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# MOLECULAR ANALYSIS OF GENETIC DIVERSITY IN *BRASSICA* SPECIES

**S. J. ABBAS[1], FARHATULLAH[2], KHAN BAHADAR MARWAT[3], I. A. KHAN[1] AND IQBAL MUNIR[1]**

[1]*Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University, Peshawar, Pakistan,*
[2]*Department of Plant Breeding and Genetics, NWFP Agricultural University, Peshawar, Pakistan.*
[3]*Department of Weed Science, NWFP Agricultural University, Peshawar, Pakistan.*

**Abstract**

*Brassica* species are the most important source of vegetable oil and the second most important oilseed crop in the international oilseed market after soybean. Genetic improvement of the seed quality made *Brassica* a source for high quality edible oil for human consumption and high quality protein meal for feeding animals. To improve yield and quality of oil content of *Brassica*, presence of sufficient genetic diversity in the germplasm is an important prerequisite. On an average, 45.8 and 25.8 alleles were amplified using RAPD and *Brassica* specific SSR primer sets, respectively. Mean genetic distance estimates ranged from 26-89% and 5-61%, respectively. Size of scorable fragments ranged from approximately 250 to >2000 bp. A high level of genetic dissimilarity (GD= up to 100%) was estimated among the 14 genotypes. Entries were grouped in clusters using cluster analysis. On the basis of dendrogram, most diverse genotypes were identified, that can be used in future brassica breeding program.

## Introduction

The family *Brassica*ceae, containing about 350 genera and 3500 species, is one of the ten most economically important plant families with a wide range of agronomic traits (Rich, 1991; Christopher *et al.,* 2005). In Pakistan, rapeseed and mustard is the second most important source of oil after cotton. It is sown from mid September to mid November throughout the country. In the season of 2006-07, total area under rapeseed and mustard cultivation was 265.8 thousand hectares with a total production of 221.0 thousand tonnes and an average yield of 831 Kg per hectare. In North West Frontier Province (NWFP), total area under rapeseed and mustard cultivation was 119.6 thousand hectares with a total production of 9.4 thousand tonnes with an average yield of 480 kg per hectare (Anon., 2006-2007).

The basic chromosome number for *Brassica* (and the tribe itself) is controversial, with number for X ranging from 7-11. Molecular studies do not provide support for the ancestral status of X = 7, as it has been found to have multiple origins in separate lineages (Warwick & Black, 1993). The evolution and relationship between members of the plant genus *Brassica*. (Anon., 1935) showed that there were three different ancestral genomes *Brassica rapa* or *Brassica campestris* AA - 2n=2x=20 (Turnip, Chinese cabbage), *Brassica nigra* BB - 2n=2x=16 (Black mustard) and *Brassica oleracea* CC - 2n=2x=18 (Cabbage, kale, broccoli, cauliflower). These three species exist as separate species but they are closely related. The interspecific breeding allowed the creation of 3 new species of tetraploid, *Brassica juncea* AABB - 2n=4x=36 (Indian mustard), *Brassica napus* AACC - 2n=4x=38 (rapeseed, rutabaga) and *Brassica carinata* BBCC - 2n=4x=34 (Ethiopian mustard). Because they are derived from the genomes of 2 different species, these hybrid plants are said to be allotetraploid (contain four genomes, derived from two

different ancestral species). The relationships among the cultivated species were largely clarified by cytological work of Morinaga (1934). According to his hypothesis, the high chromosome number of species *B. napus* (2*n* = 38, AACC), *B. juncea* (2*n* = 36, AABB), and *B. carinata* (2*n* = 34, BBCC) are amphidiploids combining in pairs the chromosome sets of the low chromosome number species *B. nigra* (2*n* = 16, BB), *B. oleracea* (2*n* = 18, CC) and *B. rapa* (2*n* = 20, AA) (Ananga *et al.,* 2008).

Like any other crop species the first step in *Brassica* improvement is full assessment of the local (indigenous) materials, including collection, evaluation and molecular characterization of germplasm lines. Often, local varieties of oil seed crops are of excellent quality and flavors have a good level of resistance to pests and diseases and may be superior to exotic materials (Williams *et al*., 1991). Knowledge about germplasm diversity and genetic relationship among breeding material could be an invaluable aid in crop improvement strategies. A number of methods are currently available for analysis of genetic diversity in germplasm accessions, breeding lines and segregating populations. These methods have relied on pedigree data, morphological data, agronomic performance data, biochemical data and more recently molecular (DNA-based) data **(**Mohammadi & Prasanna, 2003). Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including (i) analysis of genetic variability in cultivars (Smith, 1984; Cox *et al*., 1986), (ii) identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection (Barrett & Kidwell, 1998), and (iii) introgressing desirable genes from diverse germplasm into the available genetic base (Thompson *et al*., 1998).

Like any other crop species, to improve quality and quantity of *Brassica spp.*, presence of sufficient genetic diversity is very important. To estimate the amount of existing genetic variability, various morphological, cytological and biochemical markers have been used in the past (Raney *et al.,* 1995; Rimmer *et al.,* 1995). These markers though successful in many cases, were either limited in number or time consuming and hence were not considered suitable for handling larger breeding populations. Recent introduction of recombinant DNA technology especially the generation of practically unlimited number of DNA marker has transformed the opportunities of utilizing Marker Assisted Selection (MAS) for crop improvement that allow to study the genetic variability of crop germplasm. Morphological traits, total seed proteins, isozymes and several types of DNA markers are well known examples (Paterson *et al.,* 1991; Shengwu, 2003). Due to the large number, easy handling, being mostly co-dominant in nature and reliable scoring methods, molecular markers are considered the best tools for determining genetic relationships in any crop species. A variety of molecular markers including Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) and RAPD have been used to study the extent of genetic variation among the diverse group of important crop species in the genus *Brassica* (Karp, 1997; Shengwu *et al.,* 2003). The use of RAPD technique for the study of genetic variation has been demonstrated as suitable in many species. Randomly Amplified Polymorphic DNA (RAPD) is a relatively recent technique and has been widely used for estimation of genetic relationships in various crops of agronomic importance (Dos Santos *et al*., 1994; Thormann *et al*., 1994; Shengwu *et al.,* 2003). During the present study, 9 parental lines of *Brassica* species along with their 5 $F_2$s were used to estimate genetic relationship using RAPD primers and SSR primer sets. The information will be used to identify genetically diverse genotypes, which then can be utilized in future breeding programs aimed at creating genetic diversity in local *Brassica* germplasm.

**Table 1. Parental species and cross ID of the *Brassica* $F_2$ populations used during present study.**

| ***Brassica napus* - Lines/ varieties** | **Pakistani *Brassica campestris* accessions named on the basis of location of origin** |
|---|---|
| 409024 | Bannu-1 |
| MLEP-048 | Buner |
| 8966-1 | Rustam (Mardan) |
| Wester | Karak-1 |
| Baro | |
| **$F_2$ Crosses ID** | **Cross/ Pedigree** |
| 01 | 409024 (*B. N)* × Bannu -1 (*B. C)* |
| 02 | MLEP-048 (*B. N)* × Buner (*B. C)* |
| 03 | 8966-1 (*B. N)* × Rustam Mardan (*B. C)* |
| 04 | Wester (*B. N)* × Karak-1 (*B. C)* |
| 05 | Bannu-1 (*B. C)* × Baro (*B. N)* |

B. N= *Brassica napus*
B. C= *Brassica campestris*

## Materials and Methods

All the plantings were done in pots in a green house of the Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University, Peshawar during growing season 2005-2006. All the recommended agricultural practices were carried out. Nine parental lines included in the present study were *Brassica napus* (line# 409024, line# MLEP-048, line # 8966-1, Baro & Wester) and indigenous *Brassica campestris* collection from districts of Buner and Rustam (Mardan) of NWFP, line # Karak-1 and line # Bannu- 1 (Table 1). Five cross combinations viz., (1) 409024 (B. N) × Bannu- 1 (B. C), (2) MLEP-048 (B. N) × Buner (B. C), (3) 8966-1 (B. N) × Mardan (B. C), (4) Wester (B. N) × Karak-1 (B. C), (5) Bannu- 1 (B. C) × Baro (B. N) were used during the present study. $F_2$ progeny of the same crosses were used for molecular analyses.

Leaf samples were used to isolate total genomic DNA following the protocol described by Weining & Langridge (1991). Ten Randomly Amplified Polymorphic DNA primers (GL- -A02, -A08, -A11, -B19, -C10, -D07, -D18, -F18, -G05 and –H12, purchased from GeneLink, Inc. NY 10532, USA, Table 1) were used.

PCR reactions were carried out in 25 µl reaction using standard protocols (Devos & Gale, 1992). Ten Simple Sequence Repeat Primers (SSR) primer sets (*NA10-C01a, NA10-C01c, NI02-D08a, NI03-H07a, NI-F02a, OL10-A03a, OL10-F11a, RA02-A04a, RA02-A04c* and *RA02-E01a)* specific for *Brassica* chromosomes were used for diversity analysis.

For SSR analysis, 1µL genomic DNA solution was amplified in 25µL reaction mixture. Depending on the primer sets used, the protocol provided by the primer set supplier was followed. All amplification reactions were performed using the GeneAmp PCR system 2700 (Applied Biosystem). The amplification products were electrophoresed on 2.0% agarose/TBE gels and visualized by staining with ethidium bromide and viewed under UV light.

For genetic diversity analysis, every scorable band was considered as single allele / locus and was scored as present (1) or absent (0). The bivariate 1-0 data were used to estimate genetic distances (G.D) following "Unweighted Pair Group of Arithmetic Mean (UPGMA)" procedures described by Nei & Li (1979) and to construct a dendrogram using computer program "PopGene32" version 1.31 http://www.ualberta.ca./~fyeh/fyeh).

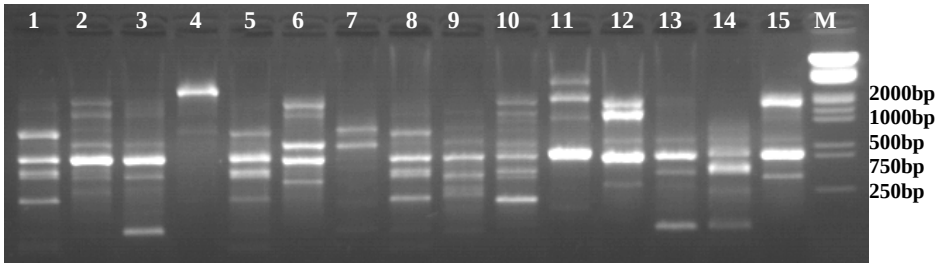


Fig. 1. PCR amplification profile of 5 $F_2$ segregating populations of *Brassica* along with 9 parental lines using RAPD primer GLA-11.
1 = 409024 (B. N*)*, 2 = 409024 (B. N*)* × Bannu- 1 (B. C*)*), 3 = Bannu- 1 (*B.* C*)*, 4 = MLEP-048 (B. N), 5 = MLEP-048 (B. N*)* × Buner (B. C*)*), 6 = Buner (B. C), 7 = (8966-1 (B. N*)*, 8 = 8966-1 (B. N*)* × Rustam Mardan (B. C*)*), 9 = Rustam Mardan (B. C*)*, 10 = Wester (B. N), 11 = Wester (B. N*)* × Karak-1 (B. C*)*), 12 = Karak-1 (B. C*)*, 13 = Bannu- 1 (B. C*)*, 14 = Bannu- 1 (B. C*)* × Baro (B. N*)*), 15 = Baro (B. N).
M = Molecular size marker (1 Kb ladder). Molecular sizes (in bp) are given on right.
B. N = *Brassica napus*
B. C = *Brassica campestris*

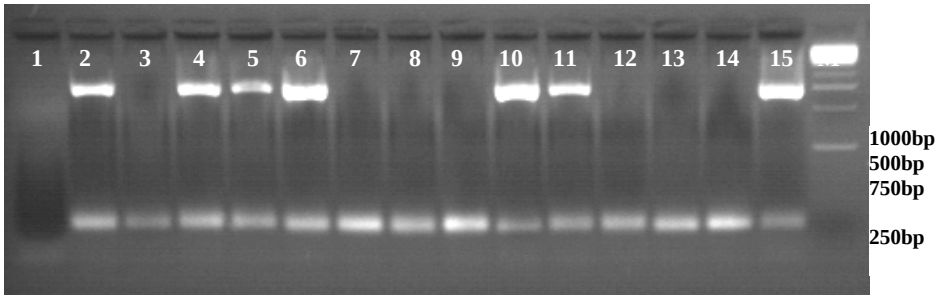


Fig. 2. PCR amplification profile of 5 $F_2$ segregating populations of *Brassica* along with 9 parental lines using SSR primer set NI-F02a.
1 = 409024 (B. N), 2 = 409024 (B. N) × Bannu- 1 (B. C)), 3 = Bannu- 1 (B. C), 4 = MLEP-048 (B. N), 5 = MLEP-048 (B. N) × Buner (B. C)), 6 = Buner (B. C), 7 = (8966-1 (B. N), 8 = 8966-1 (B. N) × Rustam Mardan (B. C)), 9 = Rustam Mardan (B. C), 10 = Wester (B. N), 11 = Wester (B. N) × Karak-1 (B. C)), 12 = Karak-1 (B. C), 13 = Bannu- 1 (B. C), 14 = Bannu- 1 (B. C) × Baro (B. N)), 15 = Baro (B. N).
M = Molecular size marker (1 Kb ladder). Molecular sizes (in bp) are given on right.
B. N = *Brassica napus*
B. C = *Brassica campestris*

## Results and Discussion

An example of PCR amplification profile of 14 *Brassica* genotypes using RAPD primer GL-A11 and SSR primer sets NI-F02a are presented in Figs. 1 and 2 respectively. Molecular sizes of amplified fragments ranged from approximately 250 to more than 2000 bp. In an earlier report Chen *et al.,* (2000) observed 900 -1600 bp fragment size amplified using RAPD primers in different sub-species of *Brassica*. During present study a total of 458 and 258 DNA fragments were amplified in 14 genotypes using 10 RAPD and 10 SSR primers sets, giving an average of 45.8 and 25.8 alleles per primer. The results of genetic dissimilarity analyses showed that extensive genetic diversity (average G.D. ranging from 26%-89% and 5%-61%) existed in 14 *Brassica* genotypes used during present study (Table 4 and 5). Most of the comparisons showed moderate estimates of genetic distance using "UPGMA" method. The detection of moderate level of genetic

diversity during present study was in agreement with the previous reports where RAPD and SSR markers detected high level of genetic polymorphsim (Welsh & McClelland 1990; DosSantos *et al*., 1994) in different crop species.

The bivariate (1-0) data and dissimilarity coefficient matrices of 5 $F_2$ segregating populations of *Brassica* alongwith 9 parental lines based on the data of 10 RAPD and 10 *Brassica* specific primer sets (SSR using UPGMA method (Nei & Lie, 1979) were used to construct separate dendrograms using computer program "popgene32" (Figs. 3 and 4). For the dendrogram constructed from data using RAPDs, the genotypes were grouped in 7 main groups (A, B, C, D, E, F, and G) (Fig. 3). Group A consisted of 3 genotypes and was further subdivided into one subgroup H consisting of 2 genotypes. Groups B, D, E and G were smallest and comprised of 1 genotype only. Group C was the largest comprising of 6 genotypes and groups F and H consists of 2 genotypes each. Based on the dendrogram analyses, parental line # 409024 (*Brassica napus)* and $F_2$ cross Wester (*B. N)* × Karak-1 (*B. C)* was most distantly related from one another. In genetic diversity analyses the comparisons among the 2 genotypes (parental line # 409024 (*Brassica napus)* and $F_2$ cross Wester (*B. N)* × Karak-1 (*B. C)*) showed high estimate of genetic distance (GD = 77 %). This finding was further strengthened by average genetic diversity analyses (Table 4) where the two genotypes showed higher levels of genetic dissimilarity with rest of the genotypes used during present studies.

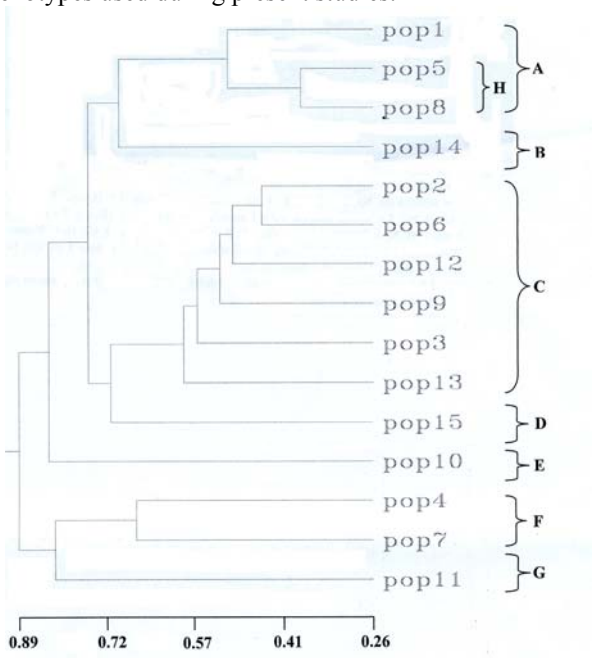


Fig. 3. Dendrogram constructed for 5 $F_2$ segregating populations of *Brassica* along with 9 parental lines using 10 RAPD primers.

1 = 409024 (B. N*)*, 2 = 409024 (B. N*)* × Bannu- 1 (B. C)), 3 = Bannu- 1 (*B. C)*, 4 = MLEP-048 (B. N), 5 = MLEP-048 (B. N*)* × Buner (B. C)), 6 = Buner (B. C), 7 = (8966-1 (B. N*)*, 8 = 8966-1 (B. N*)* × Rustam Mardan (B. C*)*), 9 = Rustam Mardan (B. C*)*, 10 = Wester (B. N), 11 = Wester (B. N*)* × Karak-1 (B. C)), 12 = Karak-1 (B. C*)*, 13 = Bannu- 1 (B. C*)*, 14 = Bannu- 1 (B. C*)* × Baro (B. N)), 15 = Baro (B. N).

B. N = *Brassica napus*

B. C = *Brassica campestris*

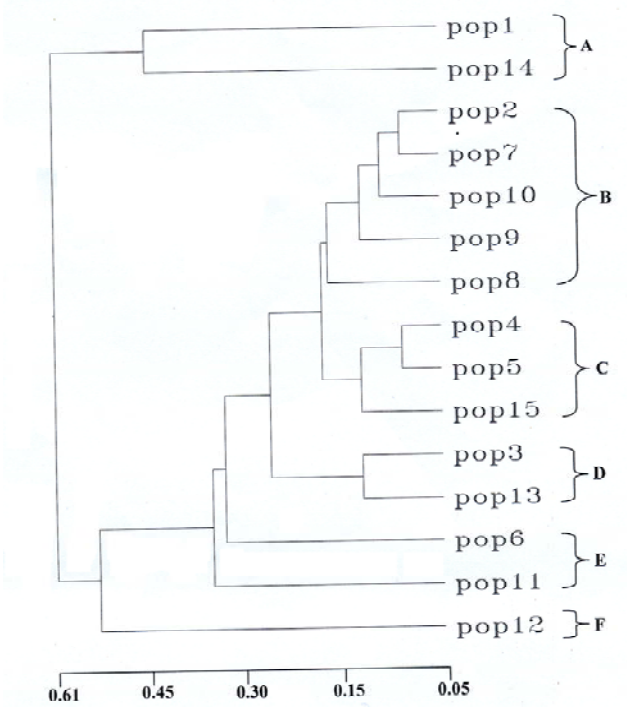


Fig. 4. Dendrogram constructed for 5 $F_2$ segregating populations of *Brassica* along with 9 parental lines using 10 *Brassica* specific SSR primer sets.
1 = 409024 (B. N), 2 = 409024 (B. N) × Bannu- 1 (B. C)), 3 = Bannu- 1 (*B. C)*, 4 = MLEP-048 (B. N), 5 = MLEP-048 (B. N) × Buner (B. C)), 6 = Buner (B. C), 7 = (8966-1 (B. N), 8 = 8966-1 (B. N) × Rustam Mardan (B. C)), 9 = Rustam Mardan (B. C), 10 = Wester (B. N), 11 = Wester (B. N) × Karak-1 (B. C)), 12 = Karak-1 (B. C), 13 = Bannu- 1 (B. C), 14 = Bannu- 1 (B. C) × Baro (B. N)), 15 = Baro (B. N).
B. N = *Brassica napus*
B. C = *Brassica campestris*

By using SSR primers sets the 5 $F_2$ segregating populations of *Brassica* along with 9 parental lines were grouped in 6 main groups A, B, C, D, E and F (Fig. 4). Groups A, and F were smallest and comprised of 1 genotype only. Group B was the largest comprising of 5 genotypes. Group C consisted of 3 genotypes and groups D and E consist of 2 genotypes each. Based on the dendrogram analyses, parental lines # 409024 (*Brassica napus)* and # Karak-1 (*Brassica campestris)* were most distantly related to each other. Genetic diversity analyses among the 2 genotypes (parental lines # 409024 (*Brassica napus)* and # Karak-1 (*Brassica campestris)*) also showed high estimate of genetic distance (GD = 46%) (Table 5). Present findings further strengthened previous reports (Hallden *et al.,* 1994; Chen *et al*., 2000) that the RAPD and SSR markers can be used effectively to estimate genetic distances among genotypes/lines/cross combinations. However, it is suggested that more molecular data is required to have better understanding of the presence of genetic variability in *Brassica* germplasm and consequently more efficient utilization of existing variability for improvement of *Brassica* crop in Pakistan.

**Table 2. Name, sequence, size, molecular weight and %GC content of 10 RAPD primers used to study genetic diversity among segregating populations of *Brassica*.**

| S. No. | Oligo name | Sequence | Size | Molecular weight | %GC |
|---|---|---|---|---|---|
| 1. | GLA-02 | TGCCGAGCTG | 10 | 3044.01 | 70 |
| 2. | GLA-08 | GTGACGTAGG | 10 | 3108.04 | 60 |
| 3. | GLA-11 | CAATCGCCGT | 10 | 2987.98 | 60 |
| 4. | GLB-19 | ACCCCCGAAG | 10 | 2981.97 | 70 |
| 5. | GLC-10 | TGTCTGGGTG | 10 | 3090.04 | 60 |
| 6. | GLD-07 | TTGGCACGGG | 10 | 3084.03 | 70 |
| 7. | GLD-18 | GAGAGCCAAC | 10 | 3046.0 | 60 |
| 8. | GLF-18 | TTCCCGGGTT | 10 | 3010.0 | 60 |
| 9. | GLG-05 | CTGAGACGGA | 10 | 3077.02 | 60 |
| 10. | GLH-12 | ACGCGCATGT | 10 | 3028.0 | 60 |

**Table 3. Name, sequence and melting temperatures of 10 SSR primer sets used to identify genetic diversity among five $F_2$ segregating populations of *Brassica* along with 9 parental lines.**

| S.No. | Locus | Sequence (Forward primer) | Sequence (Reverse Primer) | $T_m$ |
|---|---|---|---|---|
| 1. | NA10-C01a | TTTTGTCCCACTGGGTTTTC | GGAAACTAGGGTTTTCCCTTC | 54 |
| 2. | NA10-C01c | TTTTGTCCCACTGGGTTTTC | GGAAACTAGGGTTTTCCCTTC | 54 |
| 3. | NI02-D08a | TTTAGGGAAAGCGAATCTGG | ACAACAACCCATGTCTTCCG | 54 |
| 4. | NI03-H07a | GCTGTGATTTTAGTGCACCG | AGCCGTTGATGGAATTTTTG | 53 |
| 5. | NI-F02a | TGCAACGAAAAAGGATCAGC | TGCTAATTGAGCAATAGTGATTCC | 54 |
| 6. | OL10-A03a | CTGGTTTTCTCCTTCATCAG | CTGTGTAGCTTTTAGTCTTT | 52 |
| 7. | OL10-F11a | TTTGGAACGTCCGTAGAAGG | CAGCTGACTTCGAAAGGTCC | 52 |
| 8. | RA02-A04a | AAAAACTCCTCTTCAACG | CCCAAAGTTAGGTTTTAATGTAATCTC | 51 |
| 9. | RA02-A04c | AAAAACTCCTCTTCAACG | CCCAAAGTTAGGTTTTAATGTAATCTC | 51 |
| 10. | RA02-E01a | TCTATATTAACGCGCGACGG | GCACACACACACTCAAACCC | 56 |

$T_m$ = Melting Temperature (Annealing temperature = $T_m - 5^OC$)
http://brassica.bbsrc.ac.uk

**Table 4. Average estimates of genetic distances among 5 $F_2$ segregating populations of *Brassica* along with 9 parental lines using 10 RAPD primers.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | |
| 2 | 0.51 | | | | | | | | | | | | | | |
| 3 | 0.61 | 0.32 | | | | | | | | | | | | | |
| 4 | 0.85 | 0.84 | 0.86 | | | | | | | | | | | | |
| 5 | 0.42 | 0.56 | 0.60 | 0.64 | | | | | | | | | | | |
| 6 | 0.61 | 0.30 | 0.42 | 0.79 | 0.56 | | | | | | | | | | |
| 7 | 0.74 | 0.77 | 0.59 | 0.73 | 0.76 | 0.73 | | | | | | | | | |
| 8 | 0.40 | 0.54 | 0.65 | 0.76 | **0.26** | 0.56 | 0.66 | | | | | | | | |
| 9 | 0.68 | 0.47 | 0.48 | 0.83 | 0.56 | 0.48 | 0.72 | 0.53 | | | | | | | |
| 10 | 0.79 | 0.60 | 0.65 | 0.79 | 0.69 | 0.61 | 0.78 | 0.73 | 0.66 | | | | | | |
| 11 | 0.77 | 0.70 | 0.75 | 0.86 | 0.77 | 0.66 | 0.73 | 0.75 | 0.81 | 0.75 | | | | | |
| 12 | 0.71 | 0.36 | 0.51 | 0.75 | 0.61 | 0.39 | 0.70 | 0.59 | 0.41 | 0.63 | 0.76 | | | | |
| 13 | 0.75 | 0.58 | 0.55 | **0.89** | 0.70 | 0.49 | 0.65 | 0.72 | 0.43 | 0.73 | 0.81 | 0.51 | | | |
| 14 | 0.54 | 0.55 | 0.48 | 0.67 | 0.42 | 0.63 | 0.56 | 0.54 | 0.43 | 0.73 | 0.74 | 0.58 | 0.63 | | |
| 15 | 0.71 | 0.59 | 0.60 | 0.80 | 0.71 | 0.49 | 0.84 | 0.77 | 0.66 | 0.72 | 0.77 | 0.47 | 0.58 | 0.57 | |

1 = 409024 (B. N), 2 = 409024 (B. N) × Bannu-1 (B. C)), 3 = Bannu-1 (B. C), 4 = MLEP-048 (B. N), 5 = MLEP-048 (B. N) × Buner (B. C)), 6 = Buner (B. C), 7 = (8966-1 (B. N), 8 = 8966-1 (B. N) × Rustam Mardan (B. C)), 9 = Rustam Mardan (B. C), 10 = Wester (B. N), 11 = Wester (B. N) × Karak-1 (B. C)), 12 = Karak-1 (B. C), 13 = Bannu-1 (B. C), 14 = Bannu-1 (B. C) × Baro (B. N)), 15 = Baro (B. N).
B. N = Brassica napus
B. C = Brassica campestris

**Table 5. Average estimates of genetic distances among five $F_2$ segregating populations of *Brassica* along with nine parental lines using 10 SSR primer sets.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | | |
| 2 | 0.35 | | | | | | | | | | | | | | |
| 3 | 0.34 | 0.24 | | | | | | | | | | | | | |
| 4 | 0.48 | 0.17 | 0.36 | | | | | | | | | | | | |
| 5 | 0.48 | 0.27 | 0.41 | 0.10 | | | | | | | | | | | |
| 6 | 0.56 | 0.31 | 0.45 | 0.23 | 0.18 | | | | | | | | | | |
| 7 | 0.38 | 0.08 | 0.18 | 0.20 | 0.30 | 0.33 | | | | | | | | | |
| 8 | 0.40 | 0.25 | 0.29 | 0.28 | 0.18 | 0.37 | 0.18 | | | | | | | | |
| 9 | 0.36 | 0.17 | 0.22 | 0.32 | 0.22 | 0.33 | 0.15 | 0.11 | | | | | | | |
| 10 | 0.37 | 0.07 | 0.17 | 0.22 | 0.32 | 0.38 | 0.15 | 0.31 | 0.20 | | | | | | |
| 11 | 0.25 | 0.05 | 0.22 | 0.22 | 0.22 | 0.29 | 0.14 | 0.19 | 0.08 | 0.08 | | | | | |
| 12 | 0.46 | 0.37 | 0.42 | 0.45 | 0.55 | 0.55 | 0.35 | 0.45 | 0.40 | 0.37 | 0.36 | | | | |
| 13 | 0.42 | 0.26 | 0.18 | 0.32 | 0.42 | 0.51 | 0.23 | 0.36 | 0.29 | 0.19 | 0.29 | 0.39 | | | |
| 14 | 0.40 | 0.58 | 0.47 | 0.57 | 0.57 | **0.**61 | 0.55 | 0.52 | 0.51 | 0.57 | 0.49 | 0.32 | 0.51 | | |
| 15 | 0.43 | 0.12 | 0.37 | 0.09 | 0.19 | 0.24 | 0.21 | 0.37 | 0.30 | 0.20 | 0.14 | 0.37 | 0.38 | 0.53 | |

1 = 409024 (B. N), 2 = 409024 (B. N) × Bannu-1 (B. C)), 3 = Bannu-1 (B. C), 4 = MLEP-048 (B. N), 5 = MLEP-048 (B. N) × Buner (B. C)), 6 = Buner (B. C), 7 = (8966-1 (B. N), 8 = 8966-1 (B. N) × Rustam Mardan (B. C)), 9 = Rustam Mardan (B. C), 10 = Wester (B. N), 11 = Wester (B. N) × Karak-1 (B. C)), 12 = Karak-1 (B. C), 13 = Bannu-1 (B. C), 14 = Bannu-1 (B. C) × Baro (B. N)), 15 = Baro (B. N).
B. N = Brassica nap
B. C = Brassica campestris

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