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RESEARCH ARTICLE

Assessment of Distinctiveness, Uniformity and Stability of Zinc Fortified Rice (*Oryza sativa*) Genotypes based on Morphological Descriptors

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Twenty five rice genotypes including indigenous and improved zinc lines were characterized using morphological descriptors adopted from the DUS guidelines of PPV & FR Authority and subsequently examined for their distinctiveness, uniformity and stability. Among the 49 assessed characters, 10 characters were monomorphic, 22 characters were dimorphic and 17 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. The present study, highlights the need for and demands of zinc rich rice genotypes in Indira Gandhi Krishi Vishwavidyalaya, Raipur to conserve and protect high value traits for future promotion and utilization. On the basis of grouping characteristics unique morphological profiles could be established for all genotypes. Though, the morphological DUS descriptors establish distinctiveness of some varieties but varieties of similar background could not be discriminated hence some other markers/ descriptors could be thought of for complementing the morphological DUS descriptors for establishing distinctiveness.

Key Words: Biofortification, DUS descriptors, Rice, Zinc fortified lines

Introduction

Micronutrient malnutrition has emerged as one of the important global problems that afflicts billions of people particularly in developing countries. Since human body cannot synthesize micronutrients, it must be made available through diet. Rice (*Oryza sativa*), being the staple food for almost two thirds of the population, plays a pivotal role in Indian economy. Moreover, India ranks first in the world in area of rice cultivation with 42.96 million ha and second in production with 158.75 million tons (Faostat, 2016 http://www.fao.org/faostat/en/#data/QC).

Zinc deficiency is a major cause of stunting among children (Brown *et al.*, 2004). About 165 million children with stunted growth run a risk of compromised cognitive development and physical capability (Black *et al.*, 2013, Wessells *et al.*, 2012). Biofortification, the delivery of micronutrients via staple food crops, has been proposed to complement existing efforts for the alleviation of micronutrient deficiency (Bouis *et al.*, 2011).

Varietal improvement has been carried out towards Zn biofortification in rice. Some indigenous lines with high zinc concentration have been identified at Indira Gandhi Agriculture University, Raipur. There are a number of indigenous rice varieties currently under production whose identity and distinctiveness need to be established by various approaches. Morphological descriptors provide unique identification of cultivated varieties but they not only reflect the genetic constitution of the cultivar but also the environmental interaction within which it is expressed. The basic objective of varietal characterization is to test the occurrence of traits that help in identifying a particular variety. 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 25 zinc rich rice genotypes for their protection under the PPV&FR Act.

Materials and Methods

The experimental material consisted of twenty five rice genotypes (table 1). The trials were conducted at Rai Mohini Devi College of Agriculture and Research Station, Ambikapur during the *kharif* seasons of 2015 and 2016 in Randomized Block Design with three replications. Each

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Table 1. Name of genotypes along with zinc concentration in polished grain of 25 rice genotypes

S. No.	Genotypes	Zinc (µg/gm)	S. No	Genotypes	Zinc (µg/gm)
1	R-GM-AS-45	19.20	14	R-RHZ-MI-31	21.30
2	R-GM-AS-40	18.20	15	R-RHZ-R56	20.90
3	R-GM-AS-41	18.85	16	R-GM-AS-43	17.70
4	GP-145-70	17.50	17	R-RHZ-GI-56	17.55
5	R-GM-ATN-47	23.60	18	Kharigilas	23.90
6	GP-145-48	25.30	19	Jeeraphool	19.10
7	GP-145-66	18.50	20	Bisni-1	14.30
8	RKVY-77	23.35	21	IET-24780	15.00
9	R-HZ-SM-14	23.70	22	Chittimuthyalu	28.50
10	R-RHZ-IB-13	25.90	23	Kalanamak	21.70
11	R-RHZ-LI-22	22.25	24	Samba Mahsuri	10.10
12	R-RHZ-HI-11	21.10	25	IR-64	20.30
13	R-RHZ-LI-23	21.90			

replication consisted of three rows of 6 m length with 30 cm X 20 cm spacing. The observations were recorded on 49 of the 62 DUS characters at specified stages of crop growth period when characteristics under study had full expression (DRR, 2002). Of the 49 morphological characteristics studied, 40 were visually assessed and 9 were measure. Similarity matrix was generated using the SimQual programme of NTSYS-pc software version 2.02 (Rohlf, 1998). The Shan similarity coefficients were used for cluster analysis and dendrogram was constructed by Unweighted Pair-Group Method with Arithmetic Average (UPGMA) (Mathew et al., 2000). Field view and agromorphological characterization for some traits presented in Fig 2.

Results and Discussion

The various morphological traits recorded among rice genotypes are presented in Table 2. The basal leaf sheath colour for the rice genotypes varied from green to purple. Genotypes R-RHZ-GI-56, Jeeraphool, Bisni-1, ITE-24780, Chittimuthyalu, Kalanamak, Samba Mahsuri, IR-64 had green basal leaf sheath colour, while R-RHZ-GI-56 and Kharigilas showed purple while all remaining genotypes showed green colour for basal leaf sheath. The anthocyanin colouration of leaf was present in genotypes GP-145-48 and Kharigilas while this was absent in rest of the genotypes. The pubescence of leaf blade surface was found to be weak in GP-145-

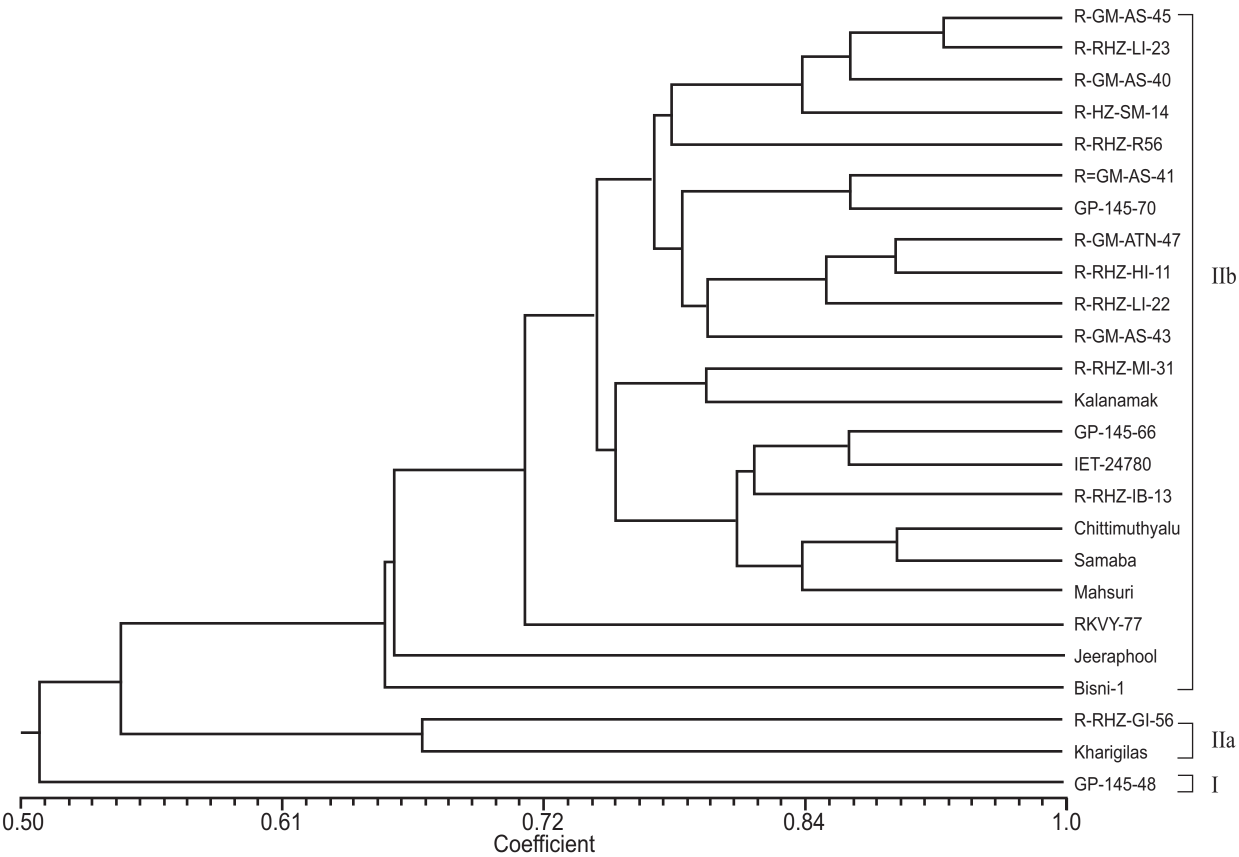

Fig 1. Dendrogram of rice genotypes based on morphological characters

Table 2. Morphological characters of rice genotypes based on DUS descriptor

S. No.	Characters	Genotypes																								
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1	Coleoptiles colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	Basal leaf sheath colour	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	3	3	1	1	1	1	1	1	1
3	Leaf intensity of green colour	3	7	5	5	7	3	5	5	7	5	5	7	5	5	7	7	3	7	5	5	5	7	5	7	5
4	Leaf anthocyanin colouration	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	1	9	1	1	1	1	1	1	1
5	Leaf distribution of anthocyanin colouration	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
6	Leaf sheath anthocyanin colouration	1	1	1	1	1	9	1	1	1	1	1	1	1	1	1	1	9	9	9	1	1	1	1	1	1
7	Leaf sheath intensity of anthocyanin colouration	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	5	5	3	1	1	1	1	1	1
8	Pubescence of blade	5	5	5	5	5	3	5	3	5	5	3	3	5	5	3	3	5	3	3	3	3	3	3	3	3
9	Leaf auricles	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	Leaf auricles colouration	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	3	2	1	1	1	1	1	1	1
11	Leaf collar	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
12	Leaf anthocyanin colouration of collar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	1	1	1	1	1	1	1
13	Leaf ligule	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	Leaf shape of ligule	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
15	Leaf colour of ligule	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	3	2	2	1	1	1	1	1	1
16	Leaf length of blade	5	5	7	5	5	5	5	7	5	5	5	5	5	5	5	5	5	7	7	5	5	5	5	5	7
17	Leaf width	3	5	3	5	5	7	5	5	3	5	3	5	3	3	3	5	3	3	5	5	5	5	3	3	5
18	Culm attitude	3	5	3	5	5	5	3	7	5	5	5	5	5	5	3	5	5	5	3	5	3	3	5	5	5
19	Time of heading (50%)	3	3	5	5	3	3	5	3	3	5	3	3	3	3	5	5	5	5	3	3	5	5	3	5	3
20	Flag leaf attitude of blade (early observation)	3	3	1	1	1	3	3	1	1	3	1	1	3	3	1	1	3	3	3	5	3	3	3	3	3
21	Spikelet density pubescence of lemma	5	3	5	1	3	1	5	5	5	1	3	3	5	3	5	5	5	7	1	1	1	5	3	3	5
22	Male sterility	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
23	Lemma anthocyanin coloration of keel	1	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1
24	Lemma anthocyanin coloration of area below apex	1	1	1	1	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1
25	Lemma anthocyanin coloration of apex	1	1	1	1	1	5	1	1	1	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1
26	Spikelet colour of stigma	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	5	1	1	1	1	1	1	1
27	Stem thickness	3	3	3	3	3	5	3	5	3	3	5	5	5	5	3	5	5	3	3	3	3	5	5	3	3
28	Stem length excluding panicle	1	1	1	5	1	7	9	7	1	1	1	1	3	3	1	3	5	9	9	7	5	1	9	1	1
29	Stem anthocyanin coloration of node	1	1	1	9	9	9	9	9	1	1	1	1	1	1	1	9	9	9	9	9	9	9	9	9	9
30	Stem intensity anthocyanin colouration of nodes	3	3	3	3	5	3	5	5	3	3	3	3	3	3	3	5	5	5	5	3	3	3	5	3	5
31	Stem anthocyanin colouration of internode	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	9	9	1	1	1	1	1	1	1
32	Panicle length of main axis	5	9	5	5	5	5	7	5	7	5	5	5	5	5	5	5	5	5	5	5	7	9	5	5	5
33	Flag leaf attitude of blade (late observation)	5	5	3	3	3	3	3	3	5	3	3	5	5	1	1	5	5	5	5	5	3	3	3	3	3
34	Panicle curvature of main axis	5	3	3	1	3	3	5	1	5	3	3	3	5	1	5	3	3	5	3	5	5	3	3	5	5
35	Panicle per plant	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	5	5	3	3	3	3	3	3	3
36	Spikelet colour of tip lemma	4	4	4	4	3	5	6	3	5	3	4	3	4	3	4	4	3	6	3	3	3	3	6	3	3
37	Lemma and palea colour	6	6	7	7	2	9	7	7	6	6	6	7	6	7	6	6	7	9	7	7	7	7	7	7	7
38	Panicle awn	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
39	Panicle colour of awn (late observation)	4	4	4	4	4	2	9	8	2	2	4	4	4	4	2	2	8	2	2	2	2	2	9	2	2
40	Panicle length of longest awn	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
41	Panicle distribution of awn	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
42	Panicle presence of secondary branches	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
43	Secondary branching	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2
44	Panicle attitude of branches	3	3	1	1	1	3	3	1	1	3	1	1	3	3	1	1	3	3	3	5	3	3	3	3	3
45	Panicle exertion	7	7	7	7	7	3	7	7	7	7	7	7	7	3	3	7	7	7	7	7	7	7	7	7	7
46	Time of maturity (days)	5	5	5	5	3	5	3	5	5	3	3	3	5	5	5	5	5	3	3	5	5	5	5	5	5
47	Leaf senescence	7	7	7	7	7	7	7	3	7	7	7	7	7	7	7	7	7	7	7	3	7	7	7	7	7
48	Sterile lemma colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
49	1000 grain weight	5	5	5	5	5	3	5	5	5	5	6	5	5	7	7	5	7	7	1	1	5	1	3	1	7

A- R-GM-AS-45, B- R-GM-AS-40, C- R-GM-AS-41, D - GP-145-70, E- R-GM-ATN-47, F- GP-145-48, G- GP-145-66, H- RKVY-77, I- R-HZ-SM-14, J- R-RHZ-IB-13, K- R-RHZ-LI-22, L- R-RHZ-HI-11, M- R-RHZ-LI-23, N- R-RHZ-MI-31, O- R-RHZ-R56, P- R-GM-AS-43, Q- R-RHZ-GI-56, R- Kharigilas, S- Jeeraphool, T- Bisni-1, U- IET-24780, V- Chittimuthyalu, W- Kalanamak, X- Samba Mahsuri, Y- IR-64

Fig.2. Field view along with Morphological observations for different characters

48, RKVY-77, R-RHZ-LI-22, RHZ-HI-11, R-RHZ-R-56, R-GM-AS-43, Jeeraphool, Bisni-1, ITE-24780, Chittimuthyalu, Kalanamak, Samba Mahsuri, IR-64, medium in R-GM-AS-45, R-GM-AS-40, RGM-AS-4, GP-145-70, R-GM-ATN-47, GP-145-66, R-HZ-SM-14, RHZ-IB-13, R-RH Z-II 23, R-RHZ-MI-31, R-RHZ-GI-56, Kharigilas. GP-145-48 and R-RHZ-GI-56 had purple auricles colour Kharigilas light purple and rest of genotypes were colourless. Anthocyanin colouration of collar was found present in genotypes R-RHZ-GI-56 and Kharigilas, while absent in rest of the genotypes. Colour of ligule was light purple in Kharigilas and Jeeraphool while purple in R-RHZ-GI-56 and white in rest of the genotypes. The leaf length of blade was noted medium for R-GM-AS-45, R-GM-AS-40, GP-145-70, R-GM-ATN-47, GP-145-48, GP-145-66, R-HZ-SM-14, RHZ-IB-13, R-RHZ-LI-22, R-RHZ-HI-11, R-RH Z-II 23, R-RHZ-MI-31, R-RHZ-R-56, R-GM-AS-43, R-RHZ-GI-56, Bisni-1, ITE-24780, Chittimuthyalu, Kalanamak and Samba Mahsuri, long for RGM-AS-41, RKVY-77, Kharigilas and Jeeraphool. The leaf blade was medium R-GM-AS-45, RGM-AS-41, R-HZ-SM-14, R-RHZ-LI-22, R-RH Z-II 23, R-RHZ-MI-31, R-RHZ-R-56, R-RHZ-GI-56, Kharigilas, Kalanamak, Samba Mahsuri and broad in rest of the genotypes. The early observation for Culm attitude revealed semi erectness in R-GM-AS-45, RGM-AS-41, GP-145-66, R-RHZ-R-56, Jeeraphool, ITE-24780, Chittimuthyalu, Samba Mahsuri and IR-64, open attitude in genotypes R-GM-AS-40, GP-145-70, R-GM-ATN-47, GP-145-66, R-HZ-SM-14, RHZ-IB-13, R-RHZ-LI-22, R-RHZ-HI-11, R-RH Z-II 23, R-RHZ-MI-31, R-GM-AS-43, R-RHZ-GI-56, Karigilas, Bisni-1 and Kalanamak, and spreading type attitude present in genotypes RKVY-77. The stigma colour was found to be light green in R-RHZ-II-23, and purple in Kharigilas while white in rest of the genotypes. The lemma anthocyanin colouration of keel found to be weak in genotype GP-145-48 and Bisni-1, while absent/ very weak in all genotypes. The stem anthocyanin colouration of internode present in R-RHZ-GI-56 and Kharigilas. The panicle length in main axis measured long panicle length in three genotypes viz., GP-145-66, R-HZ-SM-14 and Bisni-1, and very long panicle present in two genotypes R-GM-AS-40 and ITE-24780. The spikelet colour of tip lemma appeared brown in twelve genotypes R-GM-ATN-47, RKVY-77, RHZ-IB-13, R-RHZ-HI-11, R-RHZ-MI-31, R-RHZ-GI-56, Jeeraphool, Bisni-1, ITE-24780, Chittimuthyalu, Samba Mahsuri, and IR-64, red for R-GM-AS-45, R-GM-AS-40, RGM-AS-41, GP-145-70, R-RHZ-LI-22, R-RH Z-II 23, R-RHZ-R-56 and R-GM-AS-43, purple colour GP-145-48 and R-HZ-SM -14, and black in genotypes GP-145-66, Kharigilas and Kalanamak. The panicle were partially exerted in four genotypes GP-145-48, R-RHZ-MI-31 and R-RHZ-R-56, and well exerted in rest of the genotypes. Based on 1000 grain weight Jeeraphool, ITE-24780, Chittimuthyalu and Samba Mahsuri had very low test weight while, GP-145-48, R-RHZ-GI-56, Chittimuthyalu, and Kalanamak had low test weight, R-GM-AS-45, R-GM-AS-40, RGM-AS-41, GP-145-70, R-GM-ATN-47, GP-145-66, RKVY-77, R-HZ-SM-14, RHZ-IB-13, R-RHZ-LI-22, R-RHZ-HI-11, R-RH Z-II 23 and R-RHZ-MI-31 had medium test weight. Similar attempts for establishment of distinctiveness have also been made in aromatic rice genotypes (Joshi *et al.*, 2011), released rice varieties (Tiwari *et al.*, 2013) and in clustered rice germplasm (Sahu *et al.*, 2014).

UPGMA Cluster Analysis

The dendrogram generated using UPGMA cluster analysis grouped studied rice genotypes into two major clusters (cluster I and II) Pairwise. Jaccard's similarity coefficient between genotypes ranged from 0.51 to 0.92. The analysis revealed wide genetic variability among the genotypes. Cluster I consisted of only one genotype GP-145-48 (25.30 µg/gm zinc) which indicates their diversity in the rice gene pool. Cluster II was further divided into 2 sub clusters and only 54% similarity were observed. Sub cluster-1, included two genotypes R-RHZ-GI-56 (20.90 µg/gm zinc) and Kharigilas (23.90 µg/gm zinc) with a genetic similarity of more than 67 %. Sub cluster-2 consisted of rest of the genotypes with a genetic similarity ranging from 65 to 92 %. A close association among the genotypes in particular clusters is largely due to common pedigree involved in breeding of the genotypes. The morphological dendrogram generated from similarity or genetic distance matrices has provided an overall pattern of variation as well as the degree of relatedness among genotypes (Tiwari *et al.*, 2013). Early maturing varieties viz, Rasi and Tulasi with a common pedigree in their breeding grouped in a sub cluster with a similarity index of more than 67 % (Ravidra Babu *et al.*, 2015). Similarly, the study on genetic diversity of Venezuelan cultivars by Herrera *et al.* (2008) and diversity of Brazilian rice cultivars by Raimondi *et al.* (2014), demonstrated the narrow genetic base of the cultivars developed in their respective countries which can lead to genetic vulnerability to any emerging stress conditions.

Zinc deficiency is probably the most widespread micronutrient deficiency in cereals. Breeding for enhancing bio-available Zinc in edible portion through increasing the concentration of metal binding protein has been needed. But for initiating any breeding programme, morphological characterization should be the first step along with screening variability for target trait before more profound biochemical or molecular studies are carried out. As a prerequisite for efficient utilization of the germplasm, it must be properly evaluated, characterized and documented so that it could be easily retrieved and used in breeding programme (Elangovan *et al.*, 2013). The present study revealed sufficient genetic variability for most of the trait observed. Zinc rich rice genotypes identified with distinct variation in this study may be used in breeding programme for introgression of zinc content gene or QTLs in the improved varieties. Notably, there were appropriate differences in zinc content suggesting the existence of genetic potential to increase the concentration of zinc in rice grain. Identified cultivar with distinct morphological variation Chittimuthyalu (28.50 µg/gm zinc), R-RHZ-IB-13 (25.90 µg/gm zinc), GP-145-48 (25.30 µg/gm zinc) and R-RHZ-SM-14 (23.70 µg/gm zinc) may be used as zinc donor in future. These unique accessions bearing special trait of interest can be further investigated to understand its inheritance pattern. The morphological dendrogram generated for similarity or genetic distance matrices has provided an overall pattern of variation as well as the degree of relatedness among rice accessions also found by Tiwari *et al.* (2013). In the era of IPR issues, characterization of germplasm and varieties assumes greater importance. The application of morphological markers is the simplest of the methods that could be repeatedly done and effective used for IPR issues (Lyngdoh *et al.*, 2007). However, morphological descriptors alone may not be sufficient for DUS criteria. Hence, some other markers/ descriptors based on DNA (Simple Sequence Repeat) and protein (SDS-PAGE) markers could be considered for complementing the morphological DUS descriptors.

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