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Biochemical characterization of indigenous and exotic malt barley genotypes

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Abstract
Barley (*Hordeum vulgare* L.) is the most popular raw material for malting and brewing purposes. In India, most of the malting and brewing industries are importing the exotic barley genotypes as the malting quality of exotic barley genotypes is considered superior to indigenous genotypes. To find out the reason behind this, a small study was planned with the objective to characterize exotic and indigenous barley genotypes for different biochemical traits important for malting. Total starch, amylose, protein, beta-glucan, beta-amylase, phenolic content and antioxidant activity were analyzed in two biological replications of 10 barley genotypes (5 each of exotic and indigenous). The results suggest that the exotic genotypes have high amylose content, low grain beta-glucan, high beta-amylase activity. Antioxidant activity was significantly high as compared to indigenous genotypes. It can be concluded that to improve the malting potential of indigenous genotypes, there is a need to breed for barley genotypes with low β-glucan content, higher levels of amylolytic enzymes which can compete with exotic genotypes and reduce the cost of importing barley in future.

Keywords: Barley, malting, starch, β-glucan, β-amylase

1. Introduction
Barley (*Hordeum valgare* L.) is one of most important ancient cereals that is grown over diverse eco-geographical environmental conditions as compared to other crop species, because of its hardiness to environmental variations (Bothmer, 1996) [1]. In terms of production and utilization barley ranks fourth after rice, wheat and maize worldwide. In India, most of barley is used as feed for animals (75%), malting (20%) and only 5% for human consumption. From last two decades, use of barley and malt-based food products has increased because of its higher dietary fibre content especially β-glucan and higher antioxidant activity (Zhao *et al*., 2008) [2]. From the ancient time, barley is the most popular raw material for malting and the demand for malt-based products is continuously increasing in India. For malting purpose hulled barley varieties are generally preferred because it prevents the growing acrospire from damage during malting and also helps in the filtration during brewing (Bhatty, 1999) [3]. An ideal malting barley genotype should fulfil numerous agronomical, malting, and brewery criteria. For malting purposes starch is the most important component of the barley grain (Fangel *et al*, 2018) [4]. Malting and brewing quality of barley is also affected by protein content and composition. For malting low protein (9-11%) and low beta-glucan content (≤ 4%) is desirable (Muller *et al*, 1995) [5]. Higher contents of protein and beta-glucan can affect the hydrolysis of starch during mashing and leads to formation of haze during storage and create problem in proper modification of endosperm during malting. India has developed very good malt barley genotypes, but the Indian industry is not using these varieties. Most of the information on factors affecting the biochemical composition of barley grain has been generated for temperate climates or extended sub-tropical climates. However, scanty information is available regarding the differences in the quality of exotic and indigenous barley at biochemical and molecular level. Keeping the above points in view, the present study was designed with the objective to characterize exotic and indigenous barley genotypes for some important biochemical traits.

2. Material and Methods
Five exotic and five indigenous barley genotypes were cultivated at the open experimental farm of Indian Institute of Wheat and Barley Research, Karnal in year 2020-21 in two biological replications. Fully matured harvested grains were used for this study. Xanadu, Andreia, Traveller, Explorer and ABI Kranti were exotic genotypes while DWRUB 52,

DWRB 91, DWRB 101, DWRB 160 and DWRB 182 were Indigenous barley genotypes.

2.1 Preparation of Sample flour
The whole grain flour of all the genotypes was prepared by Cyclotec sample mill (Model 1093, FOSS, Denmark) using 0.5mm sieve. The flours were stored in refrigerator for further analysis.

2.2 Estimation of moisture content in flours
Moisture content was measured by thermogravimetric analyzer moisture meter (Axis spolka zoo moisture meter, Model ATS120, Poland) as per given protocol.

Moisture Content (%) = {(Initial wt. of sample- Final wt. of sample)/ Initial wt. of sample}x100

2.3 Biochemical analysis of exotic and indigenous barley genotypes
Many biochemical constituents of the grain like starch, amylose, protein, β-glucan, total phenolics, antioxidant activity and β-amylase activity play important role in determining the malting quality and the quality of the final product i.e. beer. Total starch content was estimated by Anthrone method (Clegg, 1956; Hodge and Hofreiter, 1962) [6, 7]. Amylose content was quantified by iodometric method as described by Sowbhagya and Bhattacharya (1971) [8]. Total crude protein was estimated using near infrared transmittance (NIR) grain analyser (Infratech 1241, FOSS, Denmark). A Megazyme kit (K-BGLU, Mixed-Linkage β -glucan Assay) was used for estimation of beta-glucan according to McCleary and Codd (1991). Total phenolic content was determined by the Folin-Ciocalteu method (Singleton *et al*., 1999) [10] and expressed as mg Gallic acid Equivalents per gram (mg GAE/g). The free radical scavenging activity was determined by DPPH assay (Beta *et al*., 2005) [11] and expressed in % discoloration. Antioxidant activity was determined by Ferric Reducing Antioxidant Power (FRAP) as per the method of Benzie and strain, (1996) [12] and activity expressed in mmol/g. The β-amylase (BA) activity was estimated in barley flour using Megazyme kit Ltd (Beta-amylase assay kit Betamyl1-3-analysis of beta-amylase) as per the procedure of McCleary and Codd, (1989) and activity was expressed in Units/g (U/g).

2.4 Statistical analysis
Data were reported as mean of two biological replications ± standard error for two determinations of each sample. Analysis of variance (ANOVA) and Tukey's comparison test were performed using XLSTAT Software (trial version,) to identify differences between the values. Statistical significance was declared at $p<0.05$.

3. Results and Discussion
In this study, all the ten genotypes in two biological replications were analysed in duplicate for eight important biochemical parameters (Table 1) and means for the exotic and indigenous genotypes are presented in Figure 1. Starch content is the main component of grain that directly impacts the malting quality as well as yield of malt extract. Total starch content in exotic genotypes was in the range from 56.05%- 61.82% with an average of 59.11%, while in indigenous genotypes it ranged from 50.69%-59.47% with an average of 55.48%. No significance differences (<0.05) were observed among the exotic and indigenous means as well as between individual genotypes. Amylose content in exotic genotypes ranged from 21.22%-23.60% with an average of 22.78%, while in indigenous genotypes the range was 20.33%-22.03% with an average 21.12%. No significant differences (<0.05) were observed among the individual genotypes, but significant differences were observed between exotic and indigenous mean values. Physicochemical and rheological properties of starch are affected by amylose and amylopectin composition in starch. Protein content in exotic genotypes ranged from 10.20%-11.66% with an average 11.09%, while it ranged from 9.48%-12.40% with average 11.21% in indigenous genotypes. No significant differences (<0.05) were found among the exotic and indigenous mean values. Among indigenous genotypes, two genotypes had higher (>12%) protein content which is not desirable for malting purpose. Most of the exotic genotypes, however had protein content in desirable range. For brewing purposes 9-11% protein is desirable (Muller *et al*, 1995) [5], higher level of protein causes haze formation that affect the quality of final product. β-glucan content of exotic genotypes was observed from 3.78%-4.62% with an average of 4.19%, while 3.89-6.24% beta-glucan with an average 4.88% was found in indigenous genotypes. Significant differences (<0.05) were observed among the individual genotypes but the difference between exotic and indigenous means was not significant. For malting and brewing low level of beta-glucan content is highly desirable. Higher levels can lead to incomplete endosperm modification during malting and also hinder the starch hydrolysis and filtration of wort (Bamforth and Martin, 1981; Gianinetti *et al*., 2005) [14, 15]. Phenolic content of exotic genotypes was observed from 0.99-1.10 mg/g with average of 1.04 mg/g, while in indigenous genotypes it ranged from 0.74-1.18 mg/g with an average of 1.00 mg/g. Among the individual as well as exotic and indigenous genotypes no significant differences (<0.05) were observed. During malting and brewing phenolic compound played very important role in retarding the oxidation process and increase shelf life of product. DPPH radical scavenging activity of exotic genotypes were found significantly higher (37.64%) than the indigenous genotypes (31.26%). Higher antioxidant activities help in enhancing the shelf life of beers by preventing the oxidation effects during storage. FRAP values were observed from 7.20-8.33 mmol/g with an average of 7.73 mmol/g in exotic genotypes while in indigenous genotypes it ranged from 6.87-8.78 mmol/g with an average of 7.66mmol/g. No significant differences were found among the exotic and indigenous genotypes. The exotic genotypes had higher grain β-amylase activity which ranged from 15.75-28.88 U/g with an average of 23.28 U/g. In the indigenous genotypes the activity ranged from 13.95 U/g-23.34 U/g with an average of 18.89 U/g but difference was not significant. β-amylase is one of the most important enzymes that plays role in hydrolysis of starch during malting and mashing and results in the formation of the fermentable sugars (glucose, maltose, and maltotriose) which can be further utilized by yeast during brewing. The activity of this enzyme correlates with fermentable sugar production during mashing to a much greater extent than any other diastatic power enzymes in malt (Duke *et al*., 2018) [16]. Our results suggest that exotic genotypes have an advantage of low β-glucan content, high β-amylase activity and high antioxidant activity. The differences in these biochemical parameters may be the reason for their better malting quality as compared to indigenous genotypes.

Table 1: Biochemical characterization of exotic and indigenous barley genotypes

	Genotype	Total starch (%)	Amylose (%)	Protein (% dwb)	β -glucan (% dwb)	TPC (mgGAE/g)	DPPH Assay (%)	FRAP Assay (mmol/g)	Grain β-amylase (U/g flour)
Exotic genotypes	Xanadu	61.82±1.85^{a}	23.14±2.38^{a}	11.38±0.44^{a}	3.96±0.09^{a}	1.02±0.08^{a}	38.35±5.58^{ab}	7.20±0.13^{a}	22.14±2.62^{ab}
	Andreia	60.90±3.88^{a}	23.22±0.17^{a}	10.66±0.63^{a}	4.18±0.15^{a}	0.99±0.10^{a}	34.05±1.10^{ab}	7.46±0.32^{a}	22.54±4.01^{ab}
	Traveller	60.07±0.74^{a}	23.60±0.64^{a}	11.66±1.01^{a}	4.43±0.20^{a}	1.10±0.08^{a}	46.69±0.35^{b}	8.33±0.55^{a}	28.88±0.40^{b}
	Explorer	56.70±0.14^{a}	21.22±1.32^{a}	10.20±1.13^{a}	3.78±0.00^{a}	1.08±0.06^{a}	34.55±1.43^{ab}	7.45±0.91^{a}	15.75±0.85^{a}
	ABI Kranti	56.05±1.15^{a}	22.71±1.11^{a}	11.54±1.22^{a}	4.62±0.09^{ab}	1.03±0.06^{a}	34.57±4.36^{ab}	8.19±0.17^{a}	27.08±2.00^{b}
	Mean	59.11±1.15	22.78±0.41	11.09±0.28	4.19±0.15	1.04±0.02	37.64±2.39	7.73±0.22	23.28±2.28
Indigenous genotypes	DWRUB 52	55.91±4.71^{a}	20.33±0.09^{a}	9.48±0.52^{a}	4.23±0.09^{a}	1.01±0.11^{a}	31.23±3.23^{ab}	7.98±0.27^{a}	13.95±2.06^{a}
	DWRB 91	59.47±6.79^{a}	21.14±0.55^{a}	12.16±1.80^{a}	5.41±0.25^{ab}	0.96±0.04^{a}	29.45±1.93^{ab}	6.91±0.99^{a}	23.34±1.17^{ab}
	DWRB 101	50.69±5.59^{a}	22.03±1.02^{a}	11.04±0.24^{a}	4.62±0.10^{ab}	0.74±0.06^{a}	28.76±1.71^{a}	6.87±0.67^{a}	14.49±0.55^{a}
	DWRB 160	57.94±4.71^{a}	20.97±1.23^{a}	10.98±0.25^{a}	6.24±0.90^{b}	1.18±0.14^{a}	34.18±5.14^{ab}	8.78±1.57^{a}	21.93±1.60^{ab}
	DWRB 182	53.37±3.09^{a}	21.14±0.64^{a}	12.40±1.14^{a}	3.89±0.20^{a}	1.10±0.01^{a}	32.70±2.16^{ab}	7.77±0.60^{a}	20.75±0.10^{ab}
	Mean	55.48±1.57	21.12±0.27	11.21±0.52	4.88±0.42	1.00±0.08	31.26±1.00	7.66±0.36	18.89±1.95

* The values are the mean of two replications ± standard error. Means with the same letters in the same column are not significantly different ($p<0.05$).
TPC – Total Phenolic Content; dwb – Dry weight basis

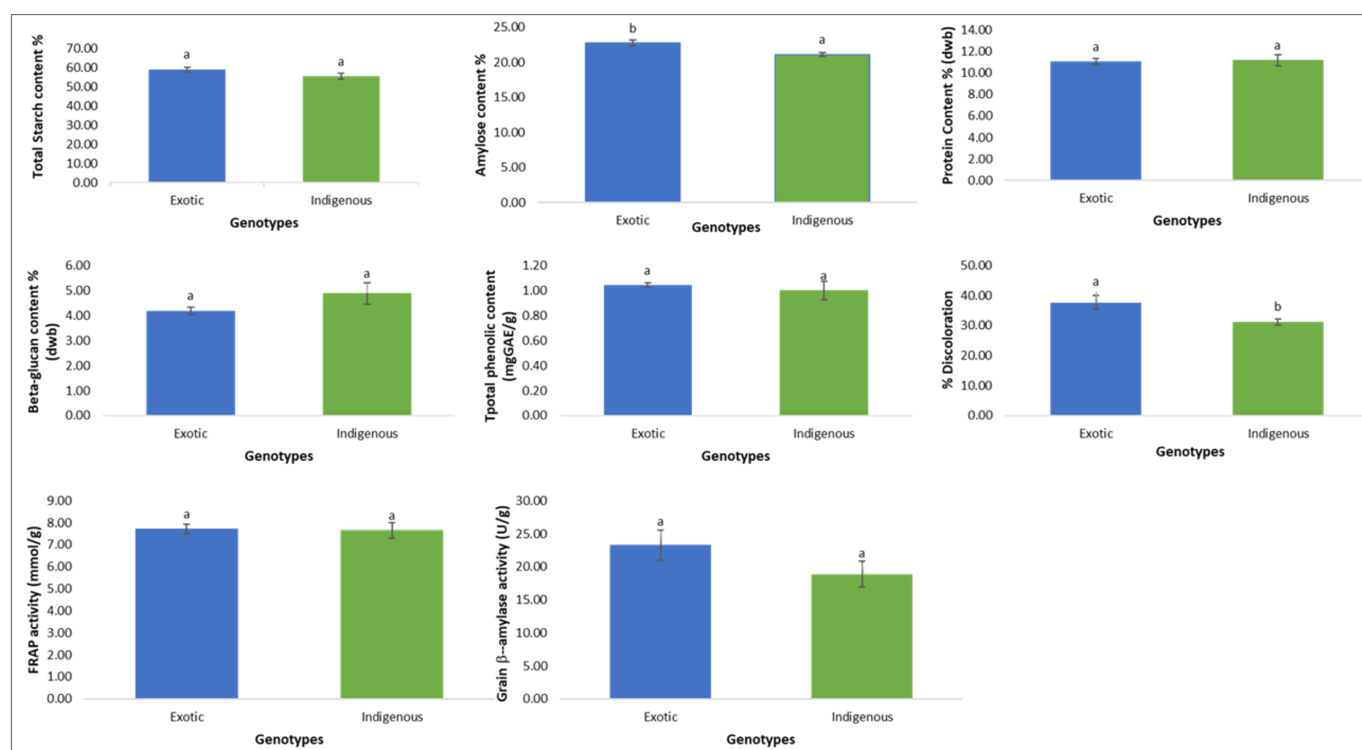

Fig 1: Differences between exotic and indigenous malt barley genotypes for various biochemical parameters. Blue- Exotic, Green- Indigenous genotypes

4. Conclusions

Based on these results it can be concluded that the exotic genotypes are better in terms of the low beta-glucan content, high amylose content and high beta-amylase activity which are the most important parameters for a good malting quality genotype. In future, further studies are required to correlates the biochemical traits with malting quality parameters and also starch properties can be studied in detail to get more insight into these difference in the malting quality of indigenous and exotic genotypes.

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6. Conflict of interest

There are no conflicts of interest.

5. References

1. Bothmer R. Distribution and habitat preferences in the genus Hordeum in Iran and Turkey. Annalen des Naturhistorischen Museums in Wien. Serie B für Botanik und Zoologie; c1996. p. 107-116.
2. Zhao H, Fan W, Dong J, Lu J, Chen J, Shan L, *et al*. Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chemistry. 2008;107(1):296-304.
3. Bhatty RS. The potential of hull-less barley. Cereal Chemistry. 1999;76(5):89-599.
4. Fangel JU, Eiken J, Sierksma A, Schols HA, Willats WGT, Harholt J. Tracking polysaccharides through the brewing process. Carbohydrate Polymers. 2018;196:465-

473.
5. Müller M, Muth JR, Gallusci P, Knudsen S, Maddaloni M, Motto M, Schmitz D, Sörensen MB, Salamini F, Wettstein DV, Thompson RD. Regulation of Storage Protein Synthesis in Cereal Seeds: Developmental and Nutritional Aspects. Journal of Plant Physiology. 1995;145(5-6):606-613.
6. Clegg KM. The application of the anthrone reagent to the estimation of starch in cereals. Journal of the Science of Food and Agriculture.1956;7(1):40-44.
7. Hodge JE, Hofreiter BT. Determination of reducing sugars and carbohydrates. Methods in Carbohydrate Chemistry. 1962;1:380-394.
8. Sowbhagya CM, Bhattacharya KR. A Simplified Colorimetric Method for Determination of Amylose Content in Rice. Starch – Stärke. 1971;23(2):53-56.
9. Mixed-linkage beta-glucan assay procedure (McCleary method 2021). www.megazyme.com
10. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology.1999;299:152-178.
11. Beta T, Nam S, Dexter JE, Sapirstein HD. Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. Cereal Chemistry. 2005;82(4):390-393. https://doi.org/10.1094/CC-82-0390
12. Benzie IFF, Strain, JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Analytical Biochemistry. 1996;239(1):70-76.
13. Beta-amylase Assay Kit Betamyl-3 -Analysis of β-amylase https://www.megazyme.com/β-amylase -assay-kit
14. Bamforth C W, Martin HL. β-glucan and β-glucan solubilase in malting and mashing. Journal of the Institute of Brewing. 1981;87(6):365-371.
15. Gianinetti A, Toffoli F, Cavallero A, Delogu G, Stanca AM. Improving discrimination for malting quality in barley breeding programmes. Field Crops Research. 2005;94(2-3):189-200.
16. Duke SH, Henson CA, Bockelman HE. Comparisons of Modern U. S. and Canadian Malting Barley Cultivars with Those from Pre-Prohibition: III. Wort Sugar Production during Mashing. Journal of the American Society of Brewing Chemists. 2018;76(2):96-111.