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DUS characterization of rice hybrids and their parental lines using morphological marker

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ABSTRACT

A plant variety is a genetically related plant group obtained by breeder, as a result of long and thoughtful process. A new cultivator is an important means of enhancing production and productivity of cultivated plants. Their fruit quality and resistance to diseases, thus helping in increasing the excellence of agriculture. Characterization of plant varieties in context of intellectual property rights is stimulated by the recent agreements within the framework of the World Trade Organization (WTO). The requirements of these activities vary, e.g. the varietal registration process involving testing for Distinctness, Uniformity and Stability (DUS) requiresa description of a newly bred variety be produced and compared to all existing varieties in common knowledge. These characters suffer from the limitations of number, interaction with the environment in which the variety grows and subjectivity in decision-making. The genuineness of variety is one of the most important characteristics of quality seed. In addition, seed certification, which forms a link between variety registration and seed production, involve assessment, varietal identification and purity to assure the quality of seed marked to farmer or grower. Characterization of four hybrids such as NARENDRA SANKAR DHAN 2, Rajlaxmi, Ajay and CR Dhan 701 their parental lines such as IR58025A, NDR3026-3IR, CRMS32A, IR42266-29-3R, CRMS31A, CRL22R and IR42266-29-3R based on distinguishing characters could differentiate the hybrids and parental lines.

Key words: *DUS Test, hybrids rice, characterization, parental lines*

Rice (*Oryza sativa* L.) is a self pollinating crop belonging to family of Gramineae and is one of the important, major staple food crops for global population and that of India (Sharma et al., 2012). In India rice production needs to be enhanced to 2.5 million tons a year to meet the demand in 2050 (Hari et al., 2013). Hybrid rice technology is considered as one of the genetically feasible and readily adoptable options to meet the increasing demand of rice, as hybrid have recorded 15-20 percent more yield as compared to inbred varieties (Hari et al., 2014). The development of one or more variety depends on the final selection of superior plants by the plant breeder who use several techniques to create the genetic variation and to select from within that variation (Singh, 2008). The release of a large number of rice hybrids has increased the task as well as the responsibilities of seed technologists in order to ensure the quality of seed. Seed technologists must be well equipped to identify different varieties and hybrids, both at field and at seed level. Varietal descriptions given by the breeders most often relate to field characters and are not sufficient to identify genotypes or seed lot adequately (Nethra, 2007). The present study was undertaken with the objective of establishing morphological markers for distinguishing rice hybrids and their parental lines.

The materials for this study comprised of four hybrids, four CMS lines and four restorer lines of rice.

The pure seeds of all these parents and hybrid of IR58025A, NDR3026-3IR, and NARENDRA SANKAR DHAN-2 were collected from Narendra Dev University of Agriculture & Technology, Kumarganj, Faizabad. Seeds of Rajalaxmi, Ajay, CR Dhan 701 and their parental lines such as CRMS32A, IR42266-29-3R, CRMS31A, CRL22R and IR42266-29-3R were obtained from ICAR-National Rice Research Institute (NRRI), Cuttack, Odisha. Ten plants selected at random from each variety were observed for various stable and distinguishable characters according to DUS guidelines (PPV and FRA, 2007). The observation of various characteristics was recorded at different stages of growth with appropriate procedures as per the DUS test guidelines of PPV & FR Act, 2001. Like UPOV, in PPV and FR Act, a variety must fulfil the criteria of distinctiveness, uniformity, stability and novelty (if new) so as to get protection under this act. The traits studied were coleoptile colour, basal leaf sheath colour, intensity of green colour of leaf, leaf anthocyanin coloration, sheath anthocyanin coloration, pubescence of leaf blade surface, leaf auricles, anthocyanin coloration of auricles, leaf collar, leaf anthocyanin coloration of collar, leaf shape of ligule, leaf colour of ligule, length leaf of blade, width of leaf blade, culm attitude, time of heading, attitude of flag leaf blade, spikelet density, pubescence of lemma, male sterility, lemma anthocyanin colouration of keel, anthocyanin colouration of area below apex, lemma anthocyanin colouration of apex, spikelet colour of stigma, stem thickness, stem length, stem anthocyanin colouration of nodes, stem anthocyanin colouration of internodes, panicle length of main axis, flag leaf attitude of blade (late observation), curvature of panicle main axis,: number of panicle per plant, spikelet colour of tip of lemma, lemma and palea colour, panicle awns, panicle colour of awns (late observation), panicle length of longest awn, panicle distribution of awns, panicle presence of secondary branching, panicle secondary branching, panicle attitude of branches, panicle exertion, time maturity (days), leaf senescence, sterile lemma colour, 1000 grain weight, grain length, grain width, decorticated grain length, decorticated grain width and decorticated grain aroma.

To establish distinctiveness among rice hybrid and parental lines fifty characters have been used. Qualitative characters are considered as morphological markers in the identification of rice hybrid and parental lines. Coleoptile colour was colourless and basal leaf sheath colour for all the rice genotypes were green and did not show any variation. However the intensity of green colour varied from medium green to dark green in different genotypes. The genotypes IR58025A, NDR3026-31R and CRL22R were classified as dark green. The rest of genotypes were medium green (Table 1).

The anthocyanin colouration of leaf was absent in all genotypes. Sheath anthocyanin colouration of leaf was absent in all genotypes. Pubescence of blade surface was not found in genotype IR58025A. The pubescence was found to be weak in five genotypes (NDR3026-31R, CRMS32A, CRMS31A and CR Dhan701 whereas the rest of genotypes showed medium pubescence of blade surface. The leaf collar was present in all genotypes studied. The leaf Anthocyanin coloration of collar was absent in all genotypes studied. Leaf auricles and ligules were present in all genotypes studied. The shape of ligule and colour of ligule also did not show any variation and found to be split and white respectively for all the genotypes. The leaf length of blade was found to be medium in four genotypes (IR58025A, CRMS32A and CRMS31A) whereas the rest of genotypes showed long leaf blade length. The leaf width of blade was medium in all genotypes. Culm attitude was found to be erect in four genotypes (NDRH-2, Rajlaxmi, Ajay and CR Dhan 701) whereas the rest of genotypes showed semi-erect of blade. The early observation on attitude of flag leaf was found to be erect in five genotypes (NDR3026-31R, CRMS32A, CRL22 R and CRMS31A) whereas the rest of genotypes as semi-erect. The density of pubescence of lemma in the spikelet was weak in six genotypes (IR58025A, CRMS32A, CRMS31A, Ajay and CR Dhan 701) which were assigned whereas the remaining genotypes recorded medium density of lemma pubescence. Male sterility was found to be present in four genotypes (IR58025A, CRMS32A and CRMS31A) whereas the rest of genotypes showed absent male sterility. Lemma anthocyanin colouration of keel and anthocyanin colouration of area below apex were absent for all the genotypes and did not show any variation. Anthocyanin colouration of apex was absent for all the genotypes

Table1. Qualitative characters in rice hybrids and their parental line.

Characters	IR58025 A	NDR3026-3IR	NDRH-2	CRMS32 A	IR42266-29-3R	RAJLAXMI
Coleoptile: Colour	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Basal leaf sheath colour	Green	Green	Green	Green	Green	Green
Leaf: Intensity of green colour	Dark	Dark	Medium	Dark	Dark	Medium
Leaf anthocyanin colouration	Absent	Absent	Absent	Absent	Absent	Absent
Leaf sheath anthocyanin colouration	Absent	Absent	Absent	Absent	Absent	Absent
Leaf pubescence of blade surface	Absent	Weak	Weak	Weak	Weak	Weak
Leaf auricle	Present	Present	Present	Present	Present	Present
Leaf Anthocynin colouration of auricle	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Leaf collar	Present	Present	Present	Present	Present	Present
Leaf Anthocynin colouration of collar	Absent	Absent	Absent	Absent	Absent	Absent
Leaf ligule	Present	Present	Present	Present	Present	Present
Shape of ligule	Split	Split	Split	Split	Split	Split
Colour of ligule	White	White	White	White	White	White
Leaf length of blade	Medium (32cm)	Long (46cm)	Long (46cm)	Medium (38cm)	Medium (32cm)	Long (55cm)
Leaf Width of blade	Narrow	Narrow	Narrow	Narrow	Narrow	Narrow
Culm altitude	Semi erect	Semi erect	Erect	Semi erect	Semi erect	Erect
Time of heading	Early	Medium	Medium	Medium	Medium	Medium
Flag leaf Attitude of blade (Early observation)	Semi erect	Erect	Semi erect	Erect	Semi erect	Semierect
Spike density ofPubescence of lemma	Weak	Medium	Medium	Weak	Medium	Medium
Male Sterility	Present	Absent	Absent	Present	Absent	Absent
Lemma AnthocyninColouration of keel	Absent	Absent	Absent	Absent	Absent	Absent
Lemma AnthocyninColouration of Apex	Absent	Absent	Absent	Absent	Absent	Absent
Spikelet : colour of stigma	White	White	White	White	White	White
Stem thickness	Medium	Thick	Thick	Thick	Thick	Thick
Stem Length	Very short (55.18cm)	Very short (63.76cm)	Very short (65cm)	Very short (55.3cm)	Very short (71.96cm)	Very short (84.08cm)
Lemma AnthocyninColouration of nodes	Absent	Absent	Absent	Absent	Absent	Absent
Lemma AnthocyninColouration of internodes	Absent	Absent	Absent	Absent	Absent	Absent
Panicle length of main axis	Medium (26.1cm)	Long (29cm)	Long (28.8cm)	Medium (24.8cm)	Long (29.5cm)	Long (29.5cm)
Fleg leaf: Attitude of blade (late observation)	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect
Panicle: Curvature of Main axis	Deflexed	Drooping	Deflexed	Straight	Drooping	Drooping
Panicle: Number per Plant	Few(9)	Medium(13)	Medium(15)	Medium(14)	Medium(16)	Medium(13)
Spikelet colour of tip oflemma	White	White	White	White	White	Yellowish
Panicle: awns	Present	Absent	Absent	Absent	Absent	Absent
Panicle length of Longest awn	Short	-	-	-	-	-
Panicle distribution of awns	Tip only	-	-	-	-	-
Panicle presence of Secondary branching	Present	Present	Present	Present	Present	Present
Panicle Secondary branching	Weak	Strong	Strong	Weak	Strong	Strong
Panicle Attitude of branches	Semi-erectto spreading	Erect to semi erect	Semi-erect	Erect to semi-erect	Semi- erect to spreading	emi-erect to spreading

and did not show any variation. The stigma colour was found to be white in all the genotypes. Stem thickness was found to be medium in three genotypes (IR58025A and CRMS31A) whereas the rest of genotypes showed stem thickness. None of the genotypes showed presence of anthocyanin coloration on nodes and internodes of stem. Flag leaf attitude of blade (late observation) was found to be semi-erect in all the genotypes. The curvature of panicle main axis at the ripening stage was drooping in five genotypes (NDR3026-31R, IR42266-29-3R, IR42266-29-3, RAJALAXMI and CR Dhan 701) whereas, four genotypes viz., IR58021A, NDRH-2, CRL22 R and Ajay showed deflexed curvature. The remaining genotypes showed straight panicle. Spikelet colour of tip of lemma was found to be yellowish in two genotypes (Rajlaxmi and Ajay) whereas, the rest of genotypes showed white colour. Lemma and palea colour was found to be gold and gold furrows on straw background in three genotypes (NDR3026-31R, IR42266-29-3R and IR42266-29-3R) whereas the rest of genotypes showed straw colour. The panicle awns were absent in the most of genotypes. Except in IR58025A, which was given a score of '9'. However, the length of longest awn was short. The panicle awns were absent in the most of genotypes except in IR58025A. However, panicle colour of awns (late observation) was yellow white. However, the panicle distribution of awns was present tip only. The presence of secondary branching of panicle was observed in all the genotypes and showed strong secondary branching in most of genotypes, except IR58025A and IR42266-29-3R for which it was weak. Based on attitude of panicle branches, three groups could be obtained. The genotypes NDR3026-31R, CRMS32A and CRL22R, had erected to semi-erect type panicle branches and semi-erect to spreading type panicle branches in IR58025A, IR42266-29-3R, CR Dhan 701 and Rajlaxmi. While the rest of the genotypes were grouped as semi-erect. The exertion of panicles was recorded as partly exerted with a score of '3' in all the CMS lines (IR58025A, CRMS31A and CRMS32A); and the remaining genotypes showed well exerted panicle. Leaf senescence was found to be late in three genotypes (IR58025A, NDR3026-31R and CRDHAN 701) whereas the rest of genotypes showed medium. Sterile Lemma colour was found to be gold background in one genotype (NDR3026-31R) whereas, the rest of genotypes showed straw colour. The decorticated grain aroma was absent in all genotypes.

Variation due to quantitative traits

The coefficient of variation ranged from 1.0 per cent (plant height) to 7.5 percent (panicle number of per plant). The time of heading among genotypes varied from 88 (IR58025A) to 115 days (CR Dhan 701). The leaf length among the genotypes ranged from 33.90 cm (IR58025A) to 45.90 cm (IR42266-29-3R). The minimum (1.17 cm) and maximum (1.60 cm) leaf width was observed in Rajlami and Ajay respectively. Plant height, panicle number per plant showed a considerable percentage of variation. Plant height was minimum (78.17 cm) in CRMS31A and maximum (124.00 cm) in NDR3026-3IR. Panicle number per plant was 10 and 15 in IR58025A and Narendra Shankar Dhan 2 respectively. The 1000 grain weight in CRMS31A was minimum (18.00 g) and the genotype Narendra Shankar Dhan 2 registered maximum grain weight (25.40g).

Several workers have suggested that, the time of heading and the plant height contribute maximum to the genetic divergence among the genotypes (Bose and Pradhan, 2005; Madhavilatha and Suneetha, 2005 and Singh et al., 2006). The length of the panicle main axis was observed as long for IR58025A, NDR3026-31R, NDRH-2, IR42266-29-3R, AJAY, IR42266-29-3R, Rajlaxmi, CRL22R and CR Dhan701 the remaining genotypes as medium and it ranged from 25.84 cm (CRMS 32A) to 30.09 cm (IR42266-29-3R).

The length of the panicle main axis and panicle number per plant directly control the yield of a particular variety. The length of panicle main axis was observed as long for IR58025A, NDR3026-31R, NDRH-2, IR42266-29-3R, Ajay, IR42266-29-3R, Rajlaxmi, CRL22R and CR Dhan701 and as medium for the remaining genotypes. Similar results were obtained by Sarma et al. (2004) where they characterized 142 ahu rice genotypes of Assam and found that eight genotypes showed more than 25 cm panicle length and the remaining genotypes recorded lesser panicle length.

In the present study, based on the number of productive tillers per plant, the genotypes were classified as few (IR58025A) and the remaining as medium. (Abbasi et al., 1995) and a great variability exists for number of tillers per plant (Zafar et al., 2004). Hence, this trait showed high variability for number of

Table 2. Qualitative characters in rice hybrids and their parental line.

Characters	CRMS31 A	IR42266-29-3R	AJAY	CRMS31 A	CRL22R	CRDHAN701
Coleoptile: Colour	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Basal leaf sheath colour	Green	Green	Green	Green	Green	Green
Leaf: Intensity of green colour	Medium	Dark	Medium	Medium	Dark	Medium
Leaf anthocyanin colouration	Absent	Absent	Absent	Absent	Absent	Absent
Leaf sheath anthocyanin colouration	Absent	Absent	Absent	Absent	Absent	Absent
Leaf pubescence of blade surface	Weak	Medium	Medium	Weak	Medium	Weak
Leaf auricle	Present	Present	Present	Present	Present	Present
Leaf Anthocynin colouration of auricle	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Leaf collar	Present	Present	Present	Present	Present	Present
Leaf Anthocynin colouration of collar	Absent	Absent	Absent	Absent	Absent	Absent
Leaf ligule	Present	Present	Present	Present	Present	Present
Shape of ligule	Split	Split	Split	Split	Split	Split
Colour of ligule	White	White	White	White	White	White
Leaf length of blade	Medium(34cm)	Medium(46cm)	Long(56cm)	Medium(38cm)	Long(52cm)	Long(55cm)
Leaf Width of blade	Narrow	Narrow	Narrow	Narrow	Narrow	Narrow
Culm altitude	Semi erect	Semi erect	Erect	Semi erect	Semi erect	Erect
Time of heading	Medium	Medium	Medium	Medium	Late	Late
Flag leaf Attitude of blade(Early observation)	Erect	Semi erect	Semi erect	Erect	Erect	Semierect
Spike density ofPubescence of lemma	Weak	Medium	Weak	Weak	Medium	Weak
Male Sterility	Present	Absent	Absent	Present	Absent	Absent
Lemma AnthocyninColouration of keel	Absent	Absent	Absent	Absent	Absent	Absent
Lemma AnthocyninColouration of Apex	Absent	Absent	Absent	Absent	Absent	Absent
Spikelet : colour of stigma	White	White	White	White	White	White
Stem thickness	Medium	Thick	Thick	Medium	Thick	Thick
Stem Length	Very short (52.98cm)	Very short (71.90cm)	Very short (75.44cm)	Very short (52.98cm)	Very short (68.28cm)	Very short (72.92cm)
Lemma AnthocyninColouration of nodes	Absent	Absent	Absent	Absent	Absent	Absent
Lemma AnthocyninColouration of internodes	Absent	Absent	Absent	Absent	Absent	Absent
Panicle length of main axis	Medium(23.5cm)	Long(29cm)	Long(30cm)	Medium(23.5cm)	Long(28.5cm)	Long(29.5cm)
Fleg leaf: Attitude of blade(late observation)	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect
Panicle: Curvature of Main axis	Straight	Drooping	Deflexed	Straight	Drooping	Deflexed
Panicle: Number per Plant	Medium(13)	Medium(12)	Medium(13)	Medium(13)	Medium(14)	Medium(13)
Spikelet colour of tip oflemma	White	White	Yellowish	White	White	White
Panicle presence of Secondary branching	Present	Present	Present	Present	Present	Present
Panicle Secondary branching	Strong	Weak	Strong	Strong	Strong	Strong
Panicle Attitude of branches	Erectto semi-erect	Semi- erect to spreading	Semi-erect	Erect to semi-erect	Semi- erect	Semi-erect to spreading
Panicle exertion	Partly esserted	Well esserted	Well esserted	Partly esserted	Well esserted	Well esserted
Time maturity (days)	Medium(125)	Medium(133)	Medium(134)	Medium(127)	Late(141)	Medium(137)
Leaf senescence	Medium	Medium	Medium	Early	Medium	Late
Sterile Lemma colour	Straw	Straw	Straw	Straw	Straw	Straw
Grain Weight of 1000 Developed grains	Low(18g)	Medium(24.5 g)	High(25.8 g)	Low(18 g)	Medium(24.5 g)	Medium(25 g)
Grain Length	Medium (9.14mm)	Medium (9.67mm)	Medium (9.71mm)	Medium (9.54mm)	Medium (9.13mm)	Medium (8.85.mm)
Grain Width	Narrow (2.34mm)	Narrow (2.45mm)	Narrow (2.34mm)	Narrow (2.38mm)	Narrow (2.31mm)	Narrow (2.24mm)

productive tillers per plant (Shah et al., 1999). The true seeds of genotypes are characterized by specific traits such as length and width, shape and size. Laboratory observations to quantify these characters include physical measurements of the length and width and thousand grain weight. Thousand grain weight has been used for characterizing rice varieties by several workers (Rosta, 1975; Liou, 1983; Bose and Pradhan, 2005; Joshi et al., 2007). This indicated that, the longer grains tend to be very narrow. Similar variations in grain length and width were observed by Gupta (1990) and Sharma et al. (1990).

The success of hybrid rice technology beside other factors depends on the production and timely supply of genetically homogenous seed to farmers. The present study establishes the morphological characteristics of seed, seedling and plant were found useful for varietal characterization in rice hybrids and parents. Some of the distinguishing characters like attitude of flag leaf blade, flag leaf length and width, days to 50 per cent flowering and maturity, stem length, degree of panicle exertion, presence of awns and seed traits such as 1000 seed weight, grain length and width and shape of grain were found to be more useful for identification and grouping of hybrids and parents to maintain genetic purity during seed production.

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