25	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.88	0.33	0.00	0.00
	b	0.75	1.00	0.64	0.00	1.00	0.00	0.00	1.00	1.00	0.00	0.13	0.67	0.00	1.00
Acp3	a	0.38	0.50	0.27	0.00	0.00	0.50	0.50	0.50	0.75	0.68	1.00	0.50	1.00	0.00
	b	0.63	0.50	0.73	1.00	0.00	0.50	0.50	0.50	0.25	0.33	0.00	0.50	0.00	1.00
Acp4	a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.50
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.50
Cat1	a	0.13	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00
	b	0.88	0.50	1.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00	1.00	0.50	0.00
Cat2	a	0.00	0.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
Cat3	a	0.17	0.25	0.17	0.00	0.00	0.00	0.00	0.33	0.13	0.20	0.50	0.50	0.00	0.50
	b	0.83	0.75	0.83	1.00	0.00	1.00	1.00	0.67	0.88	0.80	0.50	0.50	0.00	0.50
Cat4	a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.50
Est1	a	0.00	1.00	0.81	1.00	0.00	0.50	0.00	0.00	0.00	1.00	1.00	0.00	1.00	1.00
	b	0.00	0.00	0.19	0.00	0.00	0.50	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
Est2	a	0.67	0.00	0.63	0.00	0.00	0.00	0.00	1.00	0.00	0.50	0.00	0.00	1.00	1.00
	b	0.33	1.00	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00
Est3	a	1.00	0.00	0.67	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.50	0.50	0.00
	b	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.50	0.00	1.00	0.00	0.50	0.50	0.00
Est4	a	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	0.00	0.00
	b	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
Per1	a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.00
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00
Per2	a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.17	0.00	0.00	0.00	0.00
	b	0.00	1.00	1.00	0.00	0.00	1.00	0.00	0.50	1.00	0.83	1.00	1.00	0.00	0.00
Per3	a	1.00	0.00	0.78	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00
	b	0.00	1.00	0.22	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.50	1.00	0.00	0.00

Table 3. Estimates of genetic diversity and summary of results of tests for deviations from Hardy - Weinberg equilibrium in 14 populations of *B. rapa* (*A* mean allele per locus, A_e mean of effective allele number per locus, A_p number of polymorphic alleles per locus , P_p percentage of polymorphic loci, H_{obs} observed heterozygosity, H_{exp} expected heterozygosity, *F* mean of Wright's index per population, Tests number of loci for which tests could be performed, H_E number of loci with a significant excess heterozygosity, H_D number of loci with a significant deficiency of heteozygosity)

	Population	*A*	A_e	A_p	P_p	H_{obs}	H_{exp}	*F*	Tests	H_E	H_D
B. rapa ssp. *chinensis*	unknown	1.625	1.368	5	33.33	0.229	0.260	0.460	15	4 (27%)	8 (53%)
	Taiwan	1.300	1.260	3	20.00	0.250	0.217	0.240	15	3 (20%)	5 (33%)
	China	1.636	1.413	7	46.67	0.216	0.264	0.230	15	5 (33%)	7 (47%)
	U.S.A	1.000	1.000	0	0.00	0.000	0.000	0.530	15	0	8 (53%)
	Japan	1.000	1.000	0	0.00	0.000	0.000	0.730	15	0	11 (73%)
B. rapa ssp. *pekinensis*	unknown	1.286	1.286	2	13.33	0.286	0.191	0.400	15	2 (13%)	8 (53%)
	Korea	1.300	1.188	3	20.00	0.125	0.135	0.320	15	2 (13%)	6 (40%)
	China	1.400	1.380	4	26.67	0.367	0.247	0.100	15	4 (27%)	5 (33%)
	Japan	1.333	1.333	1	6.67	0.333	0.333	0.730	15	1 (7%)	12 (80%)
B. rapa ssp. *rapa*	USA	1.375	1.285	3	20.00	0.281	0.228	0.320	15	3 (20%)	7 (47%)
	unknown	1.455	1.332	5	33.33	0.218	0.263	0.200	15	5 (33%)	4 (27%)
	Egypt	1.500	1.480	5	33.33	0.400	0.373	0.060	15	4 (27%)	5 (33%)
B. rapa ssp. *oleifera ruvo-gruppe*	unknown	1.444	1.289	4	26.67	0.181	0.176	0.470	15	2	8
B. rapa ssp. *trilocularis*	unknown	1.500	1.500	4	26.67	0.500	0.294	0.200	15	4	7

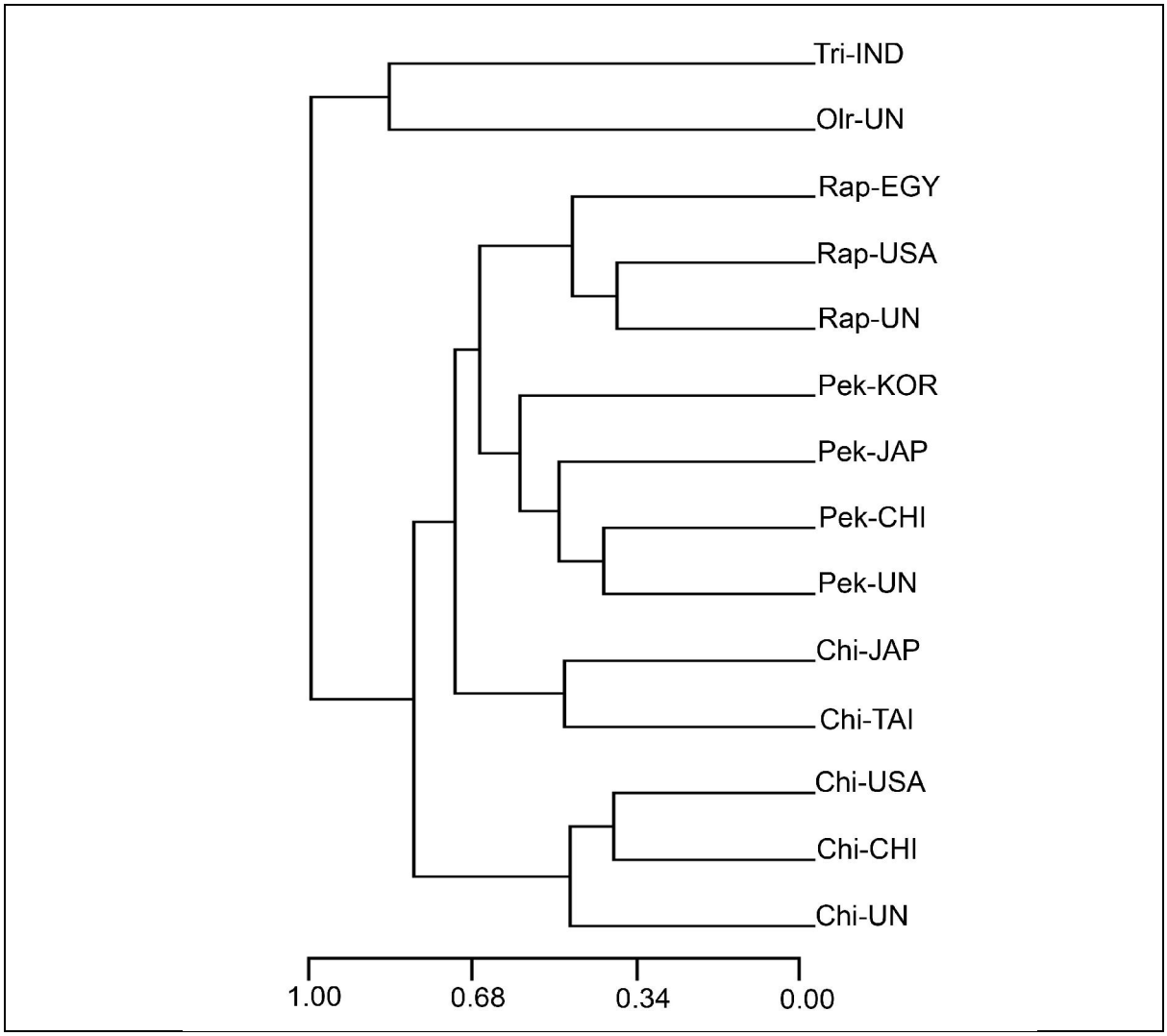

Figure 1. UPGMA clustering of the studied populations based on based on Nei's genetic distance derived from isozyme data.

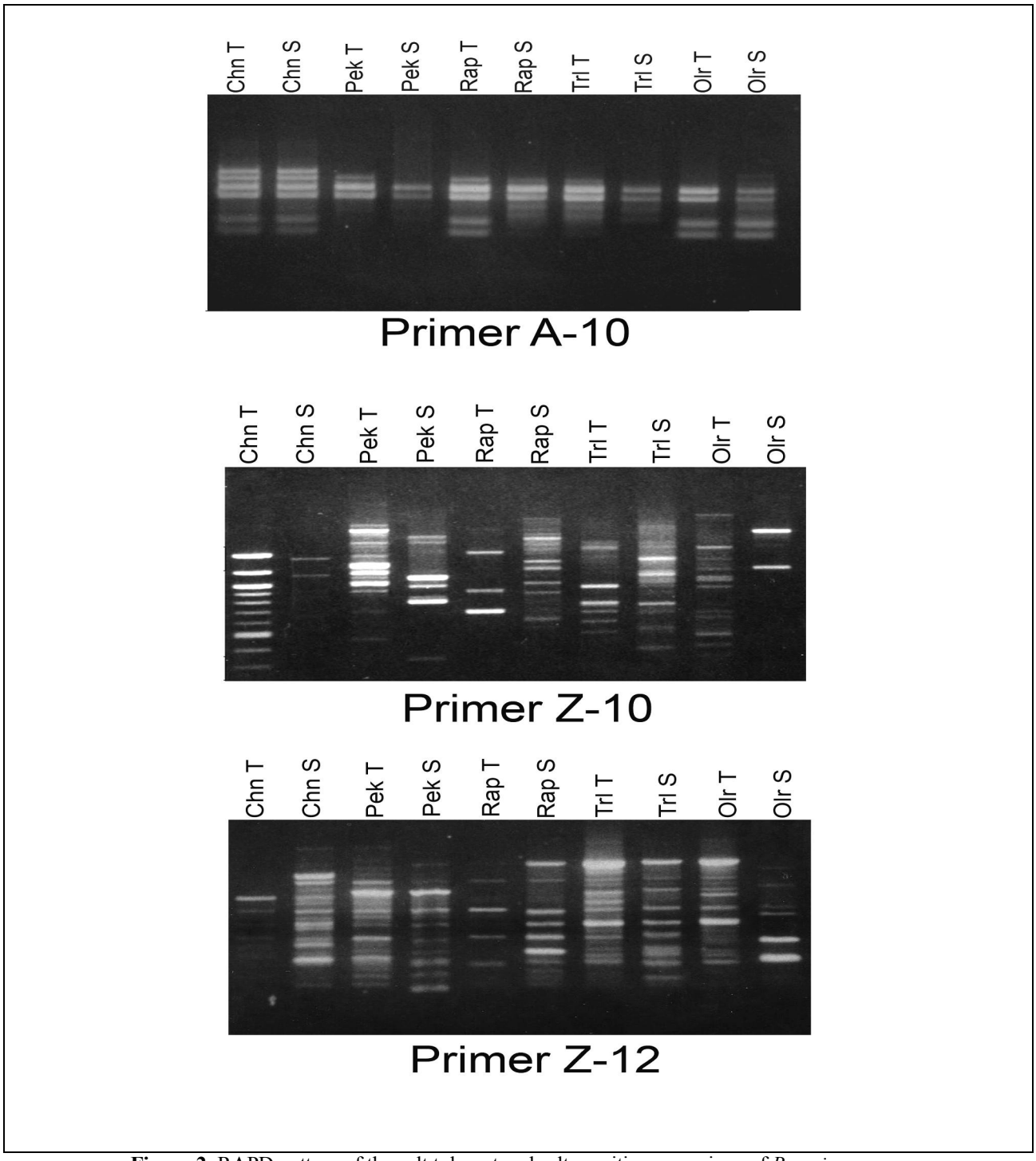

Figure 2. RAPD pattern of the salt tolerant and salt sensitive accessions of *Brassica rapa*.

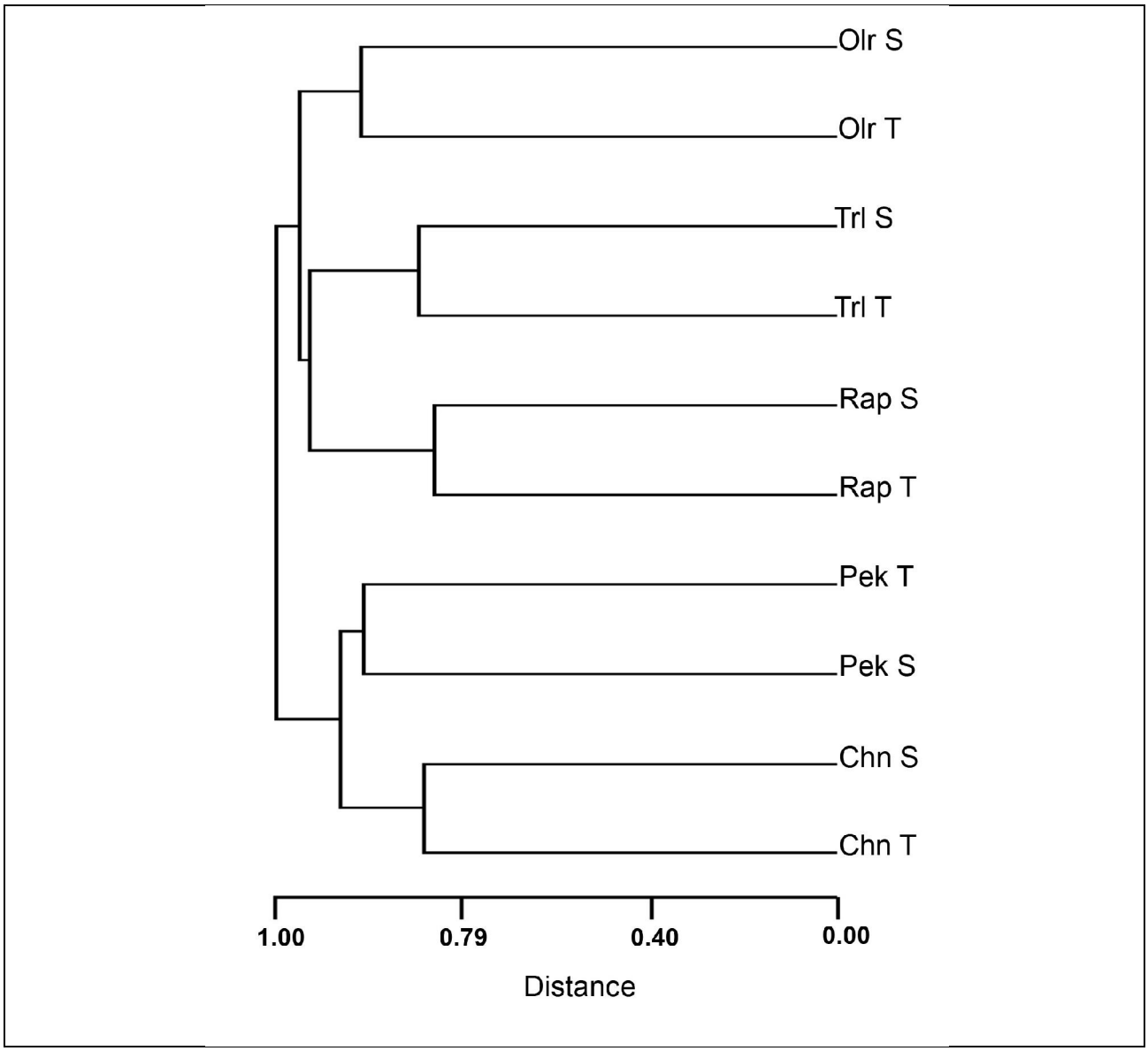

Figure 3. UPGMA clustering of the salt tolerant and sensitive accessions based on genetic distance matrix derived from RAPD data.

4. Discussion

Genetic diversity among the studied populations was assessed based on polymorphism of 30 alleles within 15 isozyme loci (Table 2). The variation among populations was observed in the presence or absence of a specific locus. Besides, the studied populations were classified into three groups based on allele frequency (populations with rare alleles, others with monomorphic loci and populations with balanced alleles). These observations can be explained on the bases of genetic divergence of the studied populations as a result of different breeding traditions in different parts of the world (Qian *et al.,* 2006; Snowdon, 2007; Zhao *et al.,* 2009).

Generally, the highest values of genetic diversity measures were observed for the Chinese populations of *B. rapas*sp. *chinensis* and *B. rapa* ssp. *chinensis* with unknown origin followed by the Chinese *B. rapa* ssp. *pekinensis* (Table 3). This indicates a considerable genetic diversity of these populations which can be expected as these leafy forms were developed in southern China (Zhao *et al.,* 2005). These results can also suggest China as an origin of the unknown population which was grouped under the same cluster with the Chinese population of *B. rapas*sp. *chinensis* (Fig 1).

Allover the studied populations, the detected isozyme loci exhibited significant decrease in

heterozygosity from Hardy-Weinberg expectation (Table 3) which indicated a tendency to increase homozygosity may be a to inbreeding due to small populations size and low gene flow. As an obligate outcrossing crop, *B. rapa* shows a severe inbreeding depression and only cultivars with a high diversity have a high expected heterozygosity(Zhao *et al.,*2005)

The genetic relationships among the studied populations were examined based on Nei's genetic distance derived fromisozymes as co-dominant genetic markers (Fig. 1). It is observed from the UPGMA clustering based on Nei's genetic distance matrix the distinctness of populations of ssp. *trilocularis* and ssp. *oleifera* in two separate clusters (Fig 1). This observation coincides with the breeding history of *B. rapa* ssp. *trilocularis*as separate breeding tradition in India that led to the development of yellow sarson(Reiner *et al.,* 1995; Snowdon, 2007; Koch and Al-Shehbaz, 2009).In addition, the separation of the populations of ssp. *oleifera* from the other studied populations which represent ssp. *chinensis*;ssp. *pekinensis*; and ssp. *rapa*indicates different geographic pedigree as suggested by (Takuno *et al.,*2007)based on AFLP that ssp. *oleifera* was originated mainly in Europe, while Asian breeding systems has resulted in ssp. *chinensis*;ssp. *pekinensis*; and ssp. *rapa*. The origin of the collected accessions of ssp. *oleifera* in the present study was unknown. Based on the findings, European origin can be suggested for these accessions.

The determination of germination potential of seeds under saline conditions could appear as a simple and useful parameter. At the germination stage, salt-tolerance is a heritable trait that can be used for the selection of salt resistant populations (Ashraf and McNeilly, 2004). There are many reports have investigated the response of *Brassica* species to salinity at different plant development stages and concluded that they maintain their degree of salinity tolerance through the plant ontogeny (reviewed by Ashraf and McNeilly, 2004)

The improvement of salinity tolerance in some important crop species could be achieve through selection and breeding, therefore, the collected accessions were salt-stressed using elevated concentrations of NaCl. There was a great variation in germination percentage among the studied accessions (Table 1). From these results there was significant variation for salt tolerance within *Brassica rapa* accessions, which can be exploited through selection and breeding to for enhancing salt tolerance of the *Brassica* crop (Ashraf and McNeilly, 2004).

In order to characterize the genome of the selected accessions based on their salinity tolerance each salt tolerant accession was compared with the most salt sensitive counterpart so that the genetic divergence could be due to salinity. This observation was confirmed since the closely related accessions were grouped when RAPD clustering is considered (Fig 3). Besides, a considerable variation between the salt tolerant and salt sensitive accessions as observed from RAPD pattern (Fig 2) as well as clustering based on RAPD fragment scoring. It can be suggested that this variation can be attributed to the genetic divergence among salt tolerant and sensitive accessions. This suggestion is based on the observation that the grouping of both tolerant and sensitive accessions representing each subspecies under one cluster at D=0.90 while at D=0.6 each accession occupied a distinctive cluster (Fig 3).

Selection of parental materials for hybridization for making new recombination, and maximizing heterotic response, should be maintained to preserve genetic diversity in germplasm resources (Maoteng *et al.,* 2010). So that salt tolerant accessions could be used as resources to improve the salt stress tolerance in *B. napus*by sequencing of characteristic bands in marker assisted selection (MAS) and follow their transport into the next generation.

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