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Research Article

Integrating DUS descriptors and phenol colour tests for comprehensive characterization and varietal identification of barley genotypes

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Abstract

Barley (*Hordeum vulgare* L.) is one of the important cereal crops which is cultivated as *rabi* crop in northern part of India *viz*., Haryana, Punjab, Himachal Pradesh, Rajasthan, Uttar Pradesh, Bihar and Madhya Pradesh. Barley is favoured as a dual crop, due to its ability to grow well in marginal soils, robust fodder properties, abundant foliage growth, ability to regenerate and modest grain yield. It is vital to understand the prevalent morphological diversity of barley to increase the selection efficiency of breeding materials with varied genetic backgrounds. Characterization and varietal identification of cultivars and genetic purity of the seed lots are pivotal for varietal improvement, varietal protection, and quality seed production. Further, several chemical tests for classifying genotypes may complement varietal identification and characterization and management of genetic resources. Considering this, morphological descriptors and phenol tests were used for distinguishing different barley genotypes during *rabi* 2021-22. *In toto*, twenty-five morphological descriptors were studied in 102 barley genotypes wherein forty genotypes were subjected to phenol colour test and modified phenol test ($CuSO_4$ & Na_2CO_3). Phenol testes were effective in categorising barley genotypes based on colour reaction in to four groups *i.e.* deep olive colour, light brown colour, brown colour, and black colour. Among forty genotypes, only one genotype *i.e.* Kedar showed black colour reaction upon exposure to phenol test. While, six genotypes showed brown colour and twelve genotypes showed light brown colour and twenty-one genotypes developed deep olive colour reaction. However, number of genotypes showing black colour reaction increased to four (Alfa 93, Dolma, Kedar, BHS352) and six (Alfa93, RD2592, Dolma, Raj Kiran, Kedar, BHS352) upon pre-soaking in $CuSO_4$ and Na_2CO_3 as compared to phenol test. Therefore, integration of phenol tests with morphological descriptors facilitates characterization and identification of barley genotypes.

Key words: DUS, Phenol test, $CuSO_4$ & Na_2CO_3

1. Introduction

Barley (*Hordeum vulgare* L.) is an earliest domesticated and important cereal grain crop, significantly contributing towards food, feed and nutritional security (Baik and Ullrich, 2008; Fatima *et al.,* 2020). Barley is one of the most genetically diverse cereal and classified as two-row or six-row barley, malt and food & feed barley depending

upon the end-use type, hulled or huskless barley due to by presence or absence of hull on the grain (Baik and Ullrich, 2008). Barley is well adapted to adverse climatic and soil conditions and mostly cultivated by small and marginal farmers in the parts of Haryana, Punjab, Himachal Pradesh, Uttarakhand, Rajasthan, Uttar Pradesh, Bihar and Madhya Pradesh. Due to its high early vigour, leaf growth, potential for regeneration and nutritional qualities, considered as dual-purpose cereal and used as fodder crop (Verma *et al.,* 2015). Further, adaptation to drought, salinity, alkalinity soil conditions, referred to be the most cosmopolitan crop (Kumar *et al.,* 2020). Additionally, barley is a crucial crop for the industrial sector and is widely used in for brewing and malting, with significantly improved production techniques to produce high-quality malt product (Kumar *et al.,* 2017). Numbers of genotypes of barley have been developed for food and feed (dual purpose) considering requirement of the farmers and malting and brewing considering demand of the brewing industry (Verma *et al.,* 2022).

DUS testing is mandatory and crucial for implementing the system of protecting plant genotypes and granting rights to breeders, farmers, or institutions. If a new variety meets the requirements for novelty, distinctness, uniformity, and stability, it must be registered under plant variety protection. If an extant variety meets certain requirements, including distinctness, uniformity and stability, it must also be registered within a certain time frame. Additionally, variety characterization and identification are now essential to guarantee seed integrity and varietal protection (Singh *et al.,* 2017). Conventionally, morphological descriptors are used to characterise and identify a variety, wherein plants of a variety were grown and observations on morphological characters were taken to establish the identity of the variety through characterization (PPV&FR Act, 2001). Although, morphological descriptors are the universally accepted markers for varietal characterization and identification, certain limitations *viz*., low level of variability observed among genotypes due to narrow genetic base, environmental factors affecting an expression of the descriptors, time and resources involved necessitates to search for complimentary methods of varietal characterization (Kumar *et al.,* 2013). Additional descriptors with significant discriminating power and reproducibility which are least influenced by the environment are always preferred. Varietal characterization and identification using different chemicals have been well established in several crops. For instance, modified phenol test in wheat (Herman, 1928), KOH test in rice (Mor *et al.* 2006), $FeSO_4$ test in foxtail millet (Pallares *et al.,* 2004), pearl millet (Varier *et al.,* 1995) and sorghum (Thangavel *et al.,* 2005) were standardised.

Phenol test is one of the chemical tests, which are considered as a primary descriptor in identification of germplasm/variety owing to its high heritability and stability of the phenol colour reaction and could serve as an excellent genetic marker (Singh *et al.,* 2017). Phenol test, which is an index of polyphenol oxidase activity, has been reported to be associated with intra-varietal diversity and have been used in ascertaining varietal purity in wheat (Singhal and Prakash, 1988) and rice (Vanangamudi,1988). Poly phenol oxidase (PPO) is a complex enzyme which react with nucleophiles *viz*., amines, thiols and phenolics forming complex dark-coloured compounds called melanin (Fuerst, 2008). (Joshi and Banerjee, 1970) clearly demonstrated that the reaction oxidizing phenol to melanin (dark colour developed in the seed coat) via orthoquinone and hydroxyquinone was catalyzed by means enzyme polyphenol oxidase/tyrosinase with the possible contribution of other phenolic substances present in the seeds and amino acids such as tyrosine and phenylalanine. Therefore, it was considered as important primary descriptor for grouping and identification of genotypes in wheat.

Hence, in the view of potential of phenol colour reaction in the DUS testing system for grouping of genotypes, research work has been undertaken with the objective of evaluating the efficiency of the phenol tests in characterization and identification of barley genotypes along with certain important morphological descriptors.

2. Material and methods:

For the present study, seeds of 102 barley genotypes were collected from Barley Unit, ICAR-Indian Institute of Wheat and Barley Research, Karnal (ICAR-IIWBR) and morphological characters were recorded on 102 barley genotypes (Table 1). However, out of 102 genotypes, 40 genotypes were selected for phenol tests for distinguishing different barley genotypes (Table 2). A total of 50 seeds were pre-soaked in distilled water for 24 hours at 25 ± 1^0C. Thereafter, the pre-soaked seeds were transferred in to petri plates saturated with 1% (w/v) phenol solution.

The petri dishes were covered and incubated at 25 ± 1^{0}C and the change in seed colour was observed after 16 hours of incubation (Singh et al. 2017). Modified phenol test was conducted as per the phenol test except that seeds were soaked in 0.4% (w/v) $CuSO_4$ and 0.6 % (w/v) Na_2CO_3 solution instead of distilled water for 16 hours. Then the seeds were placed in 1% (w/v) phenol solution, colour reaction of seed coat was observed after 24 hours of incubation. Based on the colour development in both the tests, seeds were classified in to different groups, light brown, brown, black and no reaction (Jaiswal and Agrawal,1995).

Table 1: List of barley genotypes used for DUS characterization

S. No	Genotype	S. No	Genotype	S. No	Genotype	S. No	Genotype	S. No	Genotype	S. No	Genotype	S. No	Genotype
1	ALFA93	16	BHS330	31	Gitanjali	46	K560	61	PRB502	76	RD2503	91	Sindhu
2	Amber	17	BBHS400	32	HBL113	47	K603	62	PL56	77	RD2508	92	Sonu
3	AZAD	18	BILARA2	33	HBL276	48	Kailash	63	PL172	78	RD2552	93	UPB1008
4	BCU73	19	CLIPPER	34	HBL316	49	Karan16	64	PL419	79	RD2592	94	Vijaya
5	BG25	20	DL88	35	HBL391	50	Kedar	65	PL426	80	RD2624	95	VLB1
6	BG105	21	DOLMA	36	Himani	51	Lakhan	66	PL751	81	RD2660	96	VLB56
7	BH75	22	DWR28	37	Hub113	52	LSB2	67	PL807	82	RD2668	97	VLB85
8	BH393	23	DWRUB52	38	Jagrati	53	Manjula	68	Raj Kiran	83	RD2715	98	VLB94
9	BH885	24	DWRUB92	39	JB58	54	Narboo	69	Ranjeet	84	RD2786	99	VLB118
10	BH902	25	DWRUB101	40	JB1	55	NB1	70	Ratna	85	RD2794	100	DWRB160
11	BH946	26	DWRUB123	41	Jyoti	56	NB2	71	RD31	86	RD2849	101	HBL113
12	BH959	27	DWRUB73	42	K141	57	NB3	72	RD57	87	RD2899	102	VLB130
13	BHS46	28	DWRUB91	43	K409	58	NDB1173	73	RD103	88	RD2907		
14	BHS169	29	DWRUB64	44	K508	59	NDB943	74	RD2035	89	RDB1		
15	BHS352	30	DWRUB137	45	K951	60	NDB1495	75	RD2052	90	R56		

Table 2: List of genotypes used for phenol test and modified phenol test

S. No.	Genotype	S. No	Genotype	S. No	Genotype	S. No	Genotype
1	Alfa93	11	RDB1	21	PL751	31	Raj Kiran
2	RD2715	12	K603	22	RD2592	32	Kadar
3	BH75	13	BH902	23	VLB56	33	Jagrati
4	DWRB73	14	RD2503	24	Dolma	34	RD31
5	NB1	15	Bilara2	25	PL419	35	BHS352
6	BH393	16	RD2624	26	Jyoti	36	BHS169
7	Azad	17	DWR28	27	RD2052	37	K560
8	NDB1173	18	DWRUB64	28	RD57	38	Amber
9	Vijaya	19	DWRUB52	29	NB3	39	NB2
10	RD2786	20	DWRB137	30	Ratna	40	PL426

3. Results and discussion:

3.1 Morphological descriptors:

Among the twenty-five morphological descriptors studied for DUS testing in barley, certain essential descriptors are discussed here. Barley genotypes were classified considering the spike type in two row and six row barley. Out of 102 genotypes, 20 genotypes *viz.* Alfa 93, BCU 73, DWRB91, DWRB73, DWR28, BH885, BHS400, Clipper, DWRUB52, DWRB92, DWRB101, DWRB123, HBL113, HBL 316, HBL 391, RD2668, RD2849, DWRB160, VLB130 and BCU2030 were classified as two row barley. Whereas, remaining 82 genotypes were six row barley. Flag leaf altitude was classified as erect, semi-erect (SE) and drooping. Among 102 genotypes, four genotypes *viz*., ALFA 93, HBL113, HBL 316, HBL 391 showed drooping type flag altitude wherein 20 genotypes showed SE type and remaining 78 genotypes showed erect type flag leaf altitude. Auricle anthocyanin pigmentation was classified as absent and present Auricle pigmentation was observed in case of Alfa 93, BCU 73, Clipper, HBL113, HBL 316, HBL 391, Himani, NB1, NB2, NB3, NDB1173, NDB943, PRB502, PL 807 and RD2794. Whereas, absent in 87 other genotypes. Out of 102 barley genotypes, 13 genotypes *viz*., BCU 73, NB2, NB3, NDB1173, NDB943, NDB1445, PRB502, RD2668, RD2794, RS6, UPB1008, Vijaya and VLB1 showed drooping type spike altitude. Further, 45 genotypes were identified with erect and 44 genotypes as a semi erect type. As per DUS guidelines, peduncle length is classified in to short (22 cm), medium (22-27 cm) and long (>27 cm) genotypes *viz*., BCU 73, Clipper, HBL113, HBL 391 and Kailash showed short type while 30 genotypes showed medium type and 67 genotypes showed long type peduncles.

Spike emergence was classified as very early (<65 days), early (65-75 days), medium (76-86 days), late (87-96 days) and very late (>96 days) and it varied significantly among different genotypes of barley. Spike length is categorized small (<7cm), medium (7.1-10) and long (>10cm). Spike length of 102 genotypes varied significantly. It ranged from 11.2 cm to 6.4 cm in all genotypes. The highest spike length is observed in case of Jyoti and lowest spike length is observed in BH 75. Among 102 genotypes, spike waxiness was absent in 57 barley genotypes whereas 45 genotypes showed spike waxiness.

Table 3: DUS Characterization of 102 barley genotypes during Rabi 2020-21

S. No.	Genotype	Spike Type	Auricle Pigmentation	Flag Leaf Altitude	Spike Attitude	peduncle length (cm)	Spike Length (cm)	Spike Emer. (days)	Spike Waxiness
1.	Dolma	6R	A	SE	E	29.20	9.4	98	P
2.	JB58	6R	A	S	SE	32.80	7.7	87	A
3.	BH393	6R	A	E	SE	27.20	10.8	85	A
4.	ALFA 93	2R	P	D	G	31.20	9.1	96	P
5.	BCU 73	2R	P	SE	D	28.80	9.06	86	P
6.	DWRB91	2R	A	E	E	34.40	10.2	88	A
7.	DWRB73	2R	A	SE	E	29.40	9.6	90	P
8.	AZAD	6R	A	E	E	28.60	8.2	89	A
9.	BILARA2	6R	A	E	E	32.40	10	86	A
10.	DWRUB64	6R	A	E	E	27.80	6.4	85	P
11.	AMBER	6R	A	E	E	33.60	10.4	90	A
12.	DWR28	2R	A	SE	E	36.20	10	89	A
13.	BHS169	6R	A	E	E	37.20	11.2	87	P
14.	BH75	6R	A	E	SE	27.20	9.2	81	A
15.	BHS46	6R	A	E	SE	27.40	6.6	85	A
16.	RD2715	6R	A	E	SE	29.40	10.6	86	P

17.	JYOTI	6R	A	E	SE	27.60	8	87	P
18.	RATNA	6R	A	SE	E	30.40	8.8	89	A
19.	BG25	6R	A	SE	E	33.00	9.02	90	A
20.	BG105	6R	A	SE	E	31.80	10.5	89	A
21.	BH885	2R	A	SE	E	29.40	9.1	87	A
22.	BH902	6R	A	E	E	30.20	8.6	89	A
23.	BH946	6R	A	E	SE	34.40	10.2	90	A
24.	BH959	6R	A	E	E	27.60	8.6	89	A
25.	BHS352	6R	A	E	E	30.20	7.4	92	A
26.	BHS380	6R	A	E	E	28.40	7.6	93	A
27.	BHS400	2R	A	A	E	30.80	8.6	100	A
28.	CLIPPER	2R	P	E	E	31.40	9.8	98	P
29.	DL88	6R	A	E	SE	31.60	8.2	86	A
30.	DWRUB52	2R	A	E	E	31.60	7.6	88	P
31.	DWRB92	2R	A	E	E	29.20	8.2	90	A
32.	DWRB101	2R	A	E	E	30.20	8.6	89	P
33.	DWRB123	2R	A	E	E	28.60	8.6	90	A
34.	DWRB137	6R	A	E	E	27.40	7.6	88	A
35.	GEETANJALI	6R	A	E	E	28.60	7.6	90	A
36.	HBL113	2R	P	D	SE	31.40	7.4	90	P
37.	HBL 316	2R	P	D	SE	31.60	7.8	94	P
38.	HBL 391	2R	P	D	SE	29.00	8.4	100	P
39.	HIMANI	6R	P	E	SE	30.20	9.2	88	P
40.	HUB113	6R	A	P	SE	30.60	8	90	P
41.	JAGRATI	6R	A	E	SE	36.80	8.6	87	P
42.	JB1	6R	A	E	SE	29.20	8.6	89	P
43.	K 141	6R	A	E	E	31.40	7.4	90	A
44.	K 409	6R	A	E	E	29.80	7.4	87	A
45.	K 508	6R	A	E	SE	27.20	8.4	90	P
46.	K551	6R	A	SE	SE	30.00	8.6	87	A
47.	K 560	6R	A	E	SE	28.40	7.6	88	A
48.	K 603	6R	A	E	SE	29.40	11	86	A
49.	KAILASH	6R	A	SE	SE	31.00	9.2	92	P
50.	KARAN 16	6R	A	SE	E	32.20	9	96	P
51.	KEDAR	6R	A	E	SE	30.20	8.6	90	P
52.	LAKHAN	6R	A	E	SE	31.80	8.8	89	A
53.	LSB2	6R	A	E	SE	30.20	10.6	90	P
54.	MANJULA	6R	A	E	SE	34.00	9.6	85	A
55.	NORBOO	6R	A	E	SE	31.00	10.4	97	A
56.	NB1	6R	P	E	SE	29.20	9.4	89	A

57.	NB2	6R	P	E	D	32.80	7.7	89	A
58.	NB3	6R	P	E	D	27.20	10.8	87	A
59.	NDB1173	6R	P	E	D	31.20	9.1	86	A
60.	NDB943	6R	P	E	D	28.80	9.06	85	P
61.	NDB1445	6R	A	6E	D	34.40	10.2	89	A
62.	PRB502	6R	P	E	D	29.40	9.6	92	A
63.	PL56	6R	A	E	E	28.60	8.2	93	A
64.	PL172	6R	A	SE	E	32.40	10	94	A
65.	PL 419	6R	A	SE	E	27.80	6.4	90	A
66.	PL 426	6R	A	SE	E	33.60	10.4	89	A
67.	PL 751	6R	A	E	E	36.20	10	92	A
68.	PL 807	6R	P	E	SE	37.20	11.2	94	A
69.	RAJ KIRAN	6R	A	E	E	27.20	9.2	87	A
70.	RANJEET	6R	A	E	E	27.40	6.6	89	P
71.	RD 31	6R	A	E	E	29.40	10.6	89	P
72.	RD 57	6R	A	E	E	27.60	8	90	P
73.	RD 103	6R	A	E	SE	30.40	8.8	87	A
74.	RD 2035	6R	A	SE	SE	33.00	9.02	86	P
75.	RD 2052	6R	A	E	SE	31.80	10.5	90	A
76.	RD 2503	6R	A	E	SE	29.40	9.1	90	A
77.	RD 2508	6R	A	E	SE	30.20	8.6	82	A
78.	RD2552	6R	A	E	E	34.40	10.2	90	P
79.	RD2592	6R	A	E	SE	27.60	8.6	85	A
80.	RD2624	6R	A	E	SE	30.20	7.4	89	A
81.	RD2660	6R	A	E	SE	28.40	7.6	90	P
82.	RD2668	2R	A	E	D	30.80	8.6	90	P
83.	RD2786	6R	A	E	SE	31.40	9.8	85	P
84.	RD2794	6R	P	E	DG	31.60	8.2	87	A
85.	RD2849	2R	A	E	E	31.60	7.6	88	P
86.	RD 2899	6R	A	E	E	29.20	8.2	90	P
87.	RD2907	6R	A	SE	E	30.20	8.6	89	P
88.	RDB1	6R	A	E	E	28.60	8.6	87	A
89.	RS6	6R	A	E	D	27.40	7.6	92	P
90.	SINDHU	6R	A	E	E	28.60	7.6	95	P
91.	SONU	6R	A	E	E	31.40	7.4	92	A
92.	UPB1008	6R	A	E	D	31.60	7.8	83	P
93.	VIJAYA	6R	A	E	D	29.00	8.4	83	P
94.	VLB1	6R	A	E	D	30.20	9.2	89	P
95.	VLB56	6R	A	E	SE	30.60	8	93	P
96.	VLB85	6R	A	E	SE	36.80	8.6	90	P

97.	VLB94	6R	A	E	E	29.20	8.6	90	A
98.	VLB118	6R	A	E	SE	31.40	7.4	91	A
99.	DWRB160	2R	A	SE	SE	29.80	7.4	93	A
100.	HBL713	6R	A	A	SE	27.20	8.4	98	P
101.	VLB130	2R	A	SE	SE	30.00	8.6	92	P
102.	BCU2030	2R	A	SE	SE	28.40	7.6	92	P

2R-Two row, 6R-Six row, A- Absent, P-Present, SE- Semi erect, E- Erect, D- Drooping

Characterization of barley genotypes through chemical tests

Phenol color reaction:

Traditionally varietal identification and characterization is based upon morphological characters in comparison with systematic descriptors of national set of varieties and DUS guidelines given by plant variety protection authority (Kumar *et al.,* 2021). Unlike GOT, the rationale behind chemical tests relies on the response of seed coat/pericarp composition which might be due to genetic inheritability. However, chemical tests used to differentiate varieties based upon the differences of seed coat/pericarp which are governed by the genetic constitution (McCaig *et al.,* 1999). Phenol colour reaction in wheat seeds was accidentally discovered by Pieper (1922), which was further standardized and utilized as tool for identification of wheat varieties by Hermann, 1928. In the subsequent years, phenol colour reaction test was established as standard seed testing procedure world over. Phenol colour reaction on outer glumes can also be used to distinguish varieties (Nair and Tomar, 2001).

Wheat varieties of Indian origin and accessions of different ploidy levels were tested by (Singhal and Prakash, 1988). (Nair and Tomar, 2001) for phenol reaction on grains and glumes and categorized them into four groups as black, dark brown, brown and light brown colour. Number of notified Indian durum wheat varieties have been characterized for phenol reaction by different researchers (Kundu *et al.,* 2006). Phenol reactions among genotypes in wild species, bread wheat cultivars, durum wheat and synthetic hexaploids (SH) to breed a wheat variety, that do not react to phenol and does not darken the dough. The inheritance study showed that development of phenol colour in grains was governed by three dominant alleles (Niranjana *et al.,* 2018).

Among 40 barley genotypes, 21 genotype shown deep olive colour (BH75, DWRB73, NB1, BH393, NDB1173, RD2786, RDB1, K603, BH902, RD2503, RD2624, DWR28, DWRUB52, PL51, Jyoti, RD2052, RD57, NB33, Jagrati, K560, NB2). Whereas, 12 genotype showed light brown colour (RD2715, Azad, Vijaya, DWRUB64, DWRB137, RD2592, VLB56, PL419, Raj Kiran, RD31, BHS169, PL426). Further, six genotypes showed brown colour (Alfa93, Bilara2, Dolma, Ratna, BHS352, Amber). Only one genotype resulted in black colour reaction (Kedar).

Modified phenol test using $CuSO_4$

Modified phenol test using $CuSO_4$ resulted in development of deep olive colour in twenty genotypes (BH75, DWRB73, NB1, BH393, NDB1173, Vijaya, RD2786, RDB1, K603, BH902, RD2503, RD2624, DWR28, DWRUB52, DWRB137, Jyoti, RD2052, NB3, Jagrati, RD31, K560 and NB2). Whereas, Azad, Bilara2, DWRUB64, PL751, PL419, RD57 and Ratna recorded light brown colour. Further genotypes i.e. RD2715, RD2592, VLB56, Raj Kiran, BHS169, Amber and PL426 recorded brown colour. However, four genotypes recorded as black colour (Alfa93, Dolma, Kedar, and BHS352).

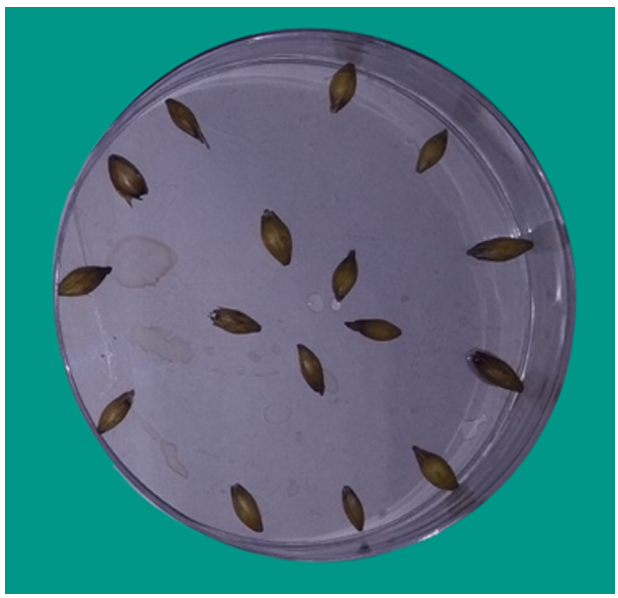
Modified phenol test using Na_2CO_3

Among 40 Barley genotypes studied, 17 genotype showed deep olive colour (BH75, DWRB73, NB1, BH393, NDB1173, RD2786, RDB1, BH902, RD2624, DWR28, DWRUB52, PL7551, Jyoti, NB3, RD31, K560, NB2), 10 genotype showed light brown colour (RD2715, Azad, Vijaya, K603, RD2503, DWRUB64, VLB56, PL419, RD2052, RD57, Jagrati), Six genotype showed brown colour (Bilara2, DWRUB52, Ratna, BHS169, Amber, PL426) remaining genotypes *viz*., Alfa93, RD2592, Dolma, Raj Kiran, Kedar, BHS352 showed black colour after exposure. Phenol colour reaction on outer glumes can also be used to distinguish genotypes (Nair and Tomar, 2001).

Table 4: Genotype characterization in barley using phenol and modified phenol test

Phenol test	Deep olive colour	Light brown colour	Brown colour	Black colour
Phenol Test (1%)	BH75, DWRB73, NB1, BH393, NDB1173, RD2786, RDB1, K603, BH902, RD2503, RD2624, DWR28, DWRUB52, PL51, Jyoti, RD2052, RD57, NB33, Jagrati, K560, NB2 **(21 genotypes)**	RD2715, Azad, Vijaya, DWRUB64, DWRB137, RD2592, VLB56, PL419, Raj Kiran, RD31, BHS169, PL426 **(12 genotypes)**	Alfa93, Bilara2, Dolma, Ratna, BHS352 and Amber **(6 genotypes)**	Kedar **(1 genotype)**
Modified phenol test using 0.4% $CuSO_4$	BH75, DWRB73, NB1, BH393, NDB1173, Vijaya, RD2786, RDB1, K603, BH902, RD2503, RD2624, DWR28, DWRUB52, DWRB137, Jyoti, RD2052, NB3, Jagrati, RD31, K560 and NB2 **(22 genotypes)**	Azad, Bilara2, DWRUB64, PL751, PL419, RD57 and Ratna **(7 genotypes)**	RD2715, RD2592, VLB56, Raj Kiran, BHS169, Amber and PL426 **(7 genotypes)**	Alfa93, Dolma, Kedar and BHS352 **(4 genotypes)**
Modified Phenol Test using 0.6 % Na_2CO_3	BH75, DWRB73, NB1, BH393, NDB1173, RD2786, RDB1, BH902, RD2624, DWR28, DWRUB52, PL7551, Jyoti, NB3, RD31, K560 and NB2 **(17 genotypes)**	RD2715, Azad, Vijaya, K603, RD2503, DWRUB64, VLB56, PL419, RD2052, RD57 and Jagrati **(11 genotypes)**	Bilara2, DWRUB52, Ratna, BHS169, Amber and PL426 **(06 genotypes)**	Alfa93,RD2592, Dolma, Raj Kiran, Kedar and BHS352 **(06 genotypes)**

BH393- Deep olive colour (Phenol)



VLB56- Light brown Colour (Phenol)

Ratna- Brown colour (Phenol)

Kedar- Black colour (Phenol)

BH75-Deep olive colour (Phenol+ $CuSO_4$)

RD57-Light brown Colour (Phenol+ $CuSO_4$)

BHS169- Brown colour (Phenol+ $CuSO_4$)

Dolma- Black colour (Phenol+ $CuSO_4$)

Jyoti-Deep olive colour (Phenol+Na_2CO_3)

RD57- Light brown Colour (Phenol+Na_2CO_3)

BHS169-Brown colour (Phenol+Na_2CO_3)

Kedar-Black colour (Phenol+Na_2CO_3)

Conclusion

The integration of DUS descriptors and phenol tests has proven to be a robust and comprehensive approach for the characterization and identification of barley genotypes. In the present study, in addition to the morphological descriptors, phenol, modified phenol using $CuSO_4$ and Na_2CO_3 tests were carried to differentiate the barley genotypes. From the present study, it is concluded that phenol colour test as well as modified phenol test using $CuSO_4$ and Na_2CO_3 were beneficial in categorising barley genotypes based on colour reaction in to four groups *i.e.* deep olive colour, light brown colour, brown colour, and black colour reaction. Further, above chemical tests were highly stable, quick, cost effective and are least influenced by the environment. The synergy between DUS characterization and phenol tests facilitates plant variety protection and barley improvement programs.

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Conflict of Interest

Authors declare that they have no conflict of interest.

Ethical Compliance Statement

NA

Author's Contribution

Conceptualization (DY and UK); Designing of experiment (UK and AKS), Execution of field/lab experiments and data collection (DY), Analysis of data and interpretation (UK, CS, AKS, SSG); Preparation of the manuscript (DY, UK, AKS, OPA), Overall editing and guidance (AKS, OPA).

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