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Effect of storage period on special DUS characters in extant varieties of rice (*Oryza sativa*) for establishing distinctiveness

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ABSTRACT

Under the PPV and FR Act 2001, a number of rice varieties have been registered based on morphological and biochemical DUS descriptors. In the present study, 61 extant varieties of rice (*Oryza sativa* L.) were characterized based on 55 morphological descriptors at different stages of plant growth. However, for the assessment of biochemical characters, namely; phenol reaction of lemma, amylose content and gelatinization temperature, the assessment is based on ripened seed onwards, and even after the seed is stored. Hence, there is a need to determine optimum period after seed ripening for recording biochemical characters to avoid confusion during grouping of varieties for establishing distinctiveness. For this, all the 61 varieties were examined to see the effect of storage period on biochemical characters. The observations were recorded beginning from freshly harvested seed (0 months) vis-à-vis stored seed up to one year (12 months). The data were recorded after every three months up to a period of one year. Results revealed that significant changes begin to occur during six months of storage and continue for remaining period. Phenol reaction of lemma colour in 26 varieties showed significant changes in colour intensity after six months of storage. Gelatinization temperature (GT) and amylose content are important cooking quality traits for rice. Among 61 varieties, 26 showed increase in Gelatinization temperature after six months of storage as ASV decreased from 6.7 to 6.0, while the remaining varieties showed constant ASV. Further, the average percentage of amylose content significantly increased after six months of storage period in all varieties. Hence, storage period significantly influences the character expression of biochemical traits. Therefore, it is recommended that the varieties should be evaluated for the biochemical assessment within six months of harvesting.

Key words: Amylose content of endosperm, Biochemical assessment, Gelatinization temperature, *Oryza sativa*, Phenol reaction of lemma, Rice, Storage period

Cultivated rice (*Oryza sativa* L.) belongs to family Poaceae and at present sustains two thirds of the world's population. Rice is a rich source of carbohydrates (71%) and proteins (7.3%) and provides vitamins, minerals and fiber. It provides 21% of the human per capita energy, and 15% of protein globally (FAO). UPOV models of plant variety protection were not suitable for Indian requirements, thus the Government of India enacted *sui generis* system on the Protection of Plant Varieties and Farmers Act (PPV and FRA) in 2001 for providing protection to plant varieties based on distinctiveness, uniformity and stability (DUS) test along with novelty (Patra 2000). The act envisages that the new and notified/extant plant varieties will be registered and protected on the basis of their morphological and biochemical characters as listed in the DUS guideline. Thus, it has become necessary for all the organizations to document and characterize all the notified/extant varieties that are in active commerce in their territories.

Under the PPV and FR Act 2001, a number of rice varieties have been registered based on morpho-physiological DUS descriptors. The plant morphological characters are recorded as per the stage of observation listed in DUS guidelines. However, for the assessment of biochemical characters, viz. amylose content, gelatinization temperature and aroma, the assessment is based on ripened seed onwards, and even after the seed is stored, which leads to biochemical changes. Hence, there is an urgent need to determine optimum period after ripening of grain for recording these observations. Biochemical parameters, viz. phenol reaction of lemma, gelatinization temperature and amylose content are the critical parameters for assessment of distinctiveness of genotypes for varietal protection, especially in case of essentially derived varieties, where there is no difference in morphological traits. Hence the present study was aimed to determine the optimum stage for assessment of

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biochemical characteristics and to study the influence of storage period on these characters.

MATERIALS AND METHODS

Breeders' seed of sixty-one extant varieties of rice (Table 1) belonging to non-basmati and basmati group was procured from the Breeder Seed Production unit of IARI New Delhi, and raised at the Seed Technology Farm in plot size of five rows with each row of four-meter length. Row to row and plant-to-plant spacing was maintained at 30 × 20 cm during *kharif* 2013 and 2014. The material was replicated thrice and all the recommended agronomic practices were followed to raise a good crop. Freshly harvested seed of all the sixty-one varieties was cleaned and packed in polythene bags of 700 gauges (moisture vapor proof material). Further the seed was stored under low temperature-low humidity (15°C-17°C and 60-70% RH) conditions for storage study. All these biochemical characters were observed up to one year of storage at three months interval, i.e. freshly harvested, three months of storage followed by observation at six, nine and twelve months of storage period. Observations were taken for following biochemical characteristics: Phenol reaction of lemma using 1.5% phenol solution (Chang and Bardenas 1965), gelatinization temperature with 1.7% KOH solution (Little *et al.* 1958) and amylose content (Juliano 1971).

Table 1 List of genotypes/extant varieties of rice

Genotype	Genotype	Genotype	Genotype
Vikramarya	NDR 359	Poornima	Sambha Mahsuri
Jaya	Pusa Sugandh 2	Mahamaya	PR 106
PR 113	Pusa Sugandh 3	Shymla	Pusa 2-21
CSR 27	Pusa Sugandh 5	Saraswati	Pusa 44
Kasturi	Mandya Vijya	IR 8	Pusa 33
Pant Dhan 11	Pant Dhan 12	Lochit	PNR 546
Vasumati	Nidhi	Krishna Veni	PNR 162
Pusa Basmati 1	Suraksha	Aruna	Sabarmati
Kranthi	Salivahana	Sugandhamati	Pusa 834
Improved Pusa Basmati 1	Taraori Basmati	CSR 13	Pusa Basmati 1121
Vikash	Annand	HMT PKV	PNR 519
Pant Dhan 4	CSR 10	Govind	JD 13
Basmati 370	VL Dhan 206	Heera	Pusa Basmati 1401
Jyoti	Vivek Dhan 62	VL Dhan 221	PNR 381
Makom	ASD 20	Rasi	JD 6
			Ravi

The data were analyzed with two factor analyses by using OPSTAT (Online Statistical Analysis Tool).

RESULTS AND DISCUSSION

Phenol reaction of lemma

The phenol colour test, which is an index of polyphenol oxidase activity, is a simple method for grouping the rice varieties into different groups on the basis of lemma colour (Nil, Light Brown, Brown, Dark Brown, Black) (Sripunitha and Sivasubramaniam 2014, Singh *et al.* 2011, Gupta *et al.* 2007, Mor *et al.* 2006). During phenol colour test, phenol gets oxidized into dark colour melanin catalyzed primarily by the enzyme 'tyrosinase' in the seed coat and is under simple genetic control (Bhowal *et al.* 1969). But there is no information about influence of storage period on lemma colour during phenol test for varietal characterization. Hence all the sixty-one varieties were examined on the basis of colour changes after every three months of storage beginning from freshly harvested seed up to a period of one year. All the 61 varieties showed intra-varietal variation and the final colour was recorded on the basis of predominant coloration of replicates by using Wilson colour chart (Royal Horticulture Society). The numerical scoring was done as 0 for nil, 1 for light brown, 2 for brown, 3 for dark brown and 4 for black respectively. Out of 61 extant rice varieties, 35 genotypes (Table 2) showed no colour change throughout the storage period, while remaining 26 genotypes exhibited significant colour change with different rate and variable time period during and after six months of storage (Table 3). The mean value of colour intensity increased from 2.07 (brown) to 3.33 (dark brown) during storage. The maximum colour intensity score (3.59) was observed in Kranthi, Pant Dhan-4, Makom, Mahamaya, Shymla, and Pusa Basmati 1401. While least (1.59) was observed for Vasumati, Taraori Basmati and VL Dhan-206. This variation could be genetically influenced as reported by Bhowal *et al.* (1969). The change in colour intensity was observed at six months in all 26 varieties during experiment. Similar study showing changes in enzymatic activity takes six months had also been reported by Ten Ching- Lee *et al.* (1965). This

Table 2 Grouping of 61 extant rice varieties based on phenol reaction of lemma

Group	Lemma reaction	Color intensity	No. of genotype	Varieties
I	Present	not changed	35	3,5,8,13,14,16,17,22,23,24,26,27,29,30,31,34, 36, 37,3938, 40,41,42, 43, 44, 45,46,50,51, 52, 53, 55, 56,59, 60
II	Present	changed after six months	26	1,2,4,6,7,9,10,11, 12,15,18,19,20, 21, 25,28,32, 33, 35, 47,48, 49, 54, 57,58,61

Table 3 Influence of storage period on colour intensity of lemma pooled for two years

Varieties	Storage period (months)/Average colour intensity					
	0	3	6	9	12	Mean
Vikramarya	2	2	3	3	3	2.63
Jaya	1	1	2	2	2	1.62
CSR 27	2	2	3	3	3	2.61
Pant Dhan 11	1	1	3	3	3	2.27
Vasumati	1	1	2	2	2	1.59
Kranthi	3	3	4	4	4	3.59
Improved Pusa Basmati 1	2	2	3	3	3	2.61
Vikash	1	1	2	2	3	1.81
Pant Dhan 4	3	3	4	4	4	3.59
Makom	3	3	4	4	4	3.59
Pusa Sugandh 3	1	2	3	3	3	2.43
Pusa Sugandh 5	1	1	2	2	3	1.81
Mandya vijya	3	3	4	4	4	3.59
Pant Dhan 12	1	1	2	3	4	2.21
Taraori Basmati	1	1	2	2	2	1.59
VL Dhan 206	1	1	2	2	2	1.59
Mahamaya	3	3	4	4	4	3.59
Shymla	3	3	4	4	4	3.59
IR 8	3	3	4	4	4	3.59
Samba Mahsuri	3	3	4	4	4	3.59
Pusa 2-21	2	2	4	4	4	3.15
Pusa 44	3	3	4	4	4	3.59
Pusa 834	3	3	3	4	4	3.41
JD 13	2	2	3	3	3	2.61
Pusa Basmati 1401	3	3	4	4	4	3.59
Ravi	1	1	2	2	3	1.85
Mean	2.07	2.10	3.10	3.17	3.33	

could be possible reason for variation in colour showed by lemma in various period of time interval.

Gelatinization temperature (GT)

Gelatinization temperature is an important cooking quality trait for the varietal characterization in rice. GT measures starch gelatinization temperature which is a chemical reaction process in which ungelatinized part of starch is changed to gelatinized part (Yamamoto *et al.* 2004). Interpretation of gelatinization temperature can be done with the help of Alkali spreading value (ASV) of starch endosperm. A low ASV corresponds to the high gelatinization temperature (Pachauri *et al.* 2013). The gelatinization parameters are important to breeders who select lines with specific starch physicochemical characteristics. All 61 genotypes were grouped in two broad classes, viz. varieties having constant ASV throughout storage period and varieties exhibiting changes in ASV value (Table 4).

It was observed that during entire storage period, 35 genotypes showed constant ASV, viz. Vikramarya, Jaya, CSR 27, Rasi while remaining 26 genotypes showed significantly decrease in ASV at six months of storage (viz. Suraksha, ASD 20) and continued up to remaining period (Table 5). The mean ASV value for 26 genotypes decreased from 6.7 to 4.5 during storage period. The ASV decreased significantly during six months of storage (6.7 to 6) resulting in more harder grain at the end of study (Table 5). The gelatinization parameters are influenced by amylopectin structure (chain length distribution), which can vary by cultivar, location and crop year (Cameron *et al.* 2008, Singh *et al.* 2006). The maximum decrease in ASV was observed in Nidhi (4.19) while least (6.25) was found in ASD 20, Poornima, PNR 162, Pusa 834 and JD 13.

Zhou *et al.* (2002, 2003) suggested that during storage, lipid hydrolysis produces free fatty acid and the complexion of free fatty acid with amylose probably occurred after gelatinization which explains the influence of storage on viscosity. Faruqa *et al.* (2015) and Wiset *et al.* (2005) also reported same findings as in present study, that the grain becomes harder and gives high GT after significant storage period (5-6 months).

Amylose content in endosperm

Amylose content is one of the important biochemical traits for varietal characterization. In DUS guideline (National DUS guideline for rice SG/ 01/ 2007), varieties are classified in to five groups on the basis of amylose content in endosperm namely: very low (<10 %), low (10-19%), medium (20-25%), high (26-30%) and very high (>30%) for varietal registration under PPV and FRA 2001. In the present study, out of 61 genotypes, 22 genotypes had low amylose content (16.2 to 19.6%). Further, 37 genotypes had medium (21.2 to 25.5%) amylose content while only two genotypes, viz. Mahamaya and Jyothi were grouped in

Table 4 Grouping of sixty-one genotypes on the basis of ASV

Group	ASV test	Consistency In ASV	No. of genotype	Varieties
I	+	Constant	35	1, 2, 4, 5, 6, 7, 9, 15,17, 25, 33, 34, 35, 36, 37, 38, 39, 40,41,42,43, 44,46,45, 47, 48, 49,50,51,52, 54,56,57,59, ,60
II	+	Decrease after six months	26	3,8, 10,11, 12, 13,14, 16, 18,19, 20, 21, 22, 23, 24, 26,27, 28, 29, 30, 31, 32,53, 55, 58 61

Factors	C.D.	SE(d)	SE(m)
Genotype (A)	0.064	0.033	0.023
Storge period (B)	0.028	0.014	0.01
Factor (A × B)	0.144	0.073	0.051

Table 5 Influence of storage period on ASV (Alkaline Spreading Value), pooled for two years

Varieties	Storage period (months)/Alkali spreading value					
	0	3	6	9	12	Mean
PR 113	7	7	7	5	5	6.19
Pusa Basmati 1	7	7	7	5	5	6.19
Improved Pusa	7	7	5	5	5	5.77
Basmati 1	7	7	5	5	5	5.79
Vikash	7	7	7	5	5	6.19
Pant Dhan 4	7	7	7	5	5	6.17
Basmati 370	7	7	5	3	3	4.97
Makom	5	5	5	3	3	4.22
Pusa Sugandh 2	7	7	5	5	5	5.83
Pusa Sugandh 3	7	7	7	5	5	6.15
Pusa Sugandh 5	7	7	5	5	5	5.79
Mandya Vijya	7	7	5	5	5	5.79
Pant Dhan 12	7	7	5	5	5	5.79
Nidhi	5	5	5	3	3	4.19
Suraksha	7	7	5	5	3	5.42
Taraori Basmati	7	7	7	5	5	6.23
Annada	7	7	7	5	5	6.24
CSR 10	7	7	7	5	5	6.23
VL Dhan 206	5	5	5	3	3	4.18
Vivek Dhan 62	5	5	5	3	sss3	4.17
ASD 20	7	7	7	5	5	6.25
Poornima	7	7	7	5	5	6.25
PNR 162	7	7	7	5	5	6.25
Pusa 834	7	7	7	5	5	6.25
JD 13	7	7	7	5	5	6.25
Ravi	7	7	5	5	5	5.85
Mean	6.7	6.7	6.0	4.6	4.5	

Factor	C.D.	SE(d)	SE(m)
Genotype (A)	0.06	0.03	0.021
storage period (B)	0.026	0.013	0.009
Factor (A × B)	0.134	0.068	0.048

Scale 1-7 was used for measuring ASV as per DUS guideline

to high category with 26.6 % and 26.1% amylose content, respectively (Table 6). The mean value of amylose content of freshly harvested paddy varieties ranged from 16-26% with the genotypic variation. The differences in amylose content were non-significant during first 3-5 months of storage; however, significant variation was recorded during and after six months of storage period (Table 7). Faruqa *et al.* (2015) also highlighted the same trend in their studies. The average increase in amylose content value was 2.5% which is significantly high enough to bring genotype under higher descriptor category. This up gradation of genotype can create problem during characterization of variety for registration under PPV and FRA 2001. In the present study, significant changes were recorded after six months of storage which was continued further. The mean value of amylose content was 21.5 % at harvesting time which increased by 22.1 % during six months of storage. However, the value of amylose content continued to increase up to 22.6 % at 12 months of storage period. Similar result was reported by Kanlayakrit and Maweang (2013), who reported that after four months of storage period, enhanced enzymatic activity occurs, which contributes in increasing amylose content, and hence, effects the hardness, gel consistency and amylograph viscosity values for cooked rice. Further, amylose content is considered as single most important characteristic for predicting rice cooking and processing quality (Juliano 1985, Webb 1985 and Lii *et al.* 1996). Amylose content is directly related to water absorption, volume expansion, fluffiness and separation of cooked grains (Delwiche *et al.* 1996) while inversely correlated to cohesiveness, tenderness and glossiness (Tester and Morrison 1990).

Hence, from the present study it was concluded that storage temperature and time are the most influential factors on the chemical, physical, and functional qualities of rice during post-harvest storage. The rate and nature of the change is primarily temperature dependent. Generally, the outer layers of seed coat are more susceptible to these reactions than the endosperm of the rice kernel. Although the physicochemical properties of paddy may exhibit greater changes, milled characteristics can also be altered during storage. Quality shifts generally occur faster with increasing temperature. Therefore, the biochemical assessment in rice

Table 6 Grouping of varieties on the basis of amylose content

Class/state	Low (10-19%)	Medium (20-25%)	High (26-30%)
Genotypes	Vikramarya, CSR 27 Pusa Sugandh 3, CSR 10 Pusa Sugandh 2, Vivek Dhan 62, ASD 20, Saraswati, Lochit, Krishna Veni, PR 106 Pusa 221, Pusa 33, PNR 162 Sabarmati, PNR 519, PNR 381, Pant Dhan 11, Vasumati Pant Dhan 4, Salivahana JD 6	Jaya, PR113, Kasturi, PB 1, IMP. PB 1, Jyothi Vikashbasmati 370, Makom NDR 359, Pusa Sugandha 5, Mandya Vijya, Pant Dhan 12 Nidhi, Surakshatarori Basmati, Annada, VL Dhan 206, Poornima, Shymlair 8, Aruna, sugandhamati. CSR 13, HMT PKV, Govind, Heera, VL Dhan 221, Rasi, Sambha Mahsuri, Pusa 44 PNR 546, Pusa 834 Pusa Basmati 1121, JD 13, Pusa Basmati 1401, Ravi, Kranthi	JYOTHI MAHAMAYA
Total	22	37	2

Table 7 Influence of storage period on average amylose content (%) pooled for two years

Varieties (V)	Storage period (months)/amylose content (%)					
	0	3	6	9	12	Mean
Vikramarya	18.800	18.800	19.300	19.300	20.100	19.260
Jaya	23.400	23.650	23.900	23.900	24.400	23.850
PR 113	22.700	22.700	22.900	22.900	23.400	22.920
CSR 27	18.600	18.600	19.600	19.600	20.100	19.300
Kasturi	24.300	24.300	24.800	24.800	25.000	24.640
Pant Dhan 11	19.600	19.600	20.300	20.300	20.800	20.120
Vasumati	19.300	19.300	20.000	20.000	20.700	19.860
PB 1	24.100	24.100	24.500	24.500	25.900	24.620
Kranthi	25.100	25.100	25.600	25.600	25.900	25.460
IMP. PB1	24.100	24.100	24.500	25.000	25.700	24.680
Vikash	25.000	25.000	25.100	25.100	25.700	25.180
Pant Dhan 4	19.300	19.300	19.900	19.900	20.400	19.760
Basmati 370	22.730	22.730	23.400	23.400	24.200	23.292
Jyothi	26.600	26.600	26.800	26.800	28.900	27.140
Makom	21.640	21.640	22.400	22.400	23.400	22.296
NDR 359	22.850	22.850	23.100	23.100	24.100	23.200
Pusa Sugandh 2	16.300	16.300	16.900	16.900	17.000	16.680
Pusa Sugandh 3	18.300	18.300	18.900	18.900	19.000	18.680
Pusa Sugandh 5	23.230	23.230	24.100	24.100	25.000	23.932
Mandya Vijya	24.500	24.500	24.700	24.700	25.000	24.680
Pant Dhan 12	22.300	22.300	22.600	22.600	23.000	22.560
Nidhi	22.940	22.940	23.100	23.100	23.700	23.156
Suraksha	24.190	24.190	25.200	25.200	25.600	24.876
Salivahana	19.450	19.450	20.400	20.400	20.600	20.060
Tarori Basmati	24.100	24.100	25.200	25.200	25.600	24.840
Annada	24.700	24.700	25.100	25.100	25.700	25.060
CSR 10	17.240	17.240	18.300	18.300	18.900	17.996
VL Dhan 206	20.040	20.040	21.500	21.500	21.900	20.996
Vivek Dhan 62	16.710	16.710	17.800	17.800	18.000	17.404
ASD 20	17.600	17.600	18.400	18.400	18.900	18.180
Poornima	25.500	25.500	26.400	26.400	26.700	26.100
Mahamaya	26.100	26.100	26.300	26.300	26.800	26.320
Shymla	23.360	23.360	24.100	24.100	24.600	23.904
Saraswati	16.200	16.200	17.500	17.500	17.900	17.060
IR 8	22.300	22.300	23.300	23.300	23.700	22.980
Lochit	17.800	17.800	18.000	18.000	18.400	18.000
Krishna Veni	18.800	18.800	19.200	19.200	20.300	19.260
Aruna	23.500	23.500	24.100	24.100	24.500	23.940
Sugandhamati	25.500	25.500	25.900	25.900	25.700	25.700
CSR 13	24.900	24.900	25.000	25.000	25.300	25.020
HMT PKV	24.500	24.500	25.000	25.000	25.000	24.800
Govind	20.900	20.900	21.000	21.000	21.400	21.040
Heera	22.600	22.600	23.000	23.000	23.400	22.920

Contd.

Table 7 (*Concluded*)

Varieties (V)	Storage period (months)/amylose content (%)					
	0	3	6	9	12	Mean
VL Dhan 221	21.200	21.200	22.100	22.100	22.500	21.820
Rasi	24.500	24.500	24.900	24.900	25.000	24.760
Sambha Mahsuri	24.800	24.800	24.900	24.900	25.000	24.880
PR 106	17.900	17.900	18.000	18.000	18.500	18.060
Pusa 221	16.260	16.260	16.900	16.900	17.000	16.664
Pusa 44	22.300	22.300	22.700	22.700	22.900	22.580
Pusa 33	18.700	18.700	19.000	19.000	19.400	18.960
PNR 546	22.300	22.300	23.000	23.000	23.600	22.840
PNR 162	18.900	18.900	19.000	19.000	19.400	19.040
Sabarmati	18.900	18.900	19.000	19.000	19.400	19.040
Pusa 834	22.800	22.800	23.000	23.000	23.100	22.940
Pusa Basmati 1121	21.900	21.900	22.000	22.000	22.300	22.020
PNR 519	17.300	17.300	18.000	18.000	18.400	17.800
JD 13	22.100	22.100	23.000	23.000	23.400	22.720
Pusa Basmati 1401	24.100	24.100	24.800	24.800	24.900	24.540
PNR 381	20.200	20.200	20.700	20.700	21.300	20.620
JD 6	19.100	19.100	19.800	19.800	20.100	19.580
Ravi	20.400	20.400	20.600	20.600	20.900	20.580
Mean	21.563	21.567	22.107	22.115	22.580	
Factors	V	M	V × M			
CD	0.004	0.001	0.008			

should be carried out within first six months of harvesting the produce for establishing distinctiveness among genotypes.

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