Pak. J. Bot., 43(4): 2075-2081, 2011.

GENETIC DIVERSITY OF NURYT-NATIONAL UNIFORM RAPE SEED YIELD TRIAL AND *BRASSICA NAPUS* VARIETIES USING RAPD MARKERS AND BIOCHEMICAL ANALYSIS

JEHAN BAKHT[1], AMNA FAREED[1], MOHAMMAD SHAFI[2], ZAHOOR AHMAD SWATI[1]

[1]*Institute of Biotechnology & Genetic Engineering, KPK Agricultural University Peshawar, Pakistan*
[2]*Department of Agronomy, KPK Agricultural University Peshawar, Pakistan*

Abstract

In Pakistan, *Brassica* is the second most important source of oil after cotton. Seventeen NURYT (National Uniform Rape Seed Yield Trial) lines and 5 *Brassica napus* varieties were assessed through RAPD primers and biochemical assays. Seven different Randomly Amplified Polymorphic DNA markers (RAPD) were employed during the present study. A total of 30 RAPD bands were scored by these primers. Size of the scorable fragments ranged from approximately 250 to 2000 bp. Diversity index was estimated to be 42%. Mean genetic distance estimates ranged between 0.10 and 1.00. For the assessment of various biochemical parameters, Near Infrared Reflectance Spectroscopy (NIRS) was used. Oil content ranged from 38.30 to 49% and protein content from 19.80 to 29.10% among the 22 genotypes. Maximum protein content was assayed in genotype RBN 3046 while minimum in Hyola 405. Glucosinolates ranged between 2 and 84% for genotype CRH 60/08 and CRH05/08 showing the maximum and minimum values respectively. Oleic acid (52 to 72.5%), linolenic acid (7.07 and 9.90%) and erucic acid content (9.57 to 38.3%) was also recorded during the present study.

Introduction

Rape seed is a member of Brassicaceae family including *Brassica compestris* and *Brassica napus* and its members contains about 350 genera and 3500 species (Christopher *et al.,* 2005). The genus *Brassica* includes economically important food crops like oilseed, vegetables and condiments. There are six species which are secondarily balanced polyploids. *Brassica carinata* (n = 17, genome BC), *B. juncea* (n = 18, genome AB) and *B. napus* (n = 19, genome AC) each originated by hybridization and polyploidization of two different elementary species (Prakash & Hinata, 1980). *B. nigra, B. carinata, B. oleraceae, B. campestris* and *B. napus* are the most commonly found species. *Brassica napus* is a self-pollinating species that can outcross for field-grown oilseed rape. Cultivars of various genetic complexities are available as *Brassica napus* cultivars are produced by both traditional and non-traditional means (Dulson *et al.,* 1998).

Improvement of chemical composition of rape seed is one of the breeding priorities for human and animal consumption. Rape seed oil is used for edible purposes as well as in industries. Quality traits like glucosinolates, erucic acid, protein and different fatty acids can be improved both by breeding and through genetic engineering of brassica. Rapeseed oil is composed of high concentration of oleic acid (60%), 10% and 20% of linolenic acid and linoleic acid. This is considered to be an ideal fatty acid composition of a vegetable oil by many nutritionists for human consumption (Rakow & Raney, 2003; Nasr *et al.,* 2006). A group of plant secondary metabolites called glucosinolates is commonly found in the family of brassica which contains an abundant of nitrogen and sulfur. Characteristic flavor, pathogen and herbivores defense system and insect attractants are the various characteristics of their degradation products. (Mithen *et al.,* 2000). Erucic acid is a monounsaturated omega-9 fatty acid, denoted 22:1 ω-9. Oil containing high erucic acid is nutritionally undesirable and there are efforts to develop varieties having low erucic acid (Downey & Rimmer, 1993). Reduction in erucic acid results in increased proportion of oleic acid but extent of its increase depends on species (Downey, 1990).

Molecular markers have been widely used to map agronomically important genes in brassica genomes and to assist rapeseed breeding and selection procedures. Genetic diversity helps a breeder in selecting the suitable parents from a population on the basis of divergence analysis for breeding programs. Genetic diversity can better help a breeder to understand the germplasm structure and predict about good combinations of off springs (Shengwu *et al.,* 2003). DNA based marker including Randomly Amplified Polymorphic DNA (RAPD) primers are widely used for detecting genetic polymorphism between genotypes at molecular level in many crop species such as brassica (Graham *et al.,* 2004; Fu *et al.,* 2006; Sargent *et al.,* 2007; Lewer *et al.,* 2008; Brennan *et al.,* 2008; Mattia *et al.,* 2008; Cerbert *et al.,* 2008). PCR based assays are easy, cheaper, faster and do not require any sequence information of the targeted genome in advance. It needs no blotting and radioactive detection (Cipriani *et al.,* 1996). Primers of short arbitrary sequence are used to generate DNA finger prints with RAPDs (Williams *et al.,* 1990). Keeping in view the importance of RAPD markers in different breeding programs, the present study was initiated (1). to determine genetic diversity in advance breeding material (NURYT material) in comparison with existing cultivars of *Brassica napus* and (2). to evaluate quality parameters (fatty acids, erucic acid, oleic acid, glucosinolates, protein and oil content) of brassica genotypes.

Materials and Methods

The present study was carried out at the Institute of Biotechnology and Genetic Engineering (IBGE) KPK Agricultural University, Peshawar Pakistan to investigate the molecular and biochemical variations among the 17 NURYT (National Uniformity Rapeseed Yield Trial) different lines and 5 varieties of *Brassica napus* (total 22 genotypes; Table 1) using seven different RAPD primers (Table 2).

Table 1. Description of brassica genotypes (lines and varieties) used in the current study.

S. No.	NURYT lines	S. No.	Lines/Varieties
1.	Hyola-405	12.	Crown OLA
2.	RBN03046	13.	HS-555
3.	Shiralee(C)	14.	RA 92
4.	RBNO3060	15.	CRH09/08
5.	CRH35/08	16.	ZR-01
6.	RBN03075	17.	OMEGA-3
7.	ROO-125/14	18.	Hagola-43
8.	CBN006	19.	Abasin -95
9.	OMEGA-2	20.	Hagola-405
10.	CRH05/08	21.	Concerde
11.	CRH06/08	22.	Shiralee

Table 2. List of primers used for genetic diversity of brassica lines and species.

S. No	Primer	Sequence	Size	TM
1.	GO9	CTGACGTCAC	10 bp	29.5 ºC
2.	GO5	CTGAGACGGA	10 bp	29.5 ºC
3.	GO8	TCACGTCCAC	10 bp	29.5 ºC
4.	A12	TCGGCGATAG	10 bp	29.5 ºC
5.	GL-18	GGCTCATGTG	10 bp	29.5 ºC
6.	GO4	AGCGTGTCTG	10 bp	29.5 ºC
7.	G-13	CTCTCCGCCA	10 bp	33.6 ºC

Preparation of DNA samples: Genomic DNA was isolated from brassica leaves according to the method of Weining and Langridge (1991). Plant material was collected in the appendorf tubes and subsequently frozen in liquid nitrogen. Leaf material was then crushed to make a fine powder. Five hundred microliter extraction buffer (1% SDS, 100 mM NaCl, 100 mM Tris, 100 mM EDTA, pH 8.5 by HCl) was added to the crushed leaf material and mixed thoroughly with equal volume (500 µl) of phenol:chloroform:isoamyl alcohol (ratio 25:24:1). Samples were centrifuged at 5000 rpm for 5 minutes in bench centrifuge. The aqueous phase was re-extracted with equal volume of chloroform, centrifuged at 5000 rpm for 5 minutes. One tenth volume of sodium acetated (pH 4.8) and equal volume of isopropanol was added and mixed gently to precipitate the DNA. The samples were kept at -20 ºC for 30 minutes and centrifuged at 5000 rpm for 5 min to pellet the DNA. The pellet was then washed with 100, 80 and 75 percent ethanol, dried and re-suspended in 50 µl of TE buffer. For complete dissolution, DNA samples were kept at 70 degree centigrade for 10 minutes. Quantity and quality of DNA sample was measured by spectrophotometer at 260 and 280 nm wave lengths.

Table 3. PCR Thermal Profile of the Reaction.

Step	Temperature	Duration
Hot start	94ºC	4 min
Denaturation	94ºC	1 min
Primer annealing	34ºC	1 min
Extension	72ºC	2 min
Total cycles (35)		
Final extension	72ºC	10 min

Polymerase chain reaction (PCR): PCR was carried out according to the protocols of Devos and Gale (1992) with certain modifications. PCR thermal profile is given in Table 3. Randomly Amplified Polymorphic DNA markers (RAPD) obtained from the Gene Link Technology USA was employed for the genetic diversity analysis (Table 2). PCR reaction was carried out in 25 µl reaction mixture, having 1 µl template DNA, 1 µl RAPD primer, 15 µl of ddH_2O and 7 µl of PCR mix (composed of 460 µl H_2O, 500 µl buffer, 10 µl of each dNTPs and 300 µl $MgCl_2$). PCR reaction mixture except DNA and primer in the above mentioned volume was pooled to sterilized PCR tubes and mixed thoroughly by gentle pipetting. DNA and primer was then added to the reaction mixture and centrifuged briefly to collect constituents and subjected to the thermal profile. The reaction was carried out in the Thermocycler (GeneAmp 2700). Amplification products were electrophoresed on 2% agarose/TBE gel and visualized by staining with ethidium bromide under the ultra violet light and photographed.

Biochemical analysis: Seventeen NURYT (National Uniform Rapeseed Yield Trial) lines were assessed for quality parameters (oil content, moisture, protein, glucosinolate, oleic acid, glucosinolates) by NIRS (Near Infrared Reflectance Spectroscopy. The samples were scanned three times to minimize sampling error.

Data analysis: Data on all unambiguous polymorphic RAPD fragments were identified and scored as presence (1) or absence (0). Similarity matrix was used for the un-weighted pair-group method with arithmetic averaging (UPGMA) (Sokal & Michener, 1958). A dendogram indicating the estimated similarity among the brassica genotypes was constructed with computer program "Popgene 32" version 1.31.

Results and Discussion

Seven primers were selected which gave reproducible and distinct polymorphic amplified products. In present study all the brassica genotypes showed various levels of genetic polymorphisim for the loci detected using all seven primers. A total of 307 alleles (bands) were

observed in 22 genotypes for the seven primers as only reproducible bands were analyzed. Among the seven primers used in the current study, primer G09 yielded on an average maximum number of bands giving an average of 5.05 bands genotype^{-1}, while primer GL13 yielded lowest number of bands genotype^{-1} on the average (Fig. 1). Molecular sizes of the RAPD amplified fragments ranged from approximately 250 to 2000 bp. Fragment sizes ranging from 900-1600 bp were reported by Chen *et al.,* (2000) when RAPD primers were used while determining the genetic diversity among *Brassica compestris* genotypes. These results agree with those reported by Cartea *et al.,* (2005) and Fu *et al.,* (2006). Absence of bands may be due to the inability of primer to anneal because of no complementary binding site (Clark & Lanigan, 1993). Reason of higher average scorable and polymorphic bands could be attributed to higher GC content (60–70%) of the primers used.

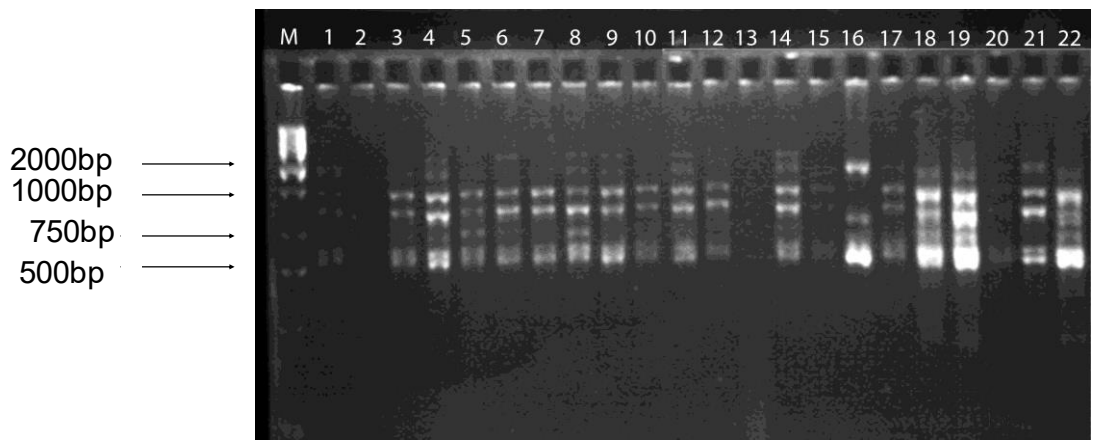

Fig. 1. PCR based DNA profile of 22 brasscia genotypes using RAPD primer G091. Hyola-405, 2. RBN03046, 3. Shiralee(C), 4. RBNO3060, 5. CRH35/08, 6. RBN03075, 7. ROO-125/14, 8. CBN006, 9. OMEGA-2, 10. CRH05/08, 11. CRH06/08, 12. Crown OLA, 13. HS-555, 14. RA 92, 15. CRH09/08, 16. ZR-01, 17. OMEGA-3, 18. Hagola-43, 19. Abasin–95, 20. Hagola-405, 21. Concerde, 22. Shiralee

Cluster analysis: These genotypes were grouped into three groups. Group A comprises of Hyola405 and CRH 09/08. Group B contained RBN03046 and HS 555 (Fig. 2). Group C1 comprises of Shiralee C, RBN03060, RBN 03075, Hyola 43, ZR- 01, CRH 35/08, Shiralee, ROO 125/14, RA 92, CBN006, OMEGAII, Crown OLA, OmegaIII, Abasin 95, Hyola 405 and Concerde while group C2 was composed of CRH 05-08. The data further suggested that there was a great diversity among the different brassica genotypes under study (Fig. 2). CRH 60/08 was found to be the most dissimilar from the rest of the population of the brassica genotypes. Maximum genetic distance was observed between CRH 09/08 and CRH 60/08. High degree of genetic diversity is probably due to the relation of CRH 60/08 to different species. Members of one group were found to be at least genetic distance with the members of the same group but distinctly related with the members of the other group. Average genetic distance was estimated to be from 10 to 100%. Results presented by the dendogram are in close agreement with the finding of the average dissimilarity matrix presented in Table 4 and Shannon's diversity index (Table 5). Similar results were also reported by Das *et al.,* (1999) who observed more or less similar ranges of genetic dissimilarities in different Brassica lines. Observed genetic variation can be attributed to the individual differences within the tested populations. Mailer *et al.,* (1994) also reported polymorphism in oilseed rape cultivars by RAPD analysis.

Biochemical analysis: Our result indicated that oil content of the 22 brassica genotypes ranged from 38 to 49% with the mean value of 45%. Velasco *et al.,* (1999) found similar results for oil content (44.3%) using NIRS while screening different quality traits in rapeseed. Similarly, oil content for brassica oilseeds ranged between 35 and 44% as reported by Downey & Rimmer (1993). However, comparatively lower percentage of oil content (ranging from 37 to 41%) was reported by Si *et al.,* (1997). Average protein content was found to be 25.23% with maximum and minimum values of 19.80 and 29.10% respectively in the present study. These results are in agreement with the findings of Velasco *et al.,* (1999) who reported that the protein value ranged from 13.4 to 28.3% in fresh seed of brassica genotypes. In the present study average glucosinolates was estimated to be 44.90 with the minimum and maximum values of 2 and 85.60 µmol g^{-1} respectively (Table 6). Level of glucosinolates varies within the same plant, in different parts of plant (Inglis *et al.,* 1992) and leaf glucosinolates quantity and profile is weakly correlated to the seed glucosinolate level in small seedlings (Glen *et al.,* 1990). Our results are supported by Velasco *et al.,* (1999) who reported a mean value of 51.2 µmol g$^{-1.}$ Glucosinolates as such do not cause harm but their break down products adversely affects iodine uptake by thyroid gland in ruminants. Our data revealed that 42.8% and 11.8% were the maximum and minimum value observed for erucic acid. Erucic acid is reported to be negatively correlated to linoleic and oleic acid in oil seed crops (Ahuja *et al.,* 1984). Our results further suggested that oleic acid content ranged from 52 to 72% of the total fatty acids (Table 6). These results are in agreement with those reported by Islam *et al.,* (2004), who measured the highest amount of oleic acid (61.6%). Monosaturated oleic acid is important from nutritional point of view because it lowers the undesirable LDL cholesterol level and also confers high stability required for healthy cooking. Maximum and minimum values observed for the linolenic acid were 5.8 and 9.9% respectively. Lavkopr *et al.,* (2006) reported similar findings and observed 3.3 to 13.1% linolenic acid in brassica cultivars.

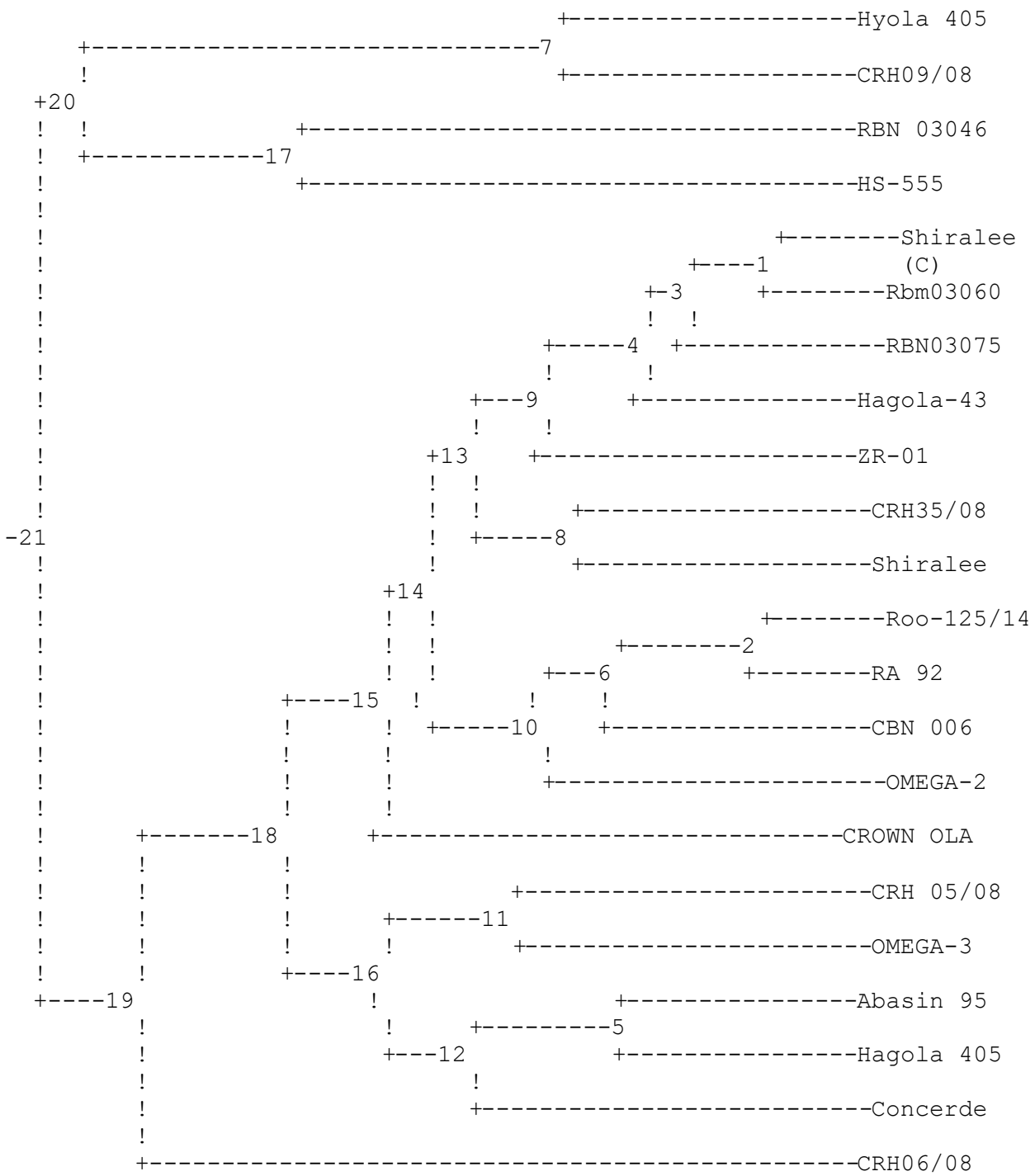

Fig. 2. Dendogram analysis of 22 brassica genotypes using seven RAPD primers by using computer program “Pop gene 32”.

Table 4. Average genetic distances (%) among all the 22 brassica genotypes using seven RAPD primers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1																					
2	62																				
3	56	69																			
4	51	76	**10**																		
5	51	40	35	40																	
6	62	62	18	14	22																
7	51	62	35	40	22	31															
8	51	91	35	22	40	31	22														
9	26	56	40	35	35	35	26	26													
10	40	62	35	40	31	31	31	40	35												
11	69	**100**	31	18	56	35	56	35	51	56											
12	56	56	56	45	35	26	26	35	40	35	62										
13	76	40	45	51	62	40	40	40	56	40	69	35									
14	56	83	31	26	35	18	10	18	22	35	40	31	35								
15	22	31	83	91	51	76	51	76	35	62	**100**	56	51	69							
16	83	56	22	26	35	26	35	26	51	56	40	40	35	31	83						
17	35	56	62	69	45	56	56	69	51	26	91	62	56	51	56	76					
18	56	83	14	18	26	18	26	26	40	26	40	31	45	22	83	22	40				
19	35	69	40	45	26	35	35	35	31	35	62	40	69	40	45	40	40	22			
20	40	51	35	40	22	22	31	40	35	22	56	26	40	35	40	39	45	26	18		
21	40	51	56	62	31	51	31	62	26	31	83	45	76	45	40	65	35	45	26	31	
22	40	76	26	31	22	22	31	31	45	22	56	35	51	35	62	39	45	18	35	31	60

Table 5. Shannon's diversity index for each RAPD primer.

Primer	Locus	Shannon index	Primer	Locus	Shannon index
G09	L1	0.586	A12	L17	0.1849
G09	L2	0.6931	A12	L18	0.689
G09	L3	0.3046	A12	L19	0.3983
G09	L4	0.536	A12	L20	0.1849
G09	L5	0.6255	A12	L21	0.1849
G09	L6	0.3983		**Mean**	**0.3284**
G09	L7	0.4741	GL18	L22	0.689
	Mean	**0.52**	GL18	L23	0.4741
G05	L8	0	GL18	L24	0.3983
G05	L9	0.3983		**Mean**	**0.520466667**
G05	L10	0.536	G04	L25	0.536
	Mean	**0.46**	G04	L26	0.6255
G08	L11	0.536	G04	L27	0.6555
G08	L12	0.6931	G04	L28	0.586
G08	L13	0.6765		**Mean**	**0.60075**
G08	L14	0.6765	G13	L29	0.3046
G08	L15	0.6555	G13	L30	0.536
G08	L16	0.6255		**Mean**	**0.4203**
	Mean	**0.64385**			
Overall mean		0.4954			
St. Dev		0.1849			
St. Dev		0.1849			

Table 6. Mean values of the different quality parameters of 22 brassica genotypes.

S. No.	Name	Oil (%)	Protein (%)	GSL ($\mu molg^{-1}$)	Oleic (%)	Linolenic acid (%)	Erucic acid (%)
	Nuryt Material (National Uniform Rapeseed Yield Trial)						
1.	Hyola-405	44.50	25.90	14.47	61.30	8.10	12.90
2.	RBN03046	41.63	28.07	53.73	62.00	7.93	22.77
3.	Shiralee(C)	45.57	25.03	63.30	**52.00**	8.00	38.30
4.	RBNO3060	41.53	28.37	38.60	62.87	7.47	27.07
5.	CRH35/08	43.07	27.90	72.57	54.23	8.77	35.73
6.	RBN03075	**38.67**	26.30	35.93	65.17	7.83	14.47
7.	ROO-125/14	41.03	27.37	72.57	57.27	**5.77**	37.00
8.	CBN006	42.77	**29.07**	84.90	55.20	6.20	**43.17**
9.	OMEGA-2	43.53	27.53	76.33	54.13	8.33	33.57
10.	CRH05/08	47.83	22.93	**2.07**	65.77	**9.90**	10.37
11.	CRH06/08	43.20	27.60	**76.93**	57.10	8.73	29.07
12.	Crown OLA	43.63	23.80	26.97	66.10	7.07	12.20
13.	HS-555	42.37	23.80	6.07	**72.20**	6.57	**9.57**
14.	RA 92	42.97	23.57	11.03	66.73	7.67	12.33
15.	CRH09/08	45.30	26.77	67.87	53.97	7.83	35.47
16.	ZR-01	48.60	23.37	58.33	56.73	8.50	36.50
17.	OMEGA-3	43.17	24.30	24.37	66.87	7.60	12.27
	***Brassica napus* varieties**						
18.	Hagola-43	48.67	21.47	45.73	57.43	8.00	33.83
19.	Abasin -95	47.43	22.50	47.43	54.93	8.23	33.30
20.	Hagola-405	48.30	**19.93**	21.23	65.13	8.27	18.53
21.	Concerde	41.67	26.27	20.20	65.67	9.27	11.80
22.	Shiralee	**48.83**	21.83	59.10	54.43	8.10	34.77

References

Ahuga, K.L., K.S. Labana, R.K. Raheja and S.S. Badwal. 1984. Oil content and fatty acid variation in mutants of *Brassica juncea*. *J. Oil Seeds Res*., 1: 71-75.

Brennan, R., J. Jorgensen, H. Christine, S. Gordon, M. Woodhead and R. Joanne. 2008. The development of a genetic linkage map of blackcurrent and the identification of regions associated with key fruit quality and agronomic traits. *Euph*., 161: 19-34.

Cartea, M.E., P. Soengas, A. Picoaga and A. Ordas. 2005. Relationships among *Brassica napus* (L) germplasm from Spain and Great Britain as determined by RAPD markers. *Genet. Resou. & Crop Evol.,* 52: 655-662.

Cebert, E., A.O. Ananga, K. Soliman, R. Kantety, K. Konan and J.W. Ochieng. 2008. Phylogenetic relationships within and among Brassica species from RAPD loci associated with blackleg resistance. *African J. Biotech.,* 7: 1287-1293.

Chen, Y.P., J.S. Cao, Y. Miao and Y.W. Zhi. 2000. Analysis of genetics polymorphisms in vegetables crops of *Brassica campestris* by RAPD markers. *J. Zhejiang Univ. Agric. & Life Sci.,* 26: 131-136.

Christopher, G.L., J.R. Andrew, A.C.L. Geraldine, J.H. Clare, B. Jacqueline, B. Gary, C.S. German and E. David. 2005. Brassica ASTRA: an integrated database for Brassica genomic research. *Nucleic Acids Res.,* 33: 656-659.

Cipriani, G., R. Dibella and R. Testolin. 1996. Screening RAPD primers for molecular taxonomy and cultivar finger printing in genus *Actinidia. Euph.,* 90: 74-169.

Clark, A.G. and C.M.S. Lanigan. 1993. Prospects for estimating nucleotide divergence with RAPDs. *Mol. Biol. & Evol.,* 10: 1096-1111.

Das, S., J. Rajagopal, P.S. Bhatia, Srivastava and M. Lakshmikumaran. 1999. Assessment of genetic variation within *Brassica campestris* cultivars using AFLP and RAPD markers. *J. Bio.Sci.,* 24: 433-440.

Devos, K.M. and M.D. Gale. 1992. The use of random polymorphic DNA markers in wheat. *Theor. & Appl. Genet.,* 84: 567-572.Dice, L.R. 1945. Measures of the amount of ecological association between species. *Ecol.*, 26: 297-302.

Downey, R.K. and S.R. Rimmer. 1993. Agronomic improvements in oil seed Brassica. *Adv. in Agron.,* 50: 1-66.

Downey, R.K. 1990. Brassica oilseed breeding, achievements and opportunities. *Plant Breed. Absts.,* 60: 1165-1170.

Dulson, J., L.S. Kott and V.L. Ripley. 1998. Efficacy of bulked DNA samples for RAPD DNA fingerprinting of genetically complex *B. napus* cultivars. *Euph.,* 102: 65–70.

Fu, J., M.F. Zhang and X.H. Qi. 2006. Genetic diversity of traditional Chinese mustard crops *Brassica juncea* as revealed by phenotypic differences and RAPD markers. *Genet. Resou. & Crop Evol.,* 53: 1513–1519.

Graham, J., K. Smith, K. MacKenzie, L. Jorgenson, C. Hackett and W. Powell. 2004. The construction of of a genetic linkage map of red raspberry based on AFLP's genomic SSR markers. *Theor. Appl. Genet.,* 109: 740-749.

Glen, D.M., H. Jones and J.K. Fieldsend. 1990. Damage to oilseed rape seedings by the field slug *Deroceras reticulatum* in relation to glucosinolate concentration. *Ann. Appl. Biol.,* 117: 197-207.

Inglis, I.R., J.T. Wadsworth, A.N. Meyer and C.G. Feare. 1992. Vertebrate damage to double zero and zero varieties of oilseed rape in relation SMCO and glucosinolate concentration in leaves. *Crop Protect.,* 11: 64-68.

Islam, M., H. Ahmad, A. Rashid, A. Khan, A. Razza and H. Derawadan. 2004. Comparative study of agronomic traits of rapeseed genotypes under Swat conditions. *Pak. J. Sci.,* 10: 31-33.

Lavkopr, R.A., P.L. Velnerus, O.T. Ichkolovr, V.L.R. Kuc and Oiskohou. 2006. Estimation of fatty acid content in intact seed of oil seed rape (*Brassica napus* L.) lines using Near-Infra red Spectroscopy. *J. Genet. & Plant Breed.,* 42: 132-136.

Lewer, K.S., C.A. Saski, B.J. Cuthbertson, D.C. Henry, M.E. Staton, D.S. Main, A.L. Dhanaraj, L.J. Rowland and J.P. Tomkins. 2008. A blackberry expressed sequence tag

library for the development of simple sequence repeat markers. *BMC Plant Biol.,* 8: 69-76.

Mailer, R.J., R. Scarth and B. Fristensky. 1994. Discrimination among cultivars of rapeseed (*Brassica napus*) using DNA polymorphism amplified from arbitrary primers. *Theor. Appl. Genet.,* 87: 697–704.

Matia, F.D., F. Grassi, S. Imazi and M. Labra. 2008. Chloroplast and nuclear DNA markers to characterize cultivated and spontaneous *Ribes. Plant Biosysts.,* 142: 204-212.

Mithen, R.F., M. Dekker, R. Verkerk, S. Rabot and I.T. Johson. 2000. The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *J. Sci. Food and Agric.,* 80: 967-984.

Nasr, N., M. Khayami, R. Heidari and R. Jamei. 2006. Genetic Diversity among Selected Varieties of *Brassica napus* (Cruciferae) Based on the Biochemical Composition of Seeds. *Just.,* 32: 37-40.

Prakash, S. and K. Hinta. 1980. Taxonomy, cytogenetics and origin of crop Brassica, a review. *Opera Bot.,* 55: 1-57.

Rakow, G. and J. P. Raney. 2003. Present status and future perspectives of breeding for seed quality in Brassica oilseed crop. Proc. 11th Int. Rape Seed Congress, Copenhagen, Denmark, Pp. 181-185.

Sargent, D.J., F. Fernandez, A. Rys, V.H. Knight, D.W. Simpson and R.K. Tobutt. 2007. Mapping of A1 confering resistance to the aphid *Amphorophora idaei dw* (dwarfing habit) in red raspberry using AFLP and microsatellite markers. *BMC Plant Biol.,* 7: 15.

Shengwu, H., J. Ovesna, L. Kucera, V. Kucera, M. Vyvadilova. 2003. Evaluation of genetic diversity of *Brassica napus* germplasm from China and Europe assessed by RAPD markers. *Plant Soil Environ.,* 49: 106–113.

Si, P., J. Rodney, Mailer, G. Nick and W.T. David. 1997. Influence of genotype and environment on oil and protein concentration of canola (*Brassica napus*) grown across southern Australia. *Austr. J. Agric.,* 54: 397-407.

Sokal, R.R. and C.D. Michener. 1958. A statistical method for evaluating systematic relationships. *Univ. Kansas Sci. Bull.,* 38: 1409-1438.

Velasco, L., C. Mollers and H.C. Becker. 1999. Estimation of seed weight, oil content and fatty acids composition in intact single seeds of rapeseed *Brassica napus* by Near Infrared Reflectance Spectroscopy. *Euph.,* 106: 79-85.

Weining, S. and P. Langrindge. 1991. Identification and mapping of polymorphism in cereals based on polymerase chain reaction. *Theor. Appl. Genet.,* 82: 537-544.

Willianms, J.G.K., A.R.K. Ublik, K.J. Livak, J.A. Rafalski and S.V. Tingeu. 1990. DNA polymorphisms amplified by the arbitrary primers are useful as genetic marker. *Nucleic Acids Res.,* 18: 6531-6535.

(Received for publication 5 August 2010)