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STUDY OF GENETIC DIVERSITY IN BUCKWHEAT USING SDS-PAGE ELECTROPHORESIS

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ABSTRACT

Buckwheat belongs to the family Polygonaceae. There has been a great deal of interest generated over the past 10 years regarding the classification of Fagopyrum species. In order to assess the genetic diversity in buckwheat germplasm using SDS-PAGE, 19 accessions of buckwheat were collected from gene bank of PGRI, NARC, Islamabad to estimate the genetic diversity among the buck wheat germplasm accessions on the basis of biochemical markers. Buckwheat accessions of different geographical origin were screened for genetic variability using 14 percent acrylamide gel concentration. Thirteen bands were found in all accessions investigated. The profile of each accession consisted of 10-13 bands. All the protein bands were polymorphic. The present study provides a comprehensive set of buckwheat accessions from various regions of the world. On the basis of cluster analysis four clusters were identified at similarity level of 1.5. Moreover, the results obtained in this study also suggest that analysis of seed protein can also provide a better understanding of genetic affinity of the germplasm. The variation present in accessions can effectively be used by the breeders and molecular biologists for the varietal development programs.

Key word: *Buckwheat, Genetic Diversity, SDS-PAGE, Cluster Analysis*

INTRODUCTION

Buckwheat is grown throughout a large area of Asia and Southeast Asia as a crop that fits the farming system on marginal and fairly unproductive land. It is used as a subsistence crop in many of the more mountainous areas where it is often grown with barley at the higher altitudes. Tartary buckwheat, because of its frost tolerance, is generally grown at the higher altitudes whereas common buckwheat is grown at the lower altitudes. In many areas the trend is for replacement of common buckwheat, which has lower yielding ability and lacks frost tolerance, with finger millet or other crops. Tartary buckwheat production in most areas appears to be remaining constant. Buckwheat (*Fagopyrum esclentum* Moench) is rich in essential amino acids (Pomeranz and Robbins 1972; Pomeranz et al., 1975) and may be milled for consumption in noodles pancakes, or porridge (Taira et al., 1974, Pomeranz et al., 1983). Interest in buckwheat has been renewed due to the increasing popularity of vegetarian diets. The distinctive test of buckwheat (Marshall and Pomeranz et al., 1982) and its high content of lysine make it a useful adjunct to cereal protein, especially in the vegetarian diet (Sure et al., 1955, Pomeranz et al., 1975). Individuals with gluten-sensitive enterpathy (including celiac disease and other gluten intolerances) are unable to tolerate storage protein from wheat, barley, rye and possibly oats and they desire high fiber cereal substitutes such as buckwheat to maintain a normal diet. Buckwheat grain is a fruit of a dicotyledonous plant and is thus taxonomically distant from wheat and the true cereals (Watson and Wrigley, 1984). In commerce, however, buckwheat has traditionally been classified with the cereals (Pomeranz et al., 1983)

The crop is not a cereal, but the seeds (strictly achenes) are usually classified among the cereal grains because of their similar usage. The grain is generally used as human food and as animal or poultry feed, with the dehulled groats being cooked as porridge and the flour used in the preparation of pancakes, biscuits, noodles, cereals, etc. The protein of buckwheat is of excellent quality and is high in the essential amino acid lysine, unlike common cereals. This, coupled with the plant's ability to do well on poorer soils, probably accounts for its widespread usage. It is also a multipurpose crop. The small leaves and shoots are used as leafy vegetables, the flowers and green leaves are used

for rutin extraction for use in medicine. The crop produces honey of very good quality. Buckwheat (*Fagopyrum esculentum* Moench) has been a crop of secondary importance in many countries and yet it has persisted through centuries of civilization and enters into the agriculture of nearly every country where cereals are cultivated. The main producers are China, Russian Federation, Ukraine and Kazakhstan. The species *F. Tataricum*, or Tartary buckwheat, is also produced in many areas of the world but generally is consumed or traded locally.

This is an annual herb, up to 1 m tall, branched, glabrous. Leaves are petiolate, blades are ovate-triangular to triangular, 2-8 cm long, with acuminate tips, bases are cordate or approximately hastate; upper leaves are smaller, sessile. Inflorescences are terminal and auxiliary, branch in dense corymbose or paniculate cyme. Flowers are white or pink, 6 mm in diameter; pedicel is 2-3 mm long, articulate; perianthsare 3 mm long; 8 nectaries are yellow, alternating with stamens; being heterostyly, capitate stigma. Achene is triquetrous, acute angle, longer than 5 mm, more than twice the length of the persistent perianths, brown or black-brown, lucid. This species is common buckwheat and is widely cultivated over the northern and to some extent the southern hemisphere. There are many cultivars or landraces in this species. Their achene forms can vary greatly, some of them being winged on the angles.

Buckwheat belongs to the family Polygonaceae. This plant group is generally referred to as the buckwheat, rhubarb or sorrel family. There has been a great deal of interest generated over the past 10 years regarding the classification of *Fagopyrum* species. Much of this has occurred as a result of Ohnishi's work in the finding of six new species in China and his work on their classification. (Ye and Guo, 1992) suggested a key to the classification of 15 species that occur in the temperate areas of Euro-Asia, with approximately 10 species occurring in China. However, the key to classification of the genus *Fagopyrum* by (Ohnishi et al., 1995) is more complete. In order to assess the genetic diversity in buckwheat germplasm using SDS-PAGE, 19 accessions of buckwheat were collected from gene bank of PGRI, NARC, Islamabad. The Present study was aimed to estimate genetic diversity among the buck wheat germplasm accessions on the basis of biochemical markers.

MATERIALS AND METHODS

The experiment was conducted in the Evaluation Laboratory of Plant Genetic Resources Institute (PGRI), National Agricultural Research Center (NARC), Islamabad. The experimental material) comprised of 20 buckwheat accessions collected from Japan, Syria and Pakistan provided by gene bank of PGRI, NARC, Islamabad.

Equipment: Equipment used for electrophoresis Bio-Rad including Centrifuge 6K12 Sigma (For centrifugation of samples), pH meter (For adjusting pH), Vertex Machine (For shaking samples), Pippets, Fume Hood, Water Distillery, Autoclave and Shaker.

Total Seed Protein Analysis: SDS-Page Electrophoresis: Here a brief description of the protocol followed for total seed protein analysis of Barley germplasm using SDS-PAGE electrophoresis is given.

Preparation of Seed Samples: Single seed of each accession was taken, crushed and grinded in mortar and pestle (Fig. 1). 10mg (0.01g) seed flour was weighed by an electronic balance and put into 1.5 ml microtube. After each sample weighing mortar and pestle were cleaned with great care so that there should not be even a single particle of last seed flour. To extract proteins from flour, 400µl of the protein extraction buffer was put into the micro tubes and mixed well by the test tube mixer (vortex). This sample was preserved in a cabinet at room temperature.

Preparation of Electrophoretic Gel: Glass plates used for electrophoresis were cleaned up from internal side with 80% Ethanol and Kim-wipe. Gaskets were sealed on glass plates with spacer; it was kept in mind that gaskets should not overlap with spacer of plates. Sets of glass plates were fixed with double clips and marked 2cm from the top. To make sure that there is no leakage; glass plate set ups were filled with water and placed for some time (Fig. 2).

Following separation gel solution was prepared after setting up the apparatus;

(A): Separation Gel with 1mm thickness (For two mini gels): 14 %

Solution A	5ml
Solution C	9.3ml
10% APS	200µl
Distilled water	5.7ml
TEMED	40µl

TEMED (N-N-N-N-Tetramethylethylenediamine) was added at the end and shaken well. Separation gel was put into the space between a set of glass plates (up to 2cm from the top). Small amount of distilled water (120µl) was added on separation gel gently to prevent gel surface from air and promote fixation. The set up was left for 30 minutes so that gel was fixed. However, it depends upon the room temperature. Sometimes it was seen that gel was fixed before 30 minutes when there were cool days. During the fixation time of separation gel, stacking gel was prepared (Fig. 2).

(B) Stacking Gel (For two mini gels)

Stacking gel	4.5%
Solution B	2.5ml
Solution C	1.5ml
10% APS	70µl
Distilled water	6.0ml
TEMED	17µl

TEMED was added at the end and shaken well. When separation gel was fixed, distilled water was removed from its top and stacking gel solution poured on it. Combs were fixed into the stacking gel. Combs were put with special care and it was confirmed that there was no any air bubble at the bottom of the combs. The

set up was left for 15 minutes so that the stacking solution became gel. Combs, clips and gaskets were removed from glass plates carefully and confirmed there was no any air bubble at this stage. Gel plates were freshly used for electrophoresis but is was also possible that these would be wrapped in aluminum foil and could be used even for one week.

Electrophoresis: Seed protein was analyzed through slab type SDS-PAGE as *per* Laemmli (1970) using 12.25 % polyacrylamide gel. Electrode buffer solution was put into the bottom pool of the apparatus. Gel plates were placed in the apparatus, here again air bubble formation was avoided. Electrode buffer solution was also put into the top pool of the apparatus; wells formed by combs were washed by syringe. Seed samples were centrifuged at 15,000 rpm for 10 minutes; 6µl of supernatant was put into wells with the help of micropipette (Fig. 3). Protein molecular weight marker was put in first well of each glass plate. The numbering of seed samples and wells were noted to avoid repetition. The apparatus was connected with + (red) and – (black) electrodes of power supply. The voltage of apparatus was kept constant at 70V and apparatus was left until a blue line of BPB came at the bottom of the gel plates.

Detection of Protein Peptides: (Staining and De-staining of Separation gel): When blue line reached at the bottom of the gel plates, electric supply was disconnected. Gel plates were taken out from the apparatus and separated by spatula. Stacking gel was removed with the help of same spatula. Separation gel was put in the box which contained staining solution. Box was put on the shaker for two hours. Staining solution was exchanged by de-staining solution and the box was shacked gently almost overnight until the background of the gel disappeared to absorb excess CBB, a piece of Kim-wipe was put in the de-staining solution check absorbance (Fig. 4).

Drying of separation gel: Wet filter paper was placed on the plate of gel dryer. Separation gel was carefully placed on the paper and covered with a wrap. It was dried in a drier for about 1.5 hours at 60°C. When gel sheet was completely dried, it was taken out while the pump was still running. All gels were dried with the same manner (Fig. 5).

RESULTS AND DISCUSSIONS

SDS bands were scored as present (1) or absent (0) for protein sample of each genotype. The 1/0 matrix was used to calculate pair wise Dice similarity coefficients and the resulting matrix was used to construct a Dendrogram using the software package NTSYS-pc 2.02k.At 1.5 levels of similarity three clusters was formed.

The first cluster was sub divided into two sub clusters. Sub cluster one comprised three genotypes 3714, 3716, 3728. The second sub cluster comprised two genotypes 3722, 3724. The second cluster composed of 9 accessions and in these nine accessions seven accessions shows high level of similarity. 3717 and 3718 were similar while accessions 3723 and 281081 were similar. In same pattern accessions 3730, 281080 and 217220 show high similarity.

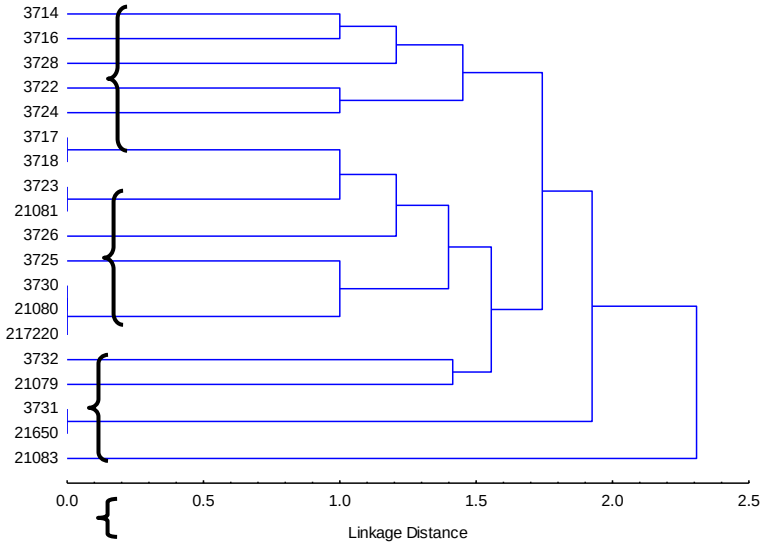

Fig. 1 Cluster analysis of 19 accessions of buckwheat using Unweighted Pair Group Average (UPGA) method.

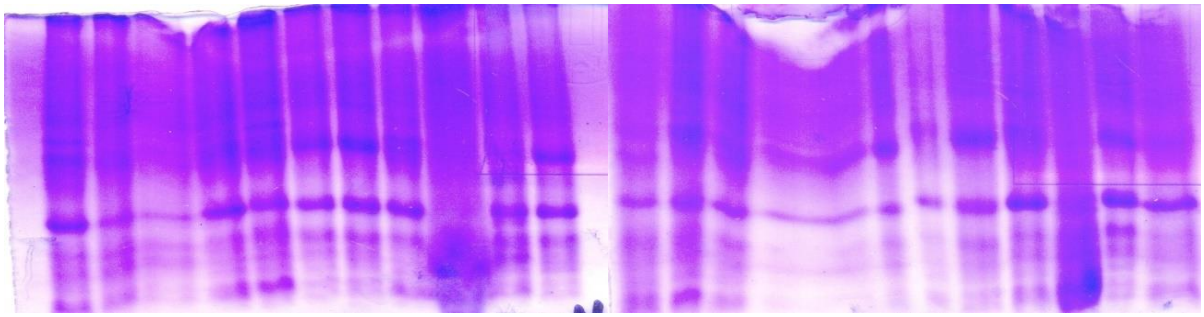

Fig. 2 Banding patteren of buck wheat accessions

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