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TOOLS FOR GENETIC PURITY TESTING OF HORTICULTURAL CROPS- A REVIEW

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ABSTRACT : The primary objective of plant breeding is to develop superior varieties. Varietal purity is one of the main quality requirements of the seed offered for sale as well as for commercial multiplication. Hence seed identity and varietal genetic purity testing is essential component of modern and effective agricultural production system. Several laboratory and field methods are used to determine the cultivar trueness and genetic purity of the seed offering genuine quality seed to the farmers for commercial cultivation. Farmers cannot distinguish the real hybrid seeds from false ones neither the poor quality ones . Hence judicious, sensible application of chemical and molecular tools for rapid genetic purity testing is of much concern for quality seeds to meet our country's food security.

Keywords: *Seed, genetic purity, grow out test, RAPD marker, electrophoresis.*

Production and distribution of high-quality seeds is fundamental to modern agricultural systems. The majority of annual crops are established each season from seeds, and seed quality can have a major impact on potential crop yield. Seeds carry the genetic traits incorporated by years of breeding and selection to create varieties that are adapted to specific production environments and will produce high yields and product quality. The genetic purity of seeds (i.e., the percentage of contamination by seeds or genetic material of other varieties or species), their physiological quality (or vigour), and the presence of weed seeds, seed borne diseases, or other materials (dirt or plant residues) contribute to overall seed quality.

Standards established for seed genetic purity?

The genetic purity of any commercial agricultural product propagated by seed begins with the purity of the seed planted. In general, the genetic purity of the seed planted must equal or exceed the final product purity standard required, as purity generally decreases with each subsequent generation of propagation. On the other hand, it is virtually impossible to assure that no off-type plants or pollens are present in the seed production field and that all handling and conveyance equipment and storage facilities are completely free of contamination, so commercial planting seed is seldom 100% pure. In practice, seed genetic purity standards have been established by state seed laws and by seed certification agencies to ensure that the purchaser receives seed that is within certain purity tolerances. These tolerances are established based on the biology of the species (i.e., self- or cross-pollinated), the type of variety (i.e., open pollinated or hybrid), and market-driven standards for final product quality.

Earlier generations of seed (e.g., foundation or registered seed) have stricter standards in order to be able to meet the certified seed purity criteria. Production of genetically pure good quality seed is exact task of seed producer which require high technical skill and high financial investment. During seed production strict attention must be given to maintenance of genetic purity and other qualities of seed. Therefore seed production must be carried out under standardized and well organized condition. It is achieved by using genetic and agronomic principles during seed production programme (Sabry *et al.*, 5).

PRINCIPLE

Genetic purity or genuineness of the cultivar is tested by means of heritable characters (morphological, physiological or chemical) of seeds, seedlings or plants.

Causes For Deterioration of Genetic Purity

The genetic purity of a variety or trueness to its type deteriorates due to several factors during the production cycles.

The following important factors are responsible are for deterioration of varieties.

1. Developmental variations

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2. Mechanical mixtures
3. Mutations
4. Natural crossing
5. Minor genetic variations
6. Selected influence of pest and diseases
7. The technique of the plant breeder

There are two main approaches for genetic or varietal purity testing:

1. The use of computerized systems to capture and process morphological information (Machine vision).
2. The use of biochemical methods to analyze various components of seeds (Chemotaxonomy).

Morphological test **(In laboratory)**

- Examination features of seeds such as length, width, thickness, shape, weight, colour, seed coat colour etc. and comparing them with those of authentic sample.
- Which are examined with naked eye / with magnified hand lens with the help of scanning electron microscope.

In field or green house condition-Grow Out test (GOT)

- The seed sample is sown in the controlled condition with the authentic sample
- Genetic purity is determined on the basis of observation made on the plant morphological characters with reference to authentic sample.
- Genetic purity is always expressed in percentage

The size of the submitted sample will be as follows: for GOT

- 1000 g- for maize, cotton, groundnut, soybean and species of other genera with seeds of similar size.
- 500 g - for sorghum wheat, paddy and species of other genera with seeds of similar size.
- 250 g - Beta sp and species of other genera with seeds of similar size.
- 100 g - for bajra, jute and species of all other genera.
- 250 tubers/ planting material – seed potato, sweet potato and other vegetatively propagating crops

METHODS TO ASSESS GENETIC PURITY

Genetic purity of a given seed lot can be assessed by using one of the following methods

1. Conventional grow out test
2. Chemical test
3. Electrophoresis method

a.Biochemical markers (Proteins and Isozymes)

b.Molecular markers (DNA)

Chemotaxonomy (Chemical test)

Chemo taxonomists have recognized two groups of compounds that are generally use full in classification of plant species

1. Episemantic or secondary metabolites (pigments or fatty acid etc.)
2. Semantides or sense carrying molecules (Proteins, Nucleic Acids)

METHODS OF TESTING BASED ON

1.Analysis of secondary compounds

2.Protein analysis

3.Nucleic acid analysis

1. Analysis of secondary compounds : These test ranges from simple colour tests to complex chromatographic separations of phenols, anthocyanin, flavonoids and other compounds.

Different tests includes

1. Phenol test
2. Peroxidase test
3. Potassium hydroxide – bleach test
4. Fluorescence test
5. Hydrochloric HCl test
6. Ferrous sulphate test
7. NaOH test
8. Anthocyanin test
9. Seedling pigmentation

2. Protein analysis : Because proteins are the direct gene product the analysis of seed, seedling proteins and enzymes is most successful and widely used. Hence much attention has been focused on seed storage proteins (Kavimandhan *et al.,* 2).

There are two primary methods

➢ Various types of gel electrophoresis

Native: separation by size and charge (charge/mass).

Denaturing: Separation by size

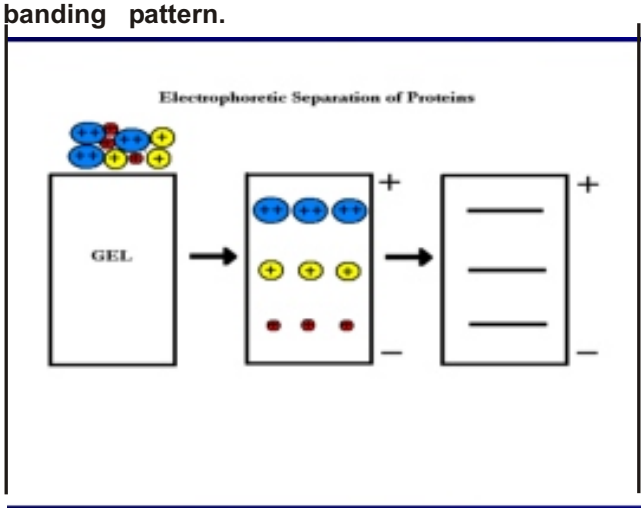
Others (IEF, 2-Dimenstional electrophoresis)

➢ High pressure liquid chromatography

What is Electrophoresis?

Migration of a charged particle through a medium (agarose, polyacrylamide, starch) under the influence of an electrical field. It is usually carried out in aqueous solution. A mixture of molecules of various sizes will migrate at different velocities and will be separated (Sinha *et al.,* 6).

The varieties are verified on the basis of banding pattern.



1. By measuring Rm of bands
2. Total number of bands
3. Presence or absence of specific band
4. Intensity of band

Gel Electrophoresis

- Native continuous system—gel and tank buffers are the same, single phase gel; examples are PAGE, agarose, and starch gels.
- Discontinuous System—gel and tank buffers are different, two phase gel (stacking gel); example is PAGE.

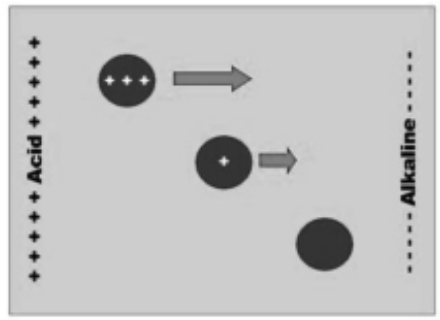
Gel Electrophoresis based on denaturation

SDS (sodium dodecyl sulphate) used to denature proteins (discontinuous system). Urea or form amide used to denature DNA or RNA.

Other types are : Isoelectric focusing: protein-separation based on isoelectric points in a pH gradient.

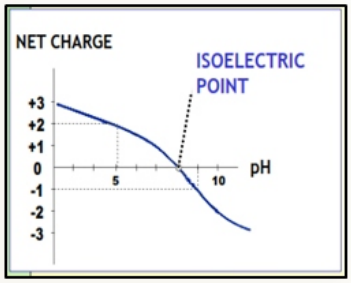
2-D electrophoresis : combination of IEF and SDS-PAGE.

Iso electric focusing : This technique relies not on the rates of mobility but on the protein's net



charge. Isozymes move through a pH gradient under the influence of an electric field (Dou *et al.,* 1).

As the enzymes move through acidic regions of the gel and enters into areas of higher alkalinity the net charge on the protein changes, eventually, it reaches a pH region where the net charge

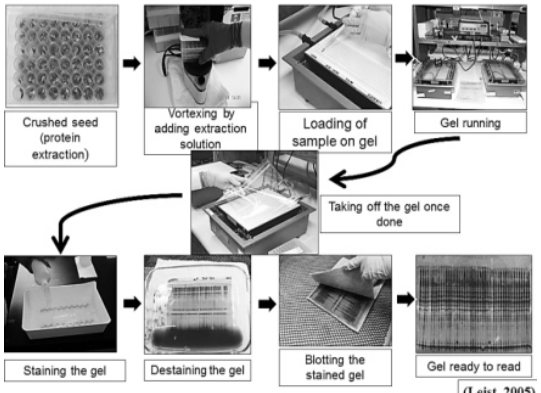


equals zero. At this point the protein will not migrate any further and is said to be "focused". IEF can be used to differentiate proteins with very subtle changes in amino acid composition.

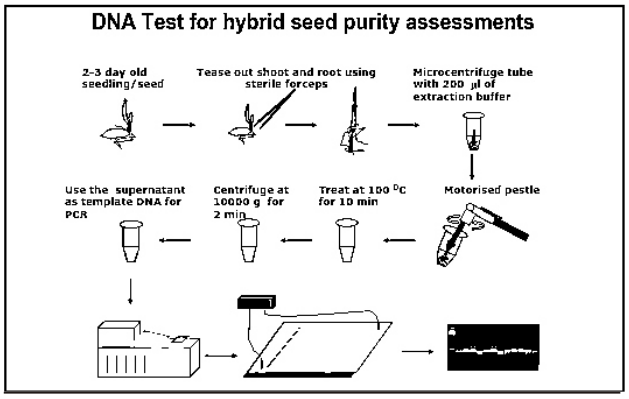
How does the pH gradient work in the gel matrix?

Carrier ampholytes are in the gel matrix that are low molecular weight and have closely related isoelectric points,When electricity is applied to the gel the ampholytes forms a pH gradient in the gel. Kumar *et al*. (3).

When an amphoteric protein from a sample is no longer charged the electrical current will not have an effect on it. Thus, the term **"FOCUSING"**.



Steps in Iso Electric Focusing



DNA Based Hybrid Purity Testing Methods

Genetic markers are any genetically determined trait (morphological, biochemical, molecular) that can distinguish among genotypes

Molecular markers : These are molecular landmarks linked to the loci of interest, which are used to identify and characterize the loci.

- biochemical constituents (Secondary metabolites)
- macromolecules (Protein & DNA)

Protein markers **-** limited application due to their stage specific expression

"DNA markers are the most suitable"

Molecular Markers (MM)

- Detect variation directly at DNA level
- Highly abundant and polymorphic
- Independent from environment and reproducible
- Expressed in all developmental stages
- Known position in the genome
- Automation possible

Markova and Stoilova (4) studied the Glumate dehydrogenase isoenzymes extracted form tomato seeds for genetic purity testing for which they used mature seeds of F_1Hybrids of tomato and their respective parental lines were used F_1-hybrid (6944 x 2413), Figure 1 and 2 show patterns and schemes of GDH of F_1 hybrids 6944 x 2413 and 2197 x 2263 and their respective parental lines. The electropherogram show the presence of three loci. *Gdh-1* locus is most active in all genotypes; however, it is invariant and cannot be used for genetic identification because it expresses an equal number (2) of homologous isoenzymes in all genotypes investigated. *Gdh-2* locus is least active but it is variant because of quality differences between the lines. The maternal (a) and the paternal (b) lines express an isoenzyrne each but with different position upon the gel, they are not homologous but products of different alleles. There are two isoenzymes in the hybrids pattern (e), homologous to the maternal and the paternal lines, respectively. *Gdh-3* locus expression is similar to that of the *Gdh-2* locus but with a considerably higher activity. The maternal and the paternal genotypes of both combinations express an isoenzymes each, the product of different alleles. The F_1 hybrids have two isoenzymes, homology

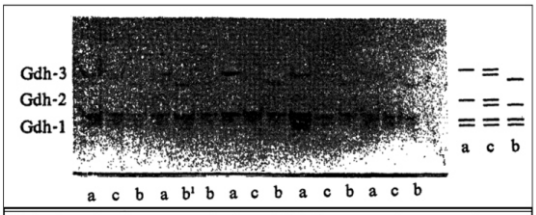


Fig 1: Electrophoretic patterns and scheme of GDH in tomato seeds a-maternal parent line 6944; b- paternal line 2413; C-F_1 hybrid; B^1-contamination.

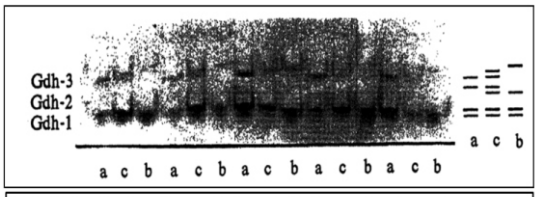


Fig 2: Electrophoretic patterns and scheme of GDH in tomato seeds a-maternal parent line 2197; b- paternal line 2263; C-F_1 hybrid.

being found with the allele expression of the maternal parent only. Compared to *Gdh-2* locus the variant differences in *Gdh-S* locus are better expressed because of the higher isoenzymes activity. The investigated expression of *Gdh* loci in parental lines and their F_1 hybrids Showed that *Gdh-3* locus in tomato seeds from the investigated combinations can be applied as a marker of parental lines genetic identification and F_1 hybridity testing.

What is a marker?

A marker is a signpost linked to the trait/gene of interest and is co-inherited along with the trait.

Marker polymorphism = Trait/gene polymorphism

Presence of specific allele of marker = Presence of specific allele of target gene.

Polymerase Chain Reaction Technique

- It requires very little DNA (single seed or leaf)
- It's a fast, simple and accurate method
- It is highly sensitive and specific method

Reproducibility of the OPB 161193 marker (Fig. 3) was confirmed in the five independent PCR reactions. The OPB161193 marker was absent in all the 20 female plants (DVRT-1) and present in all the 20 male plants (Flora Dade) and hybrids (NTH-1). Hence, the marker could be commercially used to detect selfed-seeds in the hybrid seed lot of NTH-I. Like OPB161193, reproducibility of the OPB192186 marker was also confirmed. Thawaro and Te-chato (7) undertaken a study regarding application of molecular markers in the hybrid verification and assessment of somaclonal variation from oil palm propagated *in vitro* for which they used mature zygotic embryos of Tenera hybrid, derived from the cross 366 (D) ×72 (P) 180 days after pollination were excised by the following protocol. The mesocarp was completely removed from the fruits. The seeds were gently cracked and the embryos surrounded by kernel were carefully trimmed to form a small cube of size 5 mm×5 mm×8 mm and used as explants for culture. RAPD analysis of genomic DNA was carried out using 7 decamer random oligonucleotide primers (OPB08, OPR11, OPT06, OPT19, OPAB01, OPAB09, and OPAB14) obtained from Operon Tech. The RAPD analysis was performed according to the method of Saichon. Each amplification mixture of 25 µl contained 2.5 mM MgCl , 10× *Taq* buffer, 100 µM of each dNTP, 0.3 mM of each primer, 1.5 units of

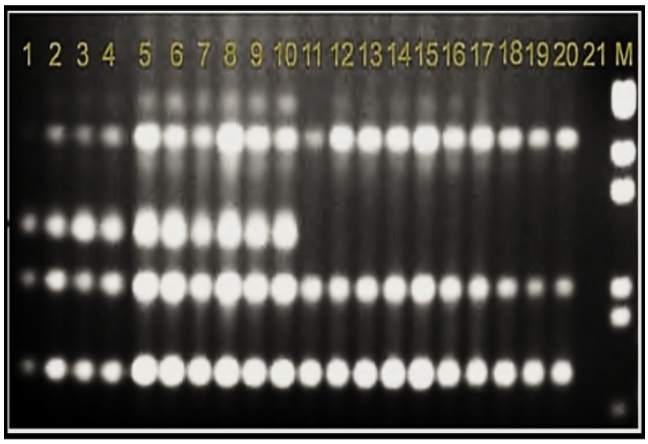


Fig 3: RAPD marker (0pb161193) present in individual male plant (Flora Dade, 1-4) Hybrids (NTH-1, lanes 5-10), and absent in female plant (DVRT-1, lanes 10-20). M.

Taq polymerase, 2 and 20 ng of template DNA. Thawaro and Te-chato (7) revealed that all primers could amplify and provided polymorphic patterns of parents. The number of bands for each primer varied from 8 to 17 with an average of 14 fragments per primer. The size of the ampli?ed products ranged from 100 to 1517 bp. A total of 97 RAPD fragments were scored from the seven random primers. The results revealed that there was only one primer, OPT06 that provided a clear DNA pattern. Results of DNA pattern of the cross 366 (D) ×72 (P) had specific fragment (650 bp) and which could be used to distinguish hybrid between dura and pisifera. All hybrids showed the DNA patterns between the two parents and more additive bands (Fig. 4).

Similarly all SSR primers could be amplified and provided polymorphic patterns of DNA from the parents. The number of bands for each primer varied from 2 to 6 with an average of 2 fragments per primer. The size of the amplified DNA ranged from 100 to 250 bp. Among those primers mEgCIR1772 provided a clear DNA pattern. This primer showed the greatest capacity for distinguishing polymorphic fragments in half-embryo cultured from the parent 366 (*Dura*) and 72 (*Pisifera*). The results from the DNA pattern of the cross 366 (D) ×72 (P) showed a specific fragment and could be used to distinguish between dura and pisifera palms. All hybrids showed the DNA patterns between the two parents and more additive bands according to SSR analysis. One hundred samples were heterozygous with primer mEgCIR1772 showing both a male parent-speci?c band at 115 and 110 bp, and a female parent-speci?c band at 108 and 105 bp (Fig. 5)

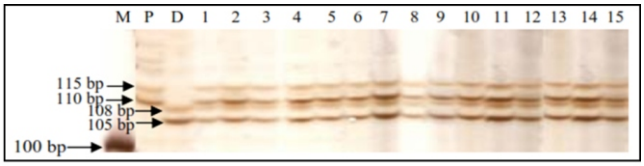


Fig. 5: SSR patterns in hybrids and parents of the cross 366 (D) ×72 (P) obtained with primers EgCIR1772.

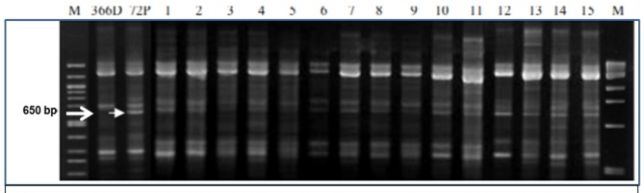


Fig. 6: RAPD pattern of somatic embryo line derived from MZE obtained with primers OPT06. The amplification products were compared on the basis of molecular size. Lane M: standard DNA (100 bp plus DNA ladder). Lane P and D: profile of DNA fragments from parents. Lane 1–15: profile of DNA fragments from hybrids.

Thawaro and Te-chato (7) reported embryos at the globular stage from the cross 366 (D) ×72 (P) were assessed using RAPD. Primers used in this technique were shown to amplify the products of DNA (Fig. 6). Among 15 somatic embryos there was no variation detected in the DNA profile. We concluded that no somaclonal variation occurred in our propagation sys-tem by RAPD marker, and no major genetic changes were observed. This augurs well for the propagation system being of use since somaclonal variation may be minimized or absent altogether.

Finally Thawaro and Te-chato (7) concluded that somaclones obtained from our protocol were uniform according to the RAPD markers. Using RAPD, various authors have reported the absence of genetic variation in trees like *Picea mariana*, *Festuca pratensis*, and *Pinus thunburghii*. Our results suggest that RAPD can be successfully used to assess genetic variations in micropropagated plants. It also demonstrates that genetic integrity of micropropagated plants should invariably be confirmed before transfer of hardened plants to yield.

Advantages of genetic purity

1. It is helpful in plant variety protection, registration, certification and patents
2. to detect the even the minute genetic differences between cultivars visa-a-versa for existence of novelty among essentially derived varieties
3. Assurance of genetic purity for ensuring better agronomic performance and predicted expectations
4. Prevention of misappropriation and willful admixture of seed/ cultivars at commercial or farmers level

CONCLUSION

- Genetic purity analysis is the important factor for quality seed
- For farmer – No loss because of poor seeds + Higher returns
- For producer – Market grip
- Technologies in hand – use for the benefit of humankind.

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