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Characterization of blackgram [*Vigna mungo* (L.) Hepper] using total seed protein separation by SDS-PAGE

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SUMMARY

Electrophoresis of seed proteins is a very useful technique for classification of the closely related genotypes of most of the agricultural crops. Genetic relationship was evaluated among thirty-four varieties of Urdbean by employing SDS-PAGE for seed protein analysis. A comparative study of electrophoretic patterns of the proteins extracted using tris and phosphate buffer were analyzed on SDS-PAGE. Proteins extracted using phosphate buffer produced quantitative and qualitative differences among the varieties whereas proteins extracted using Tris buffer did not have much variations. The differences were either in the total number of protein bands present, molecular weight or intensity of the bands. SDS-PAGE generated a total of 22 bands of which polymorphisms were observed mostly in the region between 14-38 Kda. All the varieties studied exhibited unique banding pattern for phosphate soluble proteins.

Key words : Blackgram, Genetic relationship, SDS-PAGE, Seed protein, Varietal characterization.

Urdbean or blackgram [*Vigna mungo* (L.) Hepper] belongs to the subgenus *Ceratotropis* of the genus *Vigna* and is an important pulse crop with short duration and wider adaptability. Urdbean rank fourth in terms of area and production in pulses. Currently, the area of urdbean under cultivation in India is 3.15 million hectares with an annual production of 1.33 million tonnes. It is native to North Eastern India and Burma regions of Asia. Blackgram forms an important source of proteins, mainly in vegetarian diet of the Indian subcontinent. Blackgram is generally considered to be more resistant to diseases and shattering and have higher methionine content in seed than mungbean (Santalla *et al*., 1998).

Hence, the varietal characterization and identification of urdbean is still attracting the attention of breeders, farmers, the seed industry, certification agencies, seed testing laboratories and breeders' right protection institutions with the main objectives of determining the extent to which a seed sample confirms to a given cultivar and to assure the quality of seed marketed to the consumer. Hundreds of cultivars are now included in national varietal lists, that require increasingly detailed data to distinguish one cultivar from another, thereby making identification an essential requisite for inclusion in varietal lists (Faccioli *et al*., 1995). During the seed production programme, the varietal purity is affected by a number of factors such as cross-pollination, mechanical mixtures, genetic shifts and selective influence of disease etc. The maintenance of genetic purity of released varieties is of utmost importance in seed production programs. The choice of a technique depends on the species under study and the purpose of identification. Traditional approaches to varietal identification involve the study of morphological characteristics, which is not only time consuming and costly, but also affected by the environment.

The difference between varieties should be based on the gene differences but direct comparison of genes is difficult and time consuming. Moreover, the differences can be measured by comparing the product of gene activity *i.e*. by using protein as genotype marker. The genotype controls morphological, biochemical and processing characters, some of which are constant (characters of DNA and proteins, especially kernel storage proteins), while others (for example, morphological or technological properties) may reveal slight modifications in response to environmental variation.

The attraction of utilizing biochemical methods in taxonomy is that they enable an ever-closer examination of the genotype, as opposed to the phenotype and hence environmental influences can be minimized. The relationship between chemical composition and genotype, which occurs at several different levels, was described by Zuckerkandl and Pauling (1965).

Proteins, because of their easy accessibility, are a reasonable compromise between direct studies of the genotype (primary or secondary semantides) and of the gene products (tertiary semantides) and thus protein profiles of cultivated varieties provide fingerprints that are stable descriptors of genotype. Proteins are molecules with net electrical charges that are affected by pH and

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