



Gene effect analysis of forage quality traits in barley (*Hordeum vulgare*) in Bundelkhand region of India

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ABSTRACT

Present study was carried out during winter (*rabi*) seasons of 2019–20 and 2020–21 at Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh to decipher underlying gene effects for green forage quality characters in barley (*Hordeum vulgare* L.) in Bundelkhand region of Uttar Pradesh. Eight diverse barley genotypes, viz. BH902, BH946, DWRB160, DWRB180, RD2552, RD2794, RD2899 and RD2907 were evaluated and crossed in half diallel design. A total of 28 F₁s along with 8 parents were sown in randomized complete block design (RCBD) with 3 replications. The green fodder was harvested after 55 days of sowing (DAS) and fodder quality analysis was conducted. The crude protein (CP) and nitrogen (N) contents showed mean values of 14.19 and 2.49% ranged from 12.62–15.26% and 2.22–2.68%, respectively. The characters, viz. organic matter (OM), CP, N, lignin (L) and hemicellulose (HCL) showed preponderance of additive gene effects, whereas acid detergent fibre (ADF), neutral detergent fibre (NDF) and cellulose (CL) showed the prevalence of dominance gene effects. The genotype DWRB180 and two rowed malt barley variety DWRB160 were estimated with desirable additive gene effects for forage intake and digestibility traits. The cross combinations, DWRB160/RD2899, BH946/RD2552 and RD2794/DWRB180 can be further utilized for CP and N contents, whereas, the progenies, viz. BH946/DWRB180 and RD2552/RD2907 were promising for ADF and NDF. GT biplot corroborated positive correlations of NDF with ADF (0.43**), HCL (0.88**) and CL (0.41*). The improvement in forage intake and digestibility characters like ADF, NDF and CL is suggested through complex crossing followed by selection.

Keywords: Barley, Forage quality, GT biplot, PCA

Barley (*Hordeum vulgare* L.) is a self-pollinated ancient coarse cereal crop, domesticated 10,000 years ago with wheat in the Fertile Crescent region of the world (Haas *et al.* 2019, Kumar *et al.* 2023). It is grown over a wide range of environments and marginal to sub marginal lands under rainfed conditions than any other cereal (Newton *et al.* 2011). The crop belongs to the family Poaceae, tribe triticeae and genus *Hordeum* having almost 350 species (Kumar *et al.* 2014). Barley is broadly utilized as animal feed (60%), malt production (25–30%), seed production (5%) and human food (5%) (Baik and Ullrich 2008, Kumar *et al.* 2020). It has high nutritive value coupled with high soluble fibre concentration and antioxidants helpful in lowering risk of cardio-vascular diseases and hyperlipidaemia (De Paula *et al.* 2017, Zeng *et al.* 2018). Worldwide during 2021, barley

ranked 4th in terms of production of 145.62 Mt from 48.94 Mha after maize (1210.23 Mt), rice (787.29 Mt) and wheat (770.87 Mt) (FAOSTAT 2023). The production of 1.68 Mt barley grains was estimated in India during 2022–23 from an acreage of about 6.17 lakh ha (<https://eands.dacnet.nic.in/> DES, MoA & FW 2022–2023). Barley is one of the major crops of Bundelkhand region of Uttar Pradesh (UP) and Madhya Pradesh (MP) due to its resilience against low rainfall and terminal heat.

Animal husbandry is one of the most important sources of livelihood for people in Bundelkhand region of MP and UP. Barley has low water and fertilizer requirements coupled with high regeneration capacity and can serve a potential dual-purpose crop in this dry region. It can be utilized as green fodder with limited water resources, where berseem, oat and sugarcane fodder is not available due to the water shortage (Jarial 2015). For improving the green fodder nutritional quality traits evaluation of genotypes, kind of gene effects and their segregants is utmost required. Such understanding of gene effects underlying for green fodder quality traits like crude protein, fodder intake and fodder digestibility characters will be helpful in selecting suitable

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genotypes and in designing future breeding programmes. Therefore, the present study was planned for genetic analysis of fodder quality traits in barley in Bundelkhand region of Uttar Pradesh.

MATERIALS AND METHODS

Present study was carried out during winter (*rabi*) seasons of 2019–20 and 2020–21 at Rani Lakshmi Bai Central Agricultural University, Jhansi (25°31'02.5"N, 78°33'05.11"E and at altitude of 271 m amsl), Uttar Pradesh. During *rabi*, 2019–20, 8 diverse barley genotypes, viz. BH902, BH946, DWRB160, DWRB180, RD2552, RD2794, RD2899 and RD2907 were evaluated and crossed in half diallel (excluding reciprocals) design (Griffing 1956 method II, Model I) (Table 1).

During *rabi*, 2020–2021, 28 F₁s along with 8 parents were sown in randomized complete block design (RCBD) with 3 replications in 2 m row length and 30 cm spacing. The parents were grown in paired rows of same row length and spacing. The green fodder was harvested after 55 days of sowing (DAS) and fodder quality analysis was conducted at ICAR-Indian Grassland and Fodder Research Institute, Jhansi (ICAR-IGFRI). Organic matter (OM) present in the leaves (100-ash%) was estimated following AOAC (2005). Crude protein (CP) and nitrogen contents (N) were obtained following Micro-Kjeldahl method. The acid detergent fibre (ADF%), cellulose (CL%), acid detergent lignin (L%), neutral detergent fibre (NDF%) and hemicellulose (HCL%) were computed following the procedures described by Van Soest *et al.* (1991).

Statistical analysis: The mean data of all studied traits were subjected to the statistical analysis. ANOVA, means and range were calculated following standard procedures in R 4.1.1. The heterosis, better parent heterosis and combining ability (GCA, General combining ability; SCA, Specific combining ability) were computed following standard procedures. The genotype by trait biplot (GT biplot) and correlations were developed following R Studio packages. The genotype by trait biplot was generated adopting column metric preserving singular value partitioning (SVP) and tester centred G + GE without any scaling as per Yan and Kang (2002). The principal component analysis (PCA) was carried out with covariance matrix and Eigen values were loaded for different principal components (PCs).

RESULTS AND DISCUSSION

Per se performance: The analysis of variance revealed significant differences ($P < 0.01$) for all the green fodder quality traits indicating the presence of adequate genetic variation among the treatments. The OM exhibited general mean value of 90.57%, which ranged from 89.41–91.98% among parents and F₁s. The parent DWRB180 exhibited average OM of 91.52%, followed by RD2907 (90.77%) and RD2552 (90.52%). The hybrids, viz. DWRB180/RD2907 (91.98%), BH946/DWRB180 (91.88%) and BH946/RD2552 (91.80%) depicted the highest organic matter content. The CP and N contents showed mean values of 14.19 and 2.49%, which ranged from 12.62–15.26% and 2.22–2.68%, respectively. The hybrids DWRB160/RD2899, BH946/BH902, BH902/RD2794, BH946/RD2552

Table 1 Details of parents selected for diallel mating

Parent	Symbol	Row pattern (use)	Parentage	Developed by	Trait(s)
BH946	G1	6 (Feed)	BHMS22A/BH959//RD 2552	CCS Haryana Agricultural University, Hisar, Haryana	Good agronomic base
DWRB160	G2	2 (malt)	DWRB62/DWRB73	ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana	Two-rowed malt barley with very high 1000-grain wt.
BH902	G3	6 (Feed)	BH495/RD2552	CCS Haryana Agricultural University, Hisar, Haryana	Good agronomic base
RD2552	G4	6 (Feed)	RD2035/DL472	Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan	Good agronomic base and salinity tolerant
RD2794	G5	6 (Feed)	RD2035/RD2683	Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan	Good agronomic base and salinity tolerant
RD2899	G6	6 (Feed)	RD2592/RD2035//RD2715	Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan	Good agronomic base
DWRB180	G7	6 (Feed)	P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/M111	ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana	Spot blotch resistance (genetic stock)
RD2907	G8	6 (Feed)	RD103/RD2518//RD 2592	Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan	Good agronomic base and salinity tolerant

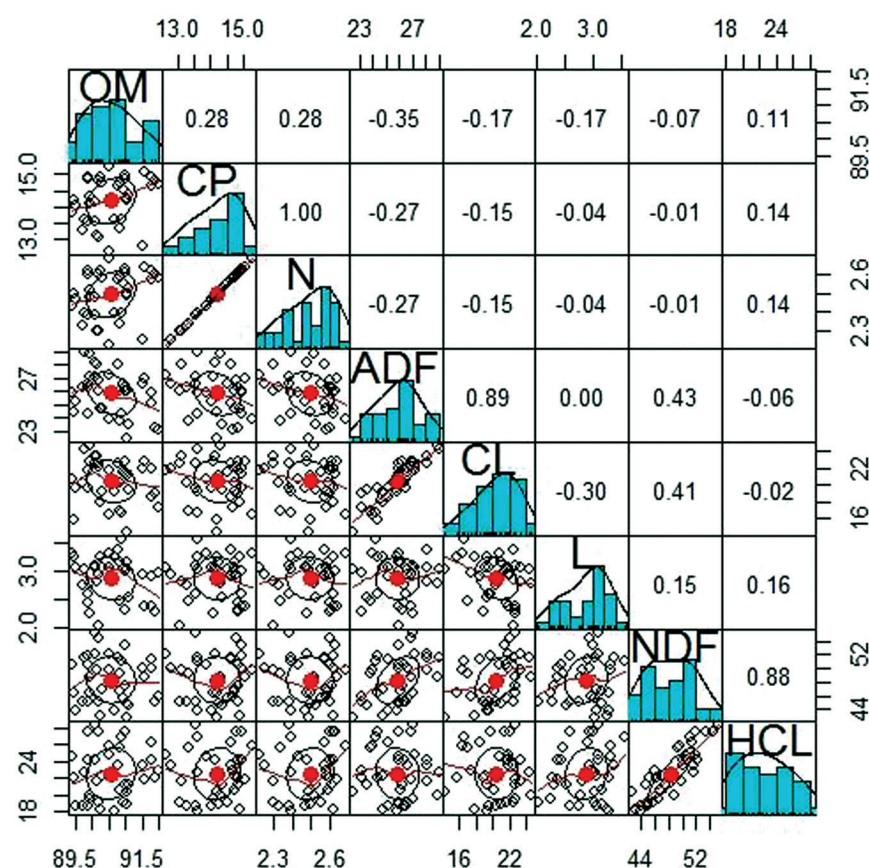


Fig. 1 Scatter plot for 8 fodder quality traits in barley.

and RD2899/RD2907 recorded higher CP and N contents. Among the parents, DWRB180 and RD2794 depicted the lowest ADF content of 26.61 and 27.05%, whereas the parental genotypes, BH902, DWRB160 and DWRB180 were promising with low NDF values. The CL, L and HCL contents ranged from 14.46–24.62%, 2.03–3.58% and 17.98–28.29%, respectively. The parent RD2794 showed the highest HCL content of 28.29%, followed by the hybrids, viz. RD2794/RD2899 (27.57%), RD2899/RD2907 (27.55%) and BH902/RD2794 (27.43%). The *per se* performances are depicted in the scatter plot (Fig. 1).

Heterosis and heterobeltiosis: The exploitation of heterosis commercially in crop plants is widely surveyed a milestone in plant breeding. The heterosis study has a direct relevance on the breeding methodology to be selected for improvement of variety and also provides details about the utility of the parents in breeding programmes. Average heterosis and heterobeltiosis for OM ranged from -1.53 (RD2899/DWRB180) to 1.86 (BH946/BH902) and -2.30 (RD2899/DWRB 180) to 1.77 (BH946/BH902), respectively. The 5 cross combinations, viz. BH946/BH 902 (1.77**), BH946/RD2552 (1.42**), RD2552/RD2899 (1.40**), BH902/RD2794 (0.98**) and BH946/RD2907 (0.24**) revealed significant and positive better parent heterosis. The better parent heterosis for CP and N ranged from -8.53–12.20 and -8.57–12.03%, respectively. The 17 and 20 hybrids showed significant and positive

heterobeltiosis for CP and N contents. The F_1 s namely, RD2552/DWRB180 (12.20**), BH946/RD2552 (9.70**), BH946/DWRB180 (8.89**), RD2794/DWRB180 (8.83**), DWRB160/DWRB180 (8.73**) and DWRB160/RD2899 (7.92**) were among the highest heterobeltiotic crosses. The hybrids such as, RD2552/RD2907 (-18.82**), followed by BH946/DWRB160 (-16.73**) and BH946/DWRB180 (-16.60**) showed negative significant heterosis for ADF, whereas, the crosses, viz. RD2552/RD2907 (-17.51**), DWRB160/RD2794 (-16.38**) and RD2552/RD2794 (-16.08**) were heterotic for NDF. The magnitude of average heterosis and heterobeltiosis depicted from -27.98 (BH946/RD2907) to 22.33 (BH902/RD2899) and -31.66 (BH946/RD2907) to 16.26 (BH902/RD2899), respectively. For HCL, the highest heterosis was manifested by the cross BH902/RD2899 (21.75**), followed by RD2899/RD2907 (15.65**) and BH946/DWRB160 (15.35) and BH902/RD2794 (13.84**).

Gene effects: In the present investigation, the mean squares due to GCA and SCA were significant for all the green fodder quality traits. The characters namely, OM, CP, N, L and HCL showed preponderance of additive gene effects with higher GCA effects. The traits, viz. ADF, NDF and CL showed the prevalence of dominance gene action with high SCA effects. For OM only the parent DWRB180 showed significant and positive GCA effects (0.46**), whereas the parental genotypes, RD2899 and RD2907 were found with significant and positive GCA effects for CP and N contents. For ADF and NDF the genotypes, DWRB180 and RD2907 were better general combiners, whereas for CL the parents DWRB160 (-0.31**), RD2552 (-0.69**) and RD2907 (-1.07**) were found with high and desirable GCA effects (Table 2). For lignin content the genotype BH946 was promising, whereas the genotypes RD2794 and RD2899 were better general combiners for HCL.

PCA, GT biplot and correlation analyses: In the present study principal component analysis (PCA) was carried out for all the fodder quality characters and initial two PCs captured significant variations (95%) of the total variations. The initial four PCs depicted nearly 66, 29, 3 and 1% of the total variations leading to the 99% of the cumulative variations (Table 3).

Here, the first PC revealed the greater variability present for NDF, HCL and CL followed by HCL in second PC. Whereas, the characters namely, ADF, L and NDF

Table 2 Estimation of GCA effects for green fodder quality traits in barley

Genotype	OM	CP	N	ADF	CL	L	NDF	HCL
BH946	0.14	0.14	0.03	0.48**	1.22**	-0.34**	0.59 **	0.12
DWRB160	-0.26	-0.30	-0.05	-0.03	-0.31**	-0.04	-1.45**	-1.46
BH902	0.09	0.05	0.01	0.69**	0.97 **	0.01	-0.33	-1.02
RD2552	0.19	-0.39	-0.07	-0.26	-0.69 **	-0.02	-0.88**	-0.62
RD2794	-0.48	-0.05	-0.01	0.21	0.24 **	0.065	1.20**	0.99**
RD2899	-0.24	0.25**	0.04**	0.043	-0.15	0.14 **	2.84**	2.79**
DWRB180	0.46 **	-0.09	-0.02	-0.62**	-0.19	0.03	-1.13**	-0.51
RD2907	0.09	0.41**	0.07**	-0.52**	-1.07**	0.15**	-0.81**	-0.29

*Significant at 5% level. OM, Organic matter; CP, Crude protein; N, Nitrogen contents; ADF, Acid detergent fibre; CL, Cellulose; L, Acid detergent lignin; NDF, Neutral detergent fibre; HCL, Hemicellulose

Table 3 PCA analysis and loading of Eigenvectors of covariance matrix

Item	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigenvalues	20.39	8.94	0.84	0.40	0.32	0.06	0.00	0.00
proportion	0.66	0.29	0.03	0.01	0.01	0.00	0.00	0.00
cumulative proportion	0.66	0.95	0.98	0.99	1.00	1.00	1.00	1.00
<i>Eigenvectors loading</i>								
Character	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
OM	-0.004	0.067	-0.552	0.279	-0.783	-0.035	0	0
CP	0.004	0.058	-0.442	-0.877	0.007	-0.061	-0.167	0.042
N	0.001	0.01	-0.077	-0.153	0.001	-0.01	0.955	-0.24
ADF	0.134	-0.484	0.398	-0.218	-0.389	-0.241	0.141	0.559
CL	0.209	-0.755	-0.452	0.163	0.299	0.258	0	0.001
L	0.01	0.033	0.233	-0.18	-0.267	0.917	-0.001	0
NDF	0.749	-0.055	0.207	-0.097	-0.183	-0.131	-0.141	-0.56
HCL	0.615	0.429	-0.19	0.121	0.204	0.109	0.141	0.56

OM, Organic matter; CP, Crude protein; N, Nitrogen contents; ADF, Acid detergent fibre; CL, Cellulose; L, Acid detergent lignin; NDF, Neutral detergent fibre; HCL, Hemicellulose.

contributed significantly in the third PC. The genotype by trait biplot substantiated the greater variability present for initial two PCs (Fig. 2). The characters, viz. NDF, ADF, HCL and CL were discriminative and representative among the considered fodder quality traits. The traits OM, CP, N and L were centered towards the origin and exhibited lower variations for these traits. The parental genotype, G5 (RD2794) and the crosses, viz. G23 (BH902/RD2794), G31 (RD2794/RD2899) and G35 (RD2899/RD2907) were marked with high vector length for HCL (Fig. 2).

OM revealed significant and negative correlation with ADF (-0.35*), and showed positive non-significant association with CP and N. CP and N content were highly positively correlated, whereas the CP showed negative non-significant correlations with ADF, NDF, L and CL. NDF exhibited significant and positive correlations with ADF (0.43**) and CL (0.41*). ADF and CL were having positive correlations, while HCL showed positive correlation with NDF (0.88**). The correlations obtained among the fodder quality traits were also corroborated in the GT biplot. The acute angles between NDF, ADF, CL and NDF and HCL revealed and confirmed positive association among these traits.

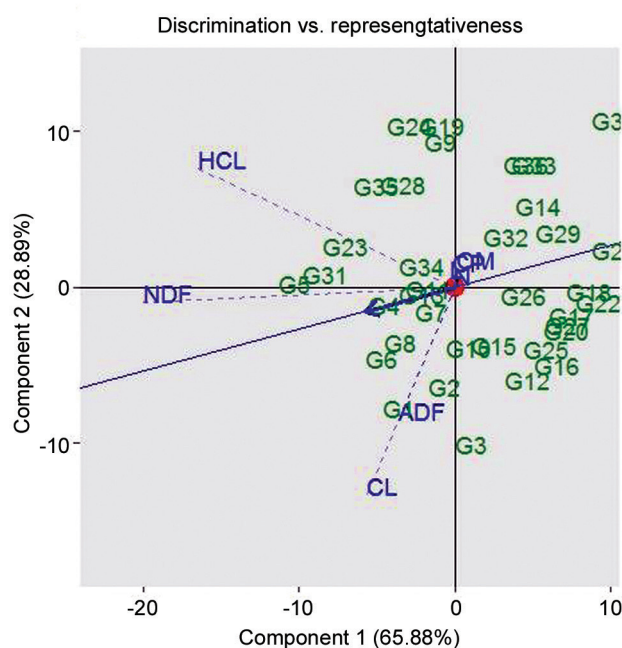


Fig. 2 GT biplot based on PC1 and PC2 for forage quality traits in barley.

Here, out of the considered 8 characters' greater variation was observed for NDF, HCL, CL and ADF, therefore these traits can be helpful in large barley germplasm classification for fodder quality analysis. The PCA analysis also substantiated for greater variations in NDF and HCL among the studied traits. NDF measures all the fibre components of forage such as HCL, CL and L and is partially digestible. The high NDF leads to decreased feed intake, and can affect the high milk production (Nair *et al.* 2018, Yang *et al.* 2021). Whereas, ADF is a direct indicator of CL and L contents in the forage, it is also less digestible and has negative association with fodder digestibility (Ball *et al.* 2001, Wang *et al.* 2016, Joshi *et al.* 2019). The factors such as rain, temperature and maturity duration etc. affect the ADF and NDF contents in the forage. In the present study the parental genotype DWRB180 showed desirable ADF, NDF, lignin and CL contents. The two-row malt barley variety DWRB160 exhibited lower NDF and lignin content. Besides CL proportion of the forage L is an important criterion as it binds up with the cellulose fraction and lower the digestibility. Hindrichsen *et al.* (2006) also reported that lignin stiffens the cell walls by cementing the matrix polysaccharides and cellulosic microfibrils, thereby leading to the lower forage digestibility. In the present investigation none of the cross was consistently good for all the fodder quality characters and suggested the role of different linkage groups for various characters. The hybrid RD2552/RD2899 depicted the highest SCA effects for OM, followed by BH946/RD2552, DWRB180/RD2907 and BH902/RD2794.

For CP and N, the crosses, viz. DWRB160/RD2899, BH946/RD2552, BH902/RD2794 and RD2794/DWRB180 were found with significant and positive SCA effects. The complex genetic nature for CP and N gene effects was corroborated as except, DWRB160/RD2899 (low $\times$ high) all other above combinations were low $\times$ low in nature. The crosses, viz. BH946/DWRB180, RD2552/RD2907 and RD2794/RD2907 exhibited significant and negative SCA effects for ADF, NDF and CL. For HCL the hybrid BH902/RD2794 depicted the highest desirable SCA effects, followed by BH946/DWRB160, RD2899/RD2907 and BH902/RD2899. The ADF and NDF contents increase with plant ageing hence barley green fodder is better for digestibility and energy for improving animal health. Barley green fodder is better than other forage crops like sorghum, maize and oat as the ADF and NDF contents were lower as also reported by Firdous and Gilani (2001), Chaudhary *et al.* (2016), Chakravarthi *et al.* (2017) and Nair *et al.* (2018). It has also been reported that green fodder harvesting at advanced tillering stage, especially in tall crop varieties is economic and better for avoiding lodging without any yield reduction (Dagar *et al.* 2022).

In conclusion, the parents DWRB180 and two-rowed malt barley variety DWRB160 were found with desirable additive gene effects for forage intake and digestibility traits. The genotypes, RD2907 and BH902 were found good general combiners for CP. The cross combinations,

DWRB160/RD2899, BH946/RD2552 and RD2794/DWRB180 can be gainfully utilized for CP and N contents, while the combinations, viz. BH946/DWRB180 and RD2552/RD2907 for ADF and NDF. The characters namely, OM, CP, N, L and HCL showed preponderance of additive gene effects and suggested for handling by pure line selection. However, the traits, viz. ADF, NDF and CL showed the prevalence of dominance gene action and can be improved through complex crossing followed by selection in barley.

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