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Isozyme Polymorphism for Genetic Purity Testing in Muskmelon(*Cucumis melo*) hybrid

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ABSTRACT The study was deliberated with the objective of applying electrophoresis for determining the genetic purity of hybrid seed of muskmelon (Punjab hybrid) and its parents MS-1 (female parent) and Hara Madhu (male parent). It was observed that there was no isozyme polymorphism with respect to four isozymes *i.e.* peroxidase, esterase, phospho-gluco-isomerase and phospho-gluco-mutase that can be applied for genetic purity testing. Therefore, it is suggested that other isozymes may be explored than these four isozymes.

Keywords: Estrerase, genetic purity, muskmelon, hybrids peroxidase.

Maintenance of genetic purity of seeds is essential to ensure maximum benefit from varieties. This is particularly relevant for hybrid seeds because these are expensive and the end user expects that every individual seed of the lot should be true to the type. However, in actual situation, hundred per cent genetic purity in a seed lot is very difficult due to lapses in maintenance of proper field isolation, purity of initial seed stocks and contamination by foreign pollen from neighboring fields or off type plants. In hybrids there is also the additional risk of obtaining selfed seeds due to incomplete male sterility or emasculation or wrong identification of male sterile and male fertile plants in hybrid seed production plot where genetic male sterility is exploited for heterosis.

The traditional method of grow-out test for genetic purity testing involves examination of plants from vegetative stage to maturity. This is time consuming as it covers the entire duration of the crop. Moreover, plant morphology characters being polygenic in nature are liable to be influenced by the environment. Hence there is a need to use alternate descriptors which are rapid, accurate and less affected by environment. Electrophoresis separation of seed/seedling proteins/isozymes can provide useful information for variety description, identification, characterization and purity testing. Using these methods, different soluble and storage protein fractions and a large number of isozymes can be separated into their components. Polymorphic protein/isozymes markers based on electrophoresis analyses are effectively used for testing the purity of commercial hybrids of tomato [1 and 2], cotton [3] and pearl millet [4]. Also the knowledge of genetic control for each of the protein/isozymes markers is required by the UPOV for inclusion in the guidelines for DUS testing. Punjab Agricultural University, Ludhiana has developed and released one hybrid of muskmelon (Punjab hybrid) using genetic male sterility which has revolutionized the muskmelon cultivation in Punjab [5]. Beside its yield superiority, it has an excellent edible quality which fetches a high premium in the market over all other varieties. Therefore, the present investigations were conducted to differentiate hybrid muskmelon *i.e.* Punjab hybrid from its parents MS-1 (female parent) and Hara Madhu (male paent) and establish hybridity on the basis of polymorphism for isozymes of peroxidase, esterase, phospho-gluco-isomerase and phospho-gluco-mutase.

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MATERIALS AND METHODS

A samolp of 50 seeds of each genotype were taken for conducting studies in sequential testing [7]. The isozymes of peroxidase (PER), esterase (EST), Phospho-Gluco-Isomerase (PGI) and Phospho-Gluco-Mutase (PGM) were tested on 7 days old seedlings grown in a germinator at ± 25°C, of Punjab Hybrid and its parental lines (MS-1 and Hara Madhu). The extractions were carried out by homogenizing 0.5 gm of plant material per sample containing 0.75 ml chilled Tris HC1 buffer (pH 7.5) solution. The homogenate was left to stand at 0°C (in ice) for 10 minutes to settle debris. The supernatant was centriruged for 3 min in cold centrifuge at 5000 rpm. The supernatant was used for analyzing enzyme activity and stored at -20°C.

The 8% polyacrylamide gels were prepared according to the method given by Shields et al. [8]. This gel was subjected to vertical gel electrophoresis for resolving isozyme bands. The electrode buffer was composed of tris and glycine with 8.3 pH and gel buffer was composed of tris (pH 8.8) and acry-bis solution. Electrophoresis was started at a voltage of 150 Volt and 12 mAmp current for first 15 minutes to elute the samples and then continued at 225 Volt and 25 mAmp until the tracking dye reached the bottom of the gel. After the run, the current was switched off and the gels were taken out and processed immediately for isozyme band determination. The gel were incubated for different isozyme activity staining systems described by Vallejos [6] and Weedan & Wendel [7] for peroxidase, esterase, and Phospho-Gluco-Isomerase and Phospho-Gluco-Mutase. When isozymes bands developed, the reaction was stopped with 7% acetic acid and photographed immediately. The relative migration (Rm) of each band was calculated as under:

$$Rm = \frac{\text{Distance migrated by the isozyme band from the origin (cm)}}{\text{Distance migrated by the tracking dye (cm)}}$$

A replicated grow out test in three replications was also planted simultaneously in the field during March, 2005. Five hundred seeds of Punjab Hybrid along with 200 seeds each of MS-1 and Hara Madhu were grown in the field for comparison purpose.

RESULTS AND DISCUSSION

The photographs of the electrophoretic patterns (plates 1-4) revealed that there was no polymorphism with respect to these four isozymes *i.e.* peroxidase, esterase, phospho-gluco-isomerase and phospho-gluco-mutase. All the bands were observed to be of same intensity and having same Rm values. As per earlier works, it was confirmed that many isozymes are tissue specific, *e.g.* peroxidase and esterase [7]. Different zymograph patterns were observed during early studies of esterase and it was demonstrated that there were zymograms differences between species and within species, as well as tissue differences [9]. Some of the plants examined included *Cucurbita andreana, Cucurbita maxima, Cucumis melo, Cucumis myriocarpus,* etc. However, *Cucumis myriocarpus* showed marked seasonal variation with highest activity in November when fruits were green and lowest activity in January when the fruits were fully ripe. In contrast, no seasonal variation in enzyme activity was observed in *Cucurbita maxima.* Similarly, no seasonal and tissue specificity was exhibited during present esterase studies in all the three genotypes under study. Polymorphism was not recorded even when different tissues at different stages (at vegetative stage and reproductive stage) were taken and tested for these isozymes. It is therefore suggested that isozymes other than these four may be explored for testing genetic purity in hybrid muskmelon.

The data obtained from the plants grown for grow-out-test in the field indicated that MS-1 plants segregated into 1:1 ratio of the male fertile and male sterile. The Hara Madhu variety was also genetically pure. The data obtained from the plants grown from seeds of Punjab Hybrid showed that seed purity was satisfactory as it had only 0.60% selfed plants.

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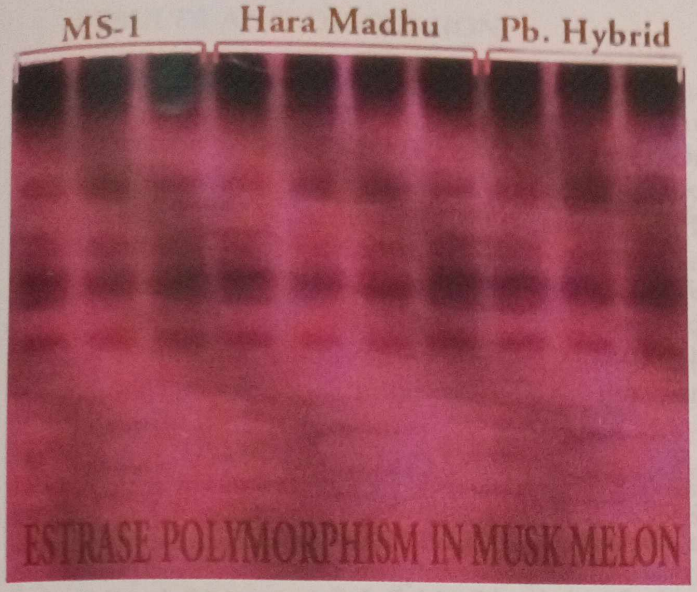

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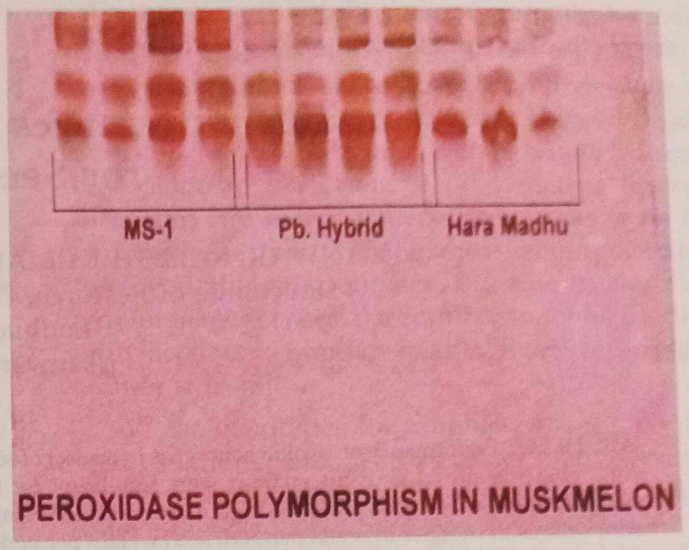

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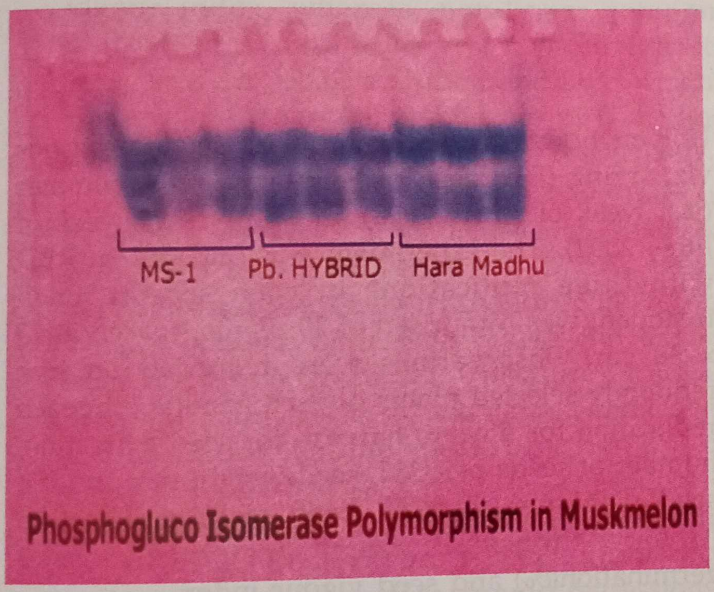

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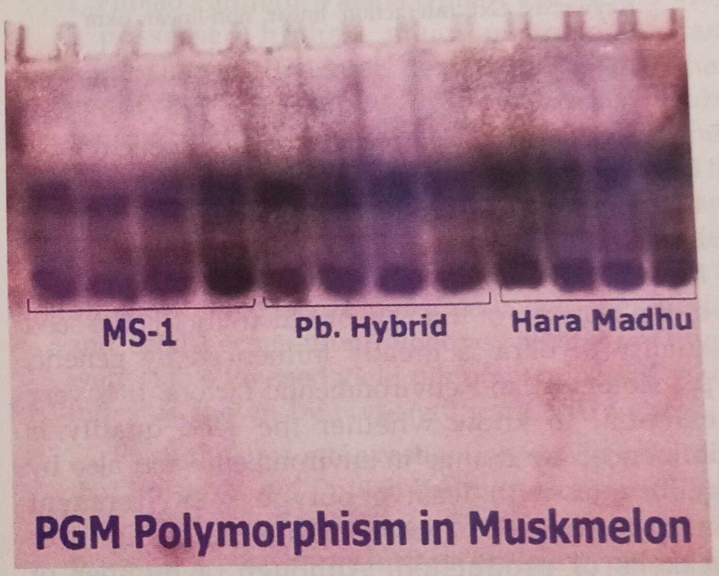

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