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Short Review article

HYBRID AND VARIETAL GENETIC PURITY TESTING METHODS FOR CROP IMPROVEMENT

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INTRODUCTION

Seeds are regarded as carriers of new technology and serve as basic catalytic input for enhancing the agricultural production. Hence the timely availability of quality seeds of the right variety in adequate quantity decides the strength of an agricultural economy. The intensive crop improvement programmes have resulted in the development of large number of hybrids and high yielding varieties in many important crops. Indian seed market is worth Rs 4,050 crores in which contribution of hybrid vegetable seed is 180 crores.

The genetic purity is one of the most important aspects of quality control. Seed quality includes good germination, purity, and vigour and seed health. With the increase in seed industry, there has been a refinement in the techniques used for testing genetic purity. Methods for testing genetic purity include different morphological, chemical, biochemical and molecular markers.

Need of seed purity testing

- To increase crop production at national level.
- To increase farmers income and standard of living.
- To make IPR (plant breeders right and plant variety protection) part strong.
- For distinctiveness, uniformity and stability (DUS) test.
- Quality control of grains for processing.
- Documentation of genetic resources.

Morphological methods

- Seed morphology
- Examination of seedling
- Examination in green houses
- Grow out test

Chemical methods

- KOH test
- NaOH test
- Ferrous sulphate colour test
- Potassium dichromate test
- Lugol's solution
- HCl
- DDT

Biochemical markers

i. Electrophoresis
   a. Polyacrylamide gel elecrophoresis (PAGE)
   b. SDS-PAGE
   c. Isoelectric focusing (IEF)
   d. Ultra thin layer isoelectric focusing (UTLIEF)

ii. Peroxidase test
iii. Phenol reaction
iv. Serological
v. Chromatography
   a. GLC
   b. HPLC
   c. RP-HPL

Muthuraj *et al.* (1999) found that 18 varieties of soybean showed off type ranging from 4 to 40 percent indicating that these varieties were not pure with respect to peroxidase activity. PAGE of the soluble proteins and esterase were found to be more useful than that of catalase and peroxidase for differentiating the hybrids from their respective parents in cotton (Agrawal *et al.*, 1988). Presence or absence of any particular band helps in demarcation and identification of variety (Singh *et al.*, 2006). Salt soluble proteins of maize were separated into numerous components and it showed great heterogeneity, which indicated that each variety possessed a characteristic PAGE pattern (Wang *et al.*, 1994). Blotting of seed proteins from isoelectrically focused gels provide multiple enzyme staining patterns without considerable loss in resolution, decrease in evolution time and cost (McDonald, 1991). Hybrid purity testing of two-line hybrid rice could be carried out before harvest (Yan *et al.*, 2006*).* UTLIEF of seed protein could replace grow out test to determine F_1 genetic purity of two-line hybrid rice. Using half seeds, the comparison between laboratory and field test was more objective and accurate than using whole seed (Zhao *et al.*, 2005). Compatible host parasite combination can also lead to change in banding pattern (Balsubramanian and Kalyansundaram, 1978).

Molecular markers

- RAPD (Random Amplification of Polymorphic DNA)
- SCAR (Sequence Characterized Amplified Region)
- SSR (Short Sequence Repeats)
- STS (Sequence Tagged Site)

Assessment of genetic purity of hybrid seed based on fertility restorer gene linked to co-dominant STMS marker was found to be more reliable than the non linked one. (Garg *et al.,*2006*).* The markers used for assessing hybrid seed purity should be selected carefully after taking into consideration the varieties grown in adjacent field that can serve as potential pollen donors (Yashitola *et al.*, 2002). Rice hybrids can be identified using RAPD markers by counting the number of bands (Santhy *et al.*, 2003). Jang *et al.* (2004) found that SCAR method was superior to RAPD markers because of simplicity in analysis of band and pellet painting can be done with SCAE markers, eliminating the need for electrophoresis.

CONCLUSION

- Combination of different methods makes them economical and accurate.
- Chemical tests create very less polymorphism and crop specific.
- Hybrid purity testing is possible before the harvesting of crop.
- Application of isozymes is limited due to their less number and crop specific nature.
- Molecular markers which create only single band for identification do not need electrophoresis to be done.
- SCAR method is superior to RAPD marker because of simplicity in analysis of band.
- Pallet painting can be a simple and easy method for testing the PCR amplified product in case of SCAR marker.
- Fertility restorer gene linked STMS markers are more reliable in purity testing as compared to non linked one.

Future thrust

- Development and standardisation of existing technology to make it an integral part in seed testing and IPR.
- Development of low cost purity testing method.
- Identification of maximum number of microsatellite loci in plants, which will help in developing maximum number of polymorphism.

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