



CHARACTERIZATION FOR DUS DESCRIPTORS AND ENVIRONMENTAL INTERACTION STUDIES FOR GRAIN PROTEIN AND STARCH CONTENT IN BARLEY (*H. vulgare*)

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SUMMARY

This study was carried out to characterize released Indian barley varieties for distinctiveness, uniformity and stability during *rabi*, 2012-13 and 2013-14 at Karnal and Faizabad. A total 79 genotypes were observed with erect growth habit, while 4 genotypes namely Alfa93, Clipper, RD2668 and Sonu were classified semi-prostrate in nature. Stem basal, auricle and upper node pigmentations were absent in most of the varieties. Only the genotypes RD 2668 and UPB 1008 were observed with short and narrow flag leaf and the cultivars BH393 and NDB943 with smooth awns. The pooled mean grain protein and starch contents were exhibited as 10.5 and 61.9%, which ranged from 8.5-13.6 and 60.2-65.7%, respectively. Grain protein content was higher in Faizabad, while the Karnal climate was more congenial for starch content. This study provides a baseline DUS data of barley varieties and will be useful for registration under Protection of Plant Varieties and Farmer's Rights (PPV&FR).

Key words: Distinctiveness, uniformity, stability, PPV&FR, AGPase, barley

Key findings: 83 barley varieties were characterized for distinctiveness, uniformity and stability (DUS) and the descriptors recorded will be helpful in selecting future parents, overview of diversity and maintaining genetic purity of the genotypes. Grain protein was higher at dry and hot environment, while starch content was more at Karnal having comparatively congenial cool climate during grain filling stage.

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INTRODUCTION

Barley is an ancient cereal grain, which with time, domestication and human need has developed mainly from a food grain to a feed and malting grain (Pourkheirandish and Komatsuda, 2007; Baik and Ullrich, 2008;

Kumar *et al.*, 2013). It is considered the fourth largest cereal crop in the world after maize, wheat and rice (Pal *et al.*, 2012). Germplasm is the base of genetic diversity, valuable source of disease and pest resistance and the primary step for the development of a food and nutritionally secure country (Brahmi *et al.*, 2004; Yadav *et*

al., 2014). India is signatory to Trade Related Aspect of Intellectual Property Rights (TRIPs), and formulated *sui generis* system for the protection of plant varieties. “Protection of Plant Varieties and Farmers Rights Act” (PPV&FR) was passed in 2001 to boost up research and protection of varieties *vis-à-vis* ensuring rights of farmers, village communities, benefit sharing and growth of seed industry (Joshi *et al.*, 2009; Patra and Chawla, 2010). Indian market is eye catching for many multi-national companies (beer, multi-grain, food products etc.), hence Indian barley germplasm needs to be protected and registered under PPV&FR. A variety should be distinct, uniform, stable (DUS) and novel to be registered under the PPV&FR act (Katiyar *et al.*, 2008; Yadav *et al.*, 2013). Therefore, we evaluated barley for 32 morphological descriptors, testing of distinctiveness, uniformity and stability. In addition, the environmental interaction and agro-ecological effects were also studied for grain protein and starch contents.

MATERIALS AND METHODS

The experimental materials comprised of 83 barley varieties, which were released after the inception of All India Coordinated Barley Improvement Project (AICBIP) for different agro-ecological conditions and purposes i.e. food, feed and malt (Table 1). During *rabi*, 2012-13, 75 genotypes were evaluated in randomized complete block design (RCBD) with 3 replications at Karnal and Faizabad locations. The experiment was repeated with inclusion of 8 new varieties namely BH 885, BH 946, BHS 400, DWRB 92, HUB 113, RD 2786, RD2794, VLB 118 during *rabi*, 2013-14 at Karnal centre, while at Faizabad, a set of 25 varieties was re-grown for characterization. The genotypes were evaluated for 32 morphological characters including growth habit (GH), stem basal pigmentation (SP), auricle anthocyanin pigmentation (AA), upper node pigmentation (UP), flag leaf attitude (FA), flag leaf sheath waxiness (FW), spike emergence (SE), spike type (ST), lateral florets (LF), spike waxiness (SW), spike colour (SC), spike attitude (SA), awn roughness (AR), flag leaf length (FL), flag

leaf breadth (FB), awn tip pigmentation (AP), spike basal sterility (SS), lemma pigmentation (LP), spike length (SL), plant height (PH), peduncle length (PL), presence of awns (PA), awn type (AT), awn length (AL), spike density (SD), hullness of grains (HG), grain colour (GC), grain shape (GS), grain weight (GW), grain surface (SG), rachilla hairs (RH) and grain crease width (CG) (Table 2). These observations were recorded on plot basis (9 sqm), while mean of 10 random competitive plants per replication was considered for flag leaf length, flag leaf breadth, spike length, plant height, peduncle length and awn length. The 2-digit code system was used to record the observations, where the first digit refers to the principal stage of development beginning with germination and ending with kernel ripening (Zadoks *et al.*, 1974). The second digit (between 0 and 9) subdivides each principal growth stage. The scale 1 to 9 was used to describe the state of each character for the purpose of digital data processing. Grain protein and starch contents of *rabi*, 2012-13 were analysed using Near Infrared Reflectance (NIR) system and the pooled analysis of variance was performed at server <http://www.iasri.res.in/sscnars> of Indian Agricultural Statistics Research Institute (IASRI).

RESULTS AND DISCUSSION

In this study, significant genetic variation was observed for all the characters, except growth habit, flag leaf sheath waxiness, awn roughness, spike basal sterility, presence of awns and awn type. The morphological descriptors on 1-9 scale are presented for some prominent malt barley varieties in Table 3 and frequency distributions with character notes of each descriptor are depicted in Table 4. In malt barley varieties, only the genotype DWRUB64 was 6-row and rest of the varieties were 2-row for spike type (Table 3). All the malt barley varieties had waxy flag leaf sheath, rough awns, medium peduncle length and intermediate spike density. Grain crease width was observed narrow in all the genotypes and rachilla hairs were rudimentary except, the variety DWR28 (Table 3).

Table 1. Name of released varieties with year and production condition.

Sr.no.	Variety	Year	Production condition*	Sr.no.	Variety	Year	Production condition*
1	KAILASH	1967	RF-TS-NHZ	43	RD 2503	1997	IR-TS-NWPZ
2	CLIPPER	1969	IR-TS-Haryana, Rajasthan, Delhi	44	RD 2508	1997	RF/IR-NWPZ
3	JYOTI	1969	RI-NEPZ	45	K 409	1997	IR-TS-U.P.
4	AMBER	1969	RF-Eastern U.P.	46	K508	1996	IR-TS-U.P.
5	RS 6	1970	RF/IR-TS-CZ	47	K551	1997	IR-TS-NEPZ
6	RATNA	1970	RF-NEPZ	48	K560	1997	RF-TS-NEPZ
7	LSB 2	1971	RF-TS-NHZ	49	DL 88	1997	IR-LS-NWPZ and IR-TS-PZ
8	RDB 1	1971	IR-TS-Rajasthan	50	RD 2552	1999	IR-TS-NWPZ, NEPZ
9	VIJAYA	1972	RF-Western U.P.	51	HBL 276	1999	RF-TS-NHZ
10	HIMANI	1973	RF-TS-NHZ	52	NB1	1999	IR-TS-U.P.
11	DOLMA	1974	RF-TS-NHZ	53	NB2	1999	IR-TS-U.P.
12	RANJIT	1974	IR-TS-Punjab	54	K 603	2000	RF-TS-NEPZ
13	BG 25	1975	IR-TS-Haryana	55	BH 393	2001	IR-TS-Haryana
14	BG 105	1975	IR-LS-Haryana	56	NB 3	2001	IR-TS/LS-UP
15	AZAD	1975	RF-Sal./alk.-U.P.	57	DWR 28	2002	IR-TS-NWPZ
16	PL 56	1975	RF-Punjab	58	RD 2624	2005	RF-TS-NWPZ
17	RD 31	1977	RF-Rajasthan	59	BHS 352	2003	RF-TS-NHZ
18	RD 57	1977	IR-Rajasthan	60	RD 2592	2004	IR-TS-Rajasthan
19	RD 103	1977	IR-TS-Rajasthan	61	NDB 1173	2005	IR-TS-sal.alk.-NWPZ/NEPZ
20	Bilara 2	1978	IR-TS-sal/alk.-Rajasthan	62	JB 58	2005	RF-TS-MP
21	Raj Kiran	1979	IR-TS-Rajasthan for C.C.N.	63	VLB 56	2005	RF-TS-Uttarakhand
22	KEDAR	1979	LS-NEPZ and TS/LS-U.P.	64	RD 2660	2006	RF-TS-NWPZ
23	SONU	1980	RF-Himachal Pradesh	65	DWRUB 52	2007	IR-TS-NWPZ
24	K 141	1982	RF-U.P.	66	RD 2668	2007	IR-TS-NWPZ
25	LAKHAN	1983	RF-U.P.	67	PL 751	2007	IR-TS-CZ
26	JAGARATI	1983	IR-U.P.	68	RD 2715	2008	IR-TS-NWPZ
27	BH 75	1983	IR-TS-Haryana	69	NDB 943	2009	IR-TS-UP
28	BHS 46	1983	RF-TS-NHZ	70	BH 902	2010	IR-TS- NWPZ
29	PL 172	1984	IR-TS-Punjab	71	BHS 380	2010	RF-TS-NHZ
30	VLB 1	1984	RF-Uttarakhand	72	DWRB 73	2011	IR-LS-NWPZ
31	RD 2052	1991	IR-Rajasthan for CCN	73	UPB 1008	2011	RF-TS-NHZ
32	MANJULA	1996	IR-LS-U.P.	74	DWRUB 64	2012	IR-LS-NWPZ
33	BHS 169	1987	RF-TS-NHZ	75	BH 885	2012	IR-TS-Haryana
34	KARAN 16	1987	IR-TS-NWPZ	76	DWRB 91	2013	IR-LS-NWPZ
35	GEETANJALI	1991	RF-U.P.	77	VLB 118	2013	RF-TS-NHZ
36	RD 2035	1994	IR-TS-NWPZ	78	RD 2786	2013	IR-TS-CZ
37	HBL 113	1995	RF-TS-NHZ	79	RD 2794	2013	IR-TS-sal.alk.-NWPZ/NEPZ
38	HBL 316	1995	RF-NHZ	80	BHS 400	2014	RF-TS-NHZ
39	Alfa 93	1995	IR-TS-NWPZ	81	DWRB 92	2014	IR-TS- NWPZ
40	PL 419	1995	RF-TS-Punjab	82	HUB 113	2014	IR-TS- NEPZ
41	PL 426	1995	IR-TS-Punjab	83	BH 946	2014	IR-TS- NWPZ
42	BCU73	1997	IR-TS-NWPZ, NEPZ, PZ				

*IR-irrigated, RF-rainfed, RI-limited irrigation, TS-timely sown, LS-late sown

NHZ-northern hills zone, NWPZ-north western plain zone, NEPZ-north eastern plain zone, CZ-central zone, PZ-peninsular zone, UP-Uttar Pradesh, CCN-cereal cyst nematode.

Table 2. List of 32 morphological descriptors with class and scale.

No.	Descriptor	Class	Scale	No.	Descriptor	Class	Scale
1	Growth habit	Erect	3	17	Spike: basal sterility	Absent	1
		Semi-prostrate	5			Present	9
		Prostrate	7				
2	Stem: Basal pigmentation	Absent	1	18	Lemma: pigmentation	Absent	3
		Present	9			Nerve pigmented	5
						Present	7
3	Auricle (Flag leaf): Anthocyanin Pigmentation	Absent	1	19	Spike: length	Small (< 7cm)	3
		Present	9			Medium (7.1-10cm)	5
						Long (>10cm)	7
4	Upper node Pigmentation	Absent	1	20	Plant: height	Very short (< 75.0 cm)	1
		Present	9			Short (75.1-85.0 cm)	3
						Medium (85.1-95.0 cm)	5
						Tall (95.1-105.0 cm)	7
5	Flag leaf attitude	Erect	1	21	Peduncle: length	Short (<22.0 cm)	3
		Semi-erect	5			Medium (22.0 -27.0 cm)	5
		Drooping	9			Long (> 27.0 cm)	7
6	Flag Leaf: Waxiness of sheath	Absent	1	22	Awns	Absent	1
		Present	9			Present	9
7	Spike emergence	Very early (<65 days)	1	23	Awns: type	Hooded	1
		Early (65-75 days)	3			Awnletted	3
		Medium (76-86days)	5			Normal	5
		Late (87-96 days)	7				
		Very late (> 96days)	9				
8	Spike type	2-row	3	24	Awn: length	Short (< 8.0 cm)	3
		6-row	7			Medium (8.0-11.0 cm)	5
						Long (>11.0 cm)	7
9	Lateral florets (2-row barley)	Rudimentary	1	25	Spike: density	Lax	3
		Developed	9			Intermediate	5
						Dense	7
10	Spike: Waxiness	Absent	1	26	Grain: hullness	Naked (hulless)	1
		Present	9			Covered (Hulled)	9
11	Spike: color	Pale green	1	27	Grain: color	Amber	1
		Green	2			Yellow	2
		Dark green	3			Purple	3
						Black	4
12	Spike: attitude	Erect	3	28	Grain: shape	Oval	1
		Semi-erect	5			Oblong	5
		Drooping	7			Elongated	7
13	Awn: roughness	Smooth	3	29	Grain: size (1000 grain weight)	Small (<30g)	1
		Rough	7			Medium (30-40g)	3
						Large (41-50g)	5
						Very large (>50g)	7
14	Flag leaf length	Short (<10cm)	3	30	Grain: surface	Smooth	1
		Medium (10-14 cm)	5			Wrinkled	9
		Long (> 14 cm)	7				
15	Flag leaf breadth	Narrow (<1.0 cm)	3	31	Rachilla hairs	Rudimentary	1
		Medium (1.0-1.5 cm)	5			Prominent	9
		Wide (>1.5 cm)	7				
16	Awn: Tip pigmentation	Absent	1	32	Grain: Crease width	Narrow	3
		Present	9			Intermediate	5
						Wide	7

Table 3. Morphological descriptors of malt barley varieties.

Variety	G H	S P	A A	U P	F A	F W	S E	S T	L F	S W	S C	S A	A R	F L	F B	A P	S S	L P	S L	P H	P L	P A	A T	A L	S D	H G	G C	G S	G W	S G	R H	C G
DWR 28	3	9	1	1	9	9	7	3	9	1	1	3	7	7	7	1	1	3	7	7	5	9	5	7	5	9	2	5	7	1	9	3
DWR B73	3	1	1	1	1	9	3	3	1	9	2	3	7	7	7	1	1	3	5	7	5	9	5	7	5	9	2	5	7	9	1	3
DWR B91	3	9	9	9	9	9	3	3	9	1	1	5	7	5	5	1	1	7	5	5	5	9	5	7	5	9	2	5	7	9	1	3
DWR B92	3	9	1	1	9	9	7	3	9	1	1	3	7	7	5	1	1	3	5	9	5	9	5	7	5	9	2	5	7	9	1	3
DWR UB52	3	1	1	1	5	9	5	3	1	9	2	3	7	7	7	1	1	3	5	7	5	9	5	7	5	9	2	5	5	9	1	3
DWR UB64	3	9	1	9	1	9	3	7	-	9	3	3	7	7	7	9	1	5	5	7	5	9	5	7	5	9	2	5	5	9	1	3

*GH-growth habit, SP-stem basal pigmentation, AA-auricle anthocyanin pigmentation, UP-upper node pigmentation, FA-flag leaf attitude, FW-flag leaf sheath waxiness, SE-spike emergence, ST-spike type, LF-lateral florets, SW-spike waxiness, SC-spike colour, SA-spike attitude, AR-awn roughness, FL-flag leaf length, FB-flag leaf breadth, AP-awn tip pigmentation, SS-spike basal sterility, LP-lemma pigmentation, SL-spike length, PH-plant height, PL-peduncle length, PA-presence of awns, AT-awn type, AL-awn length, SD-spike density, HG-hullness of grains, GC-grain colour, GS-grain shape, GW-grain weight, SG-grain surface, RH-rachilla hairs, CG-grain crease width.

Table 4. Character notes and frequency distribution for 83 barley varieties.

Descriptor	Class	Scale	Frequency	Descriptor	Class	Scale	Frequency
Growth habit	Erect	3	79	Lemma	Absent	3	50
	Semi-prostrate	5	4	pigmentation	Nerve	5	31
Stem basal pigmentation	Prostrate	7	-	Spike length	pigmentation	7	2
	Absent	1	52		Small	3	17
Auricle pigmentation	Present	9	31		Medium	5	61
Upper node pigmentation	Absent	1	66	Long	7	5	
	Present	9	17	Plant height	Very short	1	-
Flag leaf attitude	Absent	1	51		Short	3	-
	Present	9	32		Medium	5	25
Flag leaf sheath waxiness	Erect	1	23	Tall	7	50	
	Semi-erect	5	45	Very tall	9	8	
	Drooping	9	15	Peduncle length	Short	3	2
Spike emergence	Absent	1	1		Medium	5	57
	Present	9	82		Long	7	24
Spike type	Very early	1	-	Awns	Absent	1	-
	Early	3	8		Present	9	83
	Medium	5	27	Awns type	Hooded	1	-
	Late	7	39		Awnletted	3	-
	Very late	9	9		Normal	5	83
Lateral florets (2-row)	2-row	3	12	Awns length	Short	3	-
	6-row	7	71		Medium	5	11
Spike waxiness	Rudimentary	1	4	Spike density	Long	7	72
	Developed	9	8		Lax	3	11
Spike color	Absent	1	24		Grain hullness	Intermediate	5
	Present	9	59	Dense		7	9
	Spike attitude	Pale green	1	10	Grain color	Hulless	1
Green		2	71	Hulled		9	77
Dark green		3	2	Amber		1	6
Awn roughness	Erect	3	61	Grain shape	Yellow	2	77
	Semi-erect	5	17		Purple	3	-
	Drooping	7	5		Black	4	-
Flag leaf length	Smooth	1	2	Grain size	oval	1	5
	Rough	9	81		oblong	5	42
	Short	3	2		elongated	7	36
Flag leaf breadth	Medium	5	24	Grain surface	Small	1	2
	Long	7	57		Medium	3	43
	Narrow	3	2		Large	5	32
Awn tip pigmentation	Medium	5	32	Grain crease width	Very large	7	6
	Wide	7	49		Smooth	1	38
Spike basal sterility	Absent	1	61	Grain crease width	wrinkled	9	45
	Present	9	22		Narrow	3	59
Rachilla hairs	Absent	1	80	Grain crease width	Intermediate	5	23
	Present	9	3		wide	7	1
	Rudimentary	1	74				
	Prominent	9	9				

For growth habit, 79 varieties were recorded with erect plant type and 4 genotypes namely Alfa93, Clipper, RD2668 and Sonu were semi-prostrate in nature, whereas no Indian barley variety was found to be prostrate for this

character. Stem basal, auricle and upper node pigmentations were exhibited in 31, 17 and 32 varieties, respectively. Pigmentations were observed in the genotypes as Alfa93, DL88, DWRB91, HBL113, Himani, JB58, LSB2, NB1,

NB2, NB3 and NDB1173, respectively. For the flag leaf characters, most of the Indian barley varieties were characterized as semi-erect (45) with waxy flag leaf sheath (82), while 57 and 49 varieties were observed with long and wide flag leaf, respectively (Table 4). Two varieties viz. RD 2668 and UPB 1008 had short and narrow flag leaf. Flag leaf is an important selection criteria and plants with long, wide and erect flag leaf are more desirable and high yielding (Monneveux *et al.*, 2004). In 83 barley varieties, 71 were 6 row type and rest 12 genotypes were 2-row barley. In 2 row types 8 varieties were having developed lateral florets, while remaining 4 varieties viz. DWRB73, DWRUB52, RD2668 and UPB 1008 were classified with rudimentary lateral florets.

Out of 83 varieties, 50 were tall in stature and spike basal sterility was also unique feature for 3 varieties viz. HBL113, Kedar and RD2715. In this investigation, 59 genotypes were observed with waxy spikes and for other ear characters namely spike color, attitude, length and density majority of the varieties were characterized as green (71), erect (61), medium long (61) and intermediated density (63), respectively (Table 4). All the varieties considered here were having normal awns with long awn length (72) and 2 varieties viz. BH393 and NDB943 were found with smooth awns. For grain characters, 6 varieties were hullless, 77 were yellow in color and 42 genotypes were characterized with oblong grains. The grain surface of 45 varieties was observed as wrinkled and 59 genotypes were having narrow crease width (Table 4).

Barley is frequently being described as the cosmopolitan of the crops and mainly used for feed, food and malt production (Baik and Ullrich, 2008; Pal *et al.*, 2012). Owing to health conscious issues, barley has gained importance in multi grain blends owing to rich source of tocopherols, including tocopherols and tocotrienols, which are known to reduce serum LDL cholesterol through their antioxidant action (Baik and Ullrich, 2008). In these directions, all the varieties were screened for grain protein and starch contents and the pooled values of 10.5% and 61.9% were exhibited, respectively. Particularly in the hullless group, variety HBL276 exhibited the highest protein content

13.6% followed by Dolma (13.3%), NDB943 (13.0%) and BHS352 (12.0%). The variety Gitanjali (65.7%) followed by BHS352 (64.7%), NDB943 (64.3%), Karan16 (64.1%) etc. depicted high grain starch content.

In addition, the analysis of variance was also performed for grain protein and starch content in each location separately. It revealed significant differences ($P < .0001$) for both the characters indicating presence of genetic variation among the genotypes at Karnal and Faizabad locations. The pooled analysis depicted highly significant differences for grain protein and starch content, which indicated that genotypes performed differentially at both the centres. Average grain protein content at Faizabad and Karnal were observed as 11.9% and 9.1%, respectively, while starch content for these locations were exhibited as 60.9% and 63.0%, respectively. It was observed that the protein content was higher in the samples of Faizabad, whereas the Karnal climatic conditions were more suited for starch content. The congenial cool climatic conditions for starch bio-synthesis enzyme ADP-glucose pyrophosphorylase (AGPase) during grain filling stage may be the reason for high starch content at Karnal (Shewry *et al.*, 2001). The dry agro-ecology with slightly high temperature at terminal stage/grain filling activates more accumulation of sulphur poor C hordeins and resultantly increasing the overall grain protein content may be the reason for high protein in Faizabad samples (Shewry *et al.*, 2001).

After perusal of data it could also be assumed that the barley varieties with presence of basal stem/auricle/upper-node/lemma/awn pigmentations were resilient and good yielders for feed and malt barley. DWR28, DWRB 91, DWRB 92, DWRUB64 etc. for malt and RD2035 and HBL113 for feed barley are excellent examples of the above hypothesis. This study provides evidence of sufficient genetic diversity available in Indian barley germplasm, which will certainly help to select better parents and to generate precise future breeding programs. These descriptors would also be helpful to maintain the genetic purity of the genotypes. Beside this, in emerging need of intellectual property rights (IPR) regimes, this investigation would also facilitate as baseline

DUS data for varietal registration of malt barley varieties under Protection of Plant Varieties and Farmer's Rights (PPV&FR) Authority.

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