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Testing authenticity of certified paddy seed with user friendly morpho-molecular tool- kit

Parthopratim Buragohain and Sharmila Dutta Deka*

National Seed Project, Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat 785013
***E- Mail:**sharmila9368@gmail.com

Abstract
In the present investigation, twenty nine rice cultivars were characterized using grain morphological characters and molecular markers to develop a morpho- molecular tool kit for application in authenticity testing of the certified seed lot and in maintenance breeding. The study suggested that identification of rice varieties is possible with grain morphology and molecular markers. Using grain morphological characters, seven varieties were uniquely identified *viz., Prafulla, Numali, Dikhow, Dhanashree, Basundhara, Jalkuwari* and *Bhugali*. Molecular fingerprints were generated based on SSR polymorphism. Using the present set of SSR markers, eight rice varieties *viz*., *Gitesh, Jalashree, Ranjit, Jaymati, Bhugali, Ranjit sub-1, Numali,* and *Piyali*, were distinguished with RM 12, RM 25, RM 190, RM 307 and RM 287on the basis of the unique banding pattern. In the phylogenetic relationship based on grain morphology, *Dikhow* was identified as an outlier, which is unique for the grain length, whereas based on SSR data, variety *Praffulla* was identified as an outlier with unique medium grain width.

Key words: Rice, SSR marker, Diagnostic grain characters, Genetic purity

INTRODUCTION

Identification of genetic marker is important in genetic purity testing of the varieties in seed chain to track impurities in seed lot to restrict its multiplication in future generations. It is a challenging task for seed technologists the identification of rice varieties, as they are having vast similarities of grain characters. World Trade Organization (WTO) requires its member countries to accord protection of plant varieties under Trade Related Intellectual Property Right (TRIPS) agreement (1995). Another agreement, the Convention of Biological Diversity (CBD, 1993), provides sovereign rights to nations over their genetic resources. Countries can regulate access to their genetic resources and demand equitable sharing of benefits arising out of commercial exploitation of genetic resources. Enforcement of both these international agreements is possible only if the identity and ownership of the plant genotypes are established. Considering these, it is necessary for proper characterization of rice varieties based on an effective diagnostic marker system. Both quantitative and qualitative traits have been employed to characterize plant varieties and to establish uniqueness as the new varieties of a crop effectively (Liu, 2012; Singh *et al*., 2013; Samal *et al.,* 2018). Again, the description of varieties, along with the assessment of varietal identity and purity are essential for seed production and certification procedures. Usually, results of the grow out test (GOT) needs a full growing season, hence dispatching of the seed lot delays by one season. Thus, the identification of morphological marker that enables in testing of the genetic purity of the varieties in a short time is important. This will be useful in the seed chain to track impurities in seed lots to restrict its multiplication in future generations. Morphological markers are convenient and economic for screening large germplasm collection, however, have limited numbers (Singh *et al.,* 2018). The use of DNA markers to obtain a genotype specific profile has distinct advantages over morphological and biochemical markers (Dong *et al.,* 2010; Samal *et al.,* 2018). Thus, there is a need to identify, evaluate and characterize the available rice genotypes at both morphological and molecular levels to authenticate and diversify the genetic base of improved rice varieties (Pourabed *et al*., 2015; Shen *et al.,* 2013; Yan *et al.,* 2017). In this investigation, a set of a genetic markers is applied to recognize the characteristics of the phenotype and genotype of rice varieties. The present study was carried out with the objective to identify diagnostic grain characters along with molecular markers which can be employed for genetic purity testing of commercial rice varieties in the seed chain without wasting a growing season. Various measurable characters *viz.* grain size, grain length, grain width, decorticated grain length, decorticated grain width, grain colour were recorded. A set of SSR markers were employed for molecular characterization. This will help in determining varietal identity and purity and provide assurance to the farmers about the seed quality of the varieties.

MATERIALS AND METHODS

The experimental materials for the present investigation comprised of the nucleus seeds of 29 recommended varieties of rice listed in **Table 1**.

A total of 50 g of freshly harvested seeds from the seed lot were taken for each cultivar to determine seed characters in two replications. Seed characters were taken on 10 sampled seeds from each replication. Observations were recorded on eight grain morphological characters following DUS guidelines (2007) *viz-* thousand grain weight, grain length, grain width, hull /grain color, decorticated grain length, decorticated grain width, decorticated grain shape, and grain L/B ratio. Classifications of different categories within the characters are done as designated in the guideline.

The genomic DNA was extracted from young seedlings following the CTAB method developed by Murray and Thompson (1980) with little modification. PCR was performed in 20 µl reaction volumes containing 25 ng of

Table 1. List of rice varieties evaluated in the study

S. No.	Variety	Parentage
V1	Prafulla	Akisali/ Kushal
V2	Numali	APMS6B/ Pillee
V3	Shraboni	Selection from IR 50
V4	Disang	Lachit / Kalinga 3
V5	Diphalu	KMJ 1-17-2/ IET 10016
V6	Bahadur	Pankaj / Mahsuri
V7	Luit	Heera / Ananda
V8	Chilarai	IR 24 / CR 44-118-1
V9	Kapilee	Heera / Ananda
V10	Jyotiprasad	K343 – 29 -1-1 / Suweon 334
V11	Dikhow	Heera / Ananda
V12	Kanaklata	Jaya / Mahsuri
V13	Gitesh	Akisali / Kushal
V14	Piyali	Pankaj / Mahsuri
V15	Lachit	CRM 13-3241 / Kalinga II
V16	Basundhara	IET 9711 / IET 11162
V17	Kalang	Chilarai / Kalinga III
V18	Bahadur sub – 1	Pankaj / Mahsuri
V19	Moniram	Pankaj / Mahsuri
V20	Dhanshree	KMJ 1-17-2/ IET 10016
V21	Satyaranjan	IET 9711 / IET 11161
V22	Jalashree	Pankaj / FR 13A
V23	Kushal	Pankaj / Mahsuri
V24	Jalkuwari	Pankaj / FR 13A
V25	Mulagabharu	Jaya / Mahsuri
V26	Ranjit	Pankaj / Mahsuri
V27	Bhugali	Ghewbora / KMJ 1-52-2
V28	Jaymati	Jaya / Mahsuri
V29	Ranjit sub - 1	Pankaj / Mahsuri

DNA, 50 p mol each forward and reverse primer for SSR, 25 µM of dNTPs (Fermentas, Bangalore Genei) and 0.6 U of Taq Polymerase (Bangalore Genei). PCR program was followed as, initial denaturation at 94°C for 3 min followed by 35 cycles, each consisting of denaturation at 94°C for 1 min, annealing at an appropriate temperature for 1 min and elongation at 72°C for 2 min for SSR primers. A final extension step included 72°C incubation for 7 min followed by hold at 4°C. Perkin Elmer (model 9600) thermo-cycler was used to carry out the reaction. The amplified DNA fragments were resolved on ethidium bromide stained agarose gel (2.5%) in 1X TAE buffer at 50V. The gels were visualized on trans-UV and photographed in Bio-red Gel Doc XR 2.0

RESULTS AND DISCUSSION

Characterization of varieties is helpful in the maintenance of genetic purity in the seed supply chain and to deliver quality and genetically pure seeds to the farmers. Different grain parameters were estimated and the mean performance of the 29 rice varieties are given in **Table 2**. A total of seven varieties was identified uniquely on the basis of their grain traits. The variety *Dikhow* was identified uniquely on the basis of grain length, which falls in a very short category which is 3.62 mm. A total of 28 per cent of genotypes (8 genotypes) were medium grain length and the rest 69 per cent of genotypes (20 genotypes) were short grain length. The variety *Prafulla* exhibited medium grain width which is unique among the varieties. Based on decorticated grain length and width the varieties were grouped into two classes. A total of 79 per cent (23 genotypes) exhibited medium decorticated grain length and the rest 21 per cent (6 genotypes) were long decorticated grain length type. Whereas 48 per cent of the varieties were with narrow decorticated grain width and 52 per cent of the varieties exhibited medium decorticated grain width. Similar findings were reported by (Manjunatha *et al.,* 2018) describing different phenotypic classes in an expression of the trait. Five different types of shapes of the rice grains were observed based on kernel length and length/ breadth ratio. Dhanshree was the only variety with a short slender grain shape. A total of 5 varieties were basmati type, 3 varieties were of the medium slender type, 4 varieties were long bold type and the other 16 varieties were with long and slender grain type. Tiwari *et al.* (2020) reported wide variation based on grain related characters in different cultivated rice varieties. Mishra *et al.* (2015) also reported similar results with respect to decorticated grain shape. Sakthi Avinash *et al.* (2019) revealed variability and distinctness in various morphological characters in a series of mutant lines of rice cultivars and are application in distinguishing genotypes.

Phylogenetic relationship based on grain morphology was evaluated and the highest Euclidean distance (6.282) was observed between Numali and Prafulla and the least was observed between *Kapilee* and *Luit* (0.281) (**Fig. 1**). *Dikhow* was the only variety in cluster IV, an outlier that was unique for the grain length (3.62mm). Cluster I consisted of a maximum number of 23 varieties with 3 subgroups and clusters II and III consisted of 3 and 2 varieties, respectively indicating close relatedness of the varieties for most of the grain characteristics. Grouping of

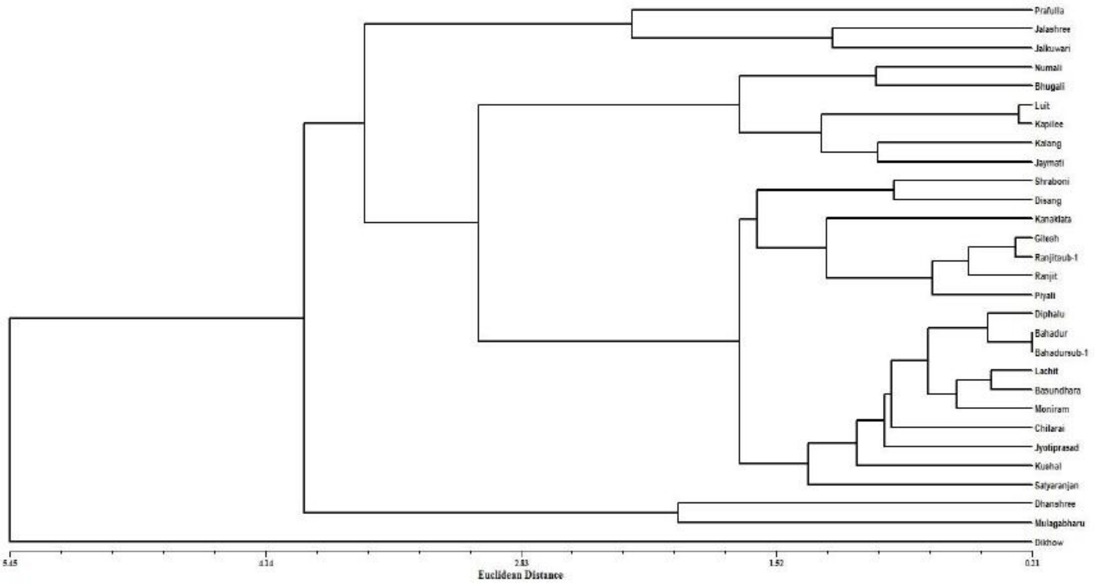

Fig. 1. Dendrogram of rice varieties based on grain morphology

Table 2. Mean performance of rice varieties based on grain traits

Varieties	Grain length (mm)	Grain width (mm)	Thousand grain weight (g)	Decorticated grain length (mm)	Decorticated grain width (mm)	Grain L/B ratio
Prafulla	7.07	3.03	22.65	5.57	2.03	2.74
Numali	9.00	1.60	25.90	7.20	1.63	4.41
Shraboni	8.72	2.10	20.74	6.53	1.90	3.64
Disang	8.10	2.07	18.68	6.50	2.00	3.25
Diphalu	7.72	2.20	21.83	6.13	1.97	3.11
Bahadur	8.00	2.17	22.53	6.10	2.10	2.90
Luit	9.00	2.13	26.38	7.13	2.07	3.44
Chilarai	7.33	2.20	25.17	6.17	2.00	3.08
Kapilee	9.10	2.20	26.13	7.17	1.90	3.77
Jyotiprasad	8.27	2.30	25.67	6.13	2.00	3.06
Dikhow	3.62	2.23	27.37	7.23	1.90	3.80
Kanaklata	7.13	2.20	19.30	5.70	1.90	3.00
Gitesh	7.40	2.20	19.27	6.10	1.90	3.21
Piyali	8.19	2.17	19.17	6.13	1.80	3.40
Lachit	7.63	2.27	23.77	6.10	1.77	3.44
Basundhara	7.90	2.37	24.23	6.13	2.13	2.87
Kalang	8.80	2.20	23.30	7.10	1.53	4.64
Bahadur sub1	7.97	2.13	23.07	6.10	1.80	3.38
Moniram	8.17	2.30	23.67	6.27	2.10	2.98
Dhanshree	7.13	1.70	17.40	5.13	1.23	4.17
Satyaranjan	8.97	2.17	21.83	6.13	2.00	3.06
Jalashree	7.11	3.10	26.40	6.10	2.00	3.05
Kushal	7.60	2.53	22.43	6.10	2.00	3.05
Jalkuwari	8.17	3.13	28.53	6.17	2.13	2.89
Mulagabharu	8.20	2.30	17.57	5.20	1.87	2.78
Ranjit	7.67	2.13	19.21	6.13	2.00	3.06
Bhugali	9.13	1.77	23.30	7.10	1.80	3.94
Jaymati	8.65	2.20	23.13	6.73	2.00	3.36
Ranjit sub-1	7.40	2.30	19.50	6.10	1.70	3.58
Mean	7.90	2.26	22.69	6.29	1.90	3.34
SEm±	0.09	0.087	0.22	0.04	-7.24	0.078
SEd±	0.13	0.12	0.31	0.05	-10.24	0.16
CD 5%	0.26	0.24	0.63	0.11	-27.30	0.43

varieties with morphological characters are reported to be useful in studying phylogenetic relationships in many studies (Tiwari *et al*., 2020)

A major challenge of the morphological marker is an effect of environment on the expression of characters hence may be biased in specific cases (Deka, 2017). Fingerprinting with molecular markers allows precise, objective and rapid variety identification hence a useful tool in the authentication of plant varieties. For molecular characterization of 29 rice varieties, 12 simple sequence repeats markers were employed. All the SSR markers were found polymorphic for the tested varieties. Out of the 12 SSR markers, RM 1 had the highest PIC value of 0.857 and the PIC value was found to be lowest in the marker RM 21 (0.131), indicating RM 1 was found to be more informative in comparison with all the other SSR markers under study **(Table 3).** The SSR polymorphism revealed that there is sufficient genetic diversity among the tested varieties. The potential of SSR markers systems to distinguish the genotypes has been reported in various studies (Deka *et al.,* 2016; Debbarma *et al.*, 2018).

Using the present set of SSR markers, eight rice varieties *viz. Gitesh, Jalashree, Ranjit, Jaymati, Bhugali, Ranjit sub-1, Numali,* and *Piyali* were uniquely identified on the basis of SSR banding pattern for markers (RM 12, RM 25, RM 190, RM 287, RM 307) (**Table 4**) and molecular fingerprints were generated based on SSR polymorphism .Hence, these markers could be successfully employed for the unique identification of rice varieties. A similar result was reported by Chen *et al.* (2017) where 30 polymorphic SSR markers were differentiated 53 rice genotypes. The genetic similarities of 29 rice varieties were calculated with the help of software NTSYs PC and with Jaccard's similarity coefficient to group the rice varieties. Based on molecular data, three major clusters were depicted in the dendrogram **(Fig.2).** Cluster I of both the dendrogram based on morphological and molecular data, nine varieties were found to be common *viz., Kushal, Moniram, Basundhara, Bahadur sub 1, Ranjit, Ranjit sub-1, Jaymoti, Kalong* and *Bhogali* (**Fig.1 & 2**).

The information generated in the present study on grain morphological characters and SSR markers for rice varieties will serve as a diagnostic tool in early and easy

Table 3. SSR markers used in the study

SSR marker	Allele size (bp)	Number of alleles	PIC
RM 1	500-1100	11	0.857
RM 11	150-800	4	0.402
RM 12	150-450	8	0.805
RM 21	200-400	3	0.131
RM 25	150-1200	5	0.719
RM 190	150-1000	5	0.519
RM 210	200-260	3	0.462
RM 215	150-700	10	0.737
RM 287	120-510	10	0.821
RM 307	150-300	4	0.505
RM 454	250-800	7	0.784
RM 474	200-310	8	0.816

Table 4. Diagnostic morphological and SSR markers identified among rice varieties

S. No.	Varieties	Traits	SSR
1	Prafulla	Medium grain width, Medium slender decorticated grain shape	-
2	Numali	Shortest grain width	Unique allele of 400 bp for RM 287
3	Dikhow	Very short grain length, Highest decorticated grain length	-
4	Gitesh	-	Unique allele of 400 bp for RM 12
5	Piyali	-	Unique allele of 1000 bp for RM 287
6	Basundhara	Highest decorticated grain width	-
7	Ranjit	-	Unique allele of 500 bp for RM 190
8	Ranjit sub-1	-	Unique allele of 1100 pb for RM 307
9	Dhanshree	Shortest decorticated grain length and width	-
10	Jalashree	-	Unique allele of 1200 bp for RM 25
11	Jalkuwari	Highest grain width	-
12	Bhugali	Highest grain length	Unique allele of 400 bp for RM 190
13	Jaymati	-	Unique allele of 900 bp for RM 190

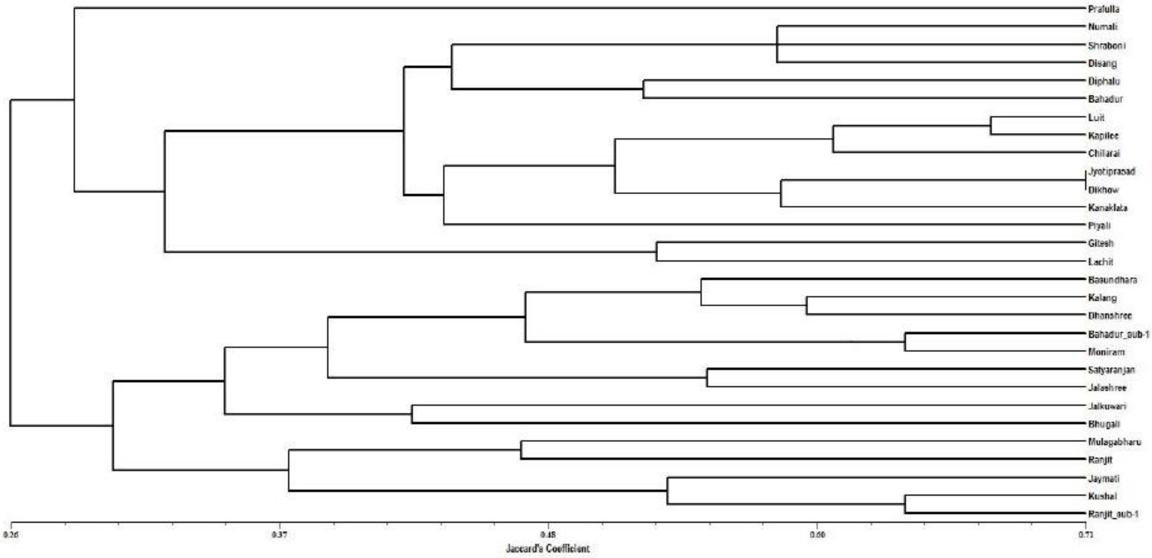

Fig. 2. Dendrogram of rice varieties based on SSR banding pattern

identification of varieties and maintenance of seed quality in the production chain and also in pre breeding and maintenance breeding activities, because of its unique nature and precise results. Such information on the crops in the commercial seed chain will serve as a valuable tool in the prevention of bio-piracy in the modern competitive seed market.

REFERENCES

Chen, C., Wenchuang He, Tondi Yacouba Nassirou, Athanase Nsabiyumva, A., Xilong Dong, A., Yawo Mawunyo Nevame Adedze, Deming Jina. 2017. Molecular characterization and genetic diversity of different genotypes of *Oryza sativa* and *Oryza glaberrima, Electronic Journal of Biotechnology.,* **30:** 48-57. [Cross Ref]

CBD. 1993. https://www.cbd.int/convention/

Debbarma Mampi, Dutta Deka, Sharmila and Sarma Debojit. 2018. Fingerprinting rice hybrids using restorer gene linked SSR markers. *Res. J. Biotech.,* **13**(4):17-23.

Deka, S. D. 2017. Taxonomic diversity of cultivated Capsicum genotypes of India. *Electronic Journal of Plant Breeding*, **8**(2): 660-667. [Cross Ref]

Dong, Y.B., Pei, X.W., Yuan, Q.H., Wu, H.J., Wang, X.J., Jia, S.R., Peng, Y.F. 2010. Ecological, morphological and genetic diversity in Oryza rufipogon Griff. (Poaceae) from Hainan Island, China. *Genet Resour Crop Evol.,* **57**:915–926. [Cross Ref]

Deka, D., Sharmila, Dadlani Malavika, Sharma Ramendranath and Talukdar Akshay 2016. DNA based Genetic Diversity and Relationship Analysis in Indian Domesticated *Capsicum spp. Res. J. Biotech.,* **11** (3) : 25-33.

Guide lines for the Conduct of Test for Distinctiveness, Uniformity and Stability on Rice (*Oryza sativaL.*). 2007. Plant Variety Journal of India. Vol. 1(1).

ManjunathaG. A. C., Elsy, R., Rajendran, P., Jiji Joseph, Rose Mary Francies and Krishnan, S. 2018. DUS Characterization of rice (*Oryza sativa* L.) landraces of wayanad, kerala. *Electronic Journal of Plant Breeding*, **9**(2): 617-630. [Cross Ref]

Mishra, P.K., Sinha, A.K. and Mallick, G.K. 2015. Diversity of grain morphology on traditional rice varieties of laterite region of West Bengal. *World J. Agril. Sci.,* **11**(1): 48-54.

Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucl Acids Res.,* **8**:4321–4325. [Cross Ref]

Pourabed Ehsan, Mohammad Reza Jazayeri Noushabadi, Seyed Hossein Jamali, Naser Moheb Alipour, Abbas Zareyan and Leila Sadeghi. 2015. Identification and DUS Testing of Rice Varieties through Microsatellite Markers. *International Journal of Plant Genomics Volume*, Article ID 965073, 1-7. [Cross Ref]

Sakthi Avinash N. P., Manonmani, K., Muthuvijayaragavan, R., Rajeswari, S., Manonmani, S. 2019. Morphological Characterization of mutant lines of Nagina 22 in rice (*Oryza sativa* L.). *Electronic Journal of Plant Breeding*, **10**(2): 559-565. [Cross Ref]

Samal, R., Roy, P.S., Sahoo, A., Kar, M.K., Patra, B.C., Marndi, B.C., Gundimeda, JNR. 2018. Morphological and molecular dissection of wild rices from eastern India suggests distinct speciation between O. rufipogon and O. nivara populations. *Sci Rep.*, **8**:2773. [Cross Ref]

Shen, X.H., Yan, S., Huang, R.L., Zhu, S., Xiong, H.L., Shen, L.J. 2013. Development of novel cytoplasmic male sterile source from Dongxiang wild rice (*Oryza rufipogon*). *Rice Sci.,* **20** : 379–382. [Cross Ref]

Singh, A., Singh, B., Panda, K., Rai, V.P., Singh, A.K., Singh, S.P., Chouhan, S.K., Rai, V., Singh, P.K., Singh, N.K. 2013. Wild rices of eastern Indo-Gangetic plains of India constitute two sub-populations harbouring rich genetic diversity. *Plant Omics J.,* **6**:121–127.

Singh, B., Singh, N., Mishra, S., Tripathi, K, Singh, B.P., Rai, V., Singh, A.K., Singh, N.K. 2018. Morphological and molecular data reveal three distinct populations of Indian wild rice *Oryza rufipogon* Griff. Species complex. *Front Plant Sci* **9**:1–18. [Cross Ref]

Tiwari Shailesh Mahesh, C. Yadav. Nilamani Dikshit., Vijay K. Yadav. Dipti R. Pani. and Latha, M. 2020. Morphological characterization and genetic identity of crop wild relatives of rice (*Oryza sativa* L.) collected from different ecological niches of India. *Genet Resour Crop Evol.,* **67**:2037–2055. [Cross Ref]

TRIPS.1995. https://www.who.int/medicines/areas/policy/wto_trips/en/

Yan, S., Zhu, S., Mao, L.H., Huang, R.L., Xiong, H.L., Shen, L.J., Shen, X.H. 2017. Molecular identification of the cytoplasmic male sterile source from Dongxiang wild rice (*Oryza rufipogon* Griff.). *J Integr Agric.,* **16**:1669–1675. [Cross Ref]

Liu Y., Xiaohong Yang, Jianhua Zhang, Junjiao Guan, Jiangmin Wang, and Hui Zhang. 2012. Distinctness Determination of DUS Test on Some Quantitative Characteristics of Rice, *Information Technology and Agricultural Engineering Springer-Verlag Berlin* Heidelberg pp 943-951. [Cross Ref]