

MULTIVARIATE ANALYSIS FOR ELEMENTAL COMPOSITION AMONG INDIGENOUS COMMON BUCKWHEAT GENOTYPES OF BALTISTAN

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ABSTRACT

Macro and microelements play fundamental role in the metabolic reactions required for growth and development. Buckwheat being used for medicinal purpose may be an important source of essential minerals and nutrients for rural communities of Pakistan. The present study was aimed to investigate elemental composition in flour samples of twelve indigenous genotypes grown in Pakistan (Baltistan) by using multivariate approach. After acid digestion, samples were subjected to atomic absorption spectrophotometry and data were analyzed statistically for basic statistics and principal component analysis (PCA). Local genotype BWB-4 showed highest P and Ca level (430 ± 4.07 and 33.91 ± 0.31 mg/100g respectively). On the other hand genotype BWA-7 recorded highest levels of Fe and Zn (2.9 ± 0.03 and 4.5 ± 0.08 mg/100g respectively). PCA extraction exhibited nine main principal components, where maximum variation was shown by the first two components (50.63%). Maximum contribution was made by genotypes with larger seed size viz. BWSN-5 followed by BWA-7 and BWZ-8. For major and trace elements the 1st component revealed a major group among all local genotypes for P and Co whereas 2nd component showed Mn as main element with greater factor loadings. Variations in the elemental composition by using multivariate analysis like PCA proved to be a suitable tool for identification of potential genotypes for future breeding programs.

Key words: Buckwheat, major and trace elements, PCA, genotypes, Pakistan.

INTRODUCTION

Common Buckwheat *Fagopyrum esculentum* (Moench) is broad-leaved herbaceous annual herb belongs to family Polygonaceae (Campbell, 1997). It is grown in the Karakoram and the Himalayas regions, extending from temperate Europe to Japan through the Indo-Myanmar region mainly for human consumption and traditional food (Ohnishi, 1994; Mehta *et al.* 2010; Zhang *et al.* 2012). These areas having diverse topography and agro-ecology, provides security for traditional farming and farmers to grow for food, animal feed and medicines (Gondola and Papp, 2010). In mountain communities of Pakistan its fresh leaves were used as vegetable, flour form seeds were traditionally used as medicine, local food and special use in different festivals (Ohnishi, 1994; Hussain *et al.* 2011). Buckwheat seeds are used for variety of purposes. Buckwheat used as raw material or main ingredients are being used as spaghetti (Biney and Beta, 2014), buckwheat green tea (Lu *et al.* 2010) and for noodle preparation (Hatcher *et al.* 2011) in different countries.

Minerals are important for various biochemical and physiological functions of biological systems (Pontieri *et al.* 2014) and its deficiency is one of the most serious challenges globally affecting human health

(Subhani *et al.* 2015). Interest for buckwheat has been developed globally, with greater emphasis on human health being important source of antioxidative substances, dietary minerals and fibers (Park *et al.* 2000; Steadman *et al.* 2001; Bonafaccia *et al.* 2003). Seeds of this species are a good source of macro and microelements (Stibilj *et al.* 2004; Christa and Soral-mietana, 2008). Many essential dietary minerals like copper, zinc and manganese have been reported (Steadman *et al.* 2001; Ahmed *et al.* 2014), however nutritional quality of food largely depends on bioavailability (Suliburska and Krejpcio, 2014). As compared to other cereals buckwheat possesses higher levels of zinc, copper, and manganese (Mazza, 1998; Steadman *et al.* 2001; Ikeda, 2002).

Production of buckwheat and genetic diversity within *Fagopyrum* species in Himalayan regions including Pakistan, India, Bhutan and Nepal has been reported by Arora *et al.* (1995). Its cultivation in Pakistan is restricted mainly to mountain areas of Gilgit-Baltistan (G.B) from 2000-3200 meter above sea level. Cultivation and utilization of common and tartary buckwheat has been reported earlier and adequately taken into agricultural farming systems in the northern Pakistan (Hushe, Kande, Rondo in Baltistan along the Indus River, Ghizer and Hunza-Nagar) than that of Garhwal and Kashmir regions (Ohnishi, 1994).

Buckwheat was mainly cultivated on 57% of total area in Ghanche as compared to Hunza-Nagar, Astore and Ghizer Districts of G. B during 2009 (Anon. 2010). Its yield in Pakistan was recorded in a range of 1221 kg/ha without fertilization and 1796 kg/ha with the application of Nitrogen and Phosphorus fertilizers (Inamullah *et al.* 2012).

Few reports in context of common buckwheat varieties from Pakistan have been reported based on phytochemicals and SDS-PAGE protein characterization (Abbasi *et al.* 2015; Hussain *et al.* 2016a). Evaluating genetic diversity of germplasm can assist in differentiating varieties with most desirable traits for the incorporation into crop improvement programs. Local genotypes of buckwheat with distinct properties could be a good source of mineral and may be utilized for breeding programs. The present study was aimed to investigate variation in major and trace elements in local common buckwheat genotypes of northern Pakistan (Baltistan) using multivariate approach.

MATERIALS AND METHODS

Plant material: Common buckwheat genotypes were grown during 2010 (July-September) with three replicates each (1×1 m) consisting of four (4) rows with 25 cm apart at Royal Orchard Khaplu, Ghanche (N35° 09.163 E76° 20.132). Standard irrigation and weeding were practiced. Seeds of all genotypes (Table 1) were harvested from each replicated plot by taking ten (10) representative plants at maturity stage (70-84) days after sowing) and its seed traits were also determined as per buckwheat descriptors for International Plant Genetic Resource Institute (IPGRI, 1994) followed by drying and storage for further analysis.

Milling of seeds: Milling process was done manually by separating seeds and hulls. Only flour fraction was prepared by grinding seeds with the help of pestle and mortar and passed through 1 mm sieve.

Reagents: Nitric Acid-Perchloric Acid (HNO₃-HClO₄) was used (2:1 ratio). To 1 liter concentrated nitric acid added 500 ml concentrated perchloric acid was mixed for acid digestion in fume hood at QAU Central Laboratory.

Standards: Working standard solutions of K, P, Mg, Ca, Fe, Co, Cu, Zn and Mn (0, 1.0, 2.0, 3.0, 4.0 and 5.0 ppm) were prepared from stock solutions to run through atomic absorption spectrophotometer. These standards were purchased from Fluka.

Analytical Procedure: The analytical procedure was carried out by wet digestion method using Nitric-Perchloric acid (HNO₃-HClO₄) digestion method (Allen, 1974). Elemental analysis was investigated according to method of Soltanpour and Schwab (1977) using Atomic

Absorption Spectrophotometer (Perkin Elmer Analyst-200, USA). Calibrating of standards for each elemental group was done and final concentration expressed as (mg/100 g).

Statistical analysis: The data from three independent replicates for each genotype were analyzed for descriptive statistics. Furthermore, the data were analyzed statistically using multivariate analysis such as Principal Components Analysis (PCA) for determining genetic diversity based on major and trace elements among local buckwheat genotypes, core contribution% of variables (major and trace elements) and contribution of observations% (genotypes). Statistically significant principal components (PCs) were selected according to Eigen significant criteria mentioned by Kaiser (1960). Data recorded were saved in MS Excel 2007 program and analyzed by the statistical software package XLSTAT (2009.1.01, Addinsoft SARL, Paris).

Table 1. List of genotypes used in the study along with the area of collection.

S. No	Genotypes	District	Seed Size*
1	BWD-1	Skardu	Small
2	BWS-2	Skardu	Large
3	BWK-3	Ganche	Large
4	BWB-4	Ganche	Small
5	BWSN-5	Ganche	Large
6	BWSI-6	Ganche	Small
7	BWA-7	Skardu	Large
8	BWZ-8	Skardu	Large
9	BWSU-10	Ganche	Large
10	BWY-11	Ganche	Large
11	BWST-14	Skardu	Small
12	BWM-16	Ganche	Large

*Seed size determined according to IPGRI, (1994).

RESULTS AND DISCUSSION

The local buckwheat genotypes evaluated for major and trace elements showed genotypic variation. The result revealed higher concentration of P (351.06 ±18.82 mg/100g) followed by K and Mg whereas among the trace elements Zn showed maximum accumulation (2.57 ±0.40 mg/100g) whereas, Co was the minimum (0.033 ±0.007 mg/100 g) among all genotypes (Table 2). The descriptive statistics for major and trace elements were presented as mean of three replicates showed that BWB-4 showed the highest P and Ca content 430±4.07 mg/100g and 33.91±0.31 mg/100g respectively. The highest levels of Fe (2.9±0.03 mg/100 g) and Zn (4.5±0.08 mg/100 g) were recorded by genotype BWA-7. Similar findings have been observed for Fe and Zn content by Bilgicli (2009), however for P, K, Mg and Ca are not in agreement with the findings. The range of P

content recorded (214.0-432.0 mg/100g) during the present investigation are also in accordance with that of Skrabanja *et al.* (2004) and Mann *et al.* (2012). Calcium content was found in a range of 14.85-33.91 across all the genotypes which are not in agreement with the findings of Ikeda *et al.* (2001) and Ikeda (2002). Data revealed that Zn content was also in a same range (2.10 mg/100g) as investigated by Bonafaccia *et al.* (2003) and Bilgicli (2009).

On the other hand Fe was lesser than that of Skrabanja *et al.* (2004) in buckwheat flour samples. The availability of some trace elements in food would also be

dependent on cell structure, food treatment and interaction with other constituents and content may also vary from optimum range (Hurrell, 1999; Steadman *et al.* 2001). The present study showed variation in mineral composition of common buckwheat genotypes. Previous studies also demonstrated variable results regarding mineral composition (Steadman *et al.* 2001; Mann *et al.*, 2012; Zhang *et al.* 2012) that might be imparted to growing condition, genetic factor, genotypes and flour types (Ikeda and Yamashita 1994; Bonafaccia *et al.* 2003; Zhao *et al.* 2009; Pandey and Awasthi, 2015).

Table 2. Descriptive statistics of elemental composition (mg/100 g) across all local genotypes of common buckwheat.

	Elements	Minimum	Maximum	Mean±SE
Major	K	165.02	276.46	199.62 ±10
	P	214.00	432.00	351.06 ±18.82
	Mg	168.50	228.00	191.60 ±5.34
	Ca	14.85	33.91	25.20 ±1.47
Trace	Fe	1.02	2.90	2.00 ±0.17
	Zn	1.02	4.50	2.57 ±0.40
	Cu	0.01	1.50	0.51 ±0.16
	Co	0.019	0.12	0.033 ±0.007
	Mn	0.64	5.00	2.80 ±0.35

K(Potassium), P(Phosphorus), Mg(Magnesium), Ca(Calcium), Fe(Iron), Zn(Zinc), Cu(Copper), Co(Cobalt), Mn(Manganese), ± represents standard error

Principal component analysis for these elements exhibited nine main principal components (PCs), where maximum variation (50.63%) was exhibited by the 1st two PCs as factor 1 (27.15%) and factor 2 (23.47%) for all variables and genotypes based variability has been presented in Bi plot (Fig. 1). The four PCs (F1-F4) possessed higher Eigen values greater than 1.0 contributing maximum variability among all factors (Fig. 2). Higher contribution by the first few PCs in total variability by PCA method were reported by Akbar *et al.* (2011); Ali *et al.* (2011), Ali *et al.* (2015) and Hussain *et al.* (2016b). The factor scores with respect to contribution% of all observations (genotypes) for all nine elements were presented in Table 3. Maximum contribution (%) shown by genotypes having larger seed size viz. BWSN-5 (62.573%) followed by genotype BWA-7 (42.157%) and BWZ-8 (33.04%) as compared to buckwheat genotypes with smaller seed size showing < 30% contribution (Table 3). PCA provides opportunity for using suitable germplasm in crop improvement for specific plant traits including mineral elements (Pecetti and Annicchiarico 1998; Sultan *et al.* 2012; Kiprotich *et al.* 2015). On the basis PCA method local genotypes

BWS-2 and BWSN-5 are distinct from other genotypes with positive factor loadings 1.42 and 4.28 respectively as compared to other genotypes on factor 1. Whereas local genotypes BWSU-10 have maximum factor loading (2.63) on factor 2 as compared to rest of genotypes (Fig. 1). Biplot for major and trace elements across all genotypes revealed that on F1 major element P showed maximum factor loading 0.802 (contributing 26.33% of variables) and on F2 it was prominent by trace element Mn with maximum loading 0.854 (Fig. 3).

PCA analyses revealed positive factor loadings for P and Co indicating as main points on the first component than that of Ca and K. On F2 it was highest for Mn, whereas major factor loadings were recorded for K and Fe on third and fourth components respectively (Table 4). Variation among genotypes by PCA method was reported due to specific traits in buckwheat crop (Yao *et al.* 2007) and in many other crop plants (Akbar *et al.* 2011; Ali *et al.* 2015). Moreover, studying nutritional traits by multivariate approach are useful tool for genetic diversity in crops aimed for breeding purposes and enhance food security (Kiprotich *et al.* 2015).

Table 3. Eigen values, factor scores with respect to contribution of observations (%) for major and trace elements among 12 local common buckwheat genotypes.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9
Eigenvalue	2.44	2.11	1.34	1.23	0.90	0.53	0.33	0.10	0.02
Variability (%)	27.15	23.47	14.93	13.69	9.97	5.87	3.66	1.07	0.17
Cumulative %	27.15	50.63	65.56	79.25	89.23	95.10	98.76	99.83	100.00
Observation	F1	F2	F3	F4	F5	F6	F7	F8	F9
BWD-1	9.802	0.271	2.990	7.280	0.463	22.444	0.748	9.994	7.750
BWS-2	6.844	11.788	18.015	16.318	32.042	0.092	0.428	3.137	2.603
BWK-3	0.926	2.595	3.771	3.601	11.145	0.404	3.473	9.600	0.660
BWB-4	0.080	29.936	4.561	0.095	2.000	9.570	8.956	0.435	29.805
BWSN-5	62.573	0.799	13.183	0.000	3.507	6.573	1.493	1.907	1.047
BWSI-6	0.639	1.231	18.097	7.605	21.784	3.463	1.825	18.702	8.365
BWA-7	0.967	7.676	1.966	42.157	5.522	0.001	11.933	0.303	0.377
BWZ-8	0.037	0.390	3.127	11.790	12.605	33.040	28.052	0.077	2.106
BWSU-10	4.834	27.320	1.239	0.722	8.100	4.311	27.675	11.667	1.258
BWY-11	0.695	0.441	0.333	1.584	2.553	7.128	14.165	16.348	26.432
BWST-14	12.549	0.333	3.391	8.808	0.118	12.723	1.032	4.081	8.273
BWM-16	0.055	17.219	29.325	0.040	0.160	0.251	0.219	23.749	11.325

* F represents factor extracted after PCA, F1 = factor 1 to F9= factor 9

Major contribution of observations (%) > 30% showed in bold for each genotype

Table 4. Factor loadings for major and trace elements across all genotypes after PCA extraction

Major elements				
	F1	F2	F3	F4
K	0.155	-0.192	0.883	0.150
P	0.802	0.340	0.048	-0.298
Mg	-0.530	-0.105	-0.181	-0.651
Ca	0.198	-0.775	-0.294	-0.338
Trace elements				
	F1	F2	F3	F4
Fe	0.213	-0.471	-0.342	0.704
Zn	-0.695	0.459	-0.381	0.282
Cu	0.483	0.421	-0.156	0.072
Co	0.834	0.098	-0.395	-0.061
Mn	-0.011	0.854	0.028	-0.026

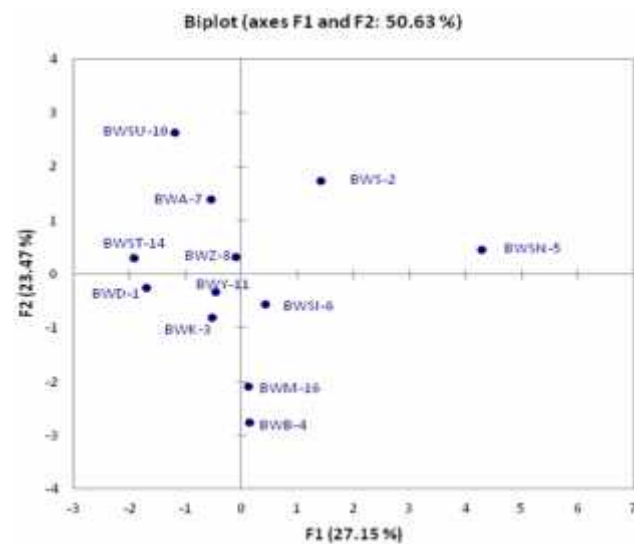
K= potassium, P = phosphorus, Mg = Magnesium, Ca= Calcium, Fe = Iron,

Zn = Zinc, Cu =Copper, Co = Cobalt, Mn =Manganese, F1=factor 1 to F4=factor 4,

Factor loadings > 0.70 are bold.

The flour can be easily incorporated with other materials for a various formulation and processing of different food products proposed by Baljeet *et al.* (2010). The addition of whole buckwheat flour in bakery products also increased K, P, Fe and Mg contents (Yildiz and Bilgicli, 2012). Buckwheat could serve as good dietary source with health beneficial effects amongst crop in mountain areas (Dogra and Awasthi, 2014) and as an optimum range of minerals (approximately 10 to 100 % of the RDA for phosphorus, magnesium, potassium, zinc and copper) would be available from flour samples of 100 g (Ikeda *et al.* 2001).

It is inferred from the present study that the 1st component provided a major grouping with perspective of P and Co in genotypes of buckwheat. The first component contributes most of the traits in crop plants as revealed from other studies (Sultan *et al.* 2012; Ali *et al.* 2015; Hussain *et al.* 2016b). Multivariate analysis like PCA proved to be a suitable tool for identification of potential genotypes for crop improvement. The genotypes with larger seed BWSN-5 and BWS-7 were distinct from others with respect to mineral composition and could be used as potential candidate in future breeding programs.

**Fig. 1. Bi plot showing contribution of major and trace elements by two main principal components (factor 1 and factor 2) among 12 common buckwheat genotypes**

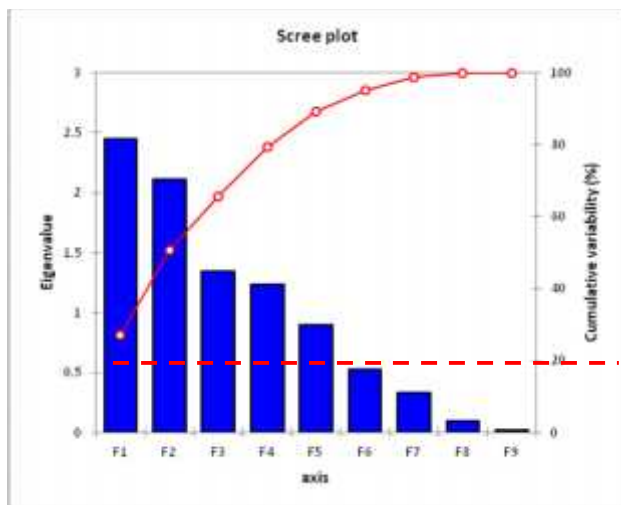


Fig. 2 Eigen value and scree plot for all factors (PCs) extracted by PCA

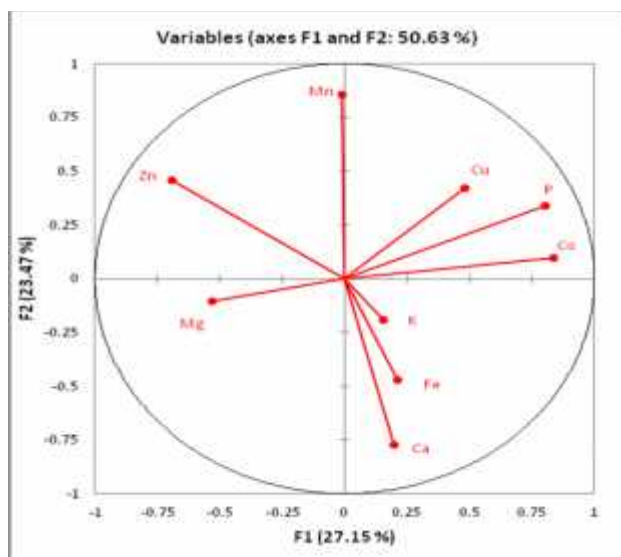


Fig. 3. Biplot for major and trace elements across all genotypes

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