

Biochemical Marker for Establishing Distinctiveness of Ethiopian Mustard (*Brassica crainata* A. Braun) Varieties as Supplementary Descriptors for Plant Variety Protection

Nitin Kumar¹, Chander Mohan^{2*} and Sunil Kumar³

¹Department of Seed Science and Technology,

G. B. Pant University of Agriculture and Technology, Pantnagar 263 145, India

²Assistant Commissioner (Seeds), DAC & FW,

Ministry of Agriculture Cooperation and Farmers Welfare, Government of India, New Delhi 110001

³Department of Vegetable Science, CoH, Banda University of Agriculture and Technology, Banda 210001 India

*Corresponding Author E-mail: chhander@yahoo.com

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ABSTRACT

India has enacted a sui generis legislation as protection of Plant Varieties and Farmer's Rights Act 2001 (PPV&FR) for the protection of plant varieties by registration. Under PPV&FR Act DUS (Distinctiveness, Uniformity and Stability) testing procedure will be performed on the basis of morphological descriptors. Utilization of biochemical marker in DUS testing for establishing distinctiveness as a supplement to morphological descriptors has been approached in this study. Ten released cultivar and advance line of Ethiopian mustard (*Brassica carinata* A. Braun) were studied for morphological descriptors and total soluble proteins as biochemical marker to unveil distinctive features. SDS-PAGE for total soluble protein analysis revealed moderate degree of polymorphism. UPGMA analysis based on SDS-PAGE banding pattern data of different proteins could discriminate only three varieties Jayanti (released cultivar), PBC-2005-1 and PBC-2006-4 (advance lines). So, it can be concluded that in situations where the morpho-physiological DUS descriptors are not able to establish distinctiveness of a variety then biochemical markers may be used as additional or supplementary descriptors for unveil distinctiveness of Ethiopian mustard.

Keywords: Ethiopian Mustard, DUS, biochemical marker, SDS-Page.

INTRODUCTION

Ethiopian mustard is an important oil crop of Ethiopian origin and it has been grown in Ethiopia as well as India since antiquity both

as an oil seed and vegetable crop. Mustard belongs to the family Cruciferae (Williams, 1989; & Hatam & Abbasi, 1994).

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It has about 338 genera and 3709 species (Warwick et al., 2006). About 159 species are included in the genus *Brassica* (Zhou, 2001; & Zhou et al., 2006). The amphidiploid *Brassica carinata* (n=17) is originated from cross between *Brassicainigra* (n=8) and *Brassica oleracea* (n=9) (Morinaga, 1934).

Among oilseed crops, rapeseed and mustard rank 3rd after soybean and oil palm in production of vegetable oils. In the production of oil seed proteins it ranks 5th (Kausar et al., 2006). Industrial uses comprise exchange of biomass to bio-energy (Ofori & Becker, 2008). In addition it is also used for food, feed and metilester which is used in biodiesel manufacture (Sabaghnia et al., 2010). This plant also part of research to develop bio-fuel for jet engions. On October 29 of 2012, the first flight of a jet aircraft powered with 100% bio-fuel, made from *Brassica carinata*, was completed*.

Many farmers in India grow Ethiopian mustard. Besides, Ethiopian mustard varieties with higher yield potential have been developed by incorporating the desirable traits. Thus, there are a number of Ethiopian mustard varieties, both traditional and improved types, currently under protection whose identity and distinctiveness need to be established by various approaches (Santhy et al., 2000).

Varietal registration has attained a critical importance all over the world including India. Testing for distinctiveness, uniformity and stability (DUS) is an essential component of variety registration procedure. In Europe, the testing procedures are determined by International union for the protection of new varieties of plants (UPOV). India has however, enacted a *sui generis* legislation as protection of Plant Varieties and Farmer's Right Act, 2001 (PPV&FR) something like UPOV Act. The PPV&FR Act recognizes the plant breeder's rights as well as rights involved in commercial exploitation of protected varieties. Like UPOV, under PPV&FR Act a variety must fulfil the criteria of (DUS) and novelty (if new) so as to get protection under this Act (Kochhar et al., 2004). There are 24 morpho-physiological characteristics of Ethiopian

mustard, which are species specific and recommended procedure for conducting trials are given in the guidelines (Anonymous, 2009). As per the DUS guidelines only morpho-physiological descriptors are used. However, serious problems may arise for establishing distinctiveness of variety only on morpho-physiological DUS descriptors as the number of candidate varieties are growing with decrease variability as well as expansion of reference collections. This study was conducted in anticipation, if the morpho-physiological DUS descriptors are not able to discriminate varieties, then biochemical markers can be considered as additional descriptors for establishing the distinctiveness of variety.

Biochemical markers, especially the electrophoretic profile of proteins, have been widely used for identification of crop varieties. Electrophoretic method have been standardized for a large number of crops and found useful for the purpose of variety identification and characterization (Dadlani, 2007). Though protein markers alone may not be sufficient in resolving the identity of a variety, these can provide useful supplementary information, which in combination with morphological descriptors will provide identification keys. The present study was conducted on 10 released as well as advance line of Ethiopian mustard varieties using biochemical (total soluble proteins) as additional markers to morphological descriptors for establishing the distinctiveness of avariety.

MATERIALS AND METHODS

Atotal number of 10 released as well as advance line were studied for 24 morpho-physiological characteristics as notified by PPV&FR Authority (Anonymous, 2009). The experiment were conducted N.E. Borlaug, Crop Research Centre, G.B.P.U.A.&T., Pantnagar during two Rabi seasons (2011-12 and 2012-13) in randomized block design with 3 replications. Each replication consisted of 6 rows of 6 m length with 45×15 cm spacing. Among the 24 morphological characteristics

studied, 8 were visually assessed and 16 measured. The observations were recorded at specified stage of crop growth period when characteristics under study had full expression.

Characterization of varieties was done according to 4 morpho-physiological grouping characteristics reported in DUS test guidelines for Ethiopian mustard (Anonymous, 2009).

Table 1: Ethiopian mustard varieties used for morpho-physiological characteristics

S. No.	Genotype	Parentage/Origin
1	Kiran Early	Selection from Kiran (Pantnagar)
2	PBC-2006-4	Selection from Kiran (Pantnagar)
3	PBC-2005-1	Selection from Kiran(Pantnagar)
4	PBC-2009-5	Selection from Kiran (Pantnagar)
5	PBC-2009-4	Selection from Kiran (Pantnagar)
6	PBC-2009-3	Selection from Kiran (Pantnagar)
7	PBC-2009-2	Selection from Kiran (Pantnagar)
8	PBC-2009-1	Selection from Kiran (Pantnagar)
9	Jayanti	(Kanpur)
10	Kiran	Selection from HC-5 (Pantnagar)

Biochemical Characterization

Total Protein Analysis by SDS-PAGE

Total proteins were extracted by hand grinding of 1 g of seed in 2 ml chilled Tris-sucrose homogenization buffer containing 0.1 M Tris, 0.4 M sucrose, 10 mM KCl, 0.1% β -mercaptoethanol and 1 mM each of MgSO₄, EDTA and PMSF. The homogenate obtained was centrifuged at 10,000 rpm at 4°C for 30 min. and the supernatant was further used for electrophoresis in a 10% SDS polyacrylamide gel.

Statistical Analysis

Varietal profile generated from total proteins were scored according to the presence (1) or absence (0) of bands and data entry was done into binary matrix as discrete variables. Jaccard's coefficient of similarity was measured and a dendrogram was generated using Unweighted Pair Group Method with Arithmetic Average (UPGMA). The computer package NTSYS-PC version 2.10d was used for cluster analysis to measure the relationship between the varieties (Rohlf, 2002).

RESULT AND DISCUSSION

The accurate description of Ethiopian mustard varieties is crucial for registration under PPV&FR Act. The identity/profile of an Ethiopian mustard variety is to be established by using a set of morphological characteristics prescribed in the DUS test guidelines on

Ethiopian mustard. Out of 24 characteristics 11 were found to be monomorphic, 8 were dimorphic and 5 were polymorphic. Maximum polymorphism was observed for leaf and seed characteristics. Low level of polymorphism especially for Siliqua characters was obtained which might be due to the fact that Ethiopian mustard cultivars were domesticated in their respective ecological zones with narrow genetic base. The low level of polymorphism was reported in sorghum local cultivars also for the DUS descriptors (Joshi et al., 2009).

Four grouping characteristics have been mentioned in the DUS test guidelines for determining distinctiveness of the varieties. Two grouping characteristics viz. number of lobes and time of flowering were monomorphic in the varieties under study. Thus, grouping of varieties was based on 2 characteristics viz. main shoot length and number of seeds per Siliqua. On the basis of grouping characters as Gazette notified by Govt. of India in the PPV&FR Act, none of the varieties could be discriminated (Fig. 1). Thus, grouping characteristics and DUS descriptors of morpho-physiological nature which were mentioned in the DUS guidelines could not establish distinctiveness for any variety. Hence, biochemical markers were considered for establishing the distinctiveness of a particular variety.

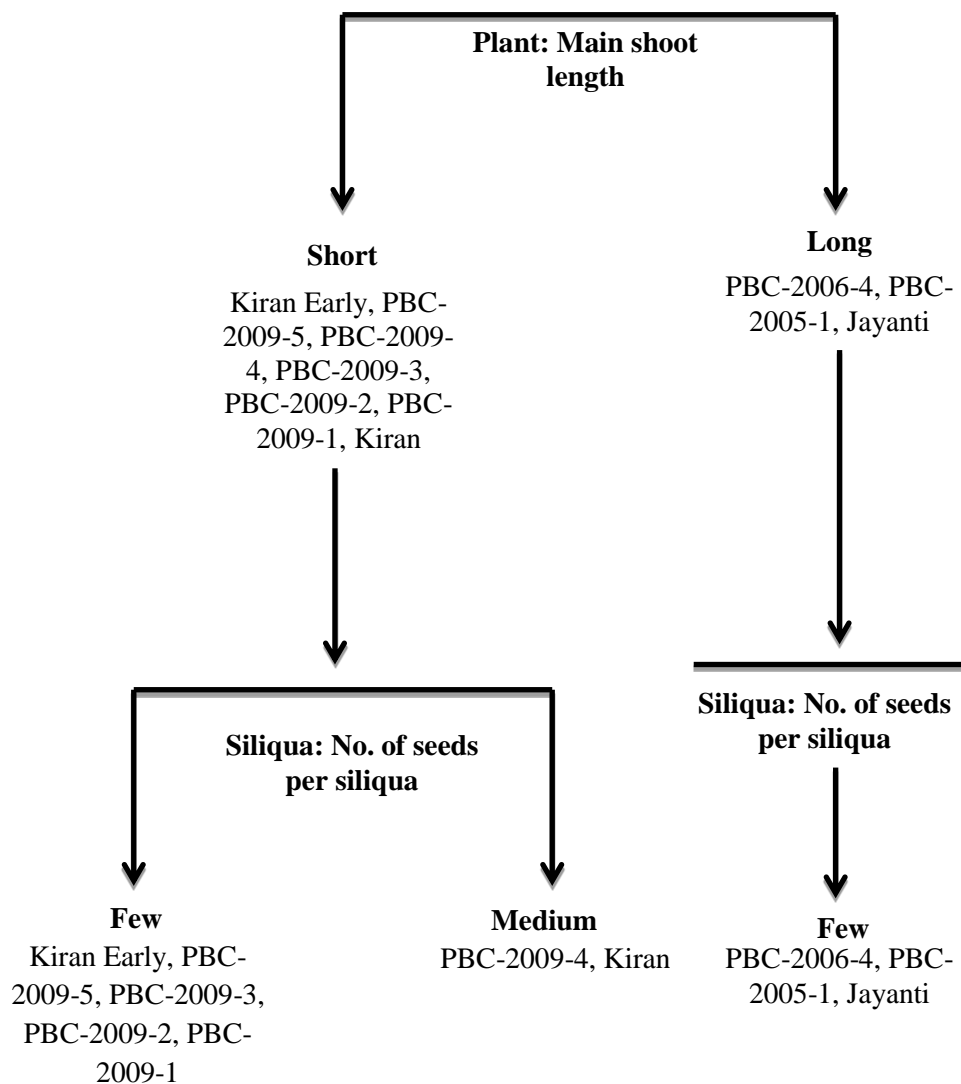


Fig. 1: Grouping of varieties based on grouping characteristics proposed in the DUS test guideline.

SDS-PAGE Analysis of Total Soluble Proteins

The electrophoresis of total soluble seed proteins revealed a total of 11 polypeptide bands, out of which 6 were polymorphic showing a moderate degree of polymorphism (Plate 1). UPGMA cluster analysis was able to individually distinguish following three varieties: PBC-2006-4, Jayanti and PBC-2005-1 (Fig. 2). PBC-2009-3, PBC-2009-4 and PBC-2009-5 exhibited 100% similarity and likewise two other pairs *viz.* PBC-2009-1 and PBC-2009-2, Kiran and Kiran Early which share common parent. Electrophoretic analysis of total soluble protein is widely recognized as technique for cultivar identification and even

UPOV has recommended SDS-PAGE for analysis of high molecular weight glutenins in wheat (Anonymous, 1994a) and hordeins in barley (Anonymous, 1994b). But in case of Ethiopian mustard from our studies it appears to be of limited use for the establishment of distinctiveness of closely related varieties.

Based on the forgoing results, it can be concluded that in situations where the morpho-physiological DUS descriptors are not able to establish distinctiveness of a variety then biochemical markers may be used as supplementary descriptors for resolving the distinctiveness of Ethiopian mustard varieties for granting plant variety protection under PPV & FR Act.

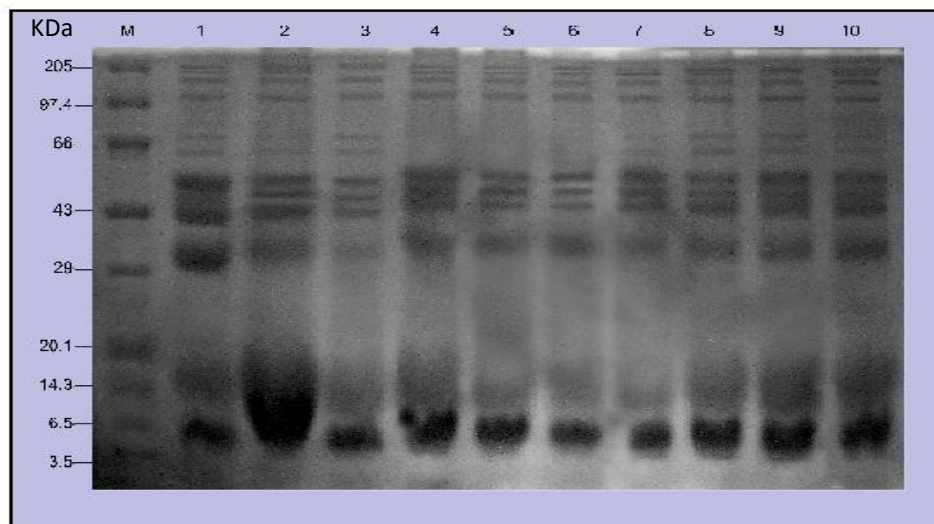


Plate 1: SDS-PAGE pattern of total soluble protein from ten Ethiopian mustard varieties (1 to 10- Names of varieties as mentioned in Table-1)

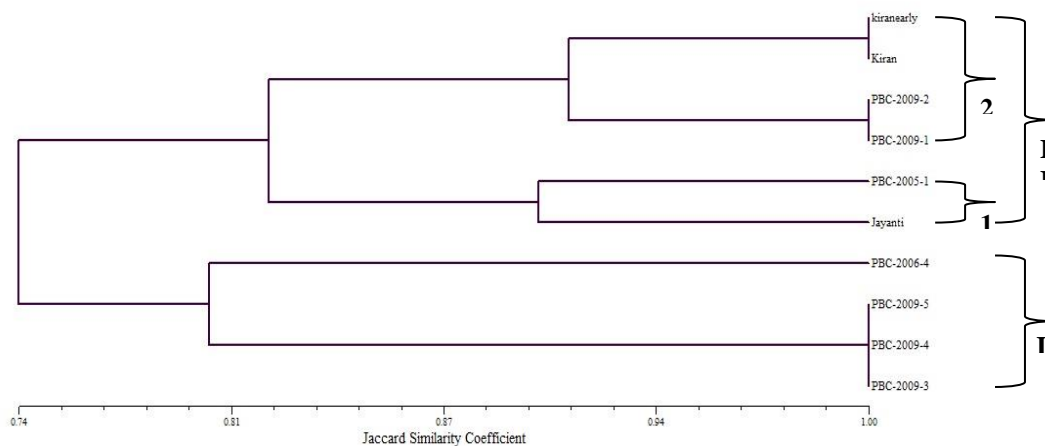


Fig. 2: UPGMA cluster analysis of ten Ethiopian mustard genotypes on the basis of SDS-PAGE of total protein profile

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