MOLECULAR CHARACTERIZATION OF DIFFERENT ADVANCED WHEAT VARIETAL LINES USING SDS-PAGE ELECTROPHORESIS DATA OF SEED STORAGE PROTEINS

***PATIL, A. S., GILANDE, H. G., SHIVANGIKAR, A. N., MANE, V. A. AND CHOUDHARY, R. S.**

K. K. WAGH COLLEGE OF AGRICULTURAL AND BIOTECHNOLOGY SARASWATINAGAR, NASHIK-422 003, MAHARASHTRA, INDIA

****Email: abhinandan_patil25@rediffmail.com***

ABSTRACT

The present study was undertaken to evaluate the genetic diversity in gluten subunits and protein content in seed endosperm of wheat for nutritional grading of 17 advanced varietal lines of wheat using SDS-PAGE, which is useful for agricultural development regarding quality food stuffs and breeding purposes. After calculating the standard factor in spectrophotometer for unit absorbance at 595 nm and the amount of protein were calculated. The wheat lines PZ-TS-5, PZ-TS-4 and PZ-TS-9 contained maximum amount of HMW-Gs proteins as compared to rest of the lines. By comparing with the six bands of the standard marker, band numbers 4 were common in all 17 wheat lines, but the other bands showed variation. Most frequent HMW protein bands had mole. Wt. 29.03 KDa marker band, while, in LMW protein bands most frequent bands were among the 14 KDa marker band. In cluster analysis, the above mentioned 3 wheat lines grouped in the same cluster showed that they were originated from the same parental line and genetically less diverged.

KEY WORDS: Cluster analysis, genetic diversity, protein content, SDS-PAGE, wheat

INTRODUCTION

Wheat is counted among the 'big three' cereal crops, with over 600 million tonnes being harvested worldwide. For example, in 2011, the total wheat harvest was about 85.93 million tonnes in India, from the production areas 29.25 million hectare. In Maharashtra, the yield of wheat was 1730 kg/ha (http://www.indiastat.com/table/agriculture/2/wheat/17195/7400/data.aspx). There is no doubt that the adaptability and high yields of wheat have contributed to its success, but these alone are not sufficient to account for its current dominance over much of the temperate world. The key characteristic which has given it an advantage over other temperate crops is the unique properties of doughs formed from wheat flours, which allow it to be processed into a range of breads and other baked products (including cakes and biscuits), pasta and noodles, and other processed foods. These properties depend on the structures and interactions of the grain storage proteins, which together form the 'gluten' protein fraction (Shewry, 2009). It is, therefore, very essential to study protein profiling of wheat varietal lines.

Variation is the key for any change, so the key for genetic change is genetic variation. The high chance of success in a breeding

program follows the availability of a wide variety of appropriate materials for the breeder to choose from. Selecting appropriate parents for breeding purpose requires a good understanding on genetic variation and on classification of germplasms.

Wheat is important due to gluten features. The sticky part is the endosperm strict proteins and it causes the stretching or expansion of fermented dough, the term is peeling. When peeling is heated to bread production, this part of protein has been linked and it holds together. Only wheat and lesser extent of rye and triticale hold the property (Arzani, 2001).

Glutenin subunits with high molecular weight can be used to identify cultivars as a supplementary indicator (Maragheh, 2013). Subunits can also are applied as an index for breeding projects to select parents and results with stronger gluten properties (Payne *et al.*, 1982). In recent years, Polyacrylamide gel electrophoresis (PAGE) grains storage proteins has been valid method for assessing the chemical diversity of the wheat. This method may be used in order to collect germplasm where have considerable genetic diversity or in areas where there is expected a large variability (Dominici and Grottanelli, 1984). In the quality modification programs, choose the lines is permissible based on glutenin components with high molecular weight that are controlled by different locus. In the present investigation 17 wheat lines were evaluated to study the genetic diversity based on seed storage proteins through constructing the dendrogram for high molecular weight (HMW) and low molecular weight (LMW) of gluten subunit bands.

MATERIALS AND METHODS

Seeds of 17 wheat lines obtained from the Department of Agriculture Botany, K. K. Wagh, College of Agriculture, Nashik, were used for molecular characterization. The well dried healthy seed was grinded to powdered form. Bradford (1976) assay was made to determine the wheat endosperm proteins. Proteins were extracted by adding 400 µl protein extraction buffer (0.05 M Tris-HCL, pH 8.0, 2% SDS, 5 M Urea, 1% β-Mercaptoethanol, 100 µl Methylene green dye) in about 10 mg powdered endosperm in 1.5 ml eppendorf, keep overnight at room temperature and spun at 13000 rpm for 15 min at room temperature and supernatant was taken into new Eppendorf to store at -20°C. Profiling of proteins was made by one dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (30% separating gel & 8.9% stacking) following Dual in a mini gel apparatus (Damania *et al.*, 1983). The gels containing protein samples with protein molecular weight markers allowed to run on 100 V until green line of Methylene Green dye reach to the bottom of the gel plates. The separating gel was kept overnight in staning solution and then destain until bands sharpened. Finally the gel was analyzed in gel documentation system (Bio Rad Instruments, Italy). In the data matrix, the presence of a band was scored as 1, whereas the absence of the band was coded as 0 in the electrophorogram. The basic data structure finally consisted of a binary (0/1) data was analyzed and genetic diversity was measured. Hierarchical cluster analysis was made by the complete linkage method with Euclidean distance proteins in different wheat lines. Cluster analysis of wheat grain storage proteins was performed on the results of SDS-PAGE (Sneath and Sokal, 1973) using the software MVSP (UPGMA) to find out the diversity among these wheat lines.

RESULTS AND DISCUSSION

Wheat has gained tremendous attention being the major food crop. A wheat improvement activity through molecular breeding is very essential for developing and releasing superior quality wheat varieties. Also the study of genetic behaviour on the basis of protein profiles of the parents for high yielding wheat lines plays very important role in

selection. It is therefore concluded that seed storage protein profiles could be useful marker in cultivar identification, registration of new varieties, cluster analysis and in studies of genetic diversity, thereby improving the efficiency of wheat breeding programs in cultivar development.

The amount of proteins was estimated in 10 mg of wheat seed sample using spectrophotometer, after calculating the standard factor for unit absorbance at 595 nm. Results revealed a wide variation in seed storage proteins in 17 wheat lines (Table 1). Protein content of these wheat varieties ranged from 32ug/ml to 103ug/ml. The minimum seed protein content was found in the variety PZ-TS-3(32 µg/ml), while the maximum protein content was observed in wheat varietyPZ-TS-5 (103µg/ml). The high protein content was also observed in the variety PZ-TS-14 (93µg/ml) and PZ-TS-6 (84µg/ml).

In this study, SDS-PAGE of grain storage proteins was performed in order to analyze molecular weight of gluten subunits and investigate genetic diversity among different wheat lines. Molecular weight analysis of proteins in the seeds of wheat varieties are given in Table 2 and the electrophorogram showing protein banding pattern of different wheat varieties are given in Figure 1. The seed storage protein patterns of each sample were determined by examining the molecular weight of the bands and intensity of staning. The relative proportion of each band was calculated on the basis of 6 bands of standard marker (in the range of 14.3-97.4 KDa). By comparing with the six bands of the standard marker, band numbers 4 were common in all 17 wheat lines, but the other bands showed variation. Most frequent HMW protein bands had molecular weight of 29.03 *KDa* marker band, while, in LMW most frequent protein bands were among the 14 *KDa* marker band. These banding patterns were confirmed with results of Zeb e*t al.* (2006), Khan *et al*. (2007), Shuaib *et al.* (2007), and Maragheh (2013).

Dendrogram obtained from cluster analysis following UPGMA method in 17 different wheat promising lines based on electrophoresis' banding patterns is given in Figure 2. Genetic diversity of European spelts wheat was evaluated by constructing the dendrogram for HMW and LMW glutenin subunit bands (Xueli *et al.*, 2005). The dendrogram as a whole revealed low genetic diversity at proteins level . The diagram revealed two main groups; the group 1 has eight lines and group 2 has nine wheat lines. At Eucladian distance of 1.75, all the varieties showed similarity, but at Eucladian distance of 1.5, all the wheat lines split into two major groups that were further divided into subgroups and mixed trend was seen in grouping of lines. All these lines posses same protein due to which they group into same cluster, but only differ in their protein concentration (µg/ml) and showing lower dissimilarity among them. Similarly, the three lines which possessed high protein content (PZ-TS-5, PZ-TS-14 and PZ-TS-6) were grouped in the same cluster showed that they were originated from the same parental line and genetically less diverged. Fufa *et al.* (2005) reported that the genetic diversity estimates based on seed storage protein were lowest because they were the major determinants of end-use quality, which is a highly selected trait.

CONCLUSION

From the results, it can be concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity, and thereby improving the efficiency of wheat breeding programs in cultivar development.

REFERENCES

Arzani, A. 2005. Breeding field crops. Isfahan University of Technology. 630. Pp. (Translated in Persian).

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**: 248-254.

Damania, A. B., Porceddu, E. and Jackson, M. T. (1983). A rapid method for the evaluation of variation in germplasm collections of cereals using polyacrylamide gel electrophoresis. *Euphytica*, **32**: 877-883.

Dominici, L. A., and Grottanelli, C. (1984). Electrophoretic Variability in landraces of *durum* wheat from Ethiopia. *J. Rachis*. **7**: 34-35.

Fufa, H., Baenziger, P. S., Beecher, I., Dweikat, V, Graybosch, R. A. and Eskridge, K. M. (2005). Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. *Euphytica*, **145**: 133-146.

http://www.indiastat.com/table/agriculture/2/wheat/17195/7400/data.aspx.

Khan, I., Shuaib, M., Ali, Z. and Zeb, A. (2007). Molecular and physiochemical Evaluation of wheat. *J. Chem. Soc. Pak.*, **29**: 301-306.

Maragheh, F. P. (2013). Compare the grouping of winter wheat lines using SDS-PAGE electrophoresis data of seed storage proteins and quantitative traits data. *J. Appl. Environ. Biol. Sci*., **3**(8): 81-87.

Payne. P. I., Holt, L. M., Worland, A. J. and Law, C. N. (1982). Structural and genetic studies on the high-molecular weight subunits of wheat glutenin. *Theor. Appl. Genet*., **63**: 129-138.

Shewry P. R. (2009). Darwin review, Wheat, J. Exp. Bot. 60, (6), 1537–1553.

Shuaib, M., Zeb, A., Ali, Z., Ali, W., Ahmed, T. and Khan, I. (2007) Characterization of wheat varieties by seed storage protein electrophoresis. *African J. Biotech*. **6**(5): 497-500.

Sneath, P. H. A. and Sokal, R. R. (1973). Numerical taxonomy: The principle and practice of numerical classification. W.F. Freeman & Co. San Francisco. p. 573.

Xueli, A., Qiaoyun, L., Yueming, Y., Yinghua, X., Hsam, S. L. K. and Zeller, F. J. (2005). Genetic diversity of European spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell.) Revealed by glutenin subunit variations at the *Glu-1* and *Glu-3* loci. *Euphytica*, **146**(3): 193-201.

Zeb, A., Zahir, A., Ahmad, T. and Abdumanon, A. (2006). Physiochemical characteristics of wheat varieties growing in the same and different ecological regions of Pakistan. *Pak. J. Bio Sci*. **9**(9): 1823-1828.

Table 1: Protein Estimation by spectrophotometer

Sr. No.	lines	Absorb At 595 nm	Protein (µg/ml)
1	PZ-TS-1	0.24	56
2	PZ-TS-2	0.34	80
3	PZ-TS-3	0.14	32
4	PZ-TS-4	0.18	42
5	PZ-TS-5	0.44	103
6	PZ-TS-6	0.36	84
7	PZ-TS-7	0.27	63
8	PZ-TS-8	0.28	65
9	PZ-TS-9	0.30	70
10	PZ-TS-10	0.22	52
11	PZ-TS-11	0.28	65
12	PZ-TS-12	0.32	75
13	PZ-TS-14	0.40	93
14	PZ-TS-15	0.26	60
15	PZ-TS-16	0.20	47
16	PZ-TS-17	0.35	82
17	PZ-TS-18	0.19	44

Table 2: Molecular weight analysis of proteins in the seeds of wheat varieties

Sr. No.	Molecular weight (KDa)	Wheat lines																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Band1	97.4	1	1	0	1	1	1	1	1	0	1	1	1	0	0	1	1	0
Band2	66	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	0	0
Band3	43	1	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0
Band4	29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Band5	20.1	1	0	1	0	1	1	1	1	0	1	1	0	0	0	0	0	0
Band6	14.3	1	1	1	1	0	0	1	0	0	0	0	1	1	0	0	0	1

Where,
1=PZ-TS-1, 2=PZ-TS-2, 3=PZ-TS-3, 4=PZ-TS-4, 5=PZ-TS-5, 6=PZ-TS-6, 7=PZ-TS-7, 8=PZ-TS-8, 9=PZ-TS-9, 10=PZ-TS-10, 11=PZ-TS-11, 12=PZ-TS-12, 13=PZ-TS-14, 14=PZ-TS-15, 15=PZ-TS-16, 16=PZ-TS-17, 17=PZ-TS-18.

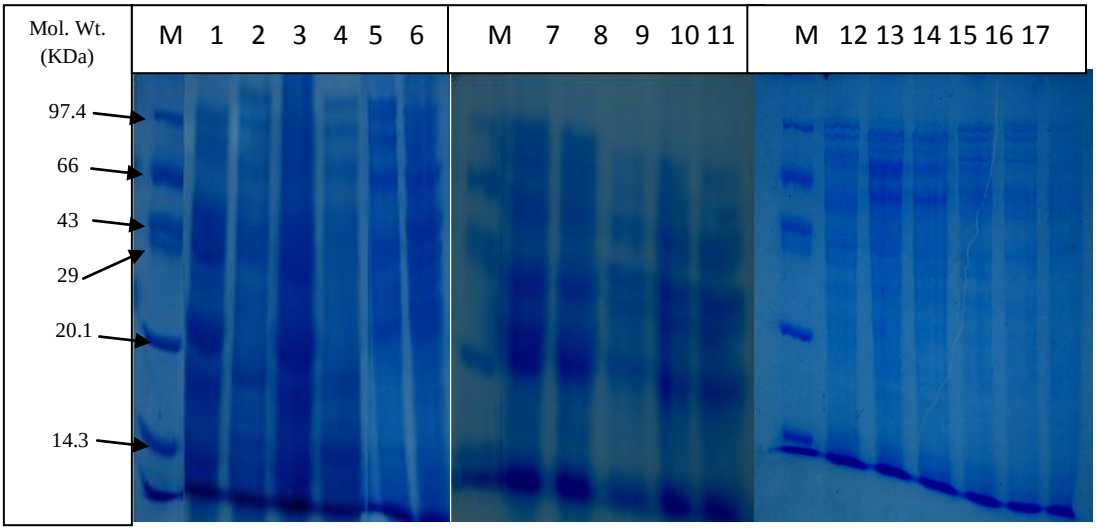

Fig. 1: Electrophorogram showing banding pattern of wheat proteins and molecular Weight markers of 17 wheat lines

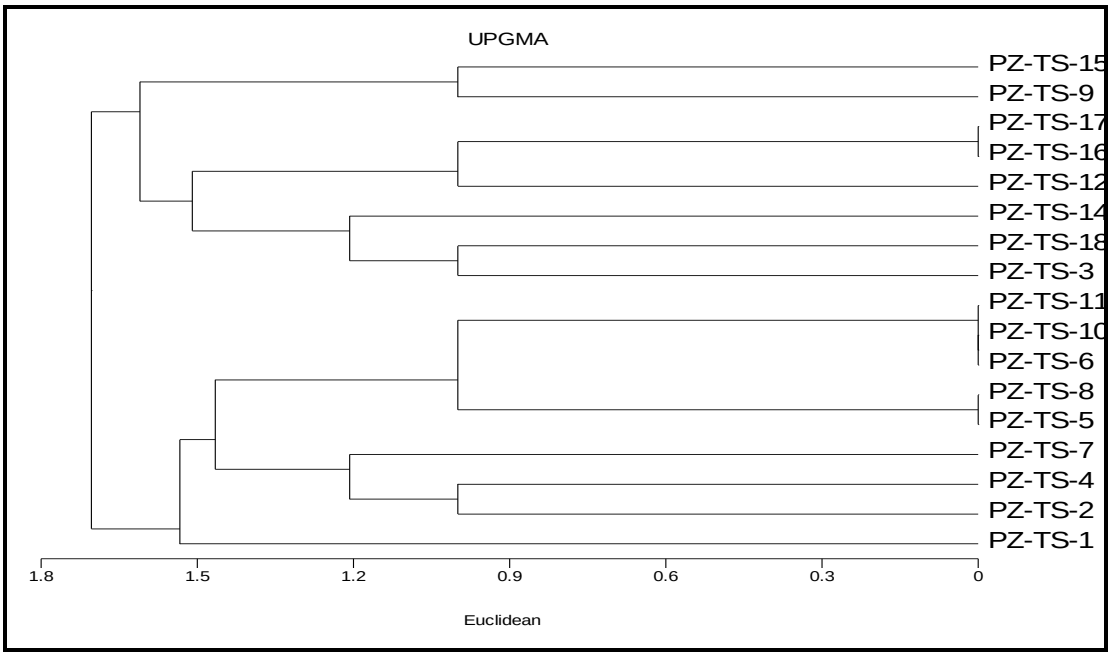

Fig. 2: Dendrogram obtained from cluster analysis following UPGMA method in 17 different wheat promising lines based on electrophoresis' banding pattern

[MS received: July 11, 2013] ***[MS accepted: August 28,2013]***