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Assessment of distinctiveness, uniformity and stability of indigenous aromatic rice *(Oryza sativa)* varieties based on morphological descriptors

ABHILASHA JOSHI[1], R C AGRAWAL[2] and H S CHAWLA[3]

G B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263 145

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ABSTRACT

Twenty indigenous aromatic rice varieties were characterized using morphological descriptors adopted from the DUS guidelines of PPV and FR Authority and subsequently examined for their distinctiveness, uniformity and stability. Among the 46 visually assessed characters, 24 characters were monomorphic, 17 characters were dimorphic and 5 characters were polymorphic indicating their potential for varietal characterization and distinctiveness. No intra-varietal variation was observed for any of the visual characteristics and expression of characters in different varieties remained same for the two consecutive years confirming the uniformity and stability of the varieties for visual characteristics. Combined over years distinctiveness (COY-D) analysis was made on 14 measurable DUS descriptors which revealed distinctiveness for all except Tilakchandan varieties 3047 and 3048 with respect to each other only. COY-D analysis was complemented with Modified Joint Regression Analysis (MJRA). The slope of the MJRA curves in both the years and regression coefficient indicated that all the considered characters were not completely independent and they are interacting with each other as well as with environment. Combined Over Years Uniformity (COY-U) analysis for five of the measurable characteristics revealed that nine out of 20 varieties were completely uniform for the characters under study. However, the other 11 varieties were non-uniform for one or two characters emphasizing the need for their further purification to attain a considerable level of homogeneity in their heterogeneous blend. On the basis of grouping characteristics unique morphological profiles could be established for seven varieties only. When all the 60 morphological descriptors were studied no further distinctive profile could be obtained for any other variety. Thus the morphological DUS descriptors could establish distinctiveness of some varieties but varieties of similar background could not be discriminated hence some other markers/ descriptors could be thought for complementing the morphological DUS descriptors for establishing distinctiveness.

Key words: Combined over years distinctiveness, Combine over years uniformity, DUS descriptors, Indigenous aromatic varieties, Rice

India being one of the secondary centres of origin of rice *(Oryza sativa* L.) diversity is blessed with a rich diversity of around 120 000 accessions of landraces, farmer's varieties and wild relatives (Bala Ravi 2004). Aromatic rice constitutes a small but special group of rice which are considered best in quality. Majority of the Indian indigenous aromatic rice are small and medium grained but may surpass basmati types in many of the quality characteristics (Singh *et al*. 2000). There are a number of indigenous aromatic rice varieties currently under production whose identity and distinctiveness need to be established by various approaches. Obviously there is a need of consolidated system in the country to protect such a vast variability present in the species and proper sharing of benefits derived out of them. In this context, Government of India under the obligation of the TRIPS agreement has enacted the Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPV&FR Act) to encourage public/private investment in research and development of new plant varieties by giving protection to different categories of plant varieties against unauthorized multiplication of seeds or propagating materials for a specified period (Anonymous 2001). The PPV&FR Act recognizes the farmers as breeders who bred new varieties as well as conserved the traditional varieties. The plant varieties must fulfill the distinctiveness, uniformity and stability (DUS) criteria for protection under the Act and hence, there is a need to characterize rice varieties according to DUS test guidelines for rice prescribed by PPV and FR Authority (Anonymous 2007).

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[2]Principal Scientist, National Bureau of Plant Genetic Resources Pusa, New Delhi

[3]Professor (e mail: chawlahs_pantnagar@rediffmail.com), Department of Genetics and Plant Breeding

The variety identification serves the important goals, such as mitigating legal claims and confirming intellectual property rights and maintenance of genetic purity. Plant morphological characters have been recognized as the universally undisputed descriptors for DUS testing and varietal characterization of crop varieties**.** Use of morphological descriptors in sequential fashion is useful and convenient to discriminate the different varieties.

Keeping this in view, the study was taken up with the objective to determine the relative extent of distinctiveness, uniformity and stability of different morphological DUS descriptors in 20 indigenous aromatic rice varieties for their protection under the PPV and FR Act.

MATERIALS AND METHODS

The experimental material consisted of 20 indigenous aromatic rice varieties (Table 1). The trials were conducted during the two *kharif* seasons of 2006 and 2007 in randomized block design with three replications. Each replication consisted of three rows of 6 m length with 30 cm × 20 cm spacing. The observations were recorded on 60 of the 62 DUS characters at specified stages of crop growth period when characteristics under study had full expression. Two characteristics, viz. polished grain: expression of white core and culm attitude (for floating rice) were not applicable to the material under study. Among the 60 morphological characteristics studied, 46 were visually assessed and 14 were measured.

For determining distinctiveness in case of visually assessed characters, differences between two varieties were considered clear if the expression of one or more characteristics fell into two different states as per test guidelines. Analysis of measurable characteristics was carried out with the help of DUSNT software (Watson *et al*. 1998) comprising of COY-D (combined over years distinctiveness) for analysis of distinctiveness (Anonymous 2008a) and COY-U (combined over years uniformity) for analysis of uniformity (Anonymous 2008b). Modified Joint Regression Analysis (MJRA) was also used as a part of COY-D analysis. This MJRA model took account of systematic annual increases or decreases in character expression across all varieties by fitting extra terms, one for each year, in the analysis of variance. Each term represented the linear regression of the observations for the year against the variety means over all years, as described by Digby (1979).

For determining uniformity where all the plants of a variety are very similar, and in particular for vegetatively propagated and self-pollinated varieties, it is possible to assess uniformity by the number of obviously different plants – "off-types" – that occur. However, where the range of variation within a variety is larger, because of the features of its propagation, and in particular for cross-pollinated, including synthetic varieties, the plants are not all very similar and it is not possible to visualize which plants should be considered as a typical or "off-types." In this case the uniformity can be assessed by considering the overall range of variation, observed across all the individual plants to determine whether it is similar to comparable varieties.

For measured characteristics, the acceptable level of variation for the variety should not significantly exceed the level of variation found in comparable varieties already known. UPOV has proposed several statistical methods for dealing with uniformity in measured quantitative characteristics. One method, which takes into account variations between years, is the combined over years uniformity (COYU) method. This techcnique adjusts for any relationship that exists between uniformity, as measured by the plant-to-plant standard deviation (SD), and the expression of the characteristic, as measured by the variety mean, before setting a standard. The technique involves ranking reference and candidate varieties by the mean value of the characteristic. Each variety's SD is taken and the mean SD of the most similar varieties is subtracted. This procedure gives, for each variety, a measure of its uniformity expressed relative to that of comparable varieties. The results for each year are combined in a variety-by-years table of adjusted SDs and analysis of variance is applied. The mean adjusted SD for the candidate is compared with the mean for the reference varieties using a standard t-test. COYU, in effect, compares the uniformity of a candidate with that of the reference varieties most similar in relation to the characteristic being assessed. The main advantages of COYU are that all varieties can be compared on the same basis and that information from several years of testing may be combined into a single criterion. This procedure gives, for each variety, a measure of its uniformity expressed relative to that of comparable varieties (Prakash *et al*. 2007).

Grouping of rice varieties was done by using grouping characteristics as mentioned in the DUS test guidelines of rice as notified by PPV and FR Authority (Anonymous 2007).

RESULTS AND DISCUSSION

The accurate description and identification of rice varieties are crucial for DUS testing. The identity/profiles of rice varieties were established by using a set of morphological characteristics prescribed in the DUS test guidelines of rice. These characteristics are useful to establish distinctiveness, uniformity and stability of a variety, based on which the variety is given protection.

Out of the 46 visually assessed DUS descriptors studied, 24 were found to be monomorphic, 17 characteristics were dimorphic and five characteristics were polymorphic (Table 1). These visually assessed characteristics did not show any variation in their states of expression over two years of study. Further, no off-type plants were observed hence, these characters were considered to be uniform. Expression of each characteristic was found to be stable in both the years for the respective varieties, thus confirming their consistency and

Table 1 Characterization of aromatic rice varieties based on visual morphological DUS descriptors

Variety	Leaf character					Panicle character								Stem character				
	Coleoptile colour	Leaf: pubescence of blade surface	Leaf width	Leaf: intensity of green colour	Leaf senescence	Panicle: curvature of main axis	Panicle: awns	Panicle: length of awns	Panicle: secondary branching	Panicle: exertion	Panicle: colour of awns	Panicle: length	Panicle: number/plant	Panicle: atitude of branches	Stem thickness	Stem length	Time of heading	Time maturity
Kalajira	1	3	3	3	5	3	1		2	5		5	3	3	5	3	7	7
Kalanamak 3131	1	3	3	3	5	5	1		2	7		7	3	3	5	5	7	7
Kalanamak 3213	1	3	5	3	5	5	1		2	7		7	3	3	5	5	7	7
Kalanamak 3216	1	3	3	3	5	5	1		2	7		7	5	3	5	5	7	7
Kalanamak 3327	1	3	3	3	5	5	1		2	7		7	5	3	5	5	7	7
Tilakchandan 3047	1	5	5	5	3	5	9	9	3	5	3	7	3	3	7	3	5	5
Tilakchandan 3048	1	5	5	5	3	5	9	9	3	5	3	7	3	3	7	3	5	5
Bindli 3192	1	5	3	5	3	5	9	7	3	5	3	5	5	3	7	3	5	5
Bindli 3173	1	5	5	5	3	5	9	7	3	5	3	7	3	3	7	3	5	5
Bindli 3193	1	5	3	5	3	5	9	7	3	5	3	5	5	3	7	3	7	7
Bindli 3255	1	5	3	5	3	5	9	7	3	5	3	5	3	3	7	3	7	7
Lalmati	3	5	3	3	3	5	1		3	5		5	3	5	5	3	5	5
Lal Ram Jawain	1	5	5	3	3	5	9	7	2	5	3	5	3	5	5	5	5	5
Sakkarchini	1	3	3	3	5	5	1		2	5		7	5	3	5	5	7	7
Chini Kapoor	1	3	3	3	5	3	1		2	5		7	3	5	5	3	7	7
Badshah Bhog	1	3	3	3	5	3	1		2	5		7	3	3	5	1	7	7
Tilakchandan	1	5	5	5	3	5	9	9	3	5	3	7	3	3	7	3	5	5
Gopal Bhog	1	3	3	3	5	5	1		2	7		7	3	3	5	3	7	7
Govind Bhog	1	3	3	3	5	5	9	7	2	7	3	5	3	3	5	3	3	3
Dubraj	1	5	5	5	3	5	9	9	3	7	1	5	5	3	7	5	7	7
	1. Colourless 3. Purple	3. Weak 5. Medium	3. Narrow 5. Medium	3. Light 5. Medium	3. Early 5. Medium	3. Semi straight 5. Deflexed	1. Absent 9. Present	7. Long 9. Very long	2. Strong 3. Clustered	5. Mostly exerted 7. Well exerted	1.Yellowish white 3. Brown	5. Medium 7. Long	3. Few 5. Medium	3. Erect to semi erect 5. Semierect	5. Medium 7. Thick	1. Very short 3. Short 5. Medium	3. Early 5. Medium 7. Late	3. Early 5. Medium 7. Late

(contd...)

(*Table 1 concluded*)

Variety	Spikelet character								Grain character								
	Lemma: anthocyanin colouration of apex	Lemma: anthocyanin colouration keel	Lemma: anthocyanin colouration below apex	Lemma and palea colour	Sterile lemma colour	Spikelet: density of pubescence of lemma	Spikelet: colour tip of lemma	Phenol reaction of lemma	1 000-grain weight	Grain length	Grain width	Décorticated grain: colour	Décorticated: grain shape	Décorticated grain: length	Décorticated grain width	Gelatinization temp.	Endo sperm content of amylose
Kalajira	7	7	7	9	4	3	6	9	1	3	1	2	3	1	3	1	5
Kalanamak 3131	7	7	7	9	4	3	6	9	1	3	3	2	3	1	3	1	7
Kalanamak 3213	7	7	7	9	4	3	6	9	1	3	1	2	3	1	3	1	5
Kalanamak 3216	7	7	7	9	4	3	6	9	1	3	3	2	3	1	3	1	5
Kalanamak 3327	7	7	7	9	4	3	6	9	1	3	3	2	3	1	3	1	7
Tilakchandan 3047	1	1	1	1	1	5	3	1	5	3	3	1	3	1	5	5	5
Tilakchandan 3048	1	1	1	1	1	5	3	1	5	3	3	1	3	1	5	5	5
Bindli 3192	1	1	1	1	3	5	3	1	3	1	5	1	2	1	5	5	5
Bindli 3173	1	1	1	1	3	5	3	1	5	3	5	1	2	1	5	5	5
Bindli 3193	1	1	1	1	3	5	3	1	3	3	5	1	2	1	5	5	5
Bindli 3255	1	1	1	1	3	5	3	1	5	3	5	1	2	1	5	5	5
Lalmati	1	1	1	4	3	5	3	9	3	3	3	1	3	1	5	5	3
Lal Ram Jawain	1	1	1	4	3	5	3	9	3	3	5	1	3	1	5	5	3
Sakkarchini	1	1	1	1	1	3	3	9	1	1	3	1	2	1	5	1	5
Chini Kapoor	1	1	1	1	3	3	2	9	1	3	3	1	3	1	3	1	5
Badshah Bhog	1	1	1	1	1	3	3	1	1	1	1	1	3	1	3	1	5
Tilakchandan	1	1	1	1	3	5	3	1	3	3	3	1	3	1	5	3	5
Gopal Bhog	1	1	1	4	4	3	6	9	1	3	1	1	3	1	3	1	5
Govind Bhog	1	1	1	1	3	3	2	1	3	5	3	1	5	3	5	3	5
Dubraj	1	1	1	1	3	5	2	9	3	3	3	1	1	1	5	1	5
	1. Absent 7. Strong	1. Absent 7. Strong	1. Absent 7. Strong	1. Straw 4. Brown furrows 9. Black	1. Red 3. Straw 4. Purple	3.Weak 5. Medium	2. Yellowish 3. Brown 6. Black	1.Absent 9. Present	1. Very low 3. Low 5. Medium	1. Very short 3. Short 5. Medium	1. Very narrow 3. Narrow 5. Medium	1. White 2. Light brown	1. Short slender 2. Short bold 3. Medium slender 5. Long slender	1. Short 3. Medium	3. Narrow 5. Medium	1. Low 3. Medium 5.High, Medium	3. Low 5. Medium 7. High

Table 2 Combined over years distinctiveness analysis of 14 measurable characteristics in aromatic rice varieties using Modified Joint Regression Analysis (MJRA)

Name	Leaf: length of blade (cm)	Leaf: width of blade (cm)	Time of heading (days)	Stem: thickness (cm)	Stem: length (excluding panicle) (cm)	Panicle: length of main axis (cm)	Panicle: no./ plant	Maturity (days)	1 000-grain weight (g)	Grain length (mm)	Grain width (mm)	Decorticated grain length (mm)	Decorticated grain width (mm)	Content of amylose (%)
Year MS	28.024	0.014	464.125	0.001	0.045	0.075	2.409	0.834	2.409	0.559	0.000	0.108	0.004	0.009
Variety MS	173.608	0.090	669.050	0.030	512.060	40.103	12.014	700.014	130.682	4.321	0.607	2.994	0.413	25.581
Var. Year MS	3.665	0.003	6.647	0.000	95.661	2.268	0.777	17.201	1.777	0.029	0.016	0.040	0.007	2.289
F1 Ratio	47.375	27.925	100.727	231.686	5.353	17.682	15.467	40.696	73.552	147.022	38.792	75.192	59.613	11.177
Var. Rep MS	1.368	0.002	1.718	0.000	99.102	1.559	0.725	1.558	0.869	0.025	0.008	0.019	0.006	2.225
F2 Ratio	2.678	1.874	3.865	1.945	0.965	1.455	1.071	11.038	2.045	1.174	2.061	2.102	1.134	1.029
Between SE	0.782	0.023	1.052	0.005	3.993	0.615	0.360	.693	0.544	0.070	0.051	0.081	0.034	0.618
Within SE	0.478	0.017	0.535	0.003	4.064	0.510	0.348	0.510	0.381	0.065	0.036	0.056	0.032	0.609
MJRA Slope 2005	0.977	1.051	1.013	1.000	0.933	1.036	1.047	0.971	0.962	1.034	1.006	0.963	1.063	0.992
MJRA slope 2006	1.023	0.949	0.987	1.000	1.065	0.964	0.952	1.029	1.038	0.966	0.994	1.037	0.937	1.008
Regr F Val	0.434	1.354	0.308	0.000	0.349	0.394	0.606	0.626	2.085	3.580	0.023	2.052	5.436	0.012
Regr Prob	51.841	25.971	58.589	98.698	56.210	53.796	44.659	43.898	16.593	7.468	87.988	16.914	3.154	91.553

$$F_1 \text{ ratio} = \frac{\text{Variety MS}}{\text{Variety} \times \text{year MS}} \qquad F_2 = \frac{\text{Variety} \times \text{year MS}}{\text{Variety} \times \text{Replication MS}}$$

stability. The stability of visually assessed characteristics can be attributed to a low genotype × environment interaction in their expression. This is due to the fact that most of the visually assessed characters are controlled by single or two genes with simple dominant or recessive relationship. Kumar *et al*. (2006) held similar views for the morphological characterization of jute varieties over three years of study.

Data recorded on 14 measurable characteristics were subjected to COY-D statistical analysis at 1% level of significance. Each variety was considered to be a candidate variety and compared to rest of the 19 varieties as reference varieties to obtain a pair-wise distinctiveness matrix using COY-D analysis. Analysis revealed that all the varieties showed distinctiveness with respect to each other except for Tilakchandan varieties 3047 and 3048 which showed non-distinctiveness with respect to each other but showed distinctiveness to other 18 varieties.

COY-D analysis of 14 measurable characteristics using MJRA model was also carried out (Table 2). F_1 ratio (ratio of variety MS to Var × Year MS) was significant for all the characters indicating less role of environment in their expression. The significant F_2 ratio (ratio of variety × year MS to var × rep MS) was observed only for the characters, namely leaf length of blade, time of heading and time of maturity indicating their inconsistent behaviour over the years because of the greater role of environment in its expression thereby limiting its scope to establish distinctiveness among the present set of 20 indigenous aromatic varieties and traits with respect to the mentioned characters. It should be tested for another year or locations before coming to any conclusion.

Table 3 Combined over years uniformity analysis of five measurable characteristics in aromatic rice varieties

Candidate variety	Leaf length (cm)	Leaf width (cm)	Stem thickness (cm)	Stem length (cm)	Panicle no./plant (cm)
Kalajira	98	99	108 1	89	108:
Kalanamak 3131	102	115 1	102	108	100
Kalanamak 3213	106	123:1	85	111	97
Kalanamak 3216	95	109	117 1	88	101
Kalanamak 3327	96	105	113 1	98	96
Tilakchandan 3047	94	101 1	88	99	99
Tilakchandan 3048	114+1	112 1	85	100	102
Bindli 3192	111:1	97	103	94	104
Bindli 3173	102	95	100	99	97
Bindli 3193	102	91	100	104	101
Bindli 3255	100	99	104	104	92
Lalmati	109	84	131+1	111 1	93
Lal Ram Jawain	92	88	95	91	93
Sakkarchini	92	104	92	101	100
Chini Kapoor	100	101	94	101	102
Badshah Bhog	100	92	82	108	100
Tilakchandan	94	89	95	90	109:
Gopal Bhog	105	104	103 1	95	106
Govind Bhog	89	95	98	108	99
Dubraj	97	97	103	100	100

*-SD exceeds over-years criterion after three years with probability 0.0100; + -SD exceeds over-years criterion after two years with probability 0.0100; : -SD not yet acceptable after two years with probability 0.0500; 1, 2, 3 – The number of occasions the within-years SD exceeds the UPOV criterion

The slope of MJRA curves in both the years and regression probability indicated that all the considered characters were not completely independent rather they are interacting with each other as well as with the environment. These results emphasize the need for testing the present experimental material in another year and other locations. Ruiz *et al.* (2001) also reported distinctness among the 16 ryegrass *(Lolium perenne* L.) varieties by using MJRA model.

COY-U analysis carried out for five measurable characteristics is shown in Table 3. It revealed that nine out of a total of 20 varieties were completely uniform for all the five measurable characteristics. However, remaining varieties were non-uniform for one or two characters. In addition to this for majority of the characters non-uniformity was within the acceptable limits of UPOV criteria (Anonymous 2008b). Furthermore, standard deviation for the character leaf length in variety Bindli 3192, for leaf width in variety Kalanamak 3213' and for panicle no./plant in varieties Kalajira and Tilakchandan were not yet acceptable even after two years with probability of 5%. This emphasizes the need for further purification of the mentioned varieties for attaining a considerable level of uniformity.

A major objective of varietal characterization is to establish the distinctiveness among the varieties so that official regulatory bodies have a basis on which they can assign rights and protect the interests of plant breeders and farmers (Joshi *et al*. 2007). Keeping this in view, varieties were characterized to establish their unique identification profiles on the basis of grouping characteristics prescribed by DUS guidelines of PPV and FR Authority, Government of India. DUS guidelines have included eight characteristics in rice as grouping characteristics. Amongst the 20 indigenous rice varieties studied it was observed that the two characteristics, viz. basal leaf: sheath colour and decorticated grain: aroma, were found to be monomorphic. Hence, the grouping of varieties was based on six characteristics. It revealed distinctive profiles for seven rice varieties only which were Govind Bhog, Lal Ram Jawain, Badshah Bhog, Lalmati, Dubraj, Sakkarchini and Kalajira (Table 4). When all the 60 morphological descriptors were considered, no further distinctive profile for any other variety was obtained. Although for some of the varieties which were discriminated by grouping characters, additional distinctive descriptors were obtained (Table 4). Thus rest of the 13 varieties remained in groups of two or three varieties. It was seen that indigenous varieties of similar origin e g Bindli and Kalanamak or basic variety Tilakchandan and its modifications Tilakchandan 3047 and Tilakchandan 3048 could not be discriminated on the basis of morphological DUS descriptors of rice. In short, the cultivars in the present study showed overlapping of morphological descriptors in various combination traits, but still the identity of some of the cultivars could be established individually. Similar attempts for establishment of distinctiveness have also been

Table 4 Morphological profiles of aromatic rice varieties based on grouping characteristics and other morphological descriptors

Variety	Characteristic
Govind Bhog	Early (time of heading)
	Early (time of maturity) *
	Medium (grain length) *
	Medium (decorticated grain length)*
Lal Ram Jawain	Medium (time of heading)
	Medium (stem length)
Badshah Bhog	Late (time of heading)
	Very short (stem length)
Lalmati	Medium (time of heading)
	Short (stem length)
	Medium slender (decorticated grain shape)
	Low (amylose content)
	Purple (coleoptile colour) *
Dubraj	Late (time of heading)
	Medium (stem length)
	Short slender (decorticated grain shape)
	Yellowish white (panicle: colour of awns) *
Sakkarchini	Late (time of heading)
	Medium (stem length)
	Short bold (decorticated grain shape)
Kalajira	Late (time of heading)
	Short (stem length)
	Medium slender (decorticated grain shape)
	Light brown (decorticated grain colour)
Tilakchandan	Medium (time of heading)
Tilakchandan 3047	Short (stem length)
Tilakchandan 3048	Medium slender (decorticated grain shape)
	Medium (amylose content)
Bindli 3173, Bindli 3192	Medium (time of heading)
	Short (stem length)
	Short bold (decorticated grain shape)
Bindli 3193, Bindli 3255	Late (time of heading)
	Short (stem length)
	Short bold (decorticated grain shape) pl check
Kalanamak 3213	Late (time of heading)
Kalanamak 3216	Medium (stem length)
	Medium slender (decorticated grain shape)
	Medium (amylose content)
Kalanamak 3131	Late (time of heading)
Kalanamak 3327	Medium (stem length)
	Medium slender (decorticated grain shape)
	High (amylose content)
Gopal Bhog, Chini Kapoor	Late (time of heading)
	Short (stem length)
	Medium slender (decorticated grain shape)
	White (decorticated grain colour)

*indicates characters other than grouping characters

made in soybean (Ravikumar and Naraayanswamy 1999), oat (Kumar *et al*. 2002), rapeseed-mustard (Gupta *et al*. 2004; Yadav 2004), pearl millet (Kumar *et al*. 2004) and jute (Kumar *et al*. 2008).

It may be concluded that the morphological DUS descriptors can be effectively used for identification and grouping of varieties and varieties satisfying the DUS criteria for these morphological descriptors could be registered under the PPV and FR Act for obtaining Plant breeders and Farmers' rights. However, morphological descriptors alone may not be sufficient for DUS criteria. Hence, some other markers/ descriptors could be considered for complementing the morphological DUS descriptors.

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