Delimitation of local mustard (*Brassica juncea*) germplasm in Sri Lanka and improvement of their nutritive quality

S. R. Weerakoon[1], M. C. M. Iqbal[2], S. Somaratne[1], P. K. D. Peiris[1], W. S. R. Wimalasuriya[1]

[1]*Department of Botany, the Open University, Nawala, Sri Lanka Email: srwee@ou.ac.lk*
[2] *Plant Reproductive Biology Division, Institute of Fundamental Studies, Kandy, Sri Lanka*

Abstract

Mustard (*Brassica juncea*) has been grown in the Indian subcontinent for hundreds of years as an oil seed crop, however, in Sri Lanka mustard is grown comparatively to a lesser extent. It is widely used as a condiment and oil is used in Ayurvedic medicines. There are *ca*. 60 mustard accessions available in Sri Lanka. However, the genetic diversity and the relationships among these mustard accessions are yet to be studied. The objective of this study was to assess the genetic divergence of local genotypes of *B. juncea* using numerical analyses of agro-morphological characters for delimitation and identification of genetically diverse and agronomically superior accessions. Thirty mustard accessions were selected and thirty five agronomic characters were measured. Data were analyzed using different multivariate statistical procedures; Cluster analyses (CA), Principle Component Analyses (PCA) and Discriminant Function Analyses (DFA). The results of the CA, PCA and DFA indicated that there is a difference in the grouping patterns of mustard accessions. Thus, there is a doubt that whether morphological characters are adequate in delimiting the mustard accessions. Therefore, the study suggests to include other sources of information such as biochemical evidence and molecular markers in characterization of mustard accessions. Local mustard consists of high amounts of unfavorable fatty acids (erucic acid) and low amounts of favorable fatty acids (oleic acid). High levels of erucic acid are associated with undesirable effects on cardiac muscles. Commercial canola (*B. napus*) varieties are nutritionally desirable for its monounsaturated property. Interspecific hybridization between six Australian commercial spring canola varieties and six local mustard varieties was successful in producing F_1 hybrid seeds. Regeneration of F_1 plants was achieved by embryo culture technique to overcome post-germination barriers using modified Lichter medium (Lichter 1982). Fatty acid analysis of F_1 seeds of all crosses with Gas Chromatography revealed a moderate amount of erucic acid (18-21%) compared to that of *B. juncea* (44-46%) and *B. napus* (0.2-0.5%). Oleic acid content in F_1 hybrids was improved (33-41%) compared to that of *B. juncea* (7-9%) and *B. napus* (43-57%). The study clearly indicated a higher possibility of transferring traits like a high level of oleic acid and a low level of erucic acid from canola to mustard and produce new mustard lines with an improved the fatty acid profile *via* interspecific hybridization.

Key words: mustard accessions, morphology, fatty acid profile, CA, PCA, DFA, interspecific hybridization, Sri Lanka

Introduction

On the basis of consumption and production of brassica oil seed crops are the third important after palm oil and soybean oil. It accounts for almost 14% of the edible vegetable oil supply of the world. Among many brassica species, *B.napus* L., *B.rapa* and *B.juncea* are considered as commercially important oil seed crops. In Sri Lanka mustard (*B.juncea*) is gown comparatively to a lesser extent and there are about sixty accessions (records of the Gene Bank of the Plant Genetic Resource Center-PGRC, Gannoruwa, Sri Lanka). These local accessions have undergone natural selection over a long period of time for desirable characters such as tolerances to drought, fungal and pest attacks.

Mustard oil contains high levels of nutritionally undesirable erucic acid. In Canada intensive breeding programs were developed to reduce the undesirable acids (erucic acid) during 1970's using the mutants of *B.napus*. Subsequently, released the first canola-quality cultivars created as a new, high-value oil and protein crop. Canola quality *B.napus* genotypes are often confined to temperate regions and they are not flowering in the tropics due to the thermo- and photosensitivity. Mustard has many advantages over canola which include more vigorous seedling growth, quicker ground covering ability, greater tolerance to heat and drought and enhanced resistance to the diseases blackleg and to pod-shattering (Burton 2004).

Recent studies have focused on the increased intake of saturated fatty acids (stearic and palmitic acids) and it's relative contribution to the increased serum cholesterol in the blood which increase the risk of coronary heart disease. The presence of polyunsaturated fatty acids (PUFA) such as linoleic and linolenic acid are considered as desirable. The available canola oil is recognized as a superior dietary oil because it contains the lowest saturated fat level of any edible vegetable oil. Advantage of the use of high levels of oleic acid, linoleic acid, linolenic acid is to prevent cardiovascular diseases by inhibiting platelet formation and reducing cholesterol level. After carried out extensive nutritional studies in India, it has prooved that linoleic acid in mustard oil is highly beneficial for vegetarians and low income section of the society.

The present research is focused on characterizing and improving oil quality of local mustards by interspecific hybridization with commercial canola varieties.

Material and Methods

The accessions of *B. juncea* obtained from the PGRC, Gannoruwa, Sri Lanka, and six commercial spring canola varieties (cvs. 'Narendra', 'Hyola', 'Monty', 'Outback', 'Karoo', 'Oscar' obtained from Westerns Australia, were grown in the pots in the green house of the Institute of Fundamental Studies (IFS), Sri Lanka. Selected vegetative and reproductive characters were rerecorded at maturity of the plants.

Interspecific crosses: Flowers of female parent were emasculated and fresh pollen from the male parent was transferred to the stigma. A total of one hundred flowers were crossed for each parental combination. The pollinated flowers were tagged and bagged. Siliques were harvested 10-21 days after pollination and were surface sterilized and opened along the suture. The seeds were taken out and the seed coat removed to culture embryos. Embryos were transferred to water agar to test for germination. Non germinated embryos were transferred to hormone–free Murashige and Skoog (1962) medium (MS) and Lichter (1982) medium with 0.5 mgl^{-1} NAA, 0.5 mgl^{-1} BAP and 3% sucrose. Culture medium was solidified with 3 gl^{-1} agar. After adjusting pH to 5.7 the medium was autoclaved at 121^{o}C for 20 min at 15 psi. The embryos were transferred to Petri dishes and maintained under fluorescent light at 26±2 ^{o}C for a photoperiod of 16 h. The developing embryos were acclimatized before potting and transfer to thee greenhouse. The inflorescences were bagged at the flowering period to ensure self-pollination. Seeds were collected for fatty acid analysis.

Fatty acids were obtained by extracting 2 g of each seed sample (F_1 hybrids) in hexane on a Wristaction shaker overnight. The extracts were evaporated to dryness in a rotavapor, at 40^{o}C. Methanolic HCl was prepared by adding 5 ml of acetylchloride slowly to cooled dry 50 ml of methanol. The seed extract (100) mg was dissolved in this reagent and the mixture was heated at 50 ^{o}C overnight. This solution containing the methyl ester was analysed by Gas Chromatography on a DB-5 column.

Morphological characterization: The seeds of each accession were sown in plastic seed beds in a plant house at the Open University of Sri Lanka. A total of five seedlings of each accession were planted in black polythene bags with standard potting mixture. Subsequently, the seedlings were (3-4 leaf stage) transferred to plastic pots with a diameter of 13 cm. Each of these replicate was arranged in Randomized Complete Block Design (RCBD). Characterization of accessions was based on different morphological traits from seedling up to the harvest of the crop (Rabbani *et al*., 1988). The dataset was subjected to Cluster Analysis, Principle Component Analysis (PCA) and Discriminant Function Analysis (DFA) in order to classify the accessions and to trace the relationships among them. Further, these results were used to explore the importance of characters in classifying mustard accessions. Statistical analyses were carried out on SPSS/PC version 13.0 (SPSS/PC, 2004).

Results

The Fatty Acid Content (FAC) of the twelve accessions of *B. juncea* was dominated by the unsaturated erucic acid and the percentage was 41%. The mean monounsaturated oleic acid was 13.8%, and this amount is undesirable for edible purposes. Polyunsaturated linolenic acid was below 14% in *B.juncea* which is desired for a better shelf-life. In both species linoleic acids were at comparable levels.

Germination of the F_1 seeds was possible on a Listure medium. Seeds germinated on culture medium were successfully acclimatized and transferred to the green house. The Table 1 shows the difference in FAC between the parental species (*B. napus* and *B. juncea*) and their F_1 hybrids.

Table 1. Fatty acid content (%) of seeds of *Brassica juncea* (ac.7700), *B.napus* canola cultivars and their F_1 hybrids

Fatty acid	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Erucic (C22:1)
B.juncea	8	16	11	46
B.napus	47.2 ± 6.0	19.5 ± 1.4	11.0 ± 1.1	0.3 ± 0.2
F_1 hybrids	34.8 ± 3.9	17.1 ±1.3	10.3 ± 0.9	18.8 ± 1.4

The dendrogram (Figure 1) obtained from cluster analysis, showed five clusters of accessions at 60% phenon level. From the results of PCA, there was a particular grouping pattern within the accessions in which certain accessions were well-separated and other accessions overlapped considerably (Figure 2). The result of DFA indicated that there are three groups of accessions (Figure 3).

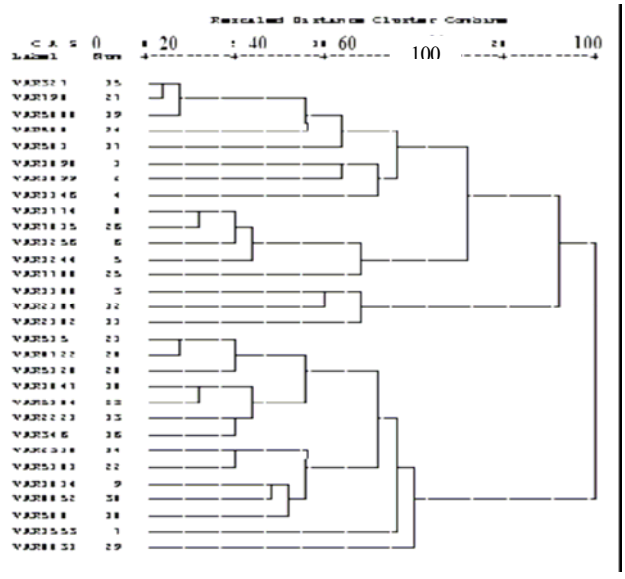

Figure 1 Dendrogram constructed from the cluster analysis

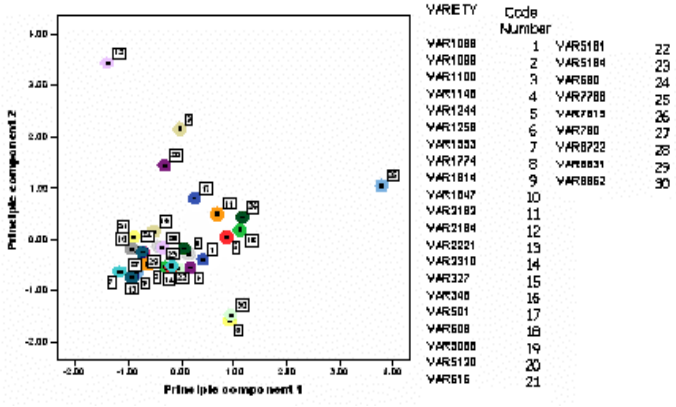

Figure 2 The "biplot" produced by plotting Principle Component axis 1 with Principle Component 2.

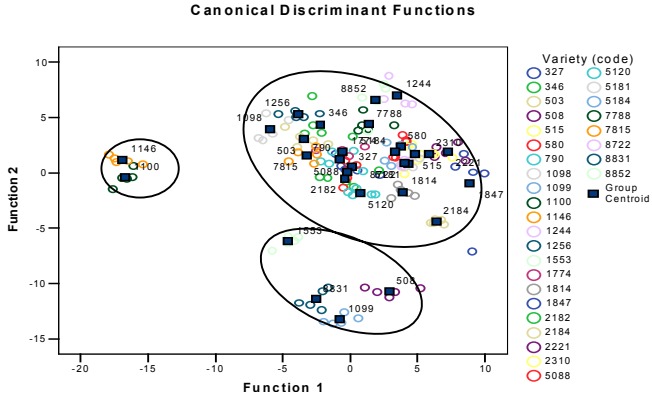

Figure 3 The scatter plot resulted from plotting Discriminant Function 1 with Discriminant Function 2.

Discussion

B.juncea grown in Sri Lanka contains nutritionally undesirable fatty acids and there is a need to improve the crop to use as a oil seed and it should be brought down to the levels of internationally and commercially accepted canola quality. By crossing *B.juncea* with *B.napus* we determine the feasibility of transferring the fatty acid profile to the canola quality. The inter-specific embryos were recovered by *in-vitro* embryo rescue, and raised them to maturity.

On cluster analysis the mustard accessions were fallen within five groups and this may be due to the similarities and relationships among accessions. However, PCA results were different from that of cluster analysis and this reflects the differences between the analytical methodologies. Since six out of thirty accessions were well-separated in PCA, these accessions could be maintained as separate accessions. Meanwhile, other accessions were broadly split into two groups with considerable overlapping. Therefore, the accessions in these two groups may be the same accessions with minor

morphological variation resulting from geographical variations and/or the difference in statistical analytical methods used in this study. The results of the DFA indicated that thirty accessions can be classified into three groups. Thus, grouping patterns of accessions were different under different statistical analytical methods.

Conclusion

Interspecific hybridization between *B.juncea* and *B.napus* produced F_1 hybrids with improved fatty acid profile. Since, there are inconsistencies in the results obtained from different statistical methods used in this study, morphological characters themselves are inadequate in characterizing mustard accessions and other sources of information such as isozymes, seed protein, seed fatty acids and molecular markers are of importance in characterization of mustard accessions grown in Sri Lanka.

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