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GENOTYPIC VARIATION AMONG INDIGENOUS COMMON BUCKWHEAT OF BALTISTAN BASED ON SDS-PAGE

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ABSTRACT

Seed proteins are important in grain quality and distinguishing genetic diversity for breeding populations. The present study was performed to evaluate genotypic diversity in protein bands in common buckwheat based on SDS-PAGE from Skardu and Ganche Districts of Gilgit-Baltistan. Seed protein profiles among indigenous genotypes (12) were assessed for genetic diversity and cluster analysis. Buckwheat protein extracts were resolved on 12.25% acrylamide in discontinuous buffer system. Protein bands produced were produced were analyzed for cluster analysis by UPGMA revealed two lineages at linkage distance of 0.39 dividing the local genotypes into 5 sub cluster groups at 0.19 (50%) linkage distances. Protein bands on SDS-PAGE were scored from 15 kDa to 72 kDa producing 37.27% (HMW) and 62.72% LMW protein bands. The ratio of HMW protein bands to total bands produced were low (0.214) as compared to LMW protein bands (0.359). The protein profiling produced revealed moderate genetic diversity between studied genotypes and could be helpful for utilization in cultivar development and conserve buckwheat germplasm.

Key words: SDS-PAGE, Protein bands, Genotypic diversity, UPGMA, Buckwheat

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench) being member of Family Polygonaceae is an unconventional crop possessing high significance due to nutritive values among plant resources of the world (Campbell, 1997; Bonafaccia *et al*., 2003). It is a multipurpose crop used for food, feed and medicine, still used as a major staple food crop in some valleys of the Karakoram region (Ohnishi, 1994; Hussain *et al*., 2011) and have medicinal as well as health promoting properties (Zhang *et al*., 2012). Buckwheat protein as compared to other crops has the advantage to contain well balanced amino acid composition and posses protein content in a range of 4.4%-15.7% (Li and Zhang, 2001; Steadman *et al*., 2001).

In order to determine diversity in plant species, use of biochemical and molecular markers are more important than morphological traits and seed proteins being insensitive to environmental variation and stable banding pattern have been used for such studies (Murphy *et al*., 1990; Javaid *et al*., 2004). Analysis of seed storage protein by SDS-PAGE is an important way of revealing the variation and relationships between and within taxa (Javaid *et al*., 2004; Zada *et al*., 2013). This method is considered as a valuable tool to study phylogenetic for native landraces and cultivars (Farsad, 2002; Tamkoc and Arslan, 2010; Peddakasim *et al*., 2015). Seed proteins have also been used for cultivars identification and numerous applications in proteomic research (Ferguson and Grabe, 1986; Sirajuddin *et al*., 2010). SDS-PAGE is a powerful tool used as genetic marker for genetic diversity in various crop plants (Javaid *et al*., 2004; Sultana *et al*., 2007; Akbar *et al*., 2012; Uddin *et al*., 2012; Iqbal *et al*., 2015).

Characterization of buckwheat by SDS-PAGE has been reported by many authors for diversity in protein content (Rogl and Javornik 1996; Li *et al*., 2008) including inheritance of pattern and intra-varietal heterogeneity for different subunits of protein (Svetek, 1994; Luthar *et al*., 2008). The knowledge on genetic diversity is also useful for selecting good lines for cultivar development, to maintain, evaluate and utilize germplasm efficiently. But due to modern cultivars, indigenous landraces and genotypes faced significant reduction in genetic diversity (Warburton *et al*., 2008). Polymorphism in buckwheat cultivars across the world has been reported earlier (Yoshioka *et al*., 2004; Rout and Chrungoo, 2007; Li *et al*., 2008). However, due to lack of scientific studies on local buckwheat landraces from Pakistani gene pool, information regarding protein profiling and diversity is lacking.

The present work will be the first attempt to focus on protein profiling based on SDS-PAGE among indigenous buckwheat genotypes of Pakistan from buckwheat growing regions and to assess genetic diversity through cluster analysis. The findings will be informative and useful for future cultivar development and conservation of germplasm resources.

MATERIALS AND METHODS

ABBREVIATION:

BPB	Bromophenol Blue
BWPE	Buckwheat protein extracts
CBB	Coomassie brilliant blue
HMW	High molecular weight protein bands,
LMW	Low molecular weight protein bands
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
UPGMA	Unweighted pair-group method of arithmetic average

Collection of plant material

Plants seed were collected from different buckwheat growing regions of Baltistan Region, Skardu and Ganche District (**Fig.1)**. The local genotypes BWD-1, BWS-2, BWK-3, BWB-4, BWSN-5, BWSI-6, BWA-7, BWZ-8, BWSU-10, BWY-11, BWST-14 and BWM-16 were grown in Royal Orchard Khaplu, Ghanche (N35° 09.163 E76° 20.132) with standard irrigation and weeding practice. Seeds of all genotypes were manually harvested at same physiological maturity stage (70 days) after sowing and dried till further use.

Protein profiling of seed:

Profiling of seed storage protein was used by SDS-PAGE in a mini gel system (Model No.AE-6530, Atto, Japan) according to method of Laemmli (1970). The gel slab consisting 12.25 % polyacrylamide was used in a discontinuous buffer system of vertical electrophoresis.

Buckwheat Protein Extracts (BWPE):

Dried seeds of each genotype after dehulling were ground to fine powder with pistil and mortar. Seed powder (0.1g) from each sample was mixed with protein extraction buffer (400 µl). The buffer contained 0.05 M Tris-Hcl (pH 8.0), 0.2% SDS, 5 M urea, 10% glycerol and 5% 2-mercaptoethanol. The samples were stored at room temperature for overnight, afterwards centrifuged at 15000 rpm for 15 minutes. The extracted supernatant was collected in 1.5 ml eppendorf tubes as BWPE and stored at 4 °C, until electrophoresis. Each sample (8 µl) mixed with BPB (2 µl) were loaded in the wells as tracking dye. The molecular weight was determined by using know molecular weight (PageRuler 10kDa-170 kDa Fermentas).

Protein Detection:

After electrophoresis, the gel was stained in a solution 0.2% (w/v) coomassie brilliant blue (CBB) R250 for 1-2 hours followed by destaining in a destaining solution of Acetic Acid:Methanol:Water (5:20:75 (v/v)).

Scoring and data analysis:

The genotype profiles produced by SDS-PAGE were scored as present (1) or absent (0) and entered in a binary data matrix. Intensity of bands was taken into consideration and classified them in to high and low molecular HMW & LMW bands respectively. Cluster analysis for all genotypes was performed based on Euclidean distance and unweighted pair-group method (UPGMA). Data were analyzed by statistical software package STATISTICA 5.5 (StatSoft Inc.) and XLSTAT 2009.1.01 (Addinsoft SARL, Paris). Estimates of genetic similarity index (Dice coefficient) also known as Nei and Li's coefficient were calculated between all pairs of genotypes (Nei and Li, 1979).

RESULTS

SDS-PAGE analysis in common buckwheat species

Proteomic assay was carried out to evaluate genetic diversity across all local buckwheat genotypes of Baltistan species *F. esculentum.* This is the first documented report of its kind among local landraces of cultivated buckwheat species from Pakistan. In the present study protein bands analyzed by SDS-PAGE showed molecular weight in a range of 15-72 kDa **(Fig. 2).** Similar findings were also reported by Maksyutova *et al*., (2005) in non-morphogenic callus-specific polypeptides in buckwheat. The findings are also in agreement with earlier reports (Li *et al*., 2008) whereas Tamami (1995) reported protein bands in range of 80-90 kDa with some unique protein bands. The electropherogram indicated high polymorphic bands (87.5%) across all genotypes from Baltistan. Protein sub-units produced on 12.25% gel slab were sub-divided into two major groups i.e., high molecular weight (HMW) and low

molecular weight (LMW) protein bands producing 37.27% and 62.72% respectively. Out of all protein sub-units, the ratio of HMW bands to total bands reported maximum (0.31) for local genotype BWD-1 whereas the ratio for LMW bands was maximum (0.44) for BWZ-8 (Table 1). Electrophoretic characterization of proteins subunits exhibited by local genotypes would also be able to identify allergenic characters related to human health (Yoshimasu *et al*., 2000).

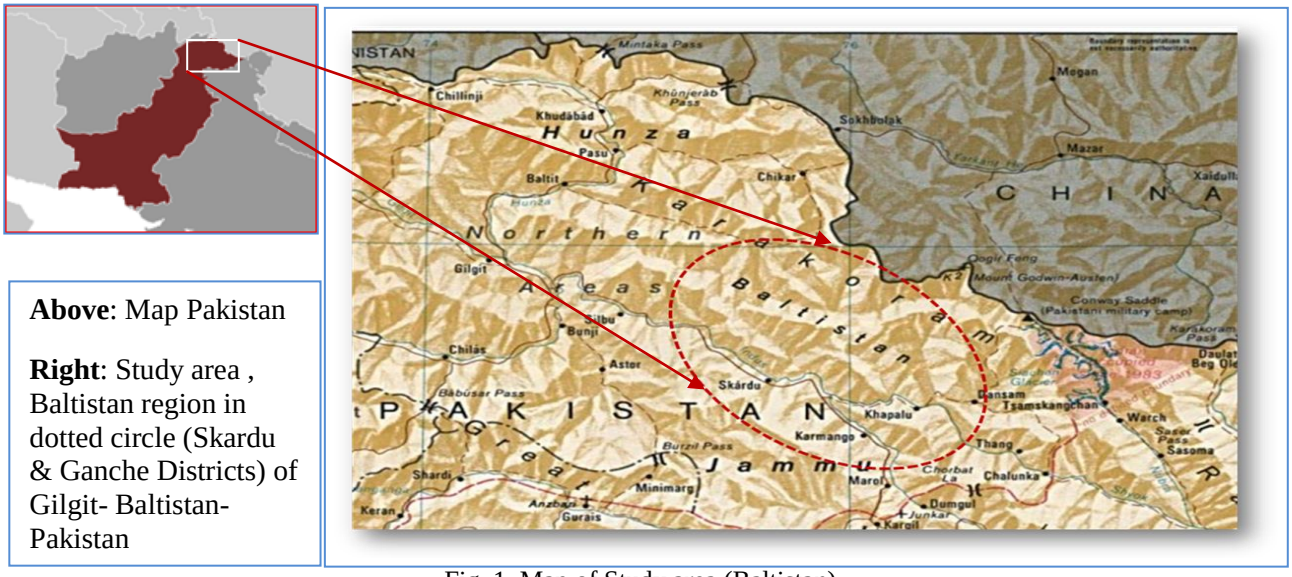

Fig. 1. Map of Study area (Baltistan).

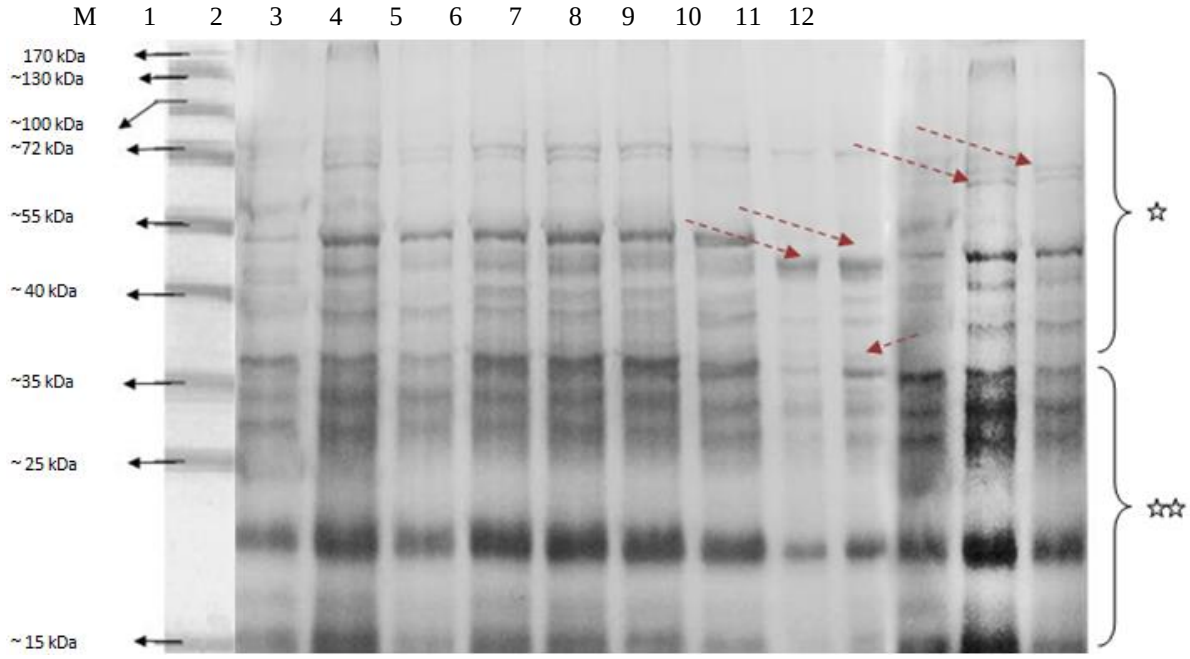

Fig. 2. Variation in protein banding patterns among 12 common buckwheat genotypes by SDS-PAGE

Number 1-12 represents local genotypes BWD-1, BWS-2, BWK-3, BWB-3, BWSN-5, BWSI-6, BWA-7, BWZ-8, BWSU-10, BWY-11, BWST-14 and BWM-16 respectively). M stands for marker (PageRuler Protein Ladder 10 - 170 kDa)
☆☆ Area of Gel having high molecular weight protein bands, ☆ Area of Gel having low molecular weight protein bands, Polymorphic bands – – ▶

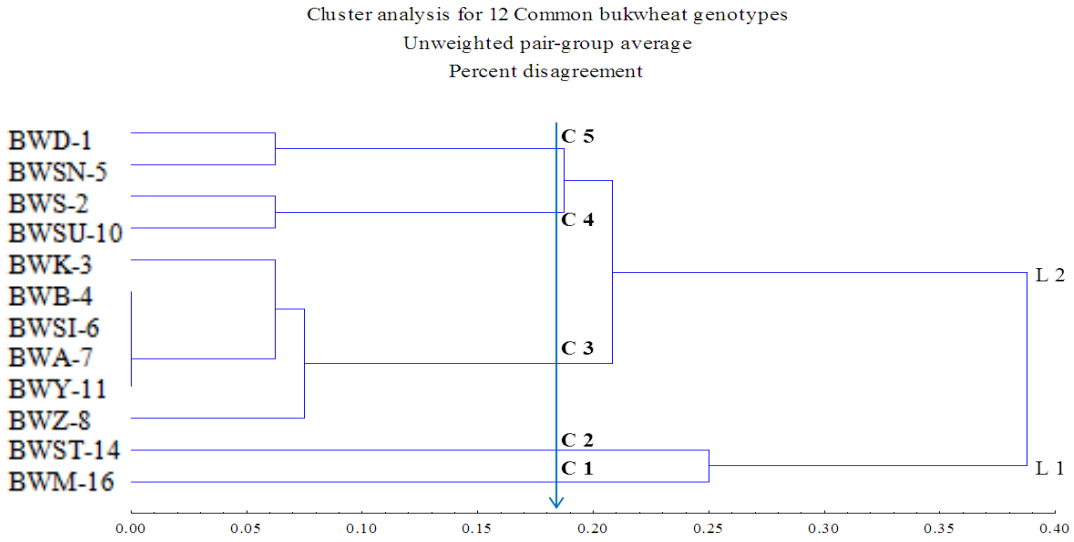

Fig. 3. Dendrogram by UPGMA among local buckwheat genotypes based on SDS-PAGE method.

Table 1. Ratio of HMW and LMW protein bands out of total bands on 12.25% gel slab.

Protein band category	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Percentage**
HMW Protein bands	**0.31**	0.25	0.13	0.25	0.25	0.25	0.13	0.13	0.25	0.25	0.19	0.19	**37.27**
LMW Protein bands	0.38	0.31	0.31	0.38	0.38	0.38	0.38	**0.44**	0.31	0.31	0.38	0.38	**62.72**

HMW= High molecular weight protein bands, LMW = Low molecular weight protein bands
Serial number represents genotypes: 1= BWD-1, 2= BWS-2, 3= BWK-3, 4 = BWB-4, 5= BWSN-5, 6 =BWSI-6, 7= BWA-7, 8= BWZ-8, 9 =BWSU-10, 10= BWY-11, 11 = BWST-14 and 12 = BWM-16.

Cluster analysis of 12 common buckwheat genotypes revealed two lineages (L 1 and L 2) at linkage distance 3.9. The cluster analysis was further divided into 3 clusters at 0.19 (50%) linkage distances. L 1 contained two cluster C 1 and C 2 having BWM-16 and BWST-14 respectively, whereas the L 2 constitute of 3 clusters, the C 3 cluster was further divided into 2 sub cluster showing 100% similarity in only four genotypes based on SDS-PAGE protein bands. Clusters C4 and C5 were further divided into 2 sub clusters at linkage distance 0.06 respectively (**Fig. 3**). Dice coefficient values generated by protein band presence or absences revealed the coefficient values in a range of 0.438-0.688 and mean value was 0.57±0.06. Cluster analysis for the genotypes showed highest percent disagreement for BWST-14 of 56 % against BWS-2 and BWSN-5 each, whereas BWST-14 also showed 50 % disagreement against BWD-1 showing different from other based on protein bands. Cluster analyses has been used to evaluate buckwheat varieties (Zeller *et al*., 2001) with varying level of diversity. The present protein profiling study in local buckwheat genotypes showed moderate level of genetic diversity with dissimilarity coefficient (0.57) across all genotypes based on Nei and Li (1979). The moderate level of genetic diversity attributes were due to geographical differentiation, preference of local farmers to use buckwheat landraces in the farming system in the study area owing to both historical reasons and favored the diversity and maintenance of these local Buckwheat genotypes. Studies from other reports also showed polymorphism in cultivated buckwheat accessions with unique protein profiling among them (Tao *et al*., 2006; Rout and Chrungoo (2007). The genotypic differences from present study also support reports of some recent research on other species based on SDS-PAGE (Iqbal *et al*., 2015; Sharma

et al., 2015). The diversity found among local genotypes revealed the diverse gene pool for exploitation in future breeding programs suggesting further research for buckwheat germplasm from other isolated areas to explore diversity and an integrated strategy for conservation and management of crop genetic resources in mountain areas of Pakistan.

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