Electrophoresis studies of cotton seed soluble protein

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Abstract

Electrophoresis is a technique which separates biological molecules on the basis of charge and mass properties. The separated molecules can be further characterized easily and precisely. The technique is fool' proof and reliable. There is hardly any biological field where this technique is not applied in one way or the other. A protein is a primary product of a structural gene and may be considered as a marker for that particular gene. As genes are a marker for that system, which may be a chromosome or the genome as a whole, hence by considering a sufficient number of protein markers, the structure of the genome can be studied to considerable degree.

Keyword: SDS-PAQE, Isoenzymes, Pisum

Introduction

A protein is a primary product of a structural gene and may be considered as a marker for that particular gene. As genes are a marker for that system, which may be a chromosome or the genome as a whole, hence by considering a sufficient number of protein markers, the structure of the genome can be studied to x considerable degree. For analyzing intra-species relationships (i.e. cultivar differentiation), it is necessary to study those proteins which can exist in multiple forms. The most commonly used are Seed Storage Proteins - which are known to be polymorphic with respect to their size, charge or both parameters in almost all species investigated. Seed and stem proteins are often used to identic epecies/cultivare and/or for the success of hybridization earlier stages of growth in many plant species using sodium dodecyi sulfate polyatrylamide gel eTectrophoresie (SDS-PAQE). Stem/leaf isozymes have also been used for this purpose and they usually give! reasonable good results to separate the plants species/cultivars. The present study was undertaken to investigate the possibility of using electrophoretic technique using leaf proteins to identify - rapeseed cultivate.

The recent rapid development of electrophoretic method for separating small amount of enzymes and seed storage proteins had led to the existence of an array of powerful techniques of great potential, for the application in plant variety identification and seed work. The electrophoretic analysis of proteins and enzymes provides a powerful means of distinguishing between and identifying varieties. It has led to many practical applications in the seed and allied industries (Cooke, 1988). From the point of view of international trade, electrophoresis can be used for testing of varietal identity and purity. This is extremely useful in a commercial quality control context in milling and malting industries. Electrophoresis can also be used in seed testing laboratories to distinguish between similar species and to identify categorically undesirable contaminations occurring in seed lots. In seed certification, protein

electrophoresis can assist in assessing the varietal purity as well as identity and it is perhaps of especially valuable in checking the identity of suspected admixtures and off types.

Biochemical Markers

Development of cultivar-specific genetic markers is desirable for variety identification and protection as well as for seed purity determination. Proteins (including isoenzymes) being the products of gene translation can be regarded as markers for the- respective structural genes. A comparison of the polymorphism, which occurs due to variation in protein composition among individuals could be reflected to the gene expression. Electrophoresis is a technique in which electric current is applied across a medium (polyacrylamide or agarose gel) for the separation of the charged molecules. The current accelerates the mobility and consequent separation of compounds, which appear as distinct bands on staining. The separation is due to differences in the size and charge of proteins involved. The relevance of electrophoresis of the seed and seedling proteins or isoenzymes for cultivar identification and genetic purity testing has been well established and thoroughly reviewed by Cooke, Smith and Smith and several others.

Seed proteins for cultivar identification

Electrophoresis of denatured seed proteins is one of the simplest and routine laboratory techniques used for cultivar identification. Considering its applicability and relevance for cultivar identification, UPOV included the electrophoresis of glutenein as a supplementary test for OUS testing of wheat and barley. ISTA (1992) recommended PAGE technique for cultivar characterization of wheat, barley, pisum and lolium. SDS-PAGE of the tris soluble proteins and salt soluble globulins have been extensively used for cuhivar identification in crops like rice (Bhowmik *et al., 1990;* Abdel *et al.,* 1993d), sunflower (Varier *et al.,* 1992; Sahoo et at, 2000), wheat (Abdel *et al.,* 1993b), sorghum (Abdel *et al.,* 1993a), maize (Wang *et aI.,* 1994a) castor (Varier *et al.,* 1999), chinese cabbage (Zheng *et al.,* 1997), pea (Mishra *et al., 1996),* Soybean (Goyal and Sharma, 1999), Pepper (Odeigah *et al.,* 1999) etc.

Biochemical markers for genetic purity testing

Good and unambiguous morphological markers are generally limited in number, and their expression depends on the developmental and growth stages in the plant. In hybrid seed production, the time between the seed harvest and subsequent sowing is normally short, and so it is difficult for seed producers to test the genetic purity of the seed by conventional methods in such a short period. The characteristics of open pollination also make control of seed purity difficult thus seed contamination can cause heavy losses for fanners. Fast, simple and reliable methods are, therefore desired for assessing the genetic purity of hybrid seed samples. For this purpose, polymorphic markers based on electrophoretic patterns of proteins and iso-enzymes have been used for genetic purity testing of various crop species.

Electrophoresis of isoenzyme analysis has been demonstrated to be useful for the genotypic characterization (Smith, 1988) and superior to field grow out tests for detecting the female selfs in hybrid seed lots (Smith and Wych, 1986). Isoenzyme analysis by electrophoresis for detection of various types of genetic contamination has been attempted by various workers in most field crops *viz.*, maize (Onnan *et al.*, 1991; Bilgen *et al.*, 1995), tomato (Zlokolika *et al.*, 1997), pearl millet (Varier *et al., 1993),* wheat (Tanyolac et *al.*, 1996), pepper (Zheng *et al.,* 1997). In cotton, Agrawal *et al.* (1988) suggested the possible use of esterase isoenzyme profile in genetic purity testing of hybrids H-4 and H-6.

Many important plant species comprise a great number of varieties or cultivars, some of which are closely related. In case of agronomically valuable crop species, it may be considerable economic

importance to be able to distinguish different cultivars, some plant species, shows distinct differences between closely related varieties or cultivars. However, some commercially important crop species here are so many cultivars listed that in spite of evident higher yield capacity, new cultivars are not readily accepted because of lack of distinctness in the traits accepted for characterization.

For registration varieties of a given species are tested for distinctness homogeneity and stability and described using certain set of morphological characteristics such as plant height, leaf colour etc. As far as possible their morphological characteristics are taken for field inspection and seed testing on the certification procedure for a guide identity check in case of doubt in certification as well as for commercial purpose. A precondition for the effective use of electrophoresis at the different level was harmonized well defined testing system which used only those gene loci and electrophoresis is method which enable a clear distinction of the bands used and descriptions of their presence or absence.

A comparison of banding pattern for the absence of a particular band or presence of additional protein band with the standard profiles gives an indication about the genetic purity of seeds. In grow out test, off types are to be detected on the basis of plant growth habit and petal colours. Cotton cultivars were successfully distinguished by fuzz characters (colour, intensity, position and length of hairs) and by esterase, catalase and peroxidase patterns given by electrophoresis of seed homogenation. The evidence obtained from the examination of seed protein spectra of gossypium species was consistent with that from genetic, cytological, morphological and phenogenetic investigation regarding the origin of new world cultivars of cotton. The electrophoretic technique though is reliable, it does not seem likely to replace grow out test. However, in specific situation disputed samples requiring a quick decision, this could be used to determine genetic purity of seed lots. The technique needs experienced man power, financial support and good quality analytical facilities. The replicated field observations are time consuming, expensive and unreliable morphology can-not provide information on the purity of specific, genetic attributes that relate to grain, quality or to pest or herbicide resistance bred into varieties. Biochemical assays, including isohytes can distinguish varieties within several species.

Conclusion

The electrophoretic patterns of cotton genotypes under study were unique. Genotypic differences were revealed by the presence or absence of particular band in the electrophoregramms. The qualitative and quantitative variations were observed in the banding patterns of cotton seed protein studied. Seed proteins that showed genetic variation may be used as probes to mark the varieties or cultivars. Electrophoresis were collaborative indicating seed protein electrophoresis can be used for testing genetic purity.

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