



## Example varieties for DUS testing in rice (*Oryza sativa*)

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Received: 2 July 2011; Revised accepted: 27 August 2012

### ABSTRACT

In order to validate the distinctiveness of rice (*Oryza sativa* L.) varieties, 106 example varieties are included in the National DUS Test Guidelines on Rice notified by the Protection of Plant Varieties and Farmers' Rights (PPV & FR) Authority. Seventy three such example varieties obtained from Nodal Officer, DUS Testing, Directorate of Rice Research, Hyderabad and respective breeders were grown in field and characterized. Twenty varieties did not conform to the distinct characteristics for which these were identified as examples. The non-conformity of the example varieties for the particular state of expression of characteristics was further examined with fresh seed lots obtained in the second year from the Directorate of Rice Research, Hyderabad. Six varieties still could not be validated for the identified example characteristics. A strict maintenance breeding of the reference varieties, including the example varieties, and use of alternate example varieties for conduct of DUS testing in rice, if needed, are suggested.

**Key words:** DUS test, Example variety, Plant variety protection, Rice

General Agreement on Tariffs and Trade (GATT) recognized agriculture as a rule-bound enterprise of investment and profit making and included it in the negotiations for the first time in Uruguay Round (1986-1994). These negotiations led to the establishment of World Trade Organization (WTO) in January 1995 of which India is a signatory. Having ratified the Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS), India made provisions for giving effect to sub-paragraph (b) of paragraph 3 of article 27 in part II of the said agreement relating to protection of plant varieties with the objective of providing an effective system of protection against unlawful commercial exploitation of new plant varieties, the rights of farmers and plant breeders and to encourage the developments of new varieties of plants. Accordingly "Protection of Plant Varieties and Farmers' Rights (PPV&FR) Act" was passed by the Government of India in 2001. The Protection of Plant Varieties and Farmers' Rights Authority established by the Government has the responsibility of implementing the provisions of this Act, which provides the unique opportunity to protect the extant, new and essentially derived varieties of plant species if these conform to the criteria as laid down under the Rules of PPV&FR Act 2001. The examination of a new plant

variety for establishment of distinctiveness, uniformity and stability is known as "Distinctiveness, Uniformity and Stability (DUS) test". The DUS tests are carried out at different centers, located at State Agricultural Universities and various crop-based institutes of Indian Council of Agricultural Research. The success of DUS test trials rest on a set of general principles and specific guidelines. The examination of a variety for DUS generates a description of the variety using its relevant characteristics (e.g. plant height, leaf shape, time of flowering etc.), by which it can be described as a variety in terms of the Act. Plant morpho-physiological characteristics have been recognized universally as undisputed descriptors for variety characterization and DUS testing of plant varieties. Use of morphological descriptors in sequential order is useful and convenient to discriminate different varieties. A new (novel) plant variety is considered for protection once it meets the requirements as laid down in the Protection of Plant Variety and Farmers' Rights Act 2001, that the variety is distinct (D) from any other variety whose existence is a matter of common knowledge in any country at the time of filing of the application and that is sufficiently uniform (U) and stable (S). The DUS test is conducted by growing the plants by the testing authority following the Test Guidelines. A variety is identified on the basis of a set of characteristics differing from other known varieties of that species. A guideline for conduct of tests for distinctiveness, uniformity and stability is required for describing a variety, assessing the level of uniformity of characteristics and the

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stability of expression of those in different growing years. In the Table of Characteristics of a crop-specific guideline for the conduct of tests for DUS, each characteristic has two or more states of expression by which varieties are described for establishment of its distinctiveness from other varieties at a given point of time. For the purpose of an objective comparison and uniform evaluation by the DUS testing personnel, example varieties are identified and included in the Table of Characteristics to exemplify the characteristic state of expression. These example varieties must exhibit the specific state of a characteristic without any ambiguity. Inadequate verification of the example varieties may lead to an incorrect conclusion on distinctiveness of candidate varieties, with negative consequences for the plant breeders' rights granted. As the introduction of an Act for Plant Variety Protection, and the Rules thereof, have only been introduced recently in India, where a larger variability exists among rice genotypes, the true character expression in the example varieties assume a greater significance. Therefore, the present study was made with the objective to validate the example varieties for the states of expression of various characteristics as provided in the Guidelines for the Conduct of Test for Distinctiveness, Uniformity and Stability on Rice (*Oryza sativa* L.) notified by the PPV& FR Authority (Anonymous 2007).

The study material comprised 57 extant rice varieties obtained from the Nodal Officer, DUS Test, Directorate of Rice Research, Hyderabad in 2009 and 16 varieties received from the respective breeders from the SAUs. A total of 73 example varieties (Table 1) were transplanted with four rows of 4 m length keeping a row to row and plant to plant spacing of 30 cm and 20 cm respectively in two replications in *kharif* season of 2009. Based on the results of 2009, fresh seed lots of 21 varieties, (that exhibited differential state of expression of characteristics), were received from the Nodal Officer, DUS Test, Directorate of Rice Research, Hyderabad in 2010. These varieties with the fresh and old seed lots were grown in the adjacent plots of two rows each of 4 m length in two replications in *kharif* 2010. The remaining varieties were grown as in the previous year. All recommended package of practices were followed in both the years.

Recording of data: A total of 41 plant morphological and 8 seed and quality characteristics were recorded, following the Guidelines for the Conduct of Test for DUS on Rice (Anonymous 2007) (henceforth referred to as DUSTG-Rice) in both the years.

## RESULTS AND DISCUSSION

The description of the 73 example varieties of rice was recorded following the DUSTG-Rice as notified by the PPV & FR Authority. Fifty seven varieties matched the state(s) of 49 characteristics for which these are identified as example variety (Table 2). Nineteen example varieties did not conform to the respective characteristic states (Table 3). The

Table 1 Example varieties validated for DUS characteristics

Example variety	Example variety	Example variety
Aditya	Kushal	Salivahana
ADT 37	Lachit	Samba Mahsuri
Annada	Madya Vijaya	Saraswati
Aruna	Mahamaya	Sasyasree
ASD 20	Makom	Shamyala
Barh-avarodhi	Mangala	Shanti
Basmati 370	Matangini	Sugandha
Basmati 386	NDR 359	Sugandhamati
Bhadra	Nidhi	Suraksha
Bhogali	Pant Dhan 11	Swarna
Chaitanya	Pant Dhan 12	Swarnadhan
CSR 10	Pant Dhan 4	Taroari Basmati
CSR 13	Phalguna	Triguna
CSR 27	PKV HMT	Tulasi
Govind	Poornima	Vandana
Heera	PR 106	Vasumati
Indira Sugandhit Dhan 1	PR 113	Vibhava
IR 24	PTB 56	Vijetha
IR 8	Pusa Basmati 1	Vikas
Jaishree	Rasi	Vikramarya
Jaya	Ravi	Vivek Dhan 62
Kasturi	Remya	VL Dhan 206
Kranti	Rongilee	VI Dhan 221
Krishna Hamsa	Sabita	VL Dhan 81
Krishnaveni		

Table 2 Example varieties validated for the state of the characteristics

Characteristic	State of expression	Example variety
Basal leaf sheath colour	Green	Rasi, Heera
	Light purple	Bhogali
	Purple lines	Mahamaya
Leaf: intensity of green colour	Light	Rasi
	Medium	Heera, Sugandha
	Dark	Swarna
Leaf: anthocyanin colouration	Absent	Sugandha
Leaf: distribution of anthocyanin colouration	Uniform	Shyamala
Leaf sheath: anthocyanin colouration	Absent	Govind
Leaf sheath: intensity of anthocyanin colouration	Weak	Rongilee
Leaf: pubescence of blade surface	Weak	Vibhava
	Medium	IR 24, Aruna
	Strong	Jaya
	Very strong	Jaishree
Leaf : auricles	Absent	Vikramarya
	Present	Jaya, Basmati 370
Leaf: anthocyanin colouration of auricles	Colourless	IR 24

Contd.

Table 2 (Contd.)

Characteristic	State of expression	Example variety
Leaf: collar	Present	Rasi, IR 24
Leaf: anthocyanin colouration of collar	Absent	Rasi, IR 24
Leaf: ligule	Present	Triguna, IR 24
Leaf: shape of ligule	Split	Vijetha, IR 24
Leaf: colour of ligule	White	Rasi
	Purple	Shyamala
Culm: attitude	Erect	Pant Dhan 11, IR 24
	Semi-erect	IR 8, VL dhan 206
Time of heading	Very early	Heera
	Early	Rasi, Ravi
	Medium	Vikas, Triguna
	Late	Basmati 370
	Very late	Sabita
Flag leaf: attitude of blade	Erect	IR 24
	Semi-erect	VL Dhan 81
Spikelet: density of pubescence of lemma	Medium	Rasi, NDR 359
Male sterility	Strong	Vasumati
Lemna: anthocyanin colouration of keel	Absent	IR 24
	Absent/very weak	IR 24, Swarnadhan
	Weak	Bhadra, Remya
	Medium	Aruna
	Strong	Makom
Lemna: anthocyanin colouration of area below apex	Absent	IR 24, VL Dhan 81
	Weak	Remya, Mandya Vijaya
	Medium	Bhadra, Aruna
	Strong	Bhogali, Makom
Lemna: anthocyanin colouration of apex	Absent	Phalguna
	Weak	Mandya Vijaya
	Strong	Shyamala
	Very strong	Rasi
Spikelet: colour of stigma	White	Jaya, Basmati 370
	Purple	Rasi, Mahamaya
Stem: thickness	Medium	Lachit, Govind
	Thick	NDR 359
Stem: anthocyanin colouration of nodes	Absent	Chaitanya, IR 24
Stem: intensity of anthocyanin colouration of nodes	Present	Amulya
	Weak	CSR 24
	Medium	Shyamala, Rongilee
	Strong	Amulya
Stem: anthocyanin colouration of internodes	Absent	IR 24, Krishnaveni
Flag leaf: attitude of blade	Erect	IR 24
	Semi-erect	VL Dhan 81
	Horizontal	VL Dhan 206
Panicle: curvature of main axis	Semi-straight	Barh Avrodhi
	Deflexed	Govind
	Drooping	Poornima, Basmati 386
Spikelet: colour of tip of lemma	Yellowish	Pant Dhan 12
	Brown	Mandya Vijaya
	Purple	Rasi

Contd.

Table 2 (Contd.)

Characteristic	State of expression	Example variety
Lemma and palea colour	Straw	Chaitanya
	Gold and gold furrows on straw background	Vibhava, Pant Dhan 11
	Brown (tawny) Reddish to light purple	Bhogali Shyamala
Panicle: awns	Absent	Jaya, Krishnaveni
	Present	Pusa Basmati 1
Panicle: colour of awns	Yellowish white	Pant Dhan 11
	Yellowish brown	Basmati 370
	Black	Bhogali
Panicle: distribution of awns	Tip only	Pant Dhan 11
	Upper half only	Basmati 370
	Whole length	Pusa Basmati 1
Panicle: presence of secondary branching	Present	Rasi, Basmati 370
Panicle: secondary branching	Weak	Basmati 386
	Strong	Pant Dhan 11, Annada
Panicle: attitude of branches	Semi-erect	Mangala
Panicle: exertion	Partly exerted	Vibhava
	Mostly exerted	Pantdhan 4, Chaitanya
	Well exerted	VL Dhan 221
Time of maturity	Very early	Heera
	Early	Rasi, Ravi
	Medium	Vikas, Triguna
	Late	Basmati 370
	Very late	Sabita
Leaf: senescence	Early	VL Dhan 81
	Medium	IR 8
	late	Basmati 370
Sterile lemma colour	Straw	Tulasi, Pant Dhan 11
	Gold	Vibhava, Shanti
	Red	Ambemohar 157
	Purple	Bhogali
Grain: weight of 1 000 fully developed grains	Very low	PKV-HMT
	Medium	Basmati 370, Basmati 386
	High	PR 113
Grain length	Short	CSR 10
	Medium	Pant Dhan 4
	Long	Basmati 386
Grain width	Narrow	Basmati 370
	Medium	Pant Dhan 4
	Broad	Kranthi
Decorticated grain: length	Medium	Sambha Mahsuri
	Long	Basmati 370
Decorticated grain: width	Narrow	Shanti
	Medium	Heera

Contd.

Table 2 (Concluded)

Characteristic	State of expression	Example variety
Decorticated grain: shape	Short bold	Salivahana
	Medium slender	Sambha Mahsuri
	Long bold	Vikramarya
	Long slender	Krishna Hamsa
Amylose content	Medium	Taroari Basmati
	High	Jaya
Gelatinization temperature	Low	Pusa Basmati 1
	Medium	Taroari Basmati

characteristics consisted both of plant (qualitative in nature) and seed (quantitative in nature). Among the plant morphological traits, majority of the example varieties failed to exhibit the identified state of expression in various leaf characteristics. Higher level of off-type plants was observed for different morphological characteristics in eight varieties, namely, Sasyashree, Mandya Vijaya, Taraori Basmati, VL-Dhan 81, Jaishree, Sugandha, Poornima and Aruna. This could be one of the reasons of mismatch in the example varieties, Aruna, Poornima and Sugandha, in which off-types were high for plant morphological traits.

The characteristic states that were recorded in the second year on the plants grown from the fresh seed lot of 21

Table 3 Comparison of expression of characteristics of example varieties of rice

Characteristic	Example variety	State of expression as per DUSTG-Rice	Observed state of expression
Basal leaf : sheath colour	Annada	Light purple	Green
	IR 24, Aruna	Uniform purple	Green
Leaf : intensity of green colour	IR 24	Dark	Medium
	Vandana	Light	Medium
Leaf : anthocyanin colouration	IR 24, Aruna	Present	Absent
Leaf : distribution of anthocyanin colouration	Vivek Dhan 62, CSR 10	On tips only	Absent
	Aruna, IR 24	On margins only	Absent
	Aruna, IR 24	Present	Absent
Leaf : sheath anthocyanin colouration	Aruna, IR 24	Medium	Absent
Leaf : sheath intensity of colouration	Sugandha,	Absent	Medium
Leaf : pubescence of blade surface	Govind	Very strong	Medium
Leaf : anthocyanin colouration of auricle	Aruna	Light purple	Colourless
Leaf : anthocyanin colouration of collar	Aruna	Present	Absent
Leaf : colour of ligule	Aruna,	Light purple	White
	IR 24	Light purple	Purple
Time of heading	Kushal	Very late	Medium
Spikelet : density of pubescence on lemma	Krishna Hamsa	Weak	Medium
	Vandana	Strong	Weak
Spikelet : colour of stigma	Poornima	Light purple	White
	IR 24	Light purple	White
Stem : intensity of anthocyanin colouration of nodes	Swaraswati	Strong	Weak
Panicle : curvature of main axis	Lachit	Semi straight	Deflexed
	ADT 37	Deflexed	Semi straight
Panicle : distribution of awn	ASD 20	upper half only	Tip only
Panicle : attitude of branches	Pant Dhan 4	Spreading	Semi-erect
Panicle : exsersion	Suraksha	Partly exerted	Mostly exerted
Time : maturity	Kushal	Very late	Medium
Grain : weight of 1 000 fully developed grains	Sugandha	Very low	High
	Mahamaya	High	Very high
	Pant Dhan 4	Very high	High
	Sugandha	Very narrow	Medium
Grain : width	Kushal	Short	Medium
Decorticated grain : length	Sugandhamati	White(155)*	Greyed white(156A)*

\* : RHS colour chart number

varieties which showed variant expression in the first year, indicated that six varieties still expressed differently for the states of expression with regard to 15 out of 33 characteristics recorded (Table 4). These example varieties also showed differential expression in other morphological characteristics (Table 5) indicating a general genetic deterioration in such varieties. Therefore, a strict maintenance of all the reference varieties, in general, and of the example varieties, in particular, is warranted for a valid DUS testing for proper implementation of PPV&FR Act.

Table 4 Example varieties exhibiting deviation in expression of characteristics in 2009 and 2010

Variety	No. of characteristics
Suraksha	7
Kasturi	11
Shanti	5
Vandana	9
Sugandha	6
ASD 20	6

Joshi *et al.* (2007) on characterization of some of the released rice varieties namely, IR 8, Jaya, PR 113, Basmati 370 and Basmati-386, reported validity of most of the example characteristics that are qualitative in nature.

The example varieties are expected to be highly uniform and stable for the expression of the characteristics at a particular state. It is more for the qualitative characteristics which are least influenced by the growing environmental conditions. If the example varieties are found to exhibit variable expression of the characteristics under different growing conditions, it is suggested that different varieties be included for different agro-ecological regions for the purpose of DUS testing.

In the DUSTG-Rice, there are 62 characteristics each with 2–9 states of expression. There are 106 example varieties identified, among which 44 varieties represent a single characteristic/state while one variety, IR 24 represents 22 characteristics/states. The number of example varieties should logically be small to reduce DUS testing cost to a minimum and for easy maintenance of the set of example varieties in a crop.

Reference varieties are of utmost importance in DUS testing for comparing characteristics states of the candidate varieties. Any chance of impurity in the reference set of varieties would lead to an inconclusive or wrong DUS test result. Therefore, maintenance of genetic purity to the highest level in the reference varieties is a basic requirement for the successful conduct of DUS test.

In view of the deviation observed for the expression of some example varieties, it is suggested that a periodical review of all example varieties be undertaken in all the DUS

Table 5 Differential expression in other characteristics other than the characteristic for which it is identified as example variety in two seed lots of example varieties of rice

Characteristics	Example variety
Leaf : intensity of green colour	Suraksha, Shanti, Vandana, ASD 20
Leaf : pubescence of blade surface	Suraksha, Kasturi, Vandana, Sugandha
Culm : attitude	Suraksha, Kasturi, Vandana, Sugandha
Time of heading	Suraksha, Vandana, Sugandha, ASD 20
Flag leaf : attitude of blade	Kasturi, Sugandha
Spikelet : density of pubescence on lemma	Suraksha, Kasturi, Vandana, Sugandha
Stem : thickness	Suraksha, Vandana, Sugandha, ASD 20
Panicle : curvature of main axis	Kasturi, Vandana
Spikelet : colour of tip of lemma	Vandana
Panicle : secondary branching	Kasturi, Vandana,
Panicle : attitude of branches	Kasturi, Vandana, ASD 20
Panicle : exsersion	Kasturi, Vandana, ASD 20
Time : maturity	Suraksha, Kasturi, Vandana, Sugandha, ASD 20, Shanti
Leaf : senescence	Suraksha, Kasturi, Vandana, ASD 20
Grain : weight of 1 000 fully developed grains	Sashyasree, Suraksha, Kasturi, Sugandha, ASD 20

test centres to validate and revise, if necessary, the set of example varieties. This exercise may also be undertaken in other plant species on a regular basis for validating the example varieties and revising the DUS test guidelines, if needed. More than one example variety for a state of expression of a characteristic is desirable in case the variety deteriorates for the given characteristic and/or it does not express in some growing condition and areas. However, a strict adherence to the maintenance breeding procedures to maintain varietal purity would of utmost importance.

#### ACKNOWLEDGEMENT

Assistance of the breeders/breeding institutes and the Nodal Officer, DUS Test, Directorate of Rice Research, Hyderabad for the supply of authentic seed samples of different rice varieties is acknowledged.

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