SEED PROTEIN PROFILE AS SUBSTITUTE FOR SORGHUM CULTIVAR IDENTIFICATION BY GROW OUT TEST (GOT)

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ABSTRACT

Conventionally Grow out Test (GOT) is used to assay the purity of sorghum hybrid seed lots on a representative sample of the seeds. In GOT, plants are grown up to maturity and several morphological and floral characteristics are assessed to distinguish the hybrid. The hybrid seed produced is not immediately distributed to the farmers for raising the crop but GOT is performed in succeeding season to check the purity of hybrid seeds. This entails a lot of cost in terms of locked-up capitals and problems of storage. With the objective of replacing the GOT with biochemical (protein) assays, A-line (cytoplasmic male sterile), B-line (maintainer), R-line (restorer) and H-line (hybrid) have been screened by means of protein markers for polymorphisms. In addition to this, eight open pollinated (pure line) varieties have also been screened by means of protein polymorphisms. A simple electrophoretic procedure for detecting purity of hybrids, their respective parents and varieties has been standardized. Seeds of the selected hybrids and their parents and varieties of sorghum (*Sorghum bicolor* (L.) Moench) have been used for assays. Electrophoretic protein profiles could be efficiently used for distinguishing varieties, hybrids and its parents and could be used as substitute of GOT.

Key words: Electrophoresis, Grow Out Test, Protein profile, Sorghum, Zymogram

INTRODUCTION

Sorghum is the fifth important cereal crop in world (FAO 2004) after rice, wheat, maize and barley. Together with maize and pearl millet, it constitutes the most important cereal crop for semi-arid tropics (SAT). It is third important cereal crop grown in India following rice and wheat. Maharashtra is the largest producer of sorghum. In self-pollinated crop like sorghum, one of the challenges is the production and supply of adequate quantities of pure hybrid seed to the farmers. Purity of sorghum hybrid seed lots is assayed conventionally by Grow Out Test (GOT) on a representative sample of the seed that is to be marketed. The GOT involves growing plants to maturity and assessing several morphological and floral characteristics that distinguish the hybrid. Moreover, GOT can be subjective; several aspects of plant phenotypes (morphology, yield etc.) can be affected by environmental conditions (Yashitola *et al.* 2002). In addition to this GOT requires large-scale field facilities.

Thus there is need for an assay to assess genetic purity of seed of hybrids and varieties that is both accurate and faster. Biochemical markers can be applied for this purpose. Several investigators have emphasized the importance of protein and enzyme electrophoresis for the identification of individuals and cultivars of different species such as wheat (Shewry *et al.* 1978[b]), barley (McDaniel 1970), oats (Cooke and Draper 1986), rice (Iwasaki *et al.* 1982), maize (Goodman and Stuber 1980), soybean (Larsen and Benson 1970), *Brassica* (Wills *et al.* 1979), and cotton (Cherry *et al.* 1970; Kapse and Nerkar 1985). Such studies authenticate the genotypic basis of qualitative variation and validate the use of protein/enzyme variation in varietal identification (McKee 1973; Douglas 1983). Proteins and enzymes are the primary products of the genes and hence are the most suited for genetic purity determination (Niejenhuis 1971).

For comparison of varieties, the tissues sampled must be of similar physiological age and condition. A simple way of achieving this will be to use seeds (Buttery and Buzzell 1968). The protein and enzyme species are also least affected by the plants growing environment (Adriaanse *et al.* 1969; Zillman and Bushuk 1979[a]; Fedak and Rajhathy 1972; Sarkar and Bose 1984; Hussain *et al.* 1986) thus imposing no serious limitation on the use of protein/ isozymes in varietal identification. The present investigation has been carried out with an objective of evaluation of seed protein as a substitute for sorghum cultivar identification by GOT.

MATERIALS AND METHODS

Sorghum Lines

The experimental material consisted of eight varieties viz., CSV 15, SPV 669, PVK 400, PVK 801 (obtained from Sorghum Research Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola), BTx623, IS 18551, R16 and E36-1 (obtained from International Crop Research Institute for Semi-Arid Tropics, Patancheru) and four hybrids (and their parents along with B-line) viz., CSH 14 (ms14A × AKR 150), CSH 9 (ms 296A × CS 3541), CSH 18 (IMS 9A × Indore 12)

and SPH 840 (ms70A × ICSR 89058) obtained from Sorghum Research Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Seeds were sown in well-isolated plot. Crossing among the respective pairs of seed parents and pollinators were effected to obtain hybrid seeds, while each A-line was maintained by crossing with its respective B-line. B-lines, R-lines and varieties were maintained by selfing. The seeds thus obtained were used for investigation. While seeds of varieties IS 18551, R 16 and E 36-1 obtained from ICRISAT were directly used for protein and enzyme extraction.

Protein Extraction

Protein were extracted from seeds of selected genotypes using 1.5 ml sodium phosphate buffer (0.1M, pH-7). The extract was transferred to 1.5 ml micro vials. The samples in tube were centrifuged at 10,000 rpm for 45 minutes at $4^{o}C$ using Remi (C-24) cooling centrifuge. The clear supernatant was collected and used as protein source for electrophoretic studies. Entire extraction procedure was done under cold condition. Proteins in the extracts were estimated by the method suggested by Lowry *et al.* (1951) using alkaline copper and Folin-phenol reagent. Bovine serum albumin was used as standard. Each sample was measured in triplicate to minimize the error.

Protein gels were stained with coomassie brilliant blue Wet gels were scanned in Bio-Rad Gel doc system and band attributes were analyzed using Gel Doc EQ software (Bio-Rad make).

Table 1. Low range protein molecular weight markers

Sr.No.	Protein molecular weight marker	Molecular weight (Da)
1	Phosphorylase b	97000
2	Serum albumin	66000
3	Ovalbumin	45000
4	Carbonic anhydrase	30000
5	Trypsin inhibitor	20100
6	α –lactalbumin	14400

Table 2. High range protein molecular weight markers

Sr.No.	Protein molecular weight marker	Molecular weight (Da)
1	Myosin	220000
2	α –2-macroglobulin	170000
3	B-galactosidase	116000
4	Transferrin	76000
5	Glutamate dehydrogenase	53000

Da - Dalton

RESULTS AND DISCUSSION

Qualitative and quantitative differences in the banding patterns of the different sorghum genotypes were analyzed. Qualitative differences were based on the presence or absence of the specific bands in the profiles. Quantitative differences were determined on the basis of staining intensities (peak intensities) of the bands in question. The peak intensities were defined as: dense (191-230), medium (151-190), light (121-150) and faint (70-120) on the basis of values of peak intensities obtained after analysis of gels in Gel doc software (Bio-Rad make). Table 3 shows the band attributes of protein. Electrophoretic patterns of water-soluble seed proteins (albumins) of the material under study were obtained by SDS-PAGE. Some bands, which were faint on the gels, are not visible in the photographs (Fig. 1 and 2). However, these bands have been depicted in zymograms (Fig. 3). Consistent results were obtained in two sample runs from the single seed extracts of ten different seed sample in each cultivar. The overall differential banding pattern of seed albumins reveals great variation in the number and intensity of bands among the different species and cultivars (Fig. 3). The number of bands in each genotype ranged from 13 to 19. No two cultivars were exactly alike, when qualitative and quantitative variations were studied in them. Thus, each genotype exhibited unique type of banding pattern. This method of study is like fingerprinting of cultivars and can be considered for determination of genetic purity of cultivars.

The qualitative results of female A-line and B-line obtained in this investigation are contradictory to that

obtained by Tripathi *et al.* (1983). In the present investigation A-line and B-line had same number of bands of seed albumins having same R_f values. Tripathi *et al.* (1983) reported different numbers of bands in A-line and B-lines.

In other crop plants also similar results were reported such as wheat (Shewry *et al.* 1978[b] and Zillman and Bushuk 1979), rice (Siddiq *et al.* 1972), maize (Scandalios 1969), beans (Hussain *et al.* 1986) and cotton (Cherry *et al.* 1970; Ibragimov *et al.* 1973; Zapruder *et al.* 1980; Kapse and Nerkar 1985).

It is estimated that for every 1% impurity in the hybrid seed, the yield reduction is 100 Kg per hectare in rice (Mao *et al.* 1996). So the hybrid sorghum seeds are beneficial to the farmers only if their genetic purity is maintained and if sufficient quantity of pure seed is available for cultivation in time. Seed certification agencies, seed companies and rules and regulations for seed production and distribution are aimed at maintaining genetic purity of the seed. Seed certification agencies and seed companies ensure the genetic purity of the hybrid seeds by conducting conventional Grow Out Test (GOT) in the field. But these grow out tests are time consuming, laborious, tedious and cumbersome. The GOT requires large-scale field facilities and also affected by natural calamities. Sometimes limited number of morphological characters may create problems in identification of genotype. Due to these factors there may be non-availability of high quality hybrid sorghum seed in time to the farmers. In India, hybrid seed production is contracted to farmers by seed companies and the produce from single farmer (2-10 Mg at an average of 2 Mg ha^{-1}) is being considered as one seed lot for purity purpose. A sample of 400 seeds is collected randomly from each seed lot for conducting GOT (Verma 1996). A similar sample size can be used for estimating seed purity by tests such as electrophoresis of proteins. These tests are easy to carry out and large number of samples can be handled within a very short period of time in laboratory.

CONCLUSIONS

Electrophoretic protein profiles could be efficiently used for distinguishing varieties, hybrids and its parents but certain limitations were also recorded like qualitative differences were limited. In many cases hybrids and its parents lacked specific bands. Quantitative differences could not be reproduced, as they were not consistent when the experiment was repeated. It was very difficult to identify and count faint bands, which would require lot of skill and efficiency. Besides these limitations if the method has been standardized according to laboratory requirement, electrophoretic protein profiles could be used as substitute of GOT.

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