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MOLECULAR AND BIOCHEMICAL ASSESSMENT OF *BRASSICA NAPUS* AND INDIGENOUS *CAMPESTRIS* SPECIES

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Abstract

In Pakistan, *Brassica* is the second most important source of oil after cotton. It contributes about 17% to the domestic production of edible oil. Parental lines along with five F_2s were assessed for biochemical parameters using Near Infrared Reflectance Spectroscopy (NIRS). Parental lines contain more oil 45.85% as compared to F_2s 42.26% while the F_2s contain more protein 25.92% as compared to the parents 23.70%. Both parents and F_2 contain high glucosinolate and fatty acids contents. Insulin Growth like Factor (IGF) primer sets were used to estimate genetic relationship among 5 F_2 segregating population of *Brassica* along with 9 parental lines. On an average 29 alleles were amplified using IGF primer sets. Mean genetic distance estimates ranged from 0.25-1.00 (G.D = 0%-100 %), 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 all genotypes. Entries were grouped in clusters using cluster analysis. On the basis of dendrogram, most diverse genotypes were identified which should be utilized in further breeding programs aimed at creating genetic variability in local germplasm.

Introduction

Brassica quality traits like oil, protein and glucosinolate contents as well as fatty acid composition can be modified by classical breeding and gene technological approaches. Near-infrared reflectance spectroscopy (NIRS) has usefully estimated quality traits in a quick and non-destructive manner in 3 g seed samples (Daun & Williams, 1995; Font *et al.,* 2006; Hom *et al.,* 2006). Genetic improvement of seed quality made oilseed rape a source for a high quality edible oil for human consumption and high quality protein meal for feeding animals. Different quality characteristics are required for use of seed for non-edible products, such as detergents, lubricants, cosmetics, hydraulic oils or biodiesel (Shahidi, 1990; Kimber & McGregor, 1995). Improvement of seed quality is one of the most important objectives in *Brassica* breeding for satisfying future edible oil requirements (Becker *et al*., 1999; Shengwu *et al.,* 2003). The functional and nutritional values of different vegetable oils are dependent on the nature of different fatty acids, which are incorporated into oil (triacylglycerols). The development of high oleic/low linolenic acid cultivars for use in frying applications and selection for low and very low (zero) content in saturated fatty acids for certain markets is prerequisite (Rakow & Raney, 2003). On the other hand, high erucic acid oilseed rape cultivars are regaining interest for industrial purposes. After oil extraction, the remaining meal contains different nutritional and anti-nutritional compounds. Among these, glucosinolates are the most important anti-nutritional compounds. The glucosinolates are nitrogen and sulphur containing natural plant products that have become increasingly important as flavour precursors, cancer prevention agents and crop protectants (Graser *et al*., 2000). They are found in all plant parts, but their quantities may vary considerably among organs (Kjaer, 1976; Font *et al.,* 2005). The content of seed glucosinolate is controlled by multiple

genes and is complexly regulated in the cell (Fenwick *et al*., 1983; Uzunova *et al*., 1995). The erucic acid (C22:1) content in seed protein is controlled by at least two genes. For erucic acid content up to 30% the alleles showed additive effect, while at higher concentration partial dominance was common (Jönsson, 1977). Fatty acid composition in oilseed rape (*B. napus*) is conditioned by the genotype of seed and not by that of the mother plant (Downey & Craig, 1964; Harvey & Downey, 1964). Some researchers showed that linolenic acid (C18:3) content was determined mainly by the genotype of the embryo, involving maternal effects and the interaction of two or three loci with some influence from environmental factors (Diepenbrock & Wilson, 1987). Chen & Gertsson (1988) reported more than 80% oleic acid (C18:1) in F_2 seeds derived from crossing of breeding line of oilseed (about 60% oleic acid) and one of resynthesized lines. To fulfil all future requirements, improvement of the seed quality is one of the most important objectives in oilseed *Brassica* breeding (Becker *et al*., 1999; Font *et al*., 2006).

Insulin-like growth factor (IGF) is a single-chain polypeptide of 70 amino acids. It is a trophic factor that circulates at high levels in the blood-stream and mediates many, if not most of the effects of growth hormone. Although the main source of IGF-1 in the serum is the liver, many other tissues synthesize it and are sensitive to its trophic action. IGF-1 was called somatomedin in the older literature. IGF-1 and insulin have similar three-dimensional structures. It appears to influence neuronal structure, functions throughout the life span and has the ability to preserve nerve cell function and promote nerve growth in experimental studies for the treatment of amyotrophic lateral sclerosis (ALS). Recently, recombinant human IGF-1 has entered the dietary supplement marketplace, as have recombinant human growth hormone and several so-called growth hormone secretagogues or releasers (Carro *et al.,* 2000).

The genetic diversity is a statistical concept referring to the variance among alleles at individual gene loci, among several loci or gene combinations, between individual plants within population, and between populations or cultivars. Methods to measure genetic diversity include classical genetic analysis to evaluate variation in single genes, multivariate analysis to analyze variation in polygenic traits, genealogical analysis, molecular analysis to detect genetic diversity with biochemical and molecular markers (Smale & McBride, 1997; Mahasi & Kamundia, 2007). 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 species. During the present study *Brassica* species were analyzed with NIRS and IGF markers. The information will be used to identify genotype having desirable characters for future breeding programs.

Materials and Methods

All the plantings were done in pots in 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, Rustam (Mardan), Karak and Bannu of North West Frontier Province (NWFP) Pakistan (Table 1). Four cross combinations of *Brassica napus* (N) x *Brassica campestris* (C) viz; 409024 × Bannu-1, (2) MLEP-048 × Buner, 8966-1 × Mardan, Wester × Karak-1 and one of C x N viz., Bannu-1 × Baro were used during present study. F_2 progeny of the same crosses were used for biochemical and molecular analyses.

Table 1. Parental species and Cross ID of the *Brassica* F_2 populations.

***Brassica napus* (N)-Lines/varieties**	**Pakistani *Brassica campestris* (C) 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 × Bannu-1
02	MLEP-048 × Buner
03	8966-1 × Rustam Mardan
04	Wester × Karak-1
05	Bannu-1 × Baro

Table 2. Name, Sequence and melting temperatures of three SSR primer sets used to identify genetic diversity among 5 F_2 segregating populations of *Brassica* along with 9 parental lines.

S. No.	Locus	Sequence (Forward primer)	Sequence (Reverse Primer)	T_m
1	*IGF1144*	GTAGCCACAGAAGACGA	CGAAGAACACAAAAACTG	46
2	*IGF1212*	TTGAGCTTGCAATGTTT	CGATTGTGGTTATCTGG	46
3	*IGF2514*	TTGTGAGAGCTTTGAGG	ACGAAACATTGTTGGA	46

T_m = Melting Temperature (Annealing temperature = T_m–5^{O}C)
http://brassica.bbsrc.ac.uk/IGF/

Biochemical analysis using NIR: To determine the following chemical constituents, whole seed samples were scanned on NIR Spectroscopy System FOSS 6500 equipped with ISI version 1.02a software of Infra Soft International according to the manufacturer's protocol. The samples were scanned thrice to minimize sampling error.

1. Oil (%)
2. Protein (%)
3. Glucosinolates content (μM g^{-1} sample).
4. Moisture (%)
5. Oleic acid content (% of the total fatty acids).
6. Linolenic acid content (% of the total fatty acids).
7. Erucic acid content (% of the total fatty acids)

Molecular analysis: Leaf samples were used to isolate total genomic DNA following the protocol described by Weining & Langridge (1991). To remove RNA, DNA was treated with 40μg RNAse-A at 37^{o}C for 1 hour and samples were stored at 4^{o}C. To use in Polymerase Chain Reaction (PCR) a 1:5 dilution of DNA was made in doubled distilled deionized and autoclaved water. Three IGF primers (IGF1144, IGF1212 and IGF2514 purchased from GeneLink, Inc. NY 10532, USA (Table 2) were used. PCR reactions were carried out in 25 μl reaction using standard protocols (Devos & Gale, 1992). 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.

Table 3. Biochemical composition of 9 *Brassica* parental lines and their 5 F_2 populations determined by NIRS

	Maximum	Minium	Mean	Variance	Std. Deviation
Nine parental lines analysis					
Oil%	49.20	42.31	45.85	4.50	2.12
Protein%	27.52	22.3	23.70	13.14	1.77
GSL µmol g^{-1}	119.8	57.89	86.94	575.23	23.98
Moisture%	8.50	5.32	6.76	1.41	1.19
Oleic acid%	57.80	32.19	45.73	59.53	7.71
Linolenic acid %	11.20	5.82	8.61	4.21	2.0
Erucic acid%	57.98	32.90	46.68	52.73	7.26
Five F_2 biochemical analysis					
Oil%	43.90	40.20	42.26	2.09	1.44
Protein%	29.60	23.50	25.92	6.44	2.54
GSL µmol g^{-1}	116.30	72.70	92.42	355.07	18.84
Moisture%	6.90	5.40	6.46	0.37	0.61
Oleic acid%	47.00	40.90	44.16	5.97	2.44
Linolenic acid %	11.30	7.30	8.94	2.19	1.48
Erucic acid%	47.50	43.60	45.60	3.19	1.79

Table 4. Average estimates of genetic distances among 5 F_2 segregating populations of *Brassica* along with 9 parental lines using 3 IGF primer sets.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1														
2	0.33													
3	0.21	0.33												
4	0.67	0.67	0.63											
5	0.93	**1.00**	0.91	0.87										
6	0.27	0.33	0.12	0.62	0.90									
7	**1.00**	**1.00**	0.95	0.50	0.37	0.93								
8	0.66	0.75	0.75	0.75	0.75	0.75	**1.00**							
9	0.50	0.56	0.49	0.72	0.90	0.47	0.75	0.75						
10	0.26	0.33	0.18	0.61	0.94	0.15	0.91	0.75	0.51					
11	0.50	0.50	0.40	0.75	0.90	0.37	0.75	0.75	0.33	0.43				
12	**0.00**	**0.00**	**0.00**	**0.00**	NA	**0.00**	NA	NA	0.67	**1.00**	NA			
13	0.22	0.33	0.12	0.62	0.95	0.21	0.93	0.75	0.51	0.12	0.43	**0.00**		
14	0.52	0.58	0.42	0.92	**1.00**	0.46	**1.00**	0.75	0.49	0.52	0.43	0.75	0.49	
15	0.50	0.50	0.45	0.50	0.87	0.43	0.50	0.75	0.25	0.41	0.25	NA	0.43	0.50

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*

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).

Results and Discussion

The oil content among 9 parents ranged from 42.31 to 49.20% with a mean value of 45.85% while the oil content in five F_2 segregating population ranged from 40.20 to 43.90% with a mean value 42.26%. Velasco *et al.,* (1999) found similar mean value of oil content (44.3%) using NIRS for screening of quality traits in rapeseed. However, comparatively lower percentage of oil content ranging from 37 to 41% was reported by Si *et al.,* (1997). These small differences may be due to variation in genotypes and / or environmental influences.

The protein content of 9 *Brassica* genotypes included in the study ranged from 22.3 to 27.52% of fresh seed with a mean value of 23.70 percent and their F_2s ranged from 23.50 to 29.60% with a mean value of 25.92%. These results are in agreement with the findings of Velasco *et al.,* (1999) who reported the protein value ranging from 13.4 to 28.3% in fresh seed of *Brassica* genotypes. This also shows that protein and oil content are inversely related, one increasing at the cost of the other.

The oleic acid content of 9 parents in our study ranged from 32.19 to 57.8% while their F_2s ranged from 40.90 to 47% of the total fatty acids. The level of oleic acid was about in the range that found by Pallot *et al.,* (1999) and Lavkopr *et al.,* (2006), who reported a range of 56 to 74% of oleic acid in *Brassica* using NIRS. However, Agnihotri (1999) reported comparatively lower values of oleic acid content ranging from 40 to 50% in fresh seed of *Brassica* spp. The monounsaturated oleic acid is important from nutritional standpoint because it lowers the undesirable LDL cholesterol level and also confers high stability required for healthy cooking.

The polyunsaturated linolenic acid of 9 parents and F_2s ranged from 5.82 to 11.2% and 7.30 to 11.30% respectively of the total fatty acids in our study. Lavkopr *et al.,* (2006) reported similar findings and observed 3.3 to 13.1% linolenic acid in *Brassica* cultivars. Several studies on recently developed low linolenic acid lines of canola have demonstrated that this type of canola oil has improved storage and frying stability.

Kaushik (1998) reported that erucic acid and glucosinolates are the two toxic substances found in rapeseed mustard seeds. In the present study, erucic acid content among the genotypes ranged from 32.9 to 57.98% while in the F_2s erucic acid content ranged from 43.60 to 47.5% of the total fatty acids. Luhs *et al*., (1999) reported that a series of alleles have been identified in *Brassica napus* and *Brassica rapa* which makes it possible to breed strains containing almost any level of erucic acid from less than 1% to about 60% of the total fatty acids. The erucic acid content variation among parental line is more because both species have different concentrations of erucic acid.

The glucosinolate content of the 9 *Brassica* genotypes ranged from 57.89 to 119.80 μmol g^{-1} of fresh seed with the mean value of 86.94 μmol g^{-1} and glucosinlate of 5 F_2 segregating population ranged from 72.70 to 116.30% μmol g^{-1} respectively. Velasco *et al.,* (1999) reported a lower mean glucosinolate value of 51.2 μmol g^{-1} of fresh rapeseed using NIRS. Bhardwaj & Hamama (2000) reported higher glucosinolates content in *Brassica napus* than *Brassica rapa* meal, 49.2 verses 43.8 μmol g^{-1}. The present quality assessment studies in *Brassica* lines and F_2 segregating population indicated the existence of a wide variation with respect to various parameters among the genotypes, which can serve a prominent role to the *Brassica* breeders in designing future breeding program.

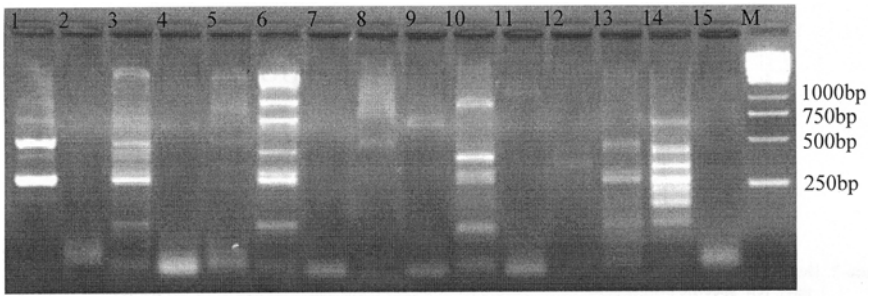

Fig. 1. PCR amplification profile of 5 F_2 segregating populations of *Brassica* along with 9 parental lines using SSR primer set IGF1144.

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*

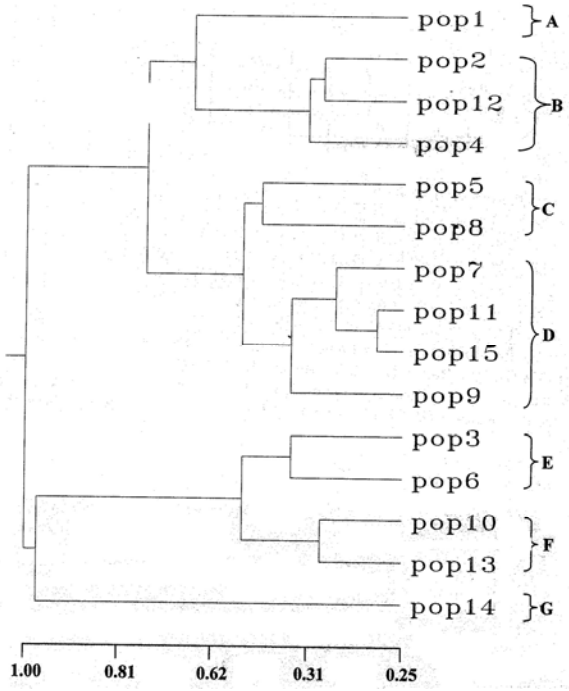

Fig. 2. Dendrogram constructed for 5 F_2 segregating populations of *Brassica* along with 9 parental lines using 3 IGF specific primer sets.

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*

For detection and estimation of genetic diversity among various genotypes included in the experiment, molecular analyses using Polymerase chain reaction was carried out. An example of PCR amplification profile of 14 *Brassica* genotypes using IGF primer IGF1144 is presented in Fig. 1 Molecular sizes of amplified fragments ranged from approximately 250 - 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 the present study, a total of 87 DNA fragments were amplified in 14 genotypes using 3 IGF primers sets, giving an average of 29 alleles per genotype per primer. The results of genetic dissimilarity analyses showed that extensive genetic diversity (average G.D. ranging from 0.25%-1.00%) existed in 14 *Brassica* genotypes used during the present study (Table 4). Most of the comparisons showed moderate estimates of genetic distance using "UPGMA" method. The detection of moderate to high level of genetic diversity during present study was in agreement with the previous reports where SSR markers detected high level of genetic polymorphsim (Welsh & McClelland, 1990; Dos Santos *et al*., 1994) in different crop species.

The bivariate data matrix and genetic dissimilarity coefficients of 5 F_2 segregating populations of *Brassica* along with 9 parental lines based on the data of 3 IGF primer sets using UPGMA method (Nei & Lie, 1979) was used to construct a separate dendrogram using computer program "popgene32" (Fig. 1). The genotypes were grouped in 7 clusters (A, B, C, D, E, F and G) comprised 1, 3, 2, 4, 2, 2 and 1 genotypes, respectively. Based on the dendrogram, parental line # 409024 and F_2 # Bannu-1 × Baro was most distantly related among the group of 14 genotypes. The results were further strengthened by previous analysis (average genetic distance analyses presented in Table 4) where high genetic distance among parental line # 409024 and F_2 population (Bannu-1 × Baro) was observed (GD=52%). For having 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 more biochemical and molecular data is required.

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References

Agnihotri, A. 1999. Screening techniques for evaluation of double low characteristics in oil seed *Brassica*. *J. Oilseed Res*., 16: 183-195.

Ananga, A.O., C. Ernst, S.R.K. Khairy, K. Koffi and W.O. Joel. 2008. Phylogenetic relationships within and among *Brassica* species from RAPD loci associated with blackleg resistance. *African J. Biotech*., 7(9): 1287-1293.

Becker, H.C., H. Löptien and G. Röbbelen. 1999. Breeding: An overview. In: *Biology of Brassica Coenospecies.* (Ed.): C. Gòmez-Campo, Elsevier, Amsterdam, pp. 413-460.

Bhardwaj, H.L. and A.A. Hamama. 2000. Oil, erucic acid and glucosinlate contents in winter hardy rapeseed germplasms. *Ind. Crops Products*, 12: 33-38.

Carro, E, A. Nuñez, S. Busiguina and I. Torres-Aleman. 2000. Circulating insulin-like growth factor 1 mediates effects of exercise on the brain. *J. Neurosci*., 20: 2926-2933.

Chen, B.Y. and B. Gertsson. 1988. Genotypes for high oleicacid content (about 80%) in the oil of rapeseed (*Brassica napus* L.). EUCARPIA *Cruciferae Newsl*., 13: 46-47.

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.

Daun, J.K. and P.C. Williams. 1995. Use of NIR spectroscopy to determine quality factors in harvest surveys of canola. In: *Groupe Consultatif International de Recherche sur le* (Ed.): Colza, Proc. 9th Int. Rapeseed Cong., Cambridge, Henry Ling, Dorchester, United Kingdom, pp. 864-866.

Devos, K.M. and M.D. Gale. 1992. The use of random amplified polymorphic DNA markers in wheat. *TAG*., 84: 567-572.

Diepenbrock, W. and R.F. Wilson. 1987. Genetic regulation of linolenic acid concentration in rapeseed. *Crop Sci*., 27:75-77.

Dos Santos, J.B., J. Nienhuis, P. Skroch, J. Tivang and M.K. Slocum. 1994. Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L., genotypes. *TAG*., 87: 909-915.

Downey, R.K. and B.M. Craig. 1964. Genetic control of fatty acid biosynthesis in rapeseed *(Brassica napus* L.). *J. Am. Oil Chem. Soc*., 41: 475-478.

Fenwick, G.R., R.K. Heaney and W.J. Mullin. 1983. Glucosinolates and their breakdown products in food and food plants. *Critical Rev. Food Sci. Nut*., 18: 123-201.

Font, R., D.R. Mercedes and D.H. Antonio. 2006. The use of near-infrared spectroscopy (NIRS) in the study of seed quality components in plant breeding programs. *Elsevier Ind. Crops Products*, 24(3): 307-313.

Font, R., M.D.R. Celestino, E. Rosa, A. Aires and A.D.H. Bailón. 2005. Glucosinolate assessment in *Brassica oleracea* leaves by near-infrared spectroscopy. *J. Agric. Sci*., 143: 65-73.

Genet, T., M.T. Labuschagne and A. Hugo. 2005. Genetic relationships among Ethiopian mustard genotypes based on oil content and fatty acid composition. *African J. Biotech*., 4: 1256-1268.

Graser, G., B. Schneider, N.J. Oldham and J. Gershenzon. 2000. The methionine chain elongation pathway in the biosynthesis of glucosinolates in *Eruca sativa (Brassicacea*e). *Archives Biochem. Biophysics*, 378: 411-419.

Halkier, B.A. and L. Du. 1997. The biosynthesis of glucosinolates. *Trends in Plant Sci*., 2: 425-431.

Harvey, B.L. and R.K. Downey. 1964. The inheritance of erucic acid content in rapeseed *(Brassica napus*). *Canadian J. Plant Sci*., 44: 104-111.

Hom, N.H., H.C. Becker and C.M. llers. 2006. Non-destructive analysis of rapeseed quality by NIRS of small seed samples and single seeds. *Euphytica*, 153: 27-34.

Ishida, M., I. Chiba and M. Kato. 1995. Evaluation of Japanese rapeseed (*Brassica napus*) germplasm for fatty acid composition and glucosinolate contents. *Proc. 9th Int. Rapeseed Cong*., 2: 398-400.

Jönsson, R. 1977. Erucic-acid heredity in rapeseed (*Brassica napus* L., and *Brassica campestris* L.). *Hereditas*, 86: 159-170.

Kaushik, N. 1998. Separation and quantification of quality parameters in rapeseed mustard. *Third International Symposium and short course on separation sciences*, 15: 23-26.

Kimber, D.S. and D.I. McGregor. 1995. The species and their origin, cultivation and world production. In: *Brassica* Oilseed; Production and Utilization. (Eds.): D.S. Kimber and D.I. McGregor. Centre for Agriculture and Biosciences International, University Press, Cambridge, pp. 1-7.

Kjaer, A. 1976. Glucosinolates in the Cruciferae. In: *The Biology and Chemistry of the Crucifera*e. (Eds.): Vaughan, J.G., A.J. Macleod and B.M.G. Jones. Academic Press, London, pp. 207-219.

Lavkopr, R.A., P.L. Velneruš, O.T. Ichkolovr, V.L.R. Kuč and Oiskohou. 2006. Estimation of fatty acid content in intact seed of oil seed rape (*Brassica napus* L.) lines using Near-Infra red Spectroscopy. *Czech J. Genet. Plant Breed*., 42(4): 132-136.

Luhs, W., V. Axel, S. Fatih and W. Friedt. 1999. Molecular genetics of erucic acid content in the genus *Brassica*. *New Horizons for an old crop.* Proc. 10th Int. Rapeseed conf.

Mahasi, M.J. and J.W. Kamundia. 2007. Cluster analysis in rapeseed (*Brassica napus* L.). *African J. Agric. Res*., 2(9): 409-411.

Nei, N. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci*., 76: 5269-5273.

Pallot, T.N., A.S. Leong, J.A. Allen, T.M. Golder, C.F. Greenwood and T. Golebiowski. 1999. Precision of fatty acid analyses using near infrared spectroscopy of whole seed *Brassica*s. 10th Int. Rapeseed Cong. Canberra, Australia.

Paterson, A.H., S.D. Tanksley and M.E. Sorrells. 1991. DNA markers in plant improvement. *Adv. Agron*., 46: 39-90.

Rakow, R. and J.P. Raney. 2003. Present status and future perspectives of breeding for seed quality in *Brassica* oilseed crops. *Proceedings of the 11th International Rapeseed Congress, Copenhangen, Denmark.* pp. 181-185.

Shahidi, F. 1990. *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing technology. Van Nostrand Reinhold: New York*. 355 p.

Shengwu, H., J. Ovesná, L. Kučera, V. Kučera and M. Vyvadilová. 2003. Evaluation of genetic diversity of *Brassica napus* germplasm from China and Europe assessed by RAPD markers. *Plant Soil Environ*., 49(3): 106-113.

Si, Ping, 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. *Aust. J. Agric. Res*., 54: 397-407.

Smale, M. and T. McBride. 1997. Understanding global trends in the use of wheat diversity and international flows of wheat genetic resources. *CIMMYT World Wheat Facts and Trends* 1995/1996.

Uzunova, M., W. Ecke, K. Weissleder and G. Röbbelen. 1995 Mapping the genome of rapeseed *(Brassica napus* L.). Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. *TAG*., 90: 194-204.

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. *Euphytica*, 106: 79-85.

Weining, S. and P. Langridge. 1991. Identification and mapping of polymorphism in cereals based on polymerase chain reaction. *TAG*., 82: 209-216.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res*., 18: 7213-7218.

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