International Journal of Agricultural Policy and Research Vol.3 (7), pp. 293-299,July 2015
Available online at http://www.journalissues.org/IJAPR/
http://dx.doi.org/10.15739/IJAPR.052

ISSN 2350-1561

Original Research Article

Nutritional characteristics and starch properties of Tibetan barley

Accepted 29 June 2015

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Nutritional imbalance and starch properties of the most crop species are mostly affected by the change of soil environment. This study was conducted to evaluate the effect of environmental conditions on nutritional (dietary elements) and microelement changes in barley grain. Barley grain is composed abundant amount of carbohydrates, proteins, vitamins and minerals along with, it is particularly good source of dietary fiber, Fe, Cu, Mn and Se for human food source and animal feed but mostly affected due acute changes in environmental conditions such as soil, water salt and cold stress. For screening and evaluation purpose, thirty three Tibetan (Tibetan) barley accessions were collected from the extremely cold region of China (Tibet) and were studied to evaluate availability and variability of microelements: Fe, Zn, Mn, Se, Cu with amylose and amylopectin ratio at the experimental site of Northwest Agriculture and Forestry University, Yangling, China during 2012 to 2013. Results indicate TB-4406, TB-664 and TB-3835 performed very well in nutritional compatibility and trace elements as compared to other thirty accessions. Among all rest thirty accessions, TB-4406 showed great performance for all five trace elements with 84.8μg kg^{-1}, 63.4, 51.0, 40.0 and 9.37 mg kg^{-1} concentration for Se, Fe, Zn, Mn and Cu respectively followed by TB-664 and TB-3835. Furthermore, the findings revealed that Tibetan barley is a highly auspicious resource for genetic diversity with higher amylose contents and nutritional value of dietary elements. It is concluded that the Tibetan barley has the potential to increase microelements and carbohydrates availability could be considered as a genetic source for barley breeding program.

Key words: Tibetan barley, nutritional value, microelements, genetic source

INTRODUCTION

Barley is one of the earliest domesticated crops in Mediterranean belt among other cereal crop, which contains sufficient quantity of nutrient for human being and large quantity of fodder for livestock purpose (Smith, 1998, Zohary et al., 2012). According to Ryu, (1979), Newman and Newman, (2006) barley is a desired need of human being in different parts of the world especially in Europe (Poland, Finland, England, and Denmark), Asia (Iran, Japan, India, Tibet region of China and Korea) and Africa (from Northern to Horn Africa). The geographic distribution of wild barley in the near East Fertile Crescent is well defined, further more (Morrel and Clegg 2007, Dai et al., 2012) added, Tibet China as additional origin of wild barley, grew up several unknown wild species of barley for its domestications and genetic resources conservation. Recently, DArT data (Dai et al., 2012) the discovery of *Hordeum agriocrithon* A ° berg and *Hordeum vulgare ssp*. spontaneum on the Qinghai-Tibet Plateau in China, are also powering up that Tibet is a second centre for domestication of barley species (Shao et al., 1975, Xu, 1975, Yang and Yen, 1985). Both types of barley (originated from East Fertile Crescent and Tibet) are differing from each other in all aspects. Morphological, archaeological cytogenetic and isozyme data have demonstrated that wild barley on the Qinghai-Tibet Plateau was different from the Fertile Crescent wild barley (Xu, 1982, Yao, 1982). The wild barley grown in Near East has capability to resist temperature and drought, as compare to the barley originated from Qinghai-Tibet Plateau, which is sensitive to survive in that type of soil and environmental conditions (Dai et al., 2012).

In food source point of view, barley contains starch (65-68%), protein (10-17%), b-glucan (4-9%), lipids (2-3%) and minerals (1.5-2.5%); and varies depending on species, crop characteristics and soil environmental conditions (Czuchajowska et al., 1998, Quinde-Axtell and Baik, 2006). Barley crop is mainly classified as hull-less and hulled barley. Hull-less or de-hulled barley requires little or no effort to remove the hull during threshing or processing and would be more suitable for soaking and cooking purpose and comparatively more nutritious than hulled barley. Along with that, hull-less barley is superior in nutritional characteristics such as protein, starch, β-glucan, total dietary fiber (TDF) and limiting amino acids compared with hulled barley (Bhatty, 1992; Edney et al., 1992; Boros, 1996). In non-traditional areas some barley species are enrich in β-glucan, which is highly effective and play vital role to reduce the risk of cholesterol synthesis. Being an inhibitor for cholesterol synthesis, importance of Tibetan barley has been increased in recent years (Konishi, 2001).

Enrichment of minerals, microelements and carbohydrates contents of cereal crops always remain important on large scale due to increasing food demand and nutritional value for many societies (Welch and Graham, 2004). For that reason several studies have been carried out in recent years in order to discover genetic diversity that provides fully sufficient opportunity to identify accessions for specific quality of food and effectiveness. This study evaluates microelements variation in Tibetan barley and express nutrients mechanisms availability and possibilities for developing elements abundant barley accessions with both high amylose and amylopectin. The information generated from this study can be very important for barley biodiversity resource conservation, nutrient source and availability under same soil environmental conditions, which has not been studied before.

MATERIALS AND METHODS

Seed accession and experimental site

In this study, field experiment was organized to evaluate Fe, Zn, Mn, Se, Cu content and amylose and amylopectin ratio in 33 Tibetan barley originated from Tibet, China in 2012 to 2013 at the experimental site of Northwest Agriculture and Forestry University, Yangling, China. Five plants were randomly selected from each cultivar as a plant sample (grain) for analyses at the harvesting time. The samples were harvested and cleaned manually to avoid any contamination of mineral element. These all samples was labeled, putted into paper envelopes and shipped to laboratory. Aliquots of grain sample about 10 gm from each accession were oven dried at 72 °C for 24 hours for removal of extra moisture content in barley grain before grinding. To observe associations among microelements concentrations and morphological traits viz., plant height (cm), spike length (cm), number of spikelet's spike^{-1}, awn length (cm), panicle length (cm), flag length (cm), flag width (cm) and tillers plant^{-1} were also carried out for Tibetan barley.

Grain sample preparation

Grain sample were processed after grinding by using Tekpa Laboratory milling system JFS-13A (with sieve 0.5 mm). The grinding mill was cleaned before and after grinding of barley grain samples.

Microelements determination methods

Ash content for microelements concentrations was determined by using the method 08-01 of the American Association of Cereal Chemists (2008). Each sample was divided into three replicates and approximately 100 mg of dry mass was taken to incinerate at 575 °C for 16 h, until light grey ash was obtained. Ash content (%) was expressed on a dry mass basis. Nutrient concentrations from the grain ash aliquots after 20 times dilution in de-ionized water was measured on atomic absorption spectrophotometer (Hitachi Z-2000, Japan) after standardizations of equipment with known standards of each microelement.

Selenium determination method

Aliquots of about 200 mg barley grain sample were used for the determination of Se content by digesting samples in concentrated nitric acid for 10 hours following a modified protocol (Zhu et al., 2008). The grain (Se) concentration was determined by using Atomic Fluorescence Spectrometer AFS-390 (Beijing Titan Instrumentals Co. Ltd, China).

Amylose and amylopectin measurement

Nearly 100 mg were used to analyses the amylose and amylopectin. The iodine-potassium iodide (IKI) protocol was adapted for standard testing (Washington et al., 2000). Absorbance were measured at 620 and 443 nm for amylose 525 and 725 nm for amylopectin by using Shimadzu UV-1800, Spectrophotometer, China to estimate amylose and amylopectin content in barley grain followed modified method of (Hovenkamp-Hermelink et al., 1987).

Soil sampling and analysis

Soil sample were collected from experimental site after crop harvest using random sampling method at the depths of 0-15 and 15-30 cm with stainless steel auger. Soil samples were sealed in plastic sampling bags and shipped to laboratory. Soil samples were air dried at room temperature for six days for removing ambient moisture

Table-1. Soil properties and nutrient content of experimental field before sowing at the depths of 0-15 and 15-30 cm

Location	Depth	Cu	Fe	Zn	Mn	P	K	OM	pH	EC
NWA&F,	0-15	0.004	0.018	0.006	0.077	0.018	0.157	1.08	8.76	0.210
University field	15-30	0.003	0.019	0.004	0.083	0.016	0.164	0.96	8.81	0.234

Symbol of variable: Cu = copper; Fe = iron; Zn = zinc; Mn = manganese; K = potassium; P = phosphorus (g kg^{-1}); OM = organic matter (%); EC = electrical conductivity (dS m^{-1}), NWA&F=North West Agriculture and Forestry.

content and were processed for next step after removing plant debris and small stones. A small proportion of soil sample was sieved from <2 mm sieve oven dried at 105°C for 12h and further ground and sieved from (0.15 mm) sieve for the determination of soil chemical properties protocol followed by (Lu, 2000), shown in Table 1.

Statistical analysis

Meanwhile data was processed in statistical software (SAS Institute, 2005). The compression between population mean followed by student t-test at ($p<0.05$). Correlation coefficient studies were conducted to assess the association map among nutrients and various plant traits. Analysis of variance was employed to test the genetic diversity between accessions, using a nested block design model.

RESULTS

Barley is considered a nutritionally enrich dietary source for human being and animal feed, keeping these views this study was focus on microelements value in different accessions of Tibetan barley under same agricultural soil environmental conditions. Study evaluates a wide range of variation in microelements content, carbohydrates and minerals.

Microelements and its availability in Tibetan barley

A wide range of variation was observed in Se, Fe, Zn, Mn and Cu concentration among 33 Tibetan barley grain samples are shown in Table 2. The maximum concentration of Grain Se in Tibetan barley was ranged up to 18 to 87 µg kg^{-1} with 38.80 mean values, while the Fe concentration of grain were from 29 to 65 mg kg^{-1} with average highest values 45.90 mg kg^{-1}. However, the Zn concentration range was within 20-55 mg kg^{-1} with 32.70 mg kg^{-1} mean value, while Mn was ranged from 19.6 to 41.2 mg kg^{-1} with 26.2 mg kg^{-1} mean value. Grain Cu concentration was ranged 0.99 to 10.99 mg kg^{-1} with 7.5 mg kg^{-1} mean value. Among 33 accessions of Tibetan barley, however three accessions TB-4406, TB-664 and TB-3835 performed very well as compared to other 30 accessions in same soil and environmental conditions. Cultivar TB-4406 showed greater performance for all five microelements (Se = 84.80 µg kg^{-1}, Fe = 63.40, Zn = 51, Mn = 40 and Cu = 9.37 mg kg^{-1}) concentration for Se, Fe, Zn, Mn and Cu respectively followed by TB-664 and TB-3835. These two accessions showed great availability of three elements. TB-3835 showed more grain Fe, Zn and Cu concentration while as TB-644 had higher concentration for Fe, Mn and Cu (Table 2). One- way ANOVA indicated highly significant difference in grain Zn, Mn and Cu concentration for Tibetan barley at ($p<0.01$) level with CV about 24%, 16% and 25% respectively. There was significant difference for grain Se and Fe concentration at the level of ($p<0.01$) among Tibetan barley. Coefficient of variance for both elements showed difference of variability about 66.4% and 18.4% respectively. Grain Se concentration among Tibetan barley showed more difference of variability as compared to other four elements (Table 2).

Availability of amylose and amylopectin in Tibetan barley

There was sufficient variability among amylose and amylopectin in Tibetan barley accessions. Among 33 accessions, amylose ranged from 11.70 to 25%, while amylopectin was ranged between 37-60% (Figure 1). The CV of Tibetan barley for amylose and amylopectin was 19.60 and 13.30% respectively. Three accessions TB-632, TB-708 and TB-3826 showed higher concentration of amylose respectively 20, 23 and 25% as compared to other accessions. However, the other 16 accessions showed greater performance of amylopectin within range of 50-60%.

Genetic relationship among Tibetan barley based on phenotypic traits

No any specific correlation was found within grain Se, Cu, Zn and Mn concentration with plant characters of Tibetan barley (Table 3 & Figure 2). Mostly trace elements was negatively correlated with plant height (cm), spike length (cm), awn length (cm), number of spikelet's $spike^{-1}$, panicle length (cm), flag width (cm), flag length (cm) and tillers $plant^{-1}$, except Fe concentration. Coefficient of variance for plant height of 33 Tibetan barley was significantly correlated with Fe concentration at 0.01%.

DISCUSSION

Nutritionally decorate barley remains always valuable for human food source due to its direct and indirect visible

Table-2.Grain microelements concentration for thirty-three Tibetan barley cultivars, values is means of (n= 3)

Accessions	Se	Fe	Zn	Mn	Cu
TB 3826	23.50	49.61	29.97	20.49	8.63
TB 708	33.26	47.15	31.17	19.60	8.27
TB 342	66.28	40.04	29.41	41.24	7.47
TB 3835	36.38	52.63	49.13	25.93	10.09
TB 3816	34.70	65.55	31.84	22.49	0.99
TB 767	39.38	45.25	29.12	20.82	7.62
TB 424	29.66	50.21	28.87	26.52	8.26
TB 625	35.91	53.50	29.54	28.80	7.28
TB 644	19.97	61.25	33.45	39.72	10.08
TB 785	56.53	45.96	29.46	27.22	4.93
TB 677	18.55	33.55	25.27	23.63	9.04
TB 2706	33.36	47.03	39.72	28.99	2.74
TB 4253	78.35	33.33	25.98	20.83	6.52
TB 4287	82.22	45.72	27.50	21.23	7.53
TB 3811	82.42	53.68	55.92	23.31	5.90
TB 3760	21.83	33.68	24.20	27.66	7.27
TB 3837	21.39	42.91	27.24	27.89	7.44
TB 386	25.88	42.57	32.80	25.67	7.51
TB 3857	22.87	44.02	28.99	23.95	7.76
TB 4406	84.87	63.49	51.00	40.04	9.37
TB 565	22.92	40.88	33.49	24.26	8.62
TB 3762	28.87	40.06	28.30	24.36	6.42
TB 4317	76.74	29.10	23.05	22.65	5.50
TB 4337	19.38	43.99	34.13	22.43	7.56
TB 4036	20.39	41.42	31.92	28.42	8.00
TB 4786	25.27	33.08	20.27	21.33	6.51
TB 758	23.13	42.22	35.15	31.70	9.42
TB 5006	35.64	51.88	35.41	26.11	7.35
TB 767	36.70	50.45	35.74	24.26	9.31
TB 121	87.16	45.56	28.83	24.68	8.63
TB 5028	19.04	50.91	46.10	31.29	8.98
TB 632	19.51	52.23	27.43	23.84	9.05
TB 3758	21.06	44.94	38.85	25.17	7.79
Mean±SE	38.8b ± 4.0	45.9a ± 1.4	32.7b ± 1.3	26.2d ± 0.7	7.5e ± 0.33
Range	18-87	29.1-65.5	20.2-55.9	19.6-41.2	0.99-10.09
C.V	66.40	18.40	24.50	16.32	25.50
ANOVA	15.5**	2.22**	4.3***	3.4***	3.5***

Concentration of microelements indicates, Se = ug kg^{-1}, Fe, Zn, Mn and Cu mg kg^{-1}

effects on growth and development of human being. Moreover, it is well known that plant breeding and genetic resource conservation technology is worthwhile and commonly adapted practice to produce high yielding capability of wild crop with enrich nutrient source to meet the targeted demand especially for microelements. Therefore it is necessary to explore possible genetic resources to add in breeding program to develop high yielding accessions with greater availability of microelements and carbohydrates (Cakmak, 2008). However, this evaluates the trace element content in different accessions of wild barley, either some cultivar have sufficient quantity essential nutrients and microelements which are highly effective source for healthy life, which mostly suppressed under climate change and stress condition in which plant survive for targeted growth (Lashari et al., 2015). According to this study, Tibetan barley offers a wide range of genetic variability and availability for Se, Zn, Fe and Mn. Similarly; wide variation for amylose and amylopectin percentage was also notated. In this study among 33 Tibetan accessions only three genotype TB-4406, TB-664 and TB-3835 showed good capability for nutrients and can be as a donor plant for breeding program with other accessions for the improvement of microelements efficiency described in Table 2. These findings were highly correlated with the findings of (Jood and Kalra, 2001), that Tibetan barley is superior in grain nutrition as compare to Hulled barley.

The genetically variation and availability in Tibetan barley particularly for Se and Fe was highly variable shown in Table 2. However, a highly significant variation was observed for Zn, Mn and Cu. Comparatively the quantity of

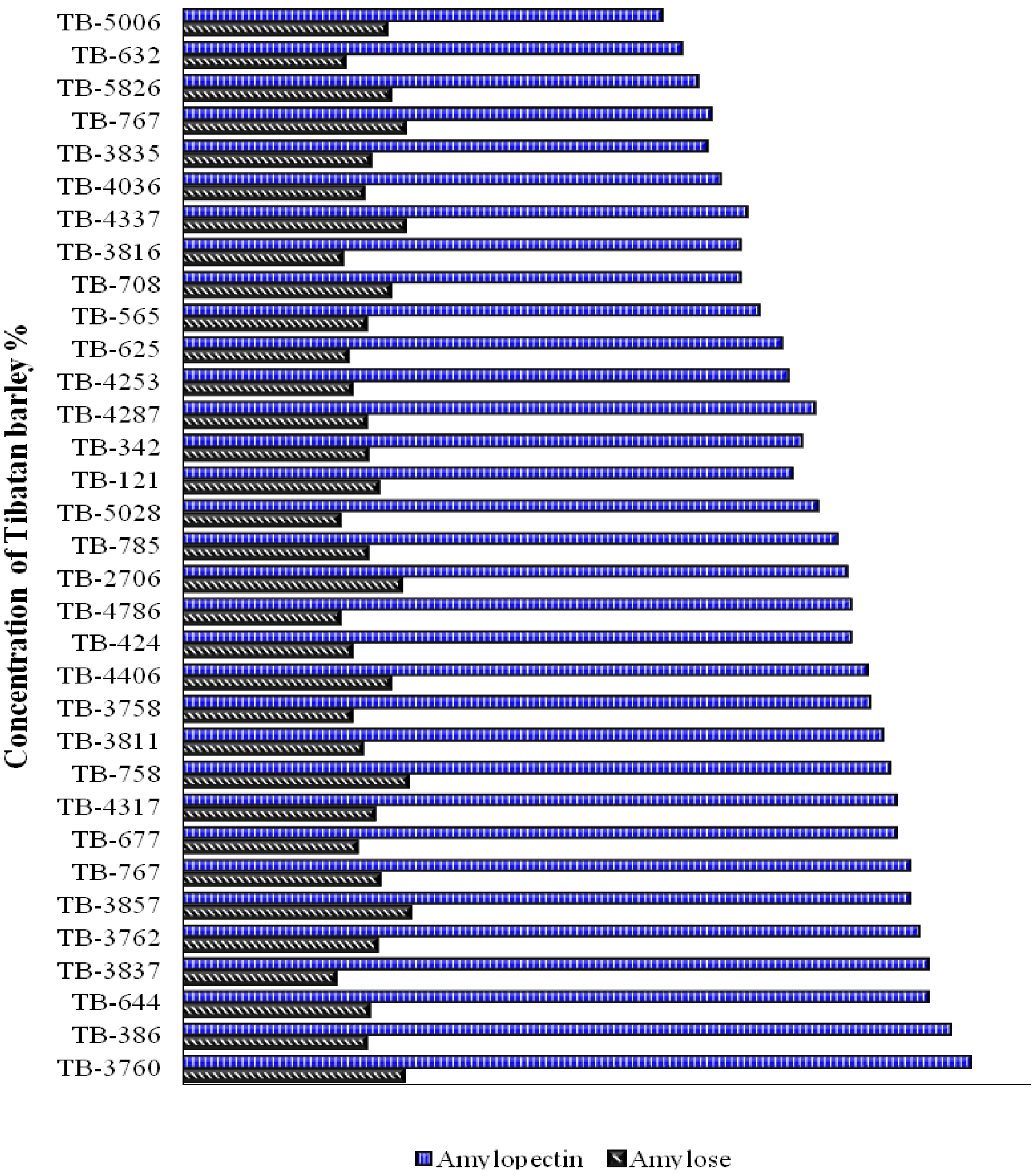

Figure-1: Amylose and amylopectin dry weight (DW) in thirty-three Tibetan barley accessions

Fe, Se, Mn, Cu, Zn, amylose and amylopectin was observed in sufficient quantity but among these all microelements the quantity of Fe was slightly higher in barley grain in compression to other microelements required for healthy diet purpose in daily life, it also increase the enzymes activity during growth and development process of plant for the next generation of the crop (Morgounoy et al., 2007). Further, the significant increase in carbohydrate content in barley grain improves the quantity of amylose in Tibetan barley accessions shown in Figure 2. Hoseney et al., (1987) explained that Tibetan barley contains significantly higher quantity of starch and nutrients as compared to hulled barley cultivar under same agro climatic conditions. The correlation indicating no any specific relationship between grains Se, Cu, Zn and Mn concentration and plant characters were observed (Table 3). Mostly trace elements were negatively correlated with plant height, spike length, awn length, number of spikelet's spike^{-1}, panicle length, flag width, flag length and number of tillers plant^{-1}, except Fe concentration. These results reveals with the finding of

Table 3. Coefficient of correlation (r) between grain Se (μg kg^{-1}), Fe, Cu, Zn, and Mn concentration and plant characters PH = plant height, SL= spike length (cm), NS = no of spikelet's $spike^{-1}$, AL = awn length, PL= panicle length, FL= flag length, FW = flag width (cm) and Tillers = tillers $plant^{-1}$ in Tibetan barley accessions.

	Se	Fe	Cu	Zn	Mn	PH	SL	NS	AL	PL	FL	FW	TP
Se	1												
Fe	.317	1											
Cu	-.179	-.222	1										
Zn	.155	.431*	-.056	1									
Mn	.176	.109	.033	.011	1								
PH	-.284	.008	.305	-.120	.036	1							
SL	-.157	-.059	.172	.114	.210	.130	1						
NS	.021	.015	-.124	-.021	.129	-.131	.086	1					
AL	-.114	.012	.106	-.234	.262	-.207	.367*	.299	1				
PL	.095	.391*	.105	-.097	.121	.462**	.026	-.189	-.231	1			
FL	-.203	-.132	-.122	.000	.219	-.251	.348*	.081	.353*	-.151	1		
FW	-.243	-.213	-.007	.001	.201	-.281	.257	-.005	.546**	-.440*	.480**	1	
TP	-.178	-.073	-.046	-.039	-.021	.164	.246	.078	-.084	-.066	-.177	.061	1

**** Correlation is significant at the p<0.01**
*** Correlation is significant at the p<0.05**

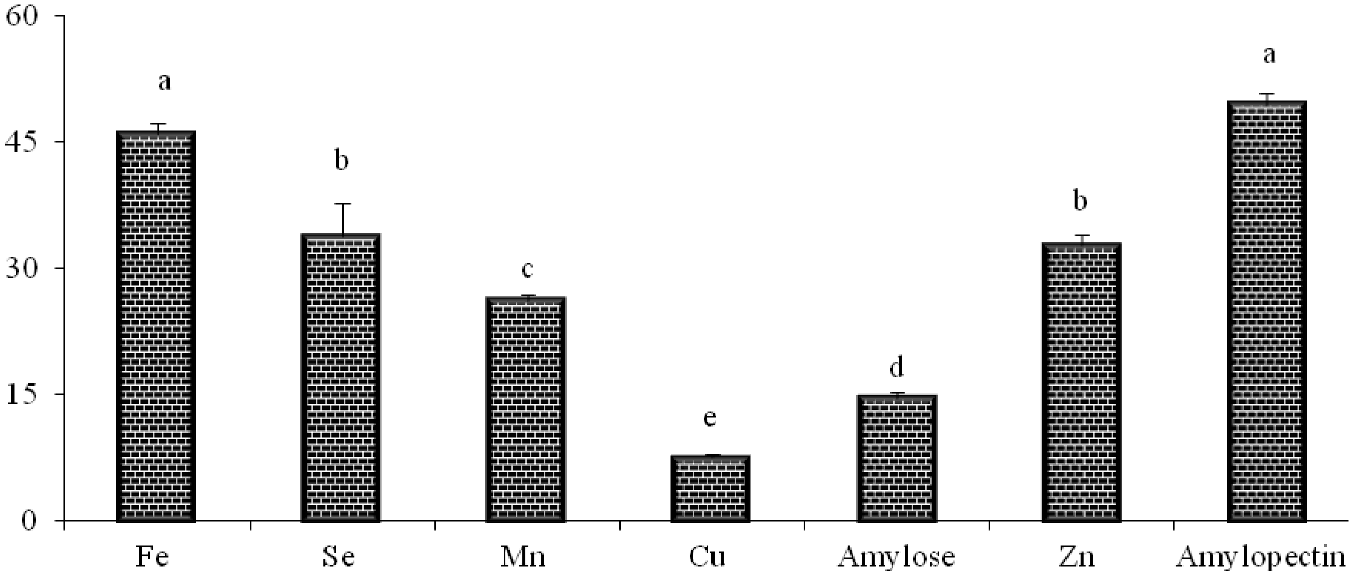

Figure-2: Comparison of Grain Se (μg kg^{-1}), Fe, Mn, Cu, Zn (mg kg^{-1}), concentration and amylose and amylopectin% in thirty three Tibetan barley cultivar. Different letters above bars indicate significant differences at 0.05 levels by t-test at. Symbols and bars represent he mean ± SD (n = 3)

(Garvin et al., 2006, Oury et al., 2006, McDonald et al., 2008). On the basis of the findings of the present study, it is concluded that Tibetan barley accessions can be a reliable resources for future barley breeding program with the objective to develop high yielding and microelements riches barley varieties for food and nutritional security of the growing population of the world

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