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Distinctiveness, uniformity and stability (DUS) characterization of wheat for identification of novel trait sources

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ABSTRACT

DUS characterization in wheat is crucial to preserve the loss of trait variability, to offer breeders and farmers rights, and to deliver high-quality seeds. In the present study, 296 wheat genotypes were characterized during Rabi seasons of years 2022 and 2023 for seventeen (17) visually assessed DUS parameters following wheat descriptor prescribed by PPV& FR Act, 2001. Out of seventeen (17) visually assessed characteristics, 2 characters (presence of awns; grain texture) were dimorphic and rest 15 characters were found to be polymorphic, indicating their potential for genotype characterization. All the genotypes showed similar expression over the years for each visual character depicting the stability of the genotypes. None of the attribute showed intra-line variation among the genotypes. Analysis of variance revealed significant differences for all the quantitative traits viz., plant height, spike length, number of spikelets per spike and number of grains per spike for two years, thereby clearly indicating the existence of high genetic variability among genotypes. The coefficient of variation for quantitative characters viz., plant height (10.87), spike length (7.16), number of spikelets per spike (5.98) indicates high magnitude of genetic variation and their scope for utilization in genotype characterization and improvement. Thus, the 17 morphological DUS descriptors used in the present study were reliable and reproducible for establishing the distinctiveness in this set of wheat genotypes. The genotypes satisfying the DUS criteria in the set of genotypes can be registered with PPV & FR authority for registration and for obtaining breeders and farmer's rights.

Key words: Descriptor traits, DUS, morphological characterization, wheat

Wheat (*Triticum sp.*) is the most widely grown of all field crops. The major target of breeding work is focused on the increase of wheat grain yield due to the predicted growth of the human population and decrease in the production area (Kant *et al.,* 2014; Guin *et al.,* 2019; Preeti *et al.,* 2016). The situation demands continuous effort in R&D for sustaining agriculture and explore every possibility to tackle any future food crisis. It occupies around 13.5 million ha in south Asia and contributes around 233.96 Mt of food grain in India (Mubrak *et al.,* 2023). For varietal development in wheat, breeders need around ten years to develop and test homozygous wheat lines, after which the genotypes undergo the process of varietal recognition. Distinctness,

Uniformity and Stability (DUS) is a basis for an intellectual property system introduced in 1961 by the International Union for the Protection of New Varieties of Plants (UPOV, 2024) for breeders' investment protection and is being adopted in our country. The DUS testing in wheat is basically done to determine how existing genotypes/ varieties differ within the same species (Distinctness), whether the characteristics used to establish Distinctness are expressed uniformly (Uniformity) and that these characteristics do not change over subsequent generations (Stability). DUS tests are performed to confirm the legal recognition of new plant varieties and to support the granting of Plant Breeders' Rights, which provide intellectual property protection for the substantial economic investment involved in modern plant breeding (Yadav et al., 2013). Based on distinctiveness, uniformity, and stability, the DUS testing process was actually initiated in 2001. Different descriptors, established based on DUS principles, are utilized for DUS testing in wheat. Plant varieties may be registered with the Protection of Plant varieties and Farmers Rights (PPV & FR), Authority, New Delhi, based on the results of DUS testing. This study reports DUS testing conducted in the field during which morphological characteristics were recorded on 296 genotypes. DUS characteristics are evaluated in the field environment or greenhouse to ensure that how a genotype is distinct from all other existing varieties, that its characteristics are uniform, and that the variety is stable in phenotypic characteristics from one generation to the next.

MATERIALS AND METHODS

Plant material: The characterization of a set of 296 wheat genotypes for important morphological traits was attempted under field conditions in the experimental research field of Division of Genetics and Plant Breeding, Faculty of Agriculture, Wadura, Sopore (latitude: 33.994007 N; longitude: 74.864334 E) Kashmir, India. The material was sown during the years 2021 and 2022in Augmented Block Design (ABD) with 4 checks (DBT-547, DBT-761, DBT-250, and DBT-259).

Investigated DUS traits: The material was characterized for seventeen (17) qualitative DUS traits (table 1) viz., ear density, presence of awn, awn colour, awn attitude, pubescence, ear colour, outer glume shoulder width, outer glume shoulder shape, glume beak length, grain shape, grain colour, grain texture, ear shape, beak shape, peduncle attitude, grain germ width, brush hair length and four (4) quantitative DUS traits including plant height, spike length, number of spikelets per spike and number of grains per spike as per the wheat DUS guidelines of PPV & FR (2007).

Statistical analysis: Analysis of variance (ANOVA) for all the characters/traits for testing variation among genotypes was carried out as per procedure suggested by Federer (1961). Minimum, maximum, mean and coefficient of variation values were calculated for following DUS traits: spike length, number of spikelets per spike, plant height, and number of grains per spike by using Microsoft Excel and R-software program (version 4.0.3) developed by R foundation (cran. project.org).

RESULTS AND DISCUSSION

Based on the distinctiveness test using visual scoring, seventeen (17) DUS characteristics viz., ear density, presence of awn, awn colour, awn attitude, pubescence, ear colour, outer glume shoulder width, outer glume shoulder shape, glume beak length, grain shape, grain colour, grain texture, ear shape, beak shape, peduncle attitude, grain germ width, brush hair length and four (4) quantitative DUS traits viz., plant height, spike length, number of spikelets per spike and number of grains per spike were recorded on 296 genotypes. The variation of traits for (17) DUS characteristics are presented in Table 1 and Figures1 (a to q).

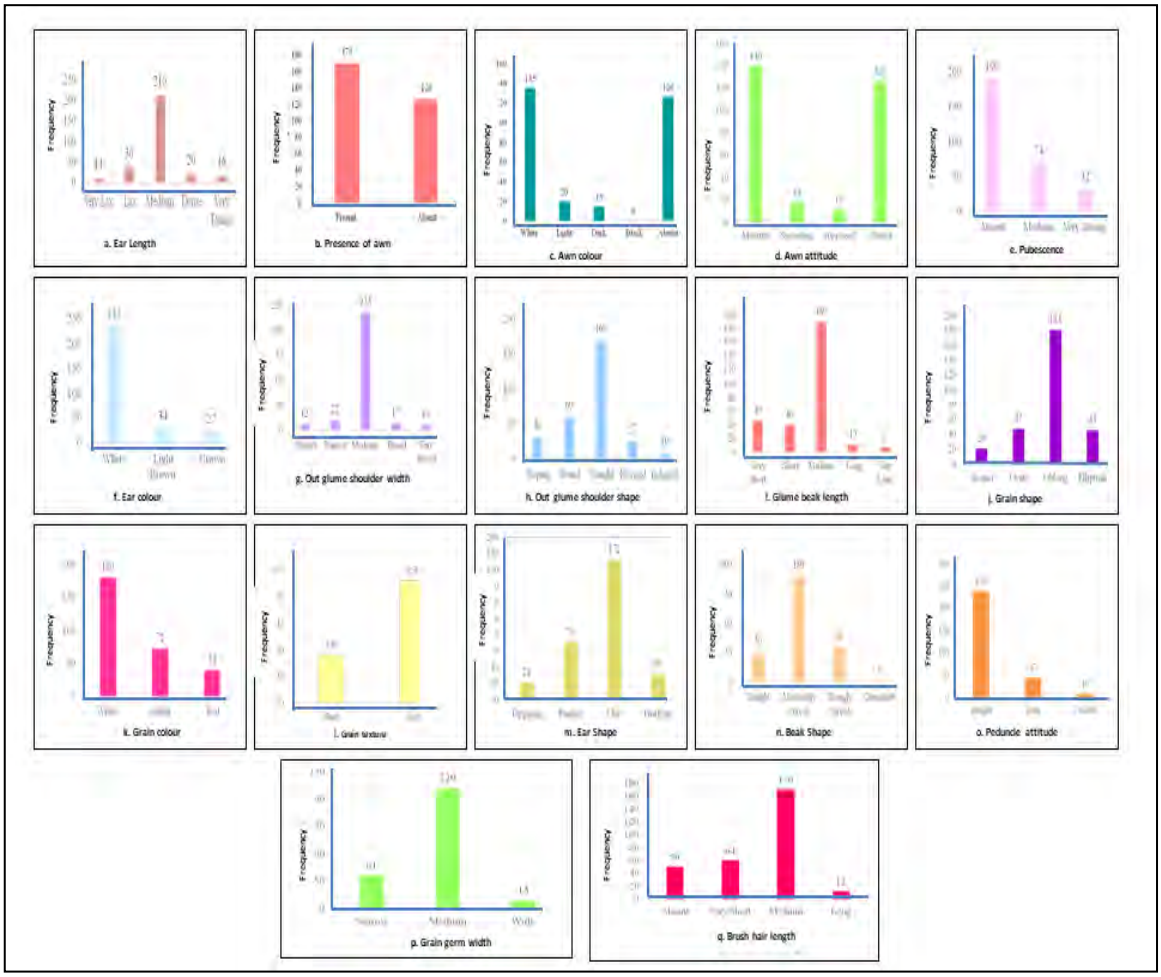

Fig. 1: Variability for 17 quantitative DUS traits (a to q): a. ear density b. presence of awn c. awn colour d. awn attitude e. pubsecencef. ear colour g. outer glume shoulder width h. outer glume shoulder shape i. glume beak length j. grain shape k. grain colour l. grain texture m. ear shape n. beak shape o. peduncle attitude p. grain germ width q. brush hair length

The DUS traits viz., presence of awn and grain texture were characterized as dimorphic traits whereas, DUS traits viz., ear density, awn colour, awn attitude, pubescence, ear colour, outer glume shoulder width, outer glume shoulder shape, glume beak length, grain shape, grain colour, ear shape, beak shape, peduncle attitude, grain germ width, brush hair length showed polymorphic variation (Fig. 2 a to f). In our study, out of the set of 296 wheat genotypes characterized for seventeen (17) DUS traits, fifteen were grouped as polymorphic and two dimorphic traits. Further the wheat material under study depicted high genetic variability for quantitative phenotypic traits viz, plant height, spike length, number of spikelets per spike and number of grains per spike. Such morphological characterization of genotypes helps create a database on which these can be distinguished and the genetic diversity existing in them can be assessed. The term DUS (Distinctness, Uniformity, and Stability) refers to the essential traits used to determine the eligibility of new plant varieties for protection under the International Union for the Protection of New Varieties of Plants (UPOV) convention (Kundu *et al.*, 2006). In wheat, these DUS traits are critical for breeders and researchers to characterize new varieties. A genotype is considered distinct if it clearly differs from all other known genotypes or varieties in at least one important trait. For wheat, distinctness can be observed through morphological characteristics which may include: plant height, leaf color and shape, glume color, ear/spike length and shape, grain color and texture (Chauhan *et al.*, 2023). They highlighted that plant height and spike morphology were effective in distinguishing different wheat genotypes in a

Fig. 2. (a to f): Variation for qualitative DUS traits viz., a) ear shape, b) awn attitude, c) ear density, d) presence of pubescence, e) awn less, f) awned in wheat genotypes

DUS test. The variability for morphological DUS traits in wheat was also demonstrated by Dhonde *et al.*, (2000). Using visual assessment and statistical methods, the study found distinct differences between genotypes based on these morphological traits. The uniformity refers to the consistency of characteristics within a single genotype. For a variety to be uniform, the plants should exhibit consistent trait expression for seed size and shape consistency, leaf architecture uniformity and grain color and weight uniformity across a representative population under normal conditions. The uniformity of a genotype is typically assessed by growing its sufficient sample and observing trait homogeneity across plants. Ahmed *et al.*, (2022) discussed the challenge of ensuring uniformity, especially in complex traits like disease resistance, where small genetic variations can lead to significant differences among plants.

Table 1: Frequency distribution of wheat genotypes for 17 DUS characteristics

S. No.	Characteristic	Class	Absolute number	Frequency (%)
1	Ear Shape	Tappering	24	8
		Parallel	70	24
		Club Shaped	172	58
		Fusiform	30	10
2	Ear Density	Very Lax	14	5
		Lax	36	12
		Medium	210	70
		Dense	20	7.6
		Very Dense	16	5.4
3	Presence of Awn	Presence	170	57
		Absence	126	43
4	Awn Colour	White	135	45.60
		Light Brown	20	6.80
		Dark Brown	15	5.06
		Black	0	0
		Absent	126	42.54
5	Awn Attitude	Appressed	12	4.05
		Medium	140	47.29
		Spreading	18	6.10
		Absent	126	42.56
6	Pubescence	Absent	190	64.18
		Medium	74	25.01
		Very strong	32	10.81
7	Ear Colour	White	235	79.4
		Light Brown	34	11.48
		Brown	27	9.12
8	Outer Glume Shoulder Width	Absent	12	4.05
		Narrow	22	7.43
		Medium	235	79.4
		Broad	17	5.74
		Very Broad	10	3.37
9	Outer Glume Shoulder Shape	Sloping	30	10.13
		Round	60	20.27
		Straight	169	57.09
		Elevated	27	9.12
		Indented	10	3.37

Table 1 continued

10	Glume Beak Length	Very Short	45	15.20
		Short	40	13.51
		Medium	189	63.85
		Long	13	4.39
		Very Long	9	2.31
11	Beak Shape	Straight	50	16.89
		Moderately curved	180	60.81
		Strongly Curved	60	20.27
		Geniculate	6	2.02
12	Peduncle Attitude	Straight	239	80.74
		Bent	47	15.87
		Crooked	10	3.37
13	Grain Germ Width	Narrow	61	20.62
		Medium	220	74.32
		Wide	15	5.06
14	Brush Hair Length	Absent	50	16.89
		Very Short	64	21.62
		Medium	170	57.43
		Long	12	4.05
15	Grain Shape	Round	20	6.75
		Ovate	47	15.87
		Oblong	184	62.16
		Elliptical	45	15.20
16	Grain Colour	White	180	60.81
		Amber	72	24.32
		Red	44	14.86
17	Grain Texture	Hard	143	48.31
		Soft	153	51.68

The analysis of variance (ANOVA) and descriptive statistics for quantitative traits was studied and is presented table 2 and 3

The stability means that the distinct characteristics of a variety remain consistent after repeated propagation over multiple generations and across different environments. A genotype or a variety is stable if its key traits viz., reproductive stability (seed propagation), consistency of yield performance and spike architecture and grain quality stability do not change significantly over time. Stability is crucial for varieties that are to be registered for commercial cultivation. A study by Destaw *et al.,* (2024) demonstrated the stability of a wheat genotypes/ varieties across multiple years and locations in terms of yield, height, and disease resistance, confirming that the variety could perform consistently under varying environmental conditions. Additionally, Wani *et al* (2020) confirmed genetic variability among bread wheat (*Triticum aestivum* L.) under temperate conditions.

Table 2: ANOVA for quantitative DUS traits

SOV	D.f	Plant Height	Spike Length	No.of Spikelets/ Spike	No. of Grains/Spike
Block (Ignoring Treatments)	11	2034.93**	6.21**	8.77**	2608.01**
Block (Eliminating Treatments)	11	0.00	0.33	0.00	0.00
Treatment (Ignorning Block)	295	188.51**	1.23**	3.98**	241.49 **
Treatment (Eliminating Blocks)	295	112.63**	1.02**	3.65**	144.24**
Treatment: Checks	3	32.75 **	12.73**	35.00**	48..75**
Treatment: Test and Test vs. Check	292	113.45**	0.88**	3.33**	145.22**
Residuals	33	0.00	0.00	0.00	0.00

ns P > 0.05; * P <= 0.05; ** P <= 0.01

Table 3: Descriptive statistics for quantitative DUS traits studied

Trait	Mean	Minimum	Maximum	GCV	PCV	C.V.
Plant height	156.04	133.57	184.30	14.29	16.53	10.87
Spike length	8.12	4.73	11.73	7.07	7.13	7.16
Number of spikelets per spike	16.72	12.57	21.75	6.85	9.01	5.98
Number of grains per spike	58. 36	28.73	82.59	27.73	27.87	2.92

CV = coefficient of variation; GCV=genotypic coefficient of variation; PCV=phenotypic coefficient of variation

CONCLUSION

DUS testing plays a pivotal role in wheat variety registration and protection. By ensuring that a new variety is distinct, uniform, and stable, breeders can ensure that it offers unique advantages while maintaining consistency over time and propagation cycles. Additionally, it can be concluded that in situations, where DUS descriptors are not able to establish distinctiveness of a genotype, molecular markers may be used as additional or complement descriptors for resolving distinctiveness of related wheat genotypes. The application of molecular markers, marker assisted selection (MAS) are thought to enhance the precision of DUS characterization and further strengthen the plant variety protection system.

CONFLICT OF INTEREST

All the authors affirm that there is no conflict of interest among them. All research activities comply with relevant legal, institutional and ethical standards.

AUTHOR CONTRIBUTION

All the authors contributed equally in the present study.

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