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Research Note

Genetic variation and evaluation of exotic barley (*Hordeum vulgare* L.) genotypes for grain protein content, starch content and agronomic traits

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Abstract
A set of 45 genotypes comprising of hulled and hulless type barley from ICARDA along with 6 check varieties (DWRUB52, DWRUB64, DWRB73, BH902, RD2035 and RD2552) was evaluated in an augmented design for grain protein content, starch content and agronomic traits during *rabi* season of 2013-14. Based on mean performance, grain protein content had a wide range of variation from 11.03-18.39% with a mean value of 12.90%. Genotype IBYT-MRA-4 had significantly higher grain protein content (18.39%) than best check RD2552 (11.78%) followed by INBYT-17 (17.89%), IBYT-LRA-M-8 (16.53%), IBON-LRA-M-52 (15.73%), IBYT-MRA-1(15.59%), IBYT-MRA-10 (15.29%), INBON-51 (15.11%) and IBYT-LRA-C-13 (14.94%). Out of these, two genotypes namely INBYT-17, INBON-51 were hulless types and can be used for improvement of food barley varieties for protein content. Starch content had a mean range values from 57.76-64.31% with a mean value of 61.21%. A hulless barley genotype namely INBON-39 was identified which had significantly higher starch content (64.31%) than best check DWRB73 (62.58%) along with more tiller number and ear length. High heritability along with high genetic advance for grain yield, thousand grain weight, ear length and grain filling period showed that these traits are under control of additive genes and hence, can be improved by selection based on phenotypic performance. Grain protein content had a negative correlation with starch content (r = -0.69) and grain yield (r = -0.39), hence simultaneous improvement for high grain protein, high starch content and high grain yield is difficult to achieve and suitable breeding strategy needs to be investigated.

Key words
Barley (*Hordeum vulgare* L.), Genetic variation, Grain protein content, Starch content, Grain yield

Barley (*Hordeum vulgare* L.) is fourth important cereal after wheat, rice and maize worldwide occupying 49.60 million ha area with 141.20 million tonnes production (www.statista.com, 2014-15). About 75% of the global barley production is used for animal feed, 20 % is malted for use in alcoholic and non-alcoholic beverages, and 5% as an ingredient in a range of food products. Grain protein content and starch content are important quality traits for breeding barley varieties to specific end uses. During malting, starch is degraded to fermentable carbohydrates by starch hydrolytic enzymes such as α -amylase, β -amylase, limit dextrinase and α -glucosidase (Fincher 1989, Zhang and Li 2009) and proteins hydrolyzed by proteases that can be further used by yeast.

A grain protein content (GPC) locus on barley chromosome 6 affect the post-anthesis flag leaf senescence, and the barley lines with high-GPC alleles senescing early (See *et al.,* 2002; Mickelson *et al.,* 2003; Heidlebaugh *et al.,* 2008; Jukanti and Fischer, 2008; Jukanti *et al.,* 2008, Lacerenza *et al.,* 2010). High-GPC allele(s) accelerates pre-anthesis plant development and anthesis occurred on average 5 days earlier in the high- than in the low-GPC line (Lacerenza *et al.,* 2010). A strong negative correlation between grain yield and protein content have been well documented in wheat (Oury and Godin, 2007; Bogard *et al.,* 2010 and Martre *et al.,* 2015).

Starch is synthesized in the pericarp and embryo of barley grain. Its presence in these tissues is transitory and little or no starch is present in any tissues of the mature grain except for the starchy endosperm where it is the major component (Tomlinson and Denyer, 2003). The endosperm of barley grain typically contains 50–60% starch, composed of two components 25% amylose and 75% amylopectin both have glucose units. Higher amylose barley contributes toward a low Glycemic Index (GI) and also promotes bowel health (Asare *et al.,* 2011). The starch content and crude protein content have a high negative correlation (-0.7) (Pasam *et al.,* 2012).

Investigation on genetic variation in barley for grain protein content, starch content and other agronomic traits had been studied by Kongl *et al.,* (1995); Koebner *et al.,* (2003) and Zeng *et al.,* (2015). Correlation analysis is important tool for indirect selection for complex traits like yield and quality traits. Correlation studies for grain protein, starch and yield traits in cereals had been reported (Simmonds 1995; Cai *et al.,* 2013; Setotaw *et al.,* 2014).

International Centre for Agricultural Research in Dry Area (ICARDA), Morocco is leading centre for developing and distributing new barley germplasm to barley breeders throughout world. These ICARDA germplasm needs to be evaluated for economically important traits as well as their adaptation to the specific regions before its use in crop improvement. At Punjab Agricultural

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University, Ludhiana, we had received a set of ICARDA barley genotypes under name of Elite International Barley Germplasm Nursery (EIBGN) from Indian Institute of Wheat and Barley Research, Karnal. The present investigation was carried out with the objective 1) to investigate genetic variation and evaluation of these exotic barley genotypes for grain protein content, starch content and agronomically important traits and 2) to find out correlation among these traits.

The materials for the present investigation comprised of 45 exotic genotypes (38 hulled and 7 hulless type) from ICARDA and 6 check varieties (DWRUB52, DWRUB64, DWRB73, BH902, RD2035 and RD2552) of barley. These were grown in an augmented block design (Federer, 1956) with 5 blocks during *rabi* season of 2013-2014. In each block, 9 barley genotypes and 5 checks were grown. There were 4 rows of 2.5 m length which were grown at a row to row distance of 30cm. Standard cultural practices for raising the barley crop were followed.

The agronomic traits such as days to heading (DTH), days to maturity (DTM) were recorded on plot basis and grain filling period (GFP) was calculated by deducting days to heading from days to maturity. The traits plant height (PH) and ear length (EL) were recorded on five random plants from the plot. Tiller numbers (TN) were counted in central one meter row length and at harvesting, grain yield (GY) was recorded. 1000 grain weight (TGW) was estimated as weight of 1000 grains in grams. Hectoliter weight or test weight (kg/hl) (TW) was determined using the apparatus developed by the Indian Institute of Wheat and Barley Research, Karnal which employs a standard container of 100 ml capacity (Mishra *et al.,* 1998). Grain protein content (GPC) and starch content (SC) was estimated using the whole grain analyzer infratech 1241 (M/S Foss Analytical AB, Sweden). The grain samples were scanned in the range of 850 to 1050 nm with a bandwidth of 7 nm. The mean data for morphological and quality characters were subjected for analysis of variance of an augmented design as suggested by Federer (1956). The data were analyzed using software *SAS* (IASRI, New Delhi) for studying analysis of variance and correlation parameter. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated according to Singh and Chaudhury (1985). Heritability in broad sense (h^2 %) was estimated according to Falconer (1989). Genetic advance (GA) as percentage of mean was calculated with the method suggested by Allard (1960); Singh and Chaudhury (1985).

The ANOVA indicated significant differences among the checks for days to maturity, grain filling period and plant height and highly significant differences for days to heading, grain yield, thousand grain weight, test weight and starch content. The analysis of variance also revealed highly significant differences among the test genotypes for all the traits studied. The mean sum of squares due to checks *vs* test genotypes were highly significant for days to heading, days to maturity, grain filling period, plant height, ear length, tiller number, grain yield, protein content and starch content. The differences among the checks *vs* test genotypes were significant for thousand grain weight (Table 1).

Mean, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2%) and genetic advance as % of mean (GA%) were given in table 2. A wide range of mean values for all the traits studied indicate the diverse genetic background of the experimental material. Based on mean performance, promising genotypes performing better than best check were identified (Table 3). Grain protein content had a wide range for mean values ranging from 11.03-18.39% with an average value of 12.90%. A wide range of variation for grain protein content was reported by Wang et al. (2015) in the cultivated and wild barley accessions ranged from 6.73 to 12.35% with a mean of 9.43%. Cai et al. (2013) also reported a wide range of the grain protein content from 8.02% to 13.50% with a mean of 10.56% in 2008 and from 8.28% to 14.45% with a mean of 10.87% in 2009 in cultivated and Tibetan wild barley accessions. The mean values for grain protein content in present material was higher than the earlier reports (Cai *et al*., 2013; Wang *et al.,* 2015). Based on mean performance, genotypes IBYT-MRA-4 had significantly higher grain protein content (18.39%) than best check RD2552 (11.78%) followed by INBYT-17 (17.89%), IBYT-LRA-M-8 (16.53%), IBON-LRA-M-52 (15.73%), IBYT-MRA-1(15.59%), IBYT-MRA-10 (15.29%), INBON-51 (15.11%) and IBYT-LRA-C-13 (14.94%).

A very low value of GCV and PCV for starch content indicated less genetic variation in studied material. Starch content had a range from 57.76-64.31 % with a mean value of 61.21%. Based on mean performance, only one genotype namely INBON-39 had significantly higher starch content (64.31%) than best check DWRB73 (62.58%).

A wide range of mean values for grain yield from 245.00-1013.33 (gm/plot) with a mean value of 651.73 (gm/plot) was recorded which indicate high genetic variation for this trait. A high GCV (24.45%) and PCV (34.13%) estimates for grain yield showed high degree of genetic variation for this trait, however a moderate level of difference among PCV (34.13%) and GCV (24.45%) estimates for grain yield indicate role of environmental variation for this trait. The traits

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such as grain filling period, ear length, tiller number, thousand grain weight also had moderate values for GCV and PCV estimates, thus have sufficient genetic variability for improvement of these traits. Low GCV and PCV values for starch content, days to maturity, plant height, days to heading and test weight showed little scope of improvement in the genotypes for these traits. High GCV and PCV estimates for grain yield, thousand grain weight, low estimates for days to heading, days to flowering and moderate estimate for plant height were reported by Yadav *et al.* (2015).

High heritability along with high genetic advance was recorded for grain filling period, ear length, grain yield and thousand grain weight, hence these traits are under control of additive genes and these can be improved by selection based on phenotypic performance. Addisu and Shumet (2015) and Yadav *et al.* (2015) also reported similar results for grain yield. Tahar et al. (2015) observed high genetic advance with moderate heritability for grain yield and thousand kernel weight in barley. Traits like days to maturity and starch content had high heritability with low genetic advance suggesting the involvement of non-additive gene action in their inheritance. The traits days to heading, plant height, tiller number, test weight and grain protein content had high heritability with moderate genetic advance. Yadav *et al.* (2015) observed moderate heritability coupled with high genetic advance for 1000 grain weight and protein content, while lower genetic advance for day to maturity and days to heading. Al-Yassin et al. (2015) reported that the highest heritable trait based on heritability as days to heading followed by yield.

Response of genotypes to photoperiod is most important trait for adaptation of exotic genotypes in new environments and also related to yield and its component traits. Out of 45 exotic barley genotypes evaluated for flowering traits, 10 genotypes were earlier in heading and 21 genotypes were earlier in maturity than Indian barley checks used (Table 3). Genotype INBON-36 had more grain filling period than best check DWRUB64. None of the genotype had significant higher grain yield than best check, although genotype IBYT-LRA-C-12 had numerically higher grain yield 1013.33 (gm/plot) than check DWRB73 (1004 gm/plot). Genotypes IBON-HI-33 and IBYT-HI-06 also had numerically higher thousand grain weight than best check DWRB73 (45.70gm). For ear length, genotypes IBYT-HI-1, IBYT-HI-14, IBON-HI-33, IBON-MRA-34, INBON-39 had significantly more ear length than best check RD2552. Genotypes IBON-LRA-M-52, IBON-MRA-78, INBON-39 had significantly more tiller number than best check BH902 and INBON-35 had significantly more test weight than best check DWRUB52. Genotypes with shorter plant height were also selected such as IBYT-LRA-C-5, IBYT-LRA-C-10, IBYT-LRA-M-8. A hulless barley genotype namely INBON-39 was identified which possess high starch content, more tillers number and ear length.

Correlation among grain protein content, starch content and agronomic traits in barley was given in table 4. Grain protein content had a highly significant negative correlation with starch content (r = -0.69), grain yield (r = -0.39), thousand grain weight (r = -0.37), test weight (r = -0.38) and a significant negative correlation with grain filling period (r = -0.31). A negative correlation between grain yield and protein content is well established in cereals (Oury and Godin 2007; Bogard *et al.,* 2010; Martre *et al.,* 2015). Starch content showed a highly significant positive correlation with test weight (r = 0.80), thousand grain weight (r = 0.44) while it showed significant positive correlation with ear length (r = 0.30) and tiller number (r =0.33). Pasam *et al.* (2012) also reported a high and negative correlation between starch content and crude protein content (-0.7), while a negative correlation was observed between thousand grain weight and crude protein content.

Days to heading had a negative correlation with grain filling period (r = -0.81), positive correlation with days to maturity (r = 0.56) and grain protein content (r= 0.32). The results were similar to earlier reports (See *et al.,* 2002; Mickelson *et al.,* 2003; Heidlebaugh *et al.,* 2008; Jukanti and Fischer, 2008; Jukanti *et al.,* 2008, Lacerenza *et al.,* 2010) which stated that the barley genotypes having high GPC alleles were earlier in heading and maturity than the low GPC alleles. Test weight had a positive correlation with thousand grain weight (r = 0.39), plant height (r = 0.29) and ear length (r = 0.31) while it had a negative correlation with days to maturity (r = -0.34). Thousand grain weight and grain yield also had a significant positive correlation.

In nut shell, tested ICARDA genotypes had immense use for improvement of protein content, starch content and agronomic traits. The genotypes having high grain protein content cannot be improved for starch content as these traits possess strong negative correlation. Eight genotypes were having high grain protein content, out of which two genotypes namely INBYT-17, INBON-51 were hulless types and can be used for improvement of food barley varieties for grain protein content. A hulless barley genotype namely INBON-39 was identified with desirable combination of starch content, tiller number and ear length which can be used for improving the existing food barley varieties for these traits.

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Table 1. Analysis of variance for grain protein content, starch content and agronomic traits in barley

	Mean Sum of squares											
Source of variation	**DF**	**DTH**	**DTM**	**GFP**	**PH**	**EL**	**TN**	**GY**	**TGW**	**TW**	**GPC**	**SC**
Block	4	2.53	23.70	23.58	36.96	1.27*	328.97	32658.33	19.53	1.15	0.96	0.71
Entries	50	69.34**	25.68**	51.55**	76.97**	1.57**	996.20**	49485.47**	46.27**	36.27**	2.95**	2.25**
Checks	5	86.59**	25.25*	52.70*	77.28*	0.46	314.77	170115.33**	145.20**	131.14**	0.81	0.34**
Test genotypes	44	64.74**	22.24**	36.01**	63.94**	1.43**	919.67**	23989.06**	33.53**	25.76**	1.19**	3.59**
Checks vs test genotypes	1	185.61**	179.24**	729.64**	648.66**	13.59**	7771.08**	568192.32**	112.49*	24.27	2.19**	2.05**
Error	20	1.69	12.12	16.68	22.13	0.35	558.11	24090.33	15.05	6.14	0.81	0.34
Critical difference (CD)												
Checks vs checks												
(P=0.05)		1.72	4.59	5.39	6.21	0.78	31.17	204.77	5.12	3.27	1.19	0.77
(P=0.01)		2.34	6.26	7.35	8.46	1.06	42.51	279.28	6.98	4.46	1.62	1.06
Genotypes within same block												
(P=0.05)		3.84	10.27	12.05	13.88	1.73	69.69	457.88	11.45	7.31	2.66	1.73
(P=0.01)		5.24	14.01	16.43	18.93	2.36	95.05	624.48	15.61	9.97	3.62	2.36
Genotypes in different blocks												
(P=0.05)		4.15	11.09	13.01	14.99	1.87	75.28	494.57	12.36	7.90	2.87	1.87
(P=0.01)		5.66	15.13	17.75	20.44	2.55	102.66	674.52	16.86	10.77	3.91	2.55
Genotypes vs checks												
(P=0.05)		3.13	8.39	9.84	11.33	1.42	56.90	373.86	9.35	5.97	2.17	1.41
(P=0.01)		4.27	11.44	13.42	15.45	1.93	77.61	509.89	12.75	8.14	2.96	1.93

*, ** Significant at5 % and 1% level of significance, respectively

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Table 2. Mean, range, genotypic (GCV %) and phenotypic (PCV %) coefficient of variability, heritability (h^2 %) and genetic advance as percentage of mean for grain protein content, starch content and agronomic traits in barley

S. No.	Trait	Mean	Range	GCV%	PCV%	h^2 %	GA %
1	Days to heading	96.29	80.70-113.37	8.54	8.65	97.56	17.38
2	Days to maturity	141.97	128.03-149.03	2.59	3.57	52.80	3.88
3	Grain filling period	45.68	29.67-61.83	12.93	15.72	67.64	21.90
4	Plant height (cm)	100.66	83.78-122.94	7.36	8.72	71.25	12.79
5	Ear length (cm)	8.76	6.46-12.52	12.61	14.30	77.71	22.90
6	Tiller no.	145.53	81.40-223.23	14.38	21.69	43.98	19.65
7	Grain Yield (Grams/plot)	651.73	245.00-1013.33	24.45	34.13	51.32	36.08
8	Thousand Grain weight (Grams)	38.44	24.53-51.67	14.54	17.70	67.47	24.60
9	Test weight (kg/hectoliter)	59.35	45.40-71.98	9.25	10.15	83.07	17.36
10	Grain protein content (%)	12.90	11.03-18.39	11.34	13.31	72.54	19.90
11	Starch content	61.21	57.76-64.31	2.26	2.45	84.89	4.29

Table 3. Promising barley genotypes identified for grain protein content, starch content and agronomic traits

Sr. No.	Trait	Best Check	Promising lines better than best check
1	Days to heading	DWRUB64 (90.60)	Early in heading: IBYT-LRA-M-11, IBYT-MRA-10, IBON-HI-5, IBON-HI-65, IBON-HI-78, INBON-35, INBON-36, INBON-109, ISEBON-27, ISEBON-33
2	Days to maturity	DWRB73 (140.00)	Early in maturity: IBYT-LRA-C-5, IBYT-LRA-C-8, IBYT-LRA-C-10, IBYT-LRA-C-11, IBYT-HI-1, IBYT-HI-11, IBYT-HI-14, IBYT-LRA-M-11, IBYT-LRA-M-12, IBYT-LRA-M-19, IBYT-LRA-M-24, INBYT-11, INBYT-17, IBYT-MRA-10, IBON-HI-5, IBON-HI-33, IBON-HI-65, IBON-HI-78, INBON-35, INBON-109, ISEBON-33
3	Grain filling period (Days)	DWRUB64 (54.20)	INBON-36
4	Plant Height (cm)	DWRUB64 (90.07)	IBYT-LRA-C-5,IBYT-LRA-C-10,IBYT-LRA-M-8
5	Ear Length (cm)	RD2035 (8.77)	IBYT-HI-1*, IBYT-HI-14*, IBON-HI-33*, IBON-MRA-34*, INBON-39*
6	Tiller no.	BH902 (143.40)	IBON-LRA-M-52*, IBON-MRA-78*, INBON-39*
7	Grain Yield (Grams/plot)	DWRUB73 (1004)	IBYT-LRA-C-12
8	Thousand Grain weight (Grams)	DWRUB73 (45.70)	IBYT-HI-06, IBON-HI-33
9	Test weight (kg/hectoliter)	DWRUB52 (65.70)	INBON-35*
10	Grain Protein content (%)	RD2552 (12.68)	IBYT-LRA-C-13*, IBYT-LRA-M-8*, INBYT-17*, IBYT-MRA-1*, IBYT-MRA-4*, IBYT-MRA-10*, IBON-LRA-M-52*, INBON-51*
11	Starch content	DWRB73 (62.58)	INBON-39*

* Significantly better than best check at 5 % level of significance, within parenthesis is the actual mean value (adjusted)

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Table 4. Correlation among grain protein content, starch content and agronomic traits in barley

Trait	DTH	DTM	GFP	PH	EL	TN	GY	TGW	TW	GPC
DTM	0.56**									
GFP	-0.81**	0.03								
PH	-0.06	-0.22	-0.08							
EL	-0.02	-0.21	-0.12	0.24						
TN	0.20	0.14	-0.14	-0.11	0.04					
GY	0.03	0.08	0.01	-0.27	-0.21	0.05				
TGW	-0.22	-0.08	0.21	0.16	0.14	0.12	0.33*			
TW	-0.19	-0.34*	0.00	0.29*	0.31*	0.26	0.00	0.39**		
GPC	0.32*	0.09	-0.31*	0.08	0.05	-0.14	-0.39**	-0.37**	-0.38**	
SC	-0.21	-0.20	0.11	0.13	0.30*	0.33*	0.09	0.44**	0.80**	-0.69**

*, ** Significant at5 % and 1% level of significance, respectively