Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 2 (17-24): 1188-1191, 2003
ISSN 1682-3974

Genetic Characterization of Some Maize (*Zea mays* L.)Varities Using SDS- PAGE

Asad Hussain Shah, Muhammad Fareed Khan and Abdul Khaliq
University College of Agriculture Rawalakot Azad Kashmir, Pakistan

Abstract: Four local and two commercial maize varieties were used as experimental material, to evaluate the biodiversity among them by using SDS-PAGE technique. Variations were observed in protein profile shown by all of them and some similarities also. Kisan-90 was different from the rest, as it was variable in the banding pattern. There were similarities in Kotli White and Sarhad White, where as they shared two common bands. Poonch Yellow and Kotli Yellow had similarity at least for one band. All the rest of accessions were different. Kisan-90 however was prominent in protein profile in the gel.

Key words: Maize, SDS-PAGE, kisan-90, kotli white, poonch white, pooch yellow, sarhad white, kotli yellow

INTRODUCTION

Maize (*Zea mays* L.) being the highest yielding cereal crop in the world, is of significant importance in the countries like Pakistan, where rapidly increasing population has already outstripped the available food supplies. In Pakistan maize is third important cereal crop after wheat and rice. It covers 0.94 m h area in Pakistan and gives grain production of 1.56 m tone. (Anonmous, 2000) Maize is also a major crop of Azad Jammu and Kashmir with about 0.122 million hectare of crop being cultivated during kharif. (Anonymous, 2002). In addition there are many other industrial uses of maize. Corn has greater potential of high yield and requires less efforts than any other cereal crop (Abdullah *et al.*, 1999).

Human beings have always depended on plants to meet a wide variety of needs. Several species were domesticated in time and thus spread with dispersing human communities, evolving variously into distinct races under diverse environmental conditions and the different methods of cultivation adopted in the various parts of the world (Larik, 1994). Germplasm is vital resource in generating new plant types having desired traits that help in increasing production and thus improve the level of human nutrition. The important germplasm has been made to destroy without to any regard to their potential, economic, aesthetic or biological significance. This biodiversity is the main hulwark between mankind and starvation. (Anonymous, 1995). The problem of erosion of crop genetic resources has arisen in part because in order to meet ever increasing food and fiber needs aggressive agricultural extension is promoting the cultivation of modern cultivars in the remotest areas of the world, resulting in the loss of land races. The cause of concern is the permanent loss of genetic material which could be used for resistance to pests and diseases and adverse climatic and soil conditions in modern advanced cultivars. They could also be used as a reservoir of genetic material which may be needed by future generations for breeding crops to meet new challenges posed by the highly unstable climatic conditions triggered by global warming (Larik, 1994). Local germplasm provides greater genetic variability and additional sources resistance to various biotic and abiotic stresses. Characterization and evaluation of crop germplasm is imperative for identification of desirable genotypes for utilization in crop improvement program (Anonymous, 1995). The genetic characterization of individuals and the interrelationship between them has for many years been determined by presence of morphological characteristics or by use of biochemical tests. Developments and refinements have led to the present day situation where a whole array of molecular technique are available to characterize the individual on genetic basis. Protein fingerprinting is one of the such techniques. It plays a vital role in the development and integration of method, based upon the integration of molecular genetics into classical breeding programs. The total cell proteins are extracted from the plant parts leaves, shoot or seeds and then subjected to electrophoresis. PAGE, has made more sophisticated studies possible. It enables us to identify variation in the physical and chemical properties of proteins (Gardner *et al*., 1991).

The carbohydrate is formed into a gel, saturated with a buffering solution and attached an electric current.

The individual protein in the protein sample can be separated from each other by passing through the gel. Protein samples are inserted in the slots at one end of the gel which are moved through the gel by electric force. The rate of movement is determined primarily by the electric change on the protein and this in turn, depends on the protein's amino acid sequence. However for resolving

proteins mixture, proteins are exposed to the ionic detergent SDS (Sodium Dodecylsulfate) before and during gel electrophoresis. SDS denatures proteins, causing multimeric proteins to dissociate into their subunits and all polypeptide chains are forced into extended confirmations with similar charge mass ratios. SDS treatments thus eliminates the effect of differences in shape, so that chain length, which reflects mass, I style sole determinate of migration rate of proteins in SDS Polyacrylamide electrophoresis. Moreover, the molecular weight of a protein can be estimated by comparing the distances that proteins of known molecular weight migrated (Lodis *et al.,* 2000). Protein fingerprinting assisted the plant breeder to select characters expressed at any stage of development by involving genotype, not the phenotype. Although the technique is presently laborious and analysis to assemble the maps is complicated. It enables that breeders to handle larger progenies with more certainty and efficiency. Polyacrylamide Gel Electrophoresis is commonly used method in Plant Breeding and Genetics Department Laboratory at University College of Agriculture Rawalakot A.K. Shah, 1999. Wallace *et al.,* 1990 used SDS- PAGE Technique for extraction and analysis of protein in maze flour. They found that this method was more quantitative than the traditional Landry Moureaux Procedure. The analysis identified a major biotin containing polypeptide in the enzyme preparation. Barros *et al.* (1990), in an experiment purified and characterized four proteinases from the endosperm of germinating maize. They used SDS PAGE for the purpose. Esquivel *et al.* (1986) collected 32 samples of crop plants, which were, threatened by genetic erosion in a province of Cuba. It was concluded that genetic diversity was great in maize. Rosales and Taba, (1988) proposed that the computerized information of a gene bank could be more helpful. Webber and Alexander, (1975) reported the genetic variation in maize on the basis of fatty acid profile. Smith *et al.* (2000), concluded that plant breeders could make the efforts in the improvement of seedling vigor including screening for genetic components of crop.

MATERIALS AND METHODS

The experimental material was comprising of six maize varieties. Four out of six were local varieties of Kotli and Poonch districts of Azad Kashmir.

The rest two were open pollinated commercial varieties. The name of the varieties are as follows:

Kotli white	(Local)
Kotli yellow	(Local)
Sarhad white	(Commercial variety)
Kisan-90	(Commercial variety)
Poonch white	(Local)
Poonch yellow	(Local)

SDS-PAGE: The SDS System developed by Weber and Osborn (1969) was used for the electrophoresis. The stacking and separating gels, staining and destaining solutions were made according to the protocol. Apparatus was assembled and gels were pored in between glass slabs. Spacers were placed in between the gels to make the space. Comb was cleaned with ethanol prior to use. It was placed just prior to polymerization of the gels. Comb was removed from the plates. The wells made in the stacking gel were washed with distilled water to avoid any unpolymerized gel.

Sample of seed protein with a drop of bromophenoleblue, was loaded in wells carefully to avoid the mixing of sample is same wells. Buffer was mounted to the gels. A current of constant voltage was given to the apparatus. The protein molecules according to the molecular weight traveled towards the opposite charge in the electric field. As soon as the dye reached to the bottom of the gel, current was stopped and glass slabs were separated from each other carefully to avoid the breakage of gels. Soon after, that gel was subjected to staining solution prepared earlier and kept on mechanical rotator for 30 to 40 minutes. After the completion of staining the gel was subjected to destaining solution, again kept on mechanical rotator for 40 minutes. After destaining the gel was analyzed visually against the transparent sheet to record the profiles.

Gel photography: The photographs of the gel were taken in the end.

RESULTS AND DISCUSSION

The banding pattern drawn from the photograph of gel is quite evident of variation among genotypes/ varieties on molecular level as well. Eight bands were visible length wise in the gel. The method has already been used by Shah (1999).

All the varieties expressed are band each at the distance of 0.5 cm (Table 1). which reflects the fact that all the varieties are belonging to same species (*Zea mays* L.). Kotli white and Kisan-90 were different from the other as they expressed one band each at the distance 0.7 cm in the protein profile. These varieties share one common band at the distance 1.2 cm, which further confirm, the presence of common genes in them. All the varieties were similar at the distance 0.8 cm in the gel and shared on

Table 1: Diagrammatic Sketch of the banding pattern of protein profiles

Varieties						
Distance traveled (cm)	Kotli White	Kotli Yellow	Sarhad White	Kisan-90	Poonch White	Poonch Yellow
0.5	+	+	+	+	+	+
0.7	+	-	-	+	-	-
0.8	+	+	+	+	+	+
1.0	-	-	-	+	-	-
1.2	+	-	-	+	-	-
1.3	-	+	-	-	-	+
1.4	+	+	+	+	+	+
1.5	-	+	-	+	+	+
1.6	+	-	+	-	-	-

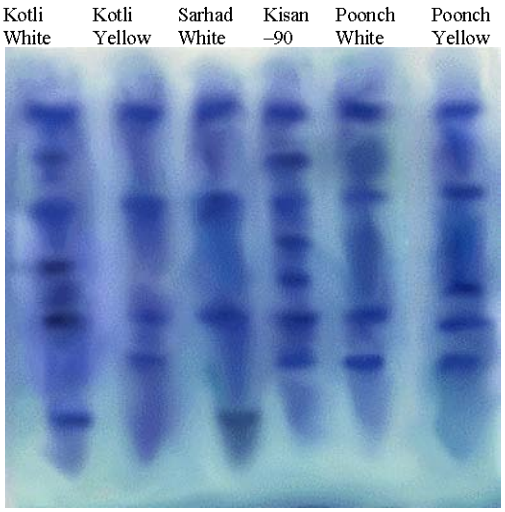

Fig. 1: Photograph of the Gel showing protein bands in the profile

common band at this distance. (Kisan–90) was different from all the others as it was prominent with solitary band in protein profile at 1.0 cm distance in the gel. A varital comparison was conducted in France by Fahmy, 1991, using SDS-PAGE. Variety Poonch yellow and Kotli yellow Poonch yellow and Kotli yellow expressed one common band at 1.3 cm distance in the gel. (Fig. 1) This common band may had reflected the common seed coat color, i.e., yellow. The same technique was used by Kazmi, 2001 to study diversity among some wheat genotypes.

Kotli white, Kotli yellow, Sarhad white, Kisan-90 and Poonch white varieties had common band at 1.4 cm distance, which is evident that these varieties have similarity on the basis of same specie.

Varieties Kotli yellow, Kisan-90, Poonch white and Poonch yellow had one band each at 1.5 cm in the gel, which is may be due to some similarity of genetic makeup.

At this distance Kotli white and Sarhad white had no common band, which reflects that, these varieties are quite different from others in plant height. These varieties had number of grains/row in close proximity with Kotli white (40.41) grains/row. Kotli white and Sarhad White had another common band at 1.6 cm distance in the gel shown in Fig. 1

These results are reflecting that some genotypes are different from one another and some are similar. However the exploitation of variable germplasm can lead to the evolution of new maize varieties.

REFERENCES

Abdullah, K.K., 1999. Corn (Maize) response to different methods of nitrogen fertilizer placement periodically at Quetta. Sharhad J. Agric., 15: 164.

Anonymous, 2002. Maize in Pakistan, Pakistan Agriculture Research Council, Islamabad, Pakistan.

Anonymous, 2001. Agric. Statistics of Pakistan, 2000.

Anonymous, 1995. Plant Genetic Resource Institute. Annual Report, pp: 4.

Anonymous, 1983. Vertification trial on maize. NARC Islamabad, Pakistan. Annual Report, pp: 80.

Larik, A.S., 1994. Genetic resources and their conservation in crop plants. Plant Breeding by National Book Foundation Islamabad, Pakistan, 235, 236, 250.

Barros, E.G.-de, Larkins and E.G.D.E. Barros, 1990. Purification and classification of Zein Degrading poteases from endosperm of germinating maize seeds. Plant Physiol., 94: 1: 297-203.

Esquivel, MA. and J.J. Perez, 1986. Germplasm collection in Western Cuba. Plant genetic resource news letter, 66: 14-15.

Fahmy, W.G., J. Aufere and E.L. Shazly, 1991. Comparison between the mechanism of protein degradation of two cereals by enzymatic and insitu methods, using gel electrophoresis. Animal Feed and Technol., 35: 115-130.

Gardner, J.H., J.M. Simons and P.D. Snorted, 1991. Principles of Genetics, pp: 567-568.

Kazmi, R.H., 2001. Screeing of wheat varieties for drought resiatance Using SDS-PAGE. B.Sc. Thesis/project Report University College of Agriculture Rawalakot, Azad Kashmir, Pakistan, pp: 13-19.

Lodis, L. Zipursky., P.B.S.l.A. Matsudair, D. Baltimore and E.J. Darnell,. 2000. Molecular cell biology Jakkon, J.R. Kiniry and G.F. Arkin, 1989.

Rosales, P.M. And S. Taba, 1988. A local germplasm bank information management system. Recent advances in the conservation and utilization of genetic resources. Proceedings of the global maize germplasm workshop CIMMYT, Mexico, pp: 6-12.

Shah, A.H., 1999. Evaluation of Biological diversity in Mentha *Spp.* from Azad Jammu and Kashmir, using protein finger printing techniques. B.Sc. Thesis/ Project Report, University College of Agriculture Rawalakot, Azad Jammu and Kahsmir, Pakistan, pp: 18–24.

Smith, 1986. Genetic diversity within the corn belt dent racial complex of maize (*Zea mays*) Maytica,.31: 349-367.

Wallace, J.C. M.A. Lops and Pavia, 1990. New Methods for extraction and quantitization of zeins revail a high content of gamma zeins in modifies opaque 2 maize. Plant Physiol., 92: 191-196.

Weber, E.L. and D.E. Alexander, 1975. Breeding for lipid composition in corn. J. Amer, Oil Chem. Soc., 52: 195–200.