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BK 306: A two-row barley (*Hordeum vulgare* L) as potential source of higher diastatic power and FAN content for malt barley improvement

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Abstract

The Indian malt industry has updated the specifications in past few years with respect to the diastatic power of the malt and free amino nitrogen content in the wort. The demand is now for higher values of malt diastatic power for better degradation of starch during the malting and brewing processes. Similarly, higher values of free amino nitrogen (FAN) content are preferred for better yeast growth during the brewing operations. We hereby report a genotype BK 306, which posses' higher diastatic power (average of 107°L) and FAN content (average of 216 ppm) as compared to the prevalent two row malt barley varieties. The genotype can be a potential source for these two traits for malt barley improvement programme of the country.

Key words: Barley, *Hordeum vulgare*, diastatic power, FAN, malt

1. Introduction

Malt is one of the major industrial products of barley and in India approximately 30% of the total barley production is utilized for this purpose (Kumar *et. al.,* 2021). Beer is one of the major products made from barley malt. In terms of volume, the Indian beer market was valued at 5,533.73 Mn ltrs in 2020 and is expected to reach 9,004.74 Mn ltrs by 2025, expanding at a compound annual growth rate (CAGR) of ~10.89% during the 2021 - 2025 period. Urbanization and change in societal perspectives, along with the launch of new low- and no-alcohol variant beer, technological advancements are a few of the significant factors that propel market growth (Source: https://www.globenewswire.com/en/news-release/2021/04/21/2213979/28124/en/ Indian-Beer-Market-Size-and-Growth-Forecasts-2021-2025-Craft-Beer-Forecast-to-Grow-at-a-Rate-of-108-in-Volume-Terms.html, Accessed on 17.01.2022). For making malt, some special physical and biochemical characteristics are desired by the industry and in past few years the demand for barley genotypes with higher malt diastatic power and higher free amino nitrogen (FAN) content in wort has increased.

The diastatic power of barley malt is the collective activity of starch degrading enzymes, which accumulates or gets activated during malting (Gibson *et. al.*, 1995). Higher diastatic power becomes more important, when adjuncts (eg wheat grains, rice flakes etc) are used for brewing and barley malt is used as source of starch degrading enzymes. Normally two row barley is used for malting especially because of higher starch content as compared to the six row type. However, two row row barley has normally lesser diastatic power vis-a-vis the six row barley. Diastatic power, like other quality attributes in barley, has been reported to be determined by a complex interaction of genetic and environmental factors (Arends *et al.,* 1995). Therefore, development of two row barley with higher diastatic power is a major challenge for the Indian malt barley programme (ICAR-IIWBR, 2021).

The nitrogenous compounds available for consumption by growing yeast (serving as catalyst in fermentation) during brewing are known as free amino nitrogen (FAN) plus ammonium ions. FAN can be defined as the sum of the individual wort amino acids and small peptides (di- and tri-peptides) plus ammonium ions. (Pugh *et al.,* 1997). Wort sugar content alone is not a good indicator of yeast fermentation performance (Ingledew, 1977). Therefore, FAN is regarded as the preferable indicator for predicting healthy yeast growth, viability, vitality, fermentation efficiency, and beer quality and stability (Stewart *et al.,* 2013). FAN is the protein degradation product of malted barley and is affected by the malt/ adjunct ratio, mashing schedule, barley variety, and malting conditions (O'Connor-Cox *et al.,* 1989). Therefore, malt barley cultivars having better FAN production capability during malting and mashing are required.

The genotype BK 306 was found to have these both traits of higher diastatic activity and higher FAN content in the preliminary studies conducted during 2019-20 at IIWBR Karnal. Therefore, the present multilocation investigation was carried out to find out the stability of these two traits across the diverse locations.

2. Materials and Methods

2.1 Grain samples

The genotype BK 306 along with the controls (current released malting barley cultivars) DWRUB 52, DWRB 91, DWRB 92, DWRB 101, RD 2849, DWRB 123, DWRB 160 and DWRB 182 was grown in rabi season (mid-November to mid-April) of 2020-21 at Karnal, Hisar, Ludhiana, Durgapura, Pantnagar and Kanpur in augmented design in single replication, with a plot size of 2.5 x 0.60 m^2 at each location. The crop was fertilized with 60 kg N (in 2 equal splits); 20 kg P and 20 kg K and all the other recommended crop management practices including weed, insect/pest were followed as and when required. The crop was harvested and thrashed mechanically; the collected grains were cleaned manually and stored in air tight bags at -20°C till further analysis. Most of the analysis was done as per EBC (2003) procedures until or otherwise stated at respective places.

2.2 Grain physical traits (Test weight, thousand grain weight, Bold grains percentage)

Test weight was estimated by using hectolitre measurement equipment designed by ICAR-IIWBR, Karnal for small grain samples and weighing on electronic balance up to 1 gram's accuracy. The test weight was then expressed as kilogram per hectolitre. For thousand grain weight (TGW), 1000 grains were counted using Contador (Pfeuffer Germany) seed counter and weighed on electronic balance up to two digits in grams. The grain plumpness was measured by using 100-gram grains on Sortimat laboratory grader (Pfeuffer GmbH, Germany) and sieved for three minutes using the sieves of 2.8 mm, 2.5 mm and 2.2 mm. The grains retained on 2.5 mm and above were considered as bold/plump grains. The grains passed through 2.2 mm sieve were designated as thin grains, while the fraction retained on 2.2 mm was considered as intermediate size grain.

2.3 Protein content

The protein content was estimated by near infrared transmittance (NIR) grain analyser (Infratech 1241, FOSS, Denmark). The values were expressed on percent dry weight basis (% dwb).

2.4 Malt preparation

The bold/plump grains (grains >2.5 mm screen) processed on Sortimat (Pfeuffer make laboratory grader) were used for malting in an automatic micro-malting system (Joe White Australia make). The malting cycle involved steeping, germination and kilning stages as per following schedule:

1. Steeping: 8 hours dip in water (temperature 18°C) with continuous aeration 6 hours air rest (temperature 18°C) →10 hours dip in water (temperature 18°C) with continuous aeration
2. Germination: 24 hrs at 18°C→24 hrs at 17°C→24 hrs at 16°C
4. Kilning: 3 hrs at 45°C→3 hrs at 50°C→3 hrs at 55°C→3 hrs at 60°C→3 hrs at 65°C→3 hrs at 70°C→3 hrs at 75°C→3 hrs at 80°C

The malt was taken out from machine after cooling to room temperature and rootlets were removed by hand rubbing. The malt samples were stored in air tight interlocking polythene bags at -20°C till further analysis.

2.5 Malt friability and homogeneity

A 50-gram quantity of malt was used in Friability meter (Pfeuffer, Germany) and machine was run for 8 minutes. The malt powder obtained was weighed on electronic balance to estimate percent friability. Thes malt fractions retained in friability meter mesh were then mixed and put on Sortimat (Pfeuffer, Germany) for one minute and fraction passing through 2.2 mm screen (plus the powdered malt) was considered homogenous malt.

2.6 Wort preparation

The malt flour was prepared in Buhler's laboratory Mill at fine grinding setting and the flour was extracted in EEC make (Australia) mashing bath for 45°C and then at 70°C for a total duration of 120 minutes. The resulting slurry was used to determine wort filtration rate and hot water extract in malt.

2.7 Filtration rate

The slurry obtained after mashing was filtered through Whatman 2555 ½ filter paper and the filtrate obtained in one hour was considered as wort filtration rate (ml/ per hour).

2.8 Hot water extract

The hot water extract or malt extract was determined using Borosil make A grade specific gravity bottles. Fifty ml of wort was kept at 18°C for 20 minutes and specific gravity was measured. The hot water extract or malt extract was computed from standard EBC table and expressed as percent fgdb (fine ground dry basis).

2.9 Diastatic Power

The analysis of diastatic power (D.P.) of malt was done as per the IOB method and expressed in °Linter value as described by Farzaneh *et al.* (2017). In brief starch degradation was done using barley malt powder as enzyme source. The free sugars were estimated through titration using Fehling solutions and methylene blue indicator.

2.10 Free Amino Nitrogen

Free Amino Nitrogen content (FAN) was determined using the method reported by Lie (1973), in brief the colour was developed in the diluted wort sample using ninhydrin and readings were taken spectrophotometrically at 570 nm. Glycine was used as standard for the calculations.

3. Results & Discussion

Barley malt is the major source of nutrition for the yeasts during the fermentation process of in brewing. The sugars and free amino acids constitute the major raw material used by the yeasts, which depends upon the starch content of grain, the starch breaking diastatic enzymes, protein content and the activities of several proteinases in grain and/or malt (Cynthia and Stanley, 2007 and De Schepper *et al.,* 2021). In recent years the brewing industry considers the higher diastatic power and free amino nitrogen as two of the important factors among the several other malt and grain quality criterions. These two parameters and some of other traits in Indian released barley cultivars and genotype BK 306 have been described and discussed in following sections:

3.1 Diastatic Power

The Diastatic Power of barley malt represents the collective activity of four starch-degrading enzymes, namely α-amylase, β-amylase, limit dextrinase, and α-glucosidase (Gibson *et al.,* 1995). The conversion of starch to fermentable products in the endosperm is primarily catalysed by α-amylase, followed by β-amylase, limit dextrinase and α-glucosidase (Bamforth, 2009). Of these enzymes, beta-amylase is laid down during grain filling and alpha-amylase, α-glucosidase and limit dextrinase are synthesised during germination, predominantly in the scutellum and aleurone layers (Arends *et al.,* 1995).

Though the malted barley is the main source in the traditional brewing of beers, but its use has been increasingly substituted by un-malted barley or other raw grain adjuncts (like wheat or rice) in recent years (Cadenas *et al.,* 2021). The incorporation of raw grains is mainly economically driven as the expenditure on malting is evaded. The use of raw grains, however, requires modifications to the brewing process to accommodate the lack of malt enzymes and the differences in structural and chemical composition between malted and raw grains (Kok *et al.,* 2018). Therefore, the barley malt with higher diastatic power is desired by the brewing industry. The genotype BK 306 has been found to have average value of 107-degree Linters (°L in IOB) or 358.5 Windisch-Kolbach units (°WK in EBC; Lintner = (°WK+16)/3.5, which is higher than the most prevalent malt barley varieties DWRUB 52 (96 °L) or DWRB 101 (97 °L) or all the other checks used in this study (Table 1). Diastatic

power is considered as a critical parameter of malt quality and normally higher diastatic power is required to get the higher malt extract from the barley grains (Cynthia and Stanley, 2007; Rani and Bhardwaj, 2021). Variation in DP of malt is affected by the complex interaction of genetic variation and environmental factors (Arends *et al.,* 1995). However, as per Fang *et al.,* (2019), diastatic power is mainly determined by genetic factors and easier to improve; thus, the genotype BK 306 can act as donor of this trait in malt barley improvement programme. Three QTLs that significantly increased diastatic power have been mapped on 1H and 4H (Cu *et al.,* 2016). This two-row genotype is progeny from the cross BK9811 / DL472 (F5-50) and having higher diastatic power in two row background can be a material for further genetic and molecular biology studies.

Table 1: Diastatic power (°L) in the malt of genotype BK 306 grown at different locations

Genotype	Hisar	Karnal	Ludhiana	Durgapura	Pantnagar	Kanpur	Average
BK 306	116	102	106	108	106	106	**107**
DWRUB 52 (c)	94	100	106	99	83	94	**96**
DWRB 91 (c)	104	109	109	109	98	102	**105**
DWRB 92 (c)	98	106	102	106	109	104	**104**
DWRB 101 (c)	104	104	96	83	100	96	**97**
RD2849 (c)	100	100	94	98	98	79	**95**
DWRB 123 (c)	111	94	94	96	109	104	**101**
DWRB-160 (c)	111	96	104	99	109	96	**103**
DWRB-182 (c)	109	109	109	90	98	98	**102**

3.2 Free Amino Nitrogen (FAN)

Free amino nitrogen is the only nitrogen source for yeast cell growth and reproduction in the wort and plays major role in metabolite changes during fermentation (Stewart *et al.,* 2013). FAN not only provides nutrition for yeast, but also constitutes the flavor substance of beer. Although higher protein content in the grains can increase the FAN content but leads to a decrease of malt extract (Qi *et al.,* 2005). Therefore, FAN in the wort is generally maintained at 180–220 mg/L (Fang *et al.,* 2019 and references there in). The genotype BK 306 has average FAN content of 216 mg/L or 216 ppm in the wort, which is in desirable range and higher than all the checks used in the study (Table 2). However, some authors have reported different ranges of FAN for optimum yeast growth. Stewart *et al.,* (2017) stated that the amount of FAN needed for the optimum yeast growth is around 130 mg/L. But the industry, especially in India, is asking for higher FAN content *i.e.,* minimum 150 mg/L (ICAR-IIWBR, 2021). Both the amount of proteins and activities of proteinases are important for higher free amino nitrogen content in the wort (Jones and Marinac, 2002; Hill and Stewart, 2019). Mainly endoproteases (primarily cysteine and metallo), carboxypeptidase, dipeptidase take part in proteolysis (Steiner *et al.,* 2012 and references therein).

Table 2: Free Amino Nitrogen (FAN content, in ppm) in the wort of genotype BK 306 grown at different locations

Genotype	Hisar	Karnal	Ludhiana	Durgapura	Pantnagar	Kanpur	Average
BK 306	201	197	206	230	244	217	**216**
DWRUB 52 (c)	192	168	257	174	198	202	**199**
DWRB 91 (c)	183	151	215	173	199	148	**178**
DWRB 92 (c)	186	173	235	187	244	143	**195**
DWRB 101 (c)	200	159	212	188	225	191	**196**
RD 2849 (c)	195	163	186	180	185	124	**172**
DWRB 123 (c)	166	119	202	176	172	140	**162**
DWRB-160 (c)	160	134	163	150	189	125	**154**
DWRB-182 (c)	171	151	218	156	219	140	**176**

3.3 Other grain and malt parameters

BK 306 has average protein content of 12.4% (at nitrogen application of 60 kg/ha), thousand grain weight of 44-gram, test weight of 63 kg/hl, bold grain percentage of 91% (Table 3). The genotype BK 306 has malt friability of 73%, malt homogeneity of 95%, hot water extract of 80 % fgdb and wort filtration rate of 294 ml/hr.

Table 3: Average values of grain and malts traits of genotype BK 306 grown at six locations

Genotype	PC#	TGW#	TW#	BG#	MFB#	MH#	HWE#	WF#
BK 306	12.4	44	63	91	73	95	80	294
DWRUB 52 (c)	12.1	45	64	74	61	92	80	243
DWRB 91 (c)	11.6	60	64	89	62	92	81	225
DWRB 92 (c)	12.6	56	63	97	61	87	79	250
DWRB 101 (c)	11.5	49	66	86	64	93	80	225
RD2849 (c)	12.5	48	65	80	53	84	77	258
DWRB 123 (c)	11.4	51	64	89	57	88	79	213
DWRB-160 (c)	11.5	58	60	92	54	89	79	188
DWRB-182 (c)	12.3	44	60	74	64	90	78	270

PC = Protein content (% dwb); TGW=Thousand grain weight (g); TW=Test weight (kg/hl); BG=Bold grains (retained over 2.5 mm sieve) percentage; MFB= Malt Friability (%); MH= Malt Homogeneity (%); HWE = Hot water extract (%fgdwb); WF = Wort filtration rate (ml/hr)

3.4 Agro morphological characters, yield and disease reaction

The agro-morphological characters have been given in table 4, the yield at different nitrogen levels in Figure 1 and disease reaction has been found as: against stripe rust 5 S* (HS); ACI of 1.3* and against leaf blight 68* (HS) (Directorate of Wheat Research, 2004). The genotype BK 306 responded upto 90 kg nitrogen/hectare and has comparable yields as compared to the released malt barley variety DWRB 101 (Fig 1).

Table 4: Agro morphological traits of genotype BK 306

S. No.	Trait	Observation
1	Growth habit	Erect
2	Stem-Basal Pigmentation	Present
3	Auricle (Flag leaf)-Anthocyanin Pigmentation	Present
4	Upper node Pigmentation	Present
5	Flag leaf attitude	Erect
6	Flag leaf-Waxiness of Sheath	Present
7	Spike emergence	Late
8	Spike type	Two-row
9	Lateral florets (two-row barley)	Developed
10	Spike-Waxiness	Present
11	Spike-colour	Green
12	Spike-attitude	Erect
13	Awn-roughness	Rough
14	Flag leaf length	Long
15	Flag leaf breadth	Narrow
16	Awn-Tip pigmentation	Present
17	Spike-basal sterility	Absent
18	Lemma-pigmentation	Absent
19	Spike length	Medium
20	Plant-height	Tall

21	Peduncle-length	Long
22	Awns	Present
23	Awns-type	Normal
24	Awn-length	Medium
25	Spike-density	Intermediate
26	Grain-hulless	Covered (Hulled)
27	Grain-colour	Yellow
28	Grain-shape	Oblong
29	Grain-size	Medium
30	Grain-surface	Wrinkled
31	Rachilla hairs	Rudimentary
32	Grain-Crease width	Intermediate
33	Flag leaf length	18.1 inches
34	Flag leaf breadth	0.8 inches
35	Spike: Basal sterility	Absent
36	Spike length	8.0 cm
37	Plant height	97.0 cm
38	Peduncle length	29.0 cm
39	Awn length	8.6 cm
40	Days to heading	90
41	Days to maturity	130

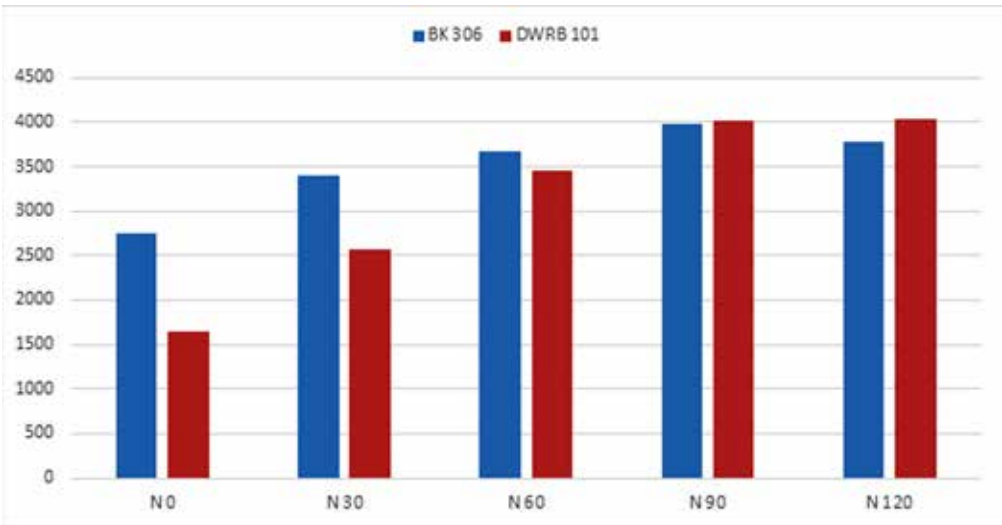

Fig. 1: Yield in kilograms of BK 306 and DWRB 101 at different nitrogen levels (pooled data of two years)

Conclusion

BK 306 can be an excellent source of higher diastatic power and FAN content for the malt barley improvement programme, and it may prove to be a very valuable research material in combination to the disease resistance to stripe rust in good agronomic background.

Conflict of Interest

Authors declare that they have no conflict of interest

Ethical Compliance Statement

NA

Author's Contribution

Dinesh Kumar (Planning & execution of experiment and writing of MS); Ramesh Pal Singh Verma (Breeder of the material, Editing of MS); Anil Kumar Khippal (Evaluation of BK 306 at different nitrogen doses); Ajit Singh Kharub (Evaluation of BK-306 at different nitrogen dotes); Charan

Singh (Recording of Agro-morphological traits of the genotype) and Gyanendra Pratap Singh (Planning and Guidance)

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