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Constraints in visual DUS characterization and differentiation of rice (*Oryza sativa* L.) cultivars

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Abstract
Rice (*Oryza sativa* L.) is one of the cultivated cereal plant species, known to show the wide diversity and adaptability to growing conditions. The Plant Variety Protection system varied considerably among different countries and was adopted as per the needs and international conventions. Under the PPV&FR Act, rice varieties are registered on the basis of Novelty, Distinctiveness, Uniformity and Stability (NDUS) characters which are taken as per the stage of observations listed in DUS guidelines. Keeping in view the importance of DUS test for cultivar characterization and differentiation, the present study was carried out to characterize the extant rice varieties for plant morphological traits and assessment the extent of variability and associated constraints in these varieties for above characters. The complete DUS database of plant morphological characters for all the rice varieties were generated as per DUS guidelines (Rice) no. SG/ 01/ 2007. The study grouped the 28 rice extant varieties into five different clusters based on these DUS parameters. The experimental material under study thus revealed considerable variability for most of the morphological characters, indicating their utility in the characterization and use in rice variety improvement programme. At the same time DUS characterization has limitation as morphological differences between the varieties is less due to narrow genetic base and requires skilled human power which is subjective in nature. Also this process is time, labour and cost intensive. Hence, the manual identification of varieties/seeds by specialized technicians is slow, has low reproducibility, and possesses a degree of subjectivity that is hard to quantify. So, it is suggested that in addition to the existing DUS guideline for establishing varietal distinctiveness, recent technologies like use of molecular markers or image analysis techniques need to be complemented with field morphological studies to have a more reliable assessment.

Keywords: DUS, Varietal characterization, differentiation, etc.

Introduction
Rice (*Oryza sativa* L.) is one of the cultivated cereal plant species, known to show the wide diversity and adaptability to growing conditions. Cultivated varieties of rice (*Oryza sativa* L.) are divided in to three sub-group *viz. indica*, *japonica*, and *javanica*. The protection of research products is necessary to provide incentive for investment as increased interest and debate over the ownership of intellectual property (e.g. Plant Property Rights) has arisen in agriculture (Jondle, 1989) [4].

The Plant Variety Protection system varied considerably among different countries and was adopted as per the needs and international conventions. The Plant Patenting Act (PPA) of US, enacted in 1930, allowed patenting of sexually propagated plant and over 6,500 of such plant patents have been granted, mostly for ornamentals and horticultural crops (Patent, 2003). Plant Variety Protection Act (PVPA) was enacted in 1970 (Anonymous, 2001) [1]. In 1985, the US Board of Patent Appeals allowed patent protection for sexually or asexually or *in vitro* propagated plants (Saha, 2001) [2]. Currently plants can be protected in the United States by PPA of 1930, the PVPA of 1970 along with its amended version in 1994, and with utility patent or in Europe by Plant Breeder's Right and (PBRs) of 1961 (Dworkin, 1988; Jondle,1989.) [3, 4]. Under section 15 of the PPV&FR Act, Novelty means commercial novelty, i.e. the propagating or harvested material of the variety should not have been sold or otherwise disposed of earlier than one year in India, or outside India, not earlier than six years in the case of trees or wines or not earlier than four years in any other case from the date of filing of application of registration. Moreover, trial of a new variety which has not been sold or otherwise disposed of shall not affect the right to protection. Distinctiveness means that a variety is clearly distinguishable by at least one essential characteristic from any other variety, whose existence is a matter of common knowledge. Uniformity implies that the variety should be sufficiently uniform in its essential characteristics subject to variation as expected from features of its propagation.

Stability requires the essential characteristics remain unchanged after repeated propagation (Dhillon *et al*., 2006) [5]. Mucha *et al*. (1994) [6] proposed the organization and methods of testing of varieties for distinctiveness, uniformity and stability (DUS) for entry in official register and for protection of Plant Breeders Right in Poland. Veress and Lazer (1997) [7] reported that the most important topic dealt by the Hungarian Institute for Agricultural Quality Control is the distinctiveness, uniformity and stability (DUS) testing in crops. Keeping in view the importance of DUS test for cultivar characterization and differentiation, the present study was carried out to characterize the extant rice varieties for plant morphological traits and assessment the extent of variability and associated constraints in these varieties for above characters.

Materials and Methods

The field experiment was conducted during *kharif* season of 2014-15 in the field of Seed Science and Technology Division and Genetics Division, Indian Agricultural Research Institute, New Delhi.

List of genotypes/extant varieties of rice under study

The experimental material comprised of twenty eight extant rice varieties which are as under:

Table 1: Experimental material comprised of twenty eight extant rice varieties which are as under

S. No	Variety Name	S. No	Variety Name
1	PNR-519	15	VIKASH
2	NIDHI	16	CSR-27
3	CSR-13	17	JAYA
4	ASD-20	18	MAKOM
5	VL DHAN-206	19	NDR-369
6	PUSA-33	20	PR-113
7	VIVEK DHAN-62	21	VASUMATI
8	TARAORO BASMATI	22	PUSA BASMATI-1121
9	PR-106	23	PUSA BASMATI-1509
10	PUSA SUGANDHA-2	24	PNR-162
11	PUSA SUGANDHA-3	25	PNR-381
12	IMPROVED PUSA BASMATI-1	26	PUSA-44
13	PUSA BASMATI-1	27	IR-64
14	PUSA BASMATI-6	28	PUSA SUGANDHA-5

Brief cultural practices

Experimental design and layout

The experimental material comprised of twenty eight extant rice varieties which are sown in nursery field of Seed Science and Technology Division, and Genetic Division, IARI New Delhi. The experiment was conducted in Randomized Block Design (RBD) with two replications during *kharif* season of 2014. The test plot details were as follows: All the 28 rice varieties were sown as per layout below: Replication = 2, Row length = 4 m, Number of rows per variety = 4 Distance of R-R = 30 cm and P-P= 20 cm is followed.

Sowing: 21 varieties were sown in nursery field of Seed Science and Technology Division, IARI New Delhi on 12-06-2015 and remaining 7 varieties namely PB-1121, PB-1509, PNR-162, PNR-381, PUSA-44, IR-64, PS-5 were sown in field of Genetic Division, IARI New Delhi at 10-06-2015.

Transplanting

Seedlings were transplanted on 15-07-2015 at Seed Science and Technology Division's field and on 13-07-2015 at Genetic Division's field IARI New Delhi.

Nursery preparation

Nursery bed was prepared by applying 15 kg N as Urea, 20 kg P_2O_5 as Single Super Phosphate and 2 Kg K_2O as Muriate of Potash per 1000 m^2 of nursery bed. FYM @ 10 t/ha was also added. Presoaked seed were sown and 30 days old single seedlings were transplanted.

Sampling procedure

The observations were made on twenty plant or plant parts, which were equally divided in two replications as per PPV&FR guidelines (Anonymous, 2007) [1].

Table 2: DUS characteristics observed in field

S. No	Variety/Character	1. Basal leaf: sheath colour	8. Leaf: Pubescence of blade surface	9. Leaf: Auricle	10. Leaf: Anthocynin coloration of auricle	14. Leaf: Shape of ligule	15. Leaf: Color of ligule	16. Leaf: Length of blade (Mean)	17. Leaf: Width of blade (Mean)
1	PNR 519	Green	Strong	Present	Colourless	Split	White	40.50 M	0.81 N
2	NIDHI	Green	Strong	Present	Colourless	Split	White	42.22 M	0.99 N
3	CSR 13	Green	Strong	Present	Colourless	Split	White	38.51 M	1.39 M
4	ASD 20	Green	Strong	Present	Colourless	Split	White	25.55 M	1.07 M
5	VL DHAN206	Green	Strong	Present	Colourless	Split	White	48.47 L	1.75 M
6	PUSA 33	Green	Strong	Present	Colourless	Split	White	40.47 M	1.52 M
7	VIVEK DHAN 62	Green	Medium	Present	Colourless	Split	White	40.49 M	1.14 M
8	TARORI BASMATI	Green	Medium	Present	Colourless	Split	White	36.36 M	1.22 M
9	PR-106	Green	Strong	Present	Colourless	Split	White	39.68 M	0.84 N
10	PS-2	Green	Strong	Present	Colourless	Split	White	40.00 M	1.69 M
11	PS-3	Green	Strong	Present	Colourless	Split	White	39.44 M	1.47 M
12	IMPROVED PB-1	Green	Medium	Present	Colourless	Split	White	35.52 M	1.58 M

13	PB 1	Green	Strong	Present	Colourless	Split	White	32.79 M	1.50 M
14	PB-6	Green	Strong	Present	Colourless	Split	White	33.61 M	1.69 M
15	VIKASH	Green	Strong	Present	Colourless	Split	White	39.72 M	1.33 M
16	CSR 27	Uniform purple	Medium	Present	Colourless	Split	White	48.53 L	1.52 M
17	JAYA	Green	Weak	Present	Colourless	Split	White	40.64 M	1.38 M
18	MAKOM	Green	Strong	Present	Colourless	Split	White	30.60 M	1.35 M

Table 2: Con….

S. No	Variety/Character	20. Time of heading	21. Flag leaf: Attitude of blade (Early observation)	22.Spikelet: Density of pubescence of lemma	26.Lemma: Anthocyanin coloration of apex	27.Spikelet : color of stigma	29.Stem: length excluding panicle (Mean)	30. Stem: Anthocyanin coloration of node	33.Panicle: Length of main axis (Mean)	34. Flag leaf: Attitude of blade (Late observation)
1	PNR 519	Early	Horizontal	Strong	Absent	White	80.59 VS	Absent	27.15 L	Horizontal
2	NIDHI	Medium	Erect	Strong	Absent	White	65.25 VS	Absent	21.68 M	Erect
3	CSR 13	Late	Erect	Strong	Absent	White	56.27 VS	Absent	19.91 S	Erect
4	ASD 20	Late	Erect	Strong	Absent	White	45.11 VS	Absent	22.28 M	Erect
5	VL DHAN 206	Late	Semi-erect	Strong	Absent	White	92.10 S	Absent	18.12 S	Semi-erect
6	PUSA 33	Late	Erect	Strong	Absent	White	75.35 VS	Absent	22.22 M	Erect
7	VIVEK DHAN 62	Medium	Semi-erect	Medium	Absent	White	92.33 S	Absent	29.47 L	Semi-erect
8	Tarori Basmati	Late	Erect	Medium	Absent	White	92.28 S	Absent	25.17 L	Erect
9	PR 106	Late	Erect	Strong	Absent	White	60.34 VS	Absent	23.38 M	Erect
10	PS-2	Very Early	Semi-erect	Medium	Absent	White	76.24 VS	Absent	27.21 L	Horizontal
11	PS-3	Very Early	Semi-erect	Medium	Absent	White	75.57 VS	Absent	29.19 L	Semi-erect
12	IMPROVED PB-1	Early	Erect	Weak	Absent	White	64.6 S	Absent	26. 5 L	Semi-erect
13	PB-1	Early	Semi-erect	Weak	Absent	White	68.52 VS	Absent	28.26 L	Horizontal
14	PB-6	Early	Semi-erect	Medium	Absent	White	69.70 VS	Absent	29.68 L	Semi-erect
15	VIKASH	Early	Erect	Strong	Absent	White	65.53 VS	Absent	25.31 L	Semi-erect
16	CSR 27	Medium	Erect	Strong	Strong	Purple	85.5 VS	Present	27.33 L	Semi-erect
17	JAYA	Medium	Semi-erect	Weak	Absent	White	65.57 VS	Absent	26.52 L	Semi-erect
18	MAKOM	Medium	Erect	Medium	Absent	White	76.48 VS	Absent	23.46 M	Semi-erect

Table 2: Con….

S. No	Variety/Character	35. Panicle: Curvature of main axis	37. Spikelet: Color of tip of lemma	39. Panicle: Awns	40. Panicle: Color of awns (Late observation)	42. Panicle: Distribution of awns	45. Panicle: Attitude of branches	46.Panicle: Exertion	49. Sterile Lemma: Color
1	PNR 519	Semi-straight	White	Present	White	Upper half only	Erect	Mostly	White
2	NIDHI	Straight	White	Present	Yellow white	Tip only	Erect	Mostly	White
3	CSR 13	Semi-straight	White	Present	White	Tip only	Semi-erect	Well	White
4	ASD 20	Straight	White	Absent	Absent	Absent	Erect	Well	White
5	VL DHAN 206	Semi-straight	White	Absent	Absent	Absent	Semi-erect	Well	White
6	PUSA-33	Straight	White	Absent	Absent	Absent	Erect	Mostly	White
7	VIVEK DHAN 62	Deflexed	White	Absent	Absent	Absent	Semi-erect	Well	White
8	TARORI BASMATI	Straight	White	Present	Yellow white	Upper half only	Spreading	Well	White
9	PR-106	Deflexed	White	Absent	Absent	Absent	Semi-erect	Mostly	White
10	PS-2	Droop	White	Absent	Absent	Absent	Erect-Semi erect	Mostly	White
11	PS-3	Droop	White	Absent	Absent	Absent	Semi erect-spreading	Mostly	White
12	IMPROVED PB-1	Deflexed	White	Present	Yellowish-white	Whole length	Spreading	Partly	White
13	PB-1	Semi-straight	White	Present	White	Whole length	Erect-Semi erect	Mostly	White
14	PB-6	Droop	White	Present	White	Whole length	Erect-Semi erect	Mostly	White
15	VIKASH	Deflexed	Yellow	Absent	Absent	Absent	Semi-erect	Mostly	White
16	CSR 27	Deflexed	Purple	Present	Light-red	Top only	Erect-semi erect	Mostly	Straw
17	JAYA	Deflexed	Brown	Absent	-	-	Semi-erect	Well	Gold
18	MAKOM	Deflexed	Brown	Absent	-	-	Erect-semi erect	Mostly	Straw

Table 2: Con….

S. no	Variety/Character	1. Basal leaf sheath color	8. Leaf: Pubescence of blade surface	9. Leaf: Auricle	10. Leaf: Anthocyanin coloration of auricle	14. Leaf: Shape of ligule	15. Leaf: Color of ligule	16. Leaf: Length of blade (Mean)	17. Leaf: Width of blade (Mean)
19	NDR-359	Green	Weak	Present	Colourless	Split	White	42.00 M	1.65 M
20	PR-113	Green	Weak	Present	Colourless	Split	White	43.63 M	1.27 M
21	VASUMATI	Green	Weak	Present	Colourless	Split	White	45.49 L	1.44 M
22	PB-1121	Green	Medium	Present	Colourless	Split	White	46.38 L	0.92 N
23	PB-1509	Green	Medium	Present	Colourless	Split	White	39.58 M	0.84 N
24	PNR-162	Green	Strong	Present	Colourless	Split	White	45.50 L	1.52 M
25	PNR-381	Green	Strong	Present	Colourless	Split	White	40.43 M	1.66 M
26	PUSA-44	Green	Medium	Present	Colourless	Split	White	38.45 M	1.03 M
27	IR-64	Green	Strong	Present	Colourless	Split	White	29.58 S	1.55 M
28	PS-5	Green	Medium	Present	Colourless	Split	White	45.78 L	1.67 M

Table 2: Con….

S. No	Variety/Character	20. Time of heading	21. Flag leaf: Attitude of blade (Early observation)	22. Spikelet: Density of pubescence of lemma	26.Lemma: Anthocyanin coloration of apex	27.Spikelet: Color of stigma	29. Stem: Length excluding panicle (Mean)	30.Stem: Anthocyanin coloration of node	33. Panicle: Length of main axis (Mean)	34. Flag leaf: Attitude of blade (Late observation)
19	NDR-359	Medium	Erect	Medium	Absent	White	73.61VS	Absent	28.31 L	Semi-erect
20	PR-113	Medium	Semi-erect	Medium	Absent	White	60.57 VS	Absent	24.31 M	Semi-erect
21	VASUMATI	Medium	Erect	Medium	Absent	White	96.49 S	Absent	28.51 L	Erect
22	PB-1121	Late	Erect	Medium	Absent	White	118.78 M	Absent	25.39 L	Erect
23	PB-1509	Medium	Semi-Erect	Weak	Absent	White	108.50 M	Absent	29.00 L	Semi-Erect
24	PNR-162	Medium	Semi-erect	Weak	Absent	White	60.57 VS	Absent	22.35 M	Semi-erect
25	PNR-381	Late	Erect	Strong	Absent	White	70.5 VS	Absent	25.39 L	Semi-Erect
26	PUSA-44	Late	Erect	Strong	Absent	White	75.63 VS	Absent	22.37 M	Semi-Erect
27	IR-64	Early	Erect	Strong	Absent	White	36.65 VS	Absent	23.56 M	Semi-Erect
28	PS-5	Early	Semi-Erect	Medium	Absent	White	97.80 S	Absent	29.20 L	Semi-Erect

Table 2: Con….

S. No	Variety / Character	35. Panicle: Curvature of main axis	37. Spikelet: Color of tip of lemma	39. Panicle: Awns	40. Panicle: Color of Awns (Late observation)	42. Panicle: Distribution of awns	45. Panicle: Attitude of branches	46. Panicle: Exertion	49. Sterile Lemma: Colour
19	NDR 359	Semi-straight	Brown	Present	Yellowish-white	Top only	Spreading	Partly	Gold
20	PR 113	Deflexed	Yellowish	Present	Yellowish-white	Top only	Semi-erect	Well	Straw
21	VASUMATI	Deflexed	Yellowish	Absent	Absent	Absent	Semi erect-Spreading	Partly	Straw
22	PB-1121	Semi-straight	White	Absent	Yellow white	Upper half only	Semi-Erect	Well	Straw
23	PB-1509	Deflexed	White	Present	Yellow white	Tip only	Semi-erect to spreding	Well	Straw
24	PNR-162	Straight	White	Absent	Absent	Absent	Erect	Mostly	White
25	PNR-381	Semi-straight	White	Present	White	Tip only	Erect	Well	White
26	PUSA-44	Straight	White	Absent	Yellow white	Tip only	Semi-erect	Well	White
27	IR 64	Straight	White	Absent	Absent	Absent	Semi-erect	Well	White
28	PS-5	Deflexed	White	Present	Yellowish-white	Upper half only	Semi-Erect	Well	Straw

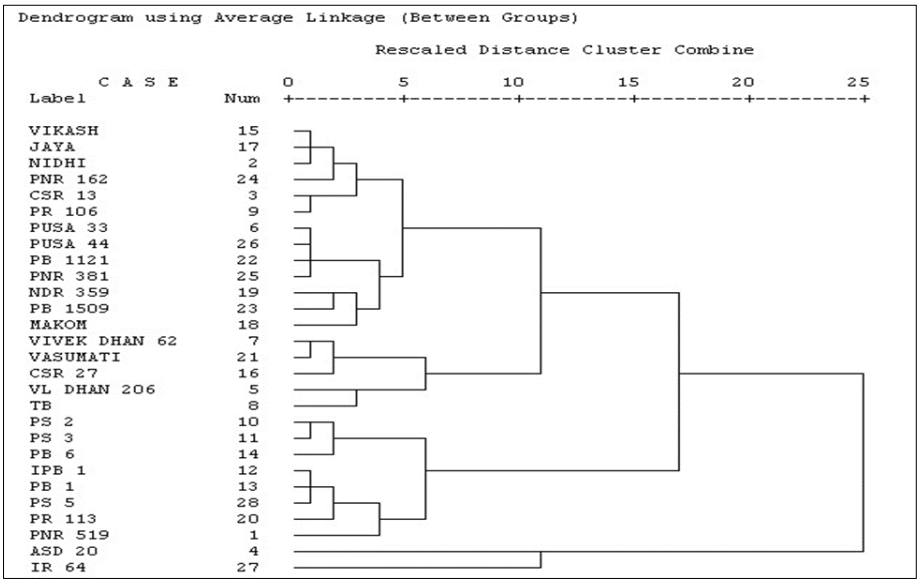

Fig 1: Dendrogram based on DUS parameters

Table 3: Clustering pattern of varieties based on DUS parameters

Cluster	Number of Varieties	Name
I	06	VIKASH, JAYA, NIDHI, PNR-162, CSR-13, PR-106
II	07	PUSA-33,PUSA-44,PB-1121,PNR-381,NDR-359,PB-1509, MAKOM
III	05	VIVEKDHAN-62, VASUMATI, CSR-27, V L DHAN-206, TARAORI BASMATI
IV	08	PS-2, PS-3, PS-5, PB-6, IMPROVED PB-1, PB-1, PR-113, PNR-519
V	02	ASD-20, IR-64

Result and Discussion

Since the objective of study was to characterize the extant rice varieties for plant morphological traits and assessment the extent of variability as per DUS guidelines and, associated constraints in these varieties for above characters. To achieve the same, the varieties of the present study were singled out using a combination of grouping characters and essential characters as listed in DUS guidelines. A total of three grouping characters and twenty four essential characters (Chang T.T. and E. A. Bardenas 1965) [8] as per the DUS guidelines (Rice) no. SG / 01 / 2007; were recorded for the

research material at various growth stages starting from basal leaf sheath colour (at seedling stage) upto grain length and width (after harvesting); represented in Table 2. Out of twenty seven morphological descriptors studied, three were found monomorphic, two were dimorphic and rests twenty two were found polymorphic in state of expression.

Similarly, Manjunatha, G. A *et al.* (2018) [9] also found that Out of 25 descriptors studied, three characteristics were found monomorphic, seven were dimorphic, six were of trimorphic, seven were tetramorphic and decorticated grain shape showed five states of expression, and lemma and palea colour recorded six states of expression. Similarly in another study a total of three characters *viz*., leaf auricles, leaf ligules and leaf shape of ligule were found to be monomorphic. The characters namely leaf anthocyanin colouration, leaf sheath anthocyanin colouration, panicle awns, panicle distribution of awns and leaf pubescence of blade surface were recorded as dimorphic. Seven traits *viz*., coleoptile colour, leaf intensity of green colour, ligule colour, leaf anthocyanin colouration of auricles, panicle exertion, leaf length of blade and leaf width of blade were of trimorphic. Five traits namely basal leaf sheath colour, culm attitude, flag leaf attitude of blade (early and late observation), panicle curvature of main axis were recorded four states of expression. The traits *viz*., spikelet colour of stigma, stem length, days to 50% flowering and time to maturity were showed five states of expression (Priyanga, R. S *et al.* 2020) [10].

The study grouped the 28 rice extant varieties into five different clusters (Table 3) based on these DUS parameters. The experimental material under study thus revealed considerable variability for most of the morphological characters, indicating their utility in the characterization and use in rice variety improvement programme.

Although characterization of varieties and variability in the expression of traits can be assessed in the varieties by DUS descriptors, but the extant/degree of expression in traits make it more subjective in nature and difficult to observe them, which can be seen in present study also. Such constraints can be classified in two groups of characters that is qualitative character and quantitative character.

Qualitative characteristics

There are 23 qualitative characters recorded among 27 total characters (Singh R.K *et al*., 2000) [11] under study, three were found monomorphic, two were dimorphic and rests eighteen were found polymorphic in state of expression. The maximum variability was recorded in ten traits: pubescence of Leaf blade surface (from weak to strong), time of heading (from very early to late), attitude of flag leaf blade (from erect to horizontal), density of pubescence of lemma (from weak to strong), curvature of panicle main axis (straight to droop), colour of tip of spikelet's lemma (from white to purple), colour of panicle's awn (from absent to light red), distribution of awns on panicle (from absent to whole length), attitude of panicle branches (from erect to spreading), panicle exertion (partly to well).

Although these characters are qualitative in nature, less influenced by environment and have stability in expression over the period of time, but some characters have more than one form of expression, such polymorphic characters are subjective in nature and there observations in field may vary from one person to another person, which can lead to different outcome also, for example pubescence of Leaf blade surface and density of pubescence of lemma are measured from weak to strong state of expression, one person may characterize a variety with weak form and another person may identify same variety with medium form of expression, same can happen with medium to strong form of expression also. So when there is no distinct expression of traits and have a degree of expression or relativity of expression, then there is possibility of different observation of trait under field condition.

Similar results was reported by Sanyal and. Joshi (2016) [12], they found that for characteristics where anthocyanin coloration and pubescence was involved like lemma: anthocyanin coloration of apex, density of pubescence of lemma and pubescence of blade surface; the categorization was subjective and varied from person to person; since the genotypes had to be grouped comparatively in to weak, medium, and strong state of expression. They also observed that characteristics like pubescence *viz*. density of pubescence of lemma, pubescence of blade surface were hard to categorize from weak to medium to strong. Hence, the character expression needs to be modified from the present stated to include only two categories *viz*. absence or presence of pubescence to rule out the ambiguity in recording the same, and to reduce the level of subjectivity involved.

Quantitative characteristics

There are 4 quantitative characters (like length of leaf blade, Width of leaf blade, length of stem excluding panicle, length of panicle main axis) recorded among 27 total characters in field condition and grain length and width (after harvesting) were recorded in present study (Ramaiah K., 1969.) [13]. Among the six quantitative characters evaluated maximum variation was observed for stem length, length of the leaf blade. Similar observation regarding variation in above quantitative characters was reported by S. K. Chakrabarty *et al.*, (2012) [14] in rice, they found that out of eight quantitative characters evaluated maximum variation for stem length, length of the leaf blade and 1000 grain weight was recorded. The quantitative characteristics or measured characteristics are more prone to environmental fluctuations during DUS test trials under open field conditions. The quantitative characteristics are governed by many genes, show Genotype X Environment interactions and have wide range of expression depending upon existing environment, location, soil type, nutrient availability etc. Thus observation of such characters also vary from location to location and existing environment, which makes varieties difficult to characterize based on uniformity and stability DUS descriptors.

The quantitative descriptors reveal not only the genetic constitution of the variety, but also the interaction of the genotypes with the environment (G x E) within which it is expressed (Lin and Bins, 1984) [15]. Morphological descriptors can provide a unique identification of cultivated varieties (Molona - Cano and Elena-Rossello, 1978). These plant characters form the basis for the breeder's selection of promising plant material. The morphological traits are used mainly for identification of genotypes and varieties. Genotype x Environment interaction effect has been found to cause aberrant means for traits, therefore, morphological data collected in field, which can provide only an initial screening of varietal identity or distinctness. It is known that multiple genotypes can give phenotypes of similar outward appearance (Ravi, 2000) [17], also the number of morphological traits is limited, most of them are multi genic, quantitative or

continuous characters, and their expression is influenced by environmental conditions (Smykal *et al*., 2008) [18].

Therefore, as per the many previous studies we also recorded following conclusions that the scope of morphological differences between the varieties is less due to narrow genetic base (Roy, S. C., & Shil, P. 2020) [19], and requires skilled human power which is subjective in nature. Also this process is time, labour and cost intensive. Hence, the manual identification of varieties/seeds by specialized technicians is slow, has low reproducibility, and possesses a degree of subjectivity that is hard to quantify. So, it is suggested that in addition to the existing DUS guideline for establishing varietal distinctiveness, recent technologies like use of molecular markers or image analysis techniques need to be complemented with field morphological studies to have a more reliable assessment. Same is also suggested by Gunjaca,J *et al.* (2008) [20] as they found that results were largely in favor of the use of molecular markers, revealing or confirming their already known advantages over morphological markers like better consistency with the pedigree, and relatively higher discriminative power. However, their integration into DUS testing protocols still depends upon resolving of several important issues. Similar suggestions were also provided by Sanyal and. Joshi (2016) [12].

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