

## Physico-Chemical and cooking characteristics of anther derived doubled haploid lines of two elite *indica* hybrid rice varieties - Ajay and Rajalaxmi

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### ABSTRACT

Grain quality is the primary determinant for market price and consumer acceptability in rice, a major food crop of the world. Despite sincere and persistent efforts by the breeders, none of the hybrids developed in India possess excellent grain quality. Doubled haploid breeding which can play a vital role in accelerating the breeding process, can also help in the development of true breeding lines having uniform grain quality. In the present study, the range of variation for grain quality present in the doubled haploid populations derived from two heterotic *indica* rice hybrids- Ajay (CRHR7) and Rajalaxmi (CRHR5) was studied. The physico-chemical characteristics and cooking quality traits were studied on twenty recombinant doubled haploid lines derived from each hybrid. The results suggest that eight derivatives from each hybrid possess all the grain quality characteristics at desired levels and some were better than their respective parents in terms of both quality and uniformity.

**Key words:** Doubled haploid, anther culture, hybrid rice, *indica*, grain quality

Rice (*Oryza sativa* L.) is the staple food of more than half of the world's population and is mostly consumed in cooked form. Hybrid rice breeding has been successful in developing superior hybrids, which have brought about dramatic increases in rice production in China and several other countries. However, the adoption rate of hybrid rice varieties in India is poor. The major constraints that limit the large scale adoption of the hybrid rice cultivars in India were the high seed cost associated with the hybrid seed and the poor grain quality of the hybrids (Janaiah, 2003). Higher yield of rice hybrids would not make hybrid rice technology acceptable unless the produce has acceptable level of grain quality.

Grain quality has always been an important consideration in rice variety selection and development and it's difficult to define it with precision as preferences for quality vary from country to country (Babu *et al.*,

2013). The kernel appearance, size, shape, nutritional value and cooking characteristics are important for judging the quality and preference of rice from one group of consumer to another (Dela Cruz and Khush, 2000; Sellappan *et al.*, 2009; Kanchana *et al.*, 2012). In particular, the cooking and eating qualities are very important determinants of cooked rice grain quality (Ge *et al.*, 2005). Rice with good grain quality can meet the consumers taste in both domestic and international markets but also can earn valuable foreign exchange (Tran and Nguyen, 2011).

The most important challenge in rice quality breeding was the development of good quality inbred varieties or hybrid combinations in *indica* rices (Hanwei and Cunshan, 2001). So far, none of the hybrids have reached the level of quality of some of the conventionally bred varieties. Doubled haploid breeding through anther culture can be used to overcome

the limitations associated with hybrid rice breeding. Anther culture technique not only can play a role in accelerating breeding progress and also can help in fixing the grain quality traits in the recombinants (Mohiuddin, 2014). Milyang-90, an anther culture derived line, with good performance and grain quality was isolated from more than 2000 lines (Chung, 1987). The breeding of Guan-18, which was having good grain quality characteristics, was a great achievement of anther culture quality breeding (Zhu *et al.*, 1990).

The primary objective of the study is to select promising doubled haploid lines having good grain quality traits from the doubled haploid populations generated from two elite heterotic F<sub>1</sub> hybrids.

## MATERIALS AND METHODS

The experimental materials were the doubled haploid lines derived from Ajay (CRHR 7) and Rajalaxmi (CRHR 5), two elite *indica* hybrid rice varieties that were developed at Central Rice Research Institute, Cuttack. These lines were evaluated in a replicated trial grown in the experimental fields along with their parents and a local control and were characterized for major morph-agronomic characters and yield. The quality analysis was conducted on the grains harvested from the crop. Twenty promising DH lines that showed high yield potential were selected from each hybrid and analyzed for grain quality traits. All the analysis was replicated and the mean data was recorded. The following parameters were analyzed.

Hulling (dehusking) of 100gm paddy seeds was done (per replication) in Satake Dehuller (Type THV 35) A. The dehusked rice kernels recovered from 100gm paddy seeds were polished in a Satake Grain Testing Mill (Type-TM 05) for 1.5 minutes. Total polished kernels were weighed and passed through rice grader having different (mm) grooves. The whole grains were then separated from the broken grains in order to quantify the head rice recovery.

Hulling percentage = (Weight of dehusked kernel / Weight of paddy) × 100

Milling percentage = (Weight of polished kernel / Weight of paddy) × 100

Head rice recovery percentage = (Weight of whole polished kernels / Weight of paddy) × 100 *Kernel*

*length and kernel breadth:* The length and breadth of 10 kernels of each variety (per replication) were recorded by means of a dial micrometer. The grain type was determined by the length and breadth of the kernel and the ratio between length and breadth.

For the estimation of the alkali spreading value, the method of Little (1958) was followed. Six whole grain milled rice samples were placed (in duplicate) in small plastic boxes containing 10ml of 1.7 percent KOH. The boxes were covered and incubated for 23 hours at 30°C. The gelatinization temperature of rice varieties, may be classified as low (55 to 69°C), intermediate (70 to 74°C) and high (> 74°C). Grains that were unaffected were given ASV of 1 and grains that were dispersed and disappeared completely were given a score of 7. The ASV value was inversely proportional to gelatinization temperature.

Amylose was determined through the method described by Juliano (1971). Whole grain milled rice was grounded to powder, made to pass through a 100 mesh sieve and 100 mg of powder was taken in a 50 ml tube and 1ml of 95% ethanol and 9ml of 1N NaOH were added. The tube was heated for 10 minutes in a boiling water bath, cooled and transferred into a 100ml volumetric flask. With several washings in distilled water, the volume was finally made up to 100ml. Five ml of aliquot was pipetted into a 100ml volumetric flask to which one ml solution of 1 N acetic acid and 2ml of iodine solution (0.2g I<sub>2</sub> + 2.0g KI in 100ml water) were added. The solution was made up to 100ml adding distilled water and was kept for 20 minutes by covering with a dark cloth. Optical density of the solution was determined in a *spectrophotometer at 620 nm wavelength*.

Five gm of whole milled rice was cooked for 20 minutes after presoaking in 15ml water for 5 minutes to determine the cooking time. The Kernel length after cooking (KLAC) was the average of the length of ten cooked kernels after cooking measured in millimeter (mm) and Elongation ratio(ER) was the average ratio of the length of cooked rice and the length of milled rice of ten kernels.

For assessing volume expansion and cooking quality, the method of Verghese (1950) was followed. Five gram of milled rice was taken in 15ml of water and then the total volume was recorded (y ml). Volume

of 5g rice was (y-15) ml. The resultant water containing tubes with the rice were cooked for 20 minutes at boiling water bath. The cooked rice was later dipped in 50ml of water (which was poured in a 100ml measuring cylinder).

The volume of cooked rice = vol. in meas. cylinder after dipping cooked rice (X) -50ml.

The volume expansion ratio is expressed as= (X -50)/(Y-15).

For determining water uptake of milled rice, the method of (Beachell and Stansel, 1963) was followed. Two grams of milled rice was taken in a 50ml centrifuge tube, ten ml of distilled water was poured into it and was kept for 30 minutes at room temperature. The resultant rice containing tubes were placed in 77°C water bath for gelatinization. After 45 minutes the tubes were kept in running tap water and then filtered into a grouch crucible. The filtrate was measured in millimeter and solid mass sedimented below was determined. Blank sample containing 10ml of distilled water was run to know the loss of water due to evaporation under the same conditions. Blank was the volume of distilled water in the tube after evaporation.

Water uptake = [Blank- (filtrate-solid mass)] ×50

All data were analyzed by the Analysis of Variance (ANOVA) procedure using CROPSTAT program (IRRI 2008).

## RESULTS AND DISCUSSION

The hulling percentage was found to be more than 76 percent in almost all the doubled haploids derived from the two hybrids with the values are in the range of 72.00% to 78.00% in CRHR 5 derivatives and 72.0% - 79.00% in the CRHR 7 derivatives. The milling percentages are in the range of 57.00%-69.00% (mean-68.1%) in CRHR 5 derivatives while in CRHR 7 doubled haploids, they are in the range of 59.00%-70.0% (mean-65.05%). Head rice recovery, one of the major criteria that determine the quality, varies depending on variety, grain type, cultural practices and post harvest conditions (Asish *et al.*, 2006; Correa *et al.*, 2007, Ghasemi *et al.*, 2008, Razavi *et al.*, 2008, Emadzade *et al.*, 2009). Of the doubled haploids analyzed, sixteen DHs of CRHR 5 and fourteen DHs of CRHR 7 have higher HRR values than the parental

values. The grain type was determined by the length and breadth of the kernel and the ratio between length and breadth. The segregation pattern of the grain types shows that the doubled haploids of CRHR 5 possesses both long slender grain and medium slender grains with a low frequency of medium bold grains and similar pattern was seen in case of CRHR 7 derivatives also (Table 5). In the doubled haploids of both the hybrids, good slender grain types are recovered having high L/B ratios. Some of the doubled haploids had exceeded the level above 3, a good attribute (Dipti *et al.*, 2002).

The alkali spreading values for most of the derivatives was in the acceptable range of 5-7 for both hybrids. Vanaja and Babu, (2003) reported that the gelatinization temperature affects water uptake, volume expansion ratio and linear kernel elongation. Amylose content was another important grain quality character. The percentage of amylose content in the grain varied from 22.4 - 24.55% and 21.8 - 24.6% in the doubled haploids of CRHR 5 and 7 respectively indicating the presence of wide variation in the doubled haploid populations. Rice varieties with amylose content of > 25% absorb more water and have a fluffy texture after cooking (Frei and Becker, 2003; Oko *et al.*, 2012). Amylose content also determines the texture of cooked rice. It is an indicator of volume expansion and water absorption during cooking (Deyner *et al.*, 2001). Intermediate amylose content (20-25%) is usually preferred in India (Subudhi *et al.*, 2012).

The kernel length after cooking (KLAC) is another important trait of grain quality. The range of kernel length after cooking was 8.32-10.13 in the derivatives of CRHR 5 and 8.64-11.23 in the derivatives of CRHR 7 with some of the doubled haploids had higher values of KLAC. The elongation ratios of the grains after cooking was within the range of 1.35-1.71 in CRHR 5 derivatives while the values were in the range of 1.44-1.77 in CRHR 7 derivatives with most of them having intermediate values. The ideal range of water uptake value was in the range of 200-300. The mean value of CRHR 5 was 210 while it was 225 in CRHR 7 and most of the doubled haploids are within the acceptable range. The appearance in the quality of cooked rice grains is associated with the amount of water uptake during the cooking process, which may be associated with the appearance of milled rice grain changes, as well as overall water absorption. The

amount of water uptake during cooking process is associated with the appearance of cooked rice (Tan *et al.*, 2000). The volume expansion ratio was within the range of 3.25-3.55 in both the hybrids. The findings support the report earlier that used anther culture technique to develop of a number of high yielding varieties with excellent eating quality characters (Watanabe *et al.*, 1998).

The present study indicates that some doubled haploid lines showed desired levels of grain quality; such as high HRR, LS or MS grain type, high KLAC, intermediate amylose content and all of them are highly uniform unlike their respective parents. Out of these twenty doubled haploids tested for each hybrid, eight DHs derived from CRHR 5 (CR 5-8, CR 5-10, CR 5-21, CR 5-49, CR 5-50, CR 5-56, CR 5-61 and CR 5-68) and eight from CRHR 7 (CR 7-5, CR 7-12, CR 7-17, CR 7-40, CR 7-41, CR 7-52, CR 7-57 and CR 7-

62) did possess desired levels of grain quality traits (Table 1 and 2). The analysis variance of the grain quality traits of the doubled haploids of CRHR 5 and CRHR 7 reveals that significant variation ( $P < 0.01$ ) was detected among the twenty DH lines for all the traits evaluated while no significant differences between the replications were observed (Table 3 and 4).

The poor cooking and eating quality of *indica* hybrid rice varieties was one of the major reasons for the poor adoption of hybrid rice technology in India. Grain quality traits like head rice recovery, grain type, cooking qualities like kernel length after cooking (KLAC) and amylose content were some of the major determinants of quality of the rice grains. In the present study, the quality traits of the doubled haploid derivatives from two elite hybrids were analyzed and the results suggest that some DH lines possess quality characters at desired levels and are superior to their respective

**Table 1.** Physico-chemical and cooking characteristics of the doubled haploids of Rajalaxmi

DH lines	Hulling	Milling	HRR	KLAC	ER	KL	KB	L/B	VE	WU	ASV	Amy %
CR-5-6	74.51	67.23	59.12	10.03	1.65	6.09	2.34	2.60	3.50	245	6	22.91
CR-5-8	77.34	69.22	60.51	8.41	1.46	5.77	2.27	2.54	3.45	195	6	22.68
CR-5-9	78.37	60.15	47.11	9.52	1.68	5.66	2.07	2.73	3.50	240	6	22.08
CR-5-10	78.53	68.51	59.11	9.13	1.60	5.70	1.94	2.94	3.50	235	5	23.62
CR-5-19	74.52	57.65	50.54	9.27	1.46	6.34	2.16	2.94	3.25	225	5	23.42
CR-5-21	78.56	68.52	58.59	9.76	1.54	6.32	2.11	3.00	3.30	220	7	22.87
CR-5-41	74.55	66.11	50.01	9.96	1.71	5.81	1.82	3.19	3.50	225	6	23.58
CR-5-49	78.43	68.51	60.54	10.04	1.70	5.90	2.22	2.66	3.50	215	7	23.13
CR-5-50	77.38	68.21	65.82	8.74	1.51	5.77	2.24	2.58	3.60	235	6	23.08
CR-5-52	76.59	67.47	65.47	9.43	1.72	5.49	1.83	3.00	3.55	190	6	24.15
CR-5-53	77.54	67.69	50.99	9.69	1.60	6.05	2.12	2.85	3.50	145	4	22.53
CR-5-56	76.22	65.71	60.28	8.32	1.38	6.04	2.07	2.92	3.50	275	7	22.16
CR-5-61	78.11	67.88	61.27	8.67	1.35	6.43	2.06	3.12	3.50	235	7	23.73
CR-5-68	75.45	64.26	60.75	9.74	1.50	6.51	2.39	2.72	3.35	200	7	24.55
CR-5-75	72.58	63.53	58.32	9.51	1.47	6.45	2.20	2.93	3.40	190	6	22.58
CR-5-78	72.88	63.26	54.88	9.48	1.66	5.71	1.92	2.97	3.30	165	5	22.98
CR-5-85	75.63	65.11	57.36	9.33	1.44	6.49	1.77	3.67	3.50	190	7	22.83
CR-5-105	76.53	67.89	63.53	9.57	1.62	5.91	1.75	3.38	3.50	295	6	22.41
CR-5-124	77.33	67.34	65.76	10.13	1.55	6.54	2.16	3.03	3.50	160	5	24.65
CR-5-129	74.38	63.21	55.98	8.46	1.35	6.28	2.09	3.00	3.45	180	6	24.41
CRHR5	78.11	65.23	54.12	9.49	1.38	6.23	2.06	3.02	3.50	245	7	26.37
Lalat	77.00	65.50	52.20	10.20	1.53	6.66	2.11	3.15	3.70	227	5	26.60
SE	±0.06	±0.2	±0.05	±0.06	±0.5	±0.066	±0.04	±0.04	±0.1	±4.5	±0.1	±0.05
LSD (5%)	0.17	0.57	0.14	0.19	0.12	0.16	0.13	0.13	0.37	13.2	3.20	0.14

Hull-Hulling(%); Mill-Milling(%); HRR- Head rice recovery(%); KL-Kernel length; KB-Kernel breadth; ASV-Alkali spreading value; WU-Water uptake (ml); KLAC- Kernel length after cooking (mm); ER- Elongation ratio; VER- Volume expansion ratio; AC- Amylose content (%).

**Table 2.** Physico-chemical and cooking characteristics of the doubled haploids of Ajay.

DH lines	Hulling	Milling	HRR	KLAC	ER	KL	KB	L/B	VE	WU	ASV	Amy %
CR-7-2	79.13	63.21	45.80	8.98	1.62	5.55	1.83	3.03	3.50	202	7	22.31
CR-7-5	76.54	66.24	58.22	10.45	1.75	5.97	1.91	3.13	3.50	270	7	23.66
CR-7-7	77.51	68.58	53.45	11.01	1.69	6.51	2.03	3.21	3.25	265	6	22.39
CR-7-12	77.66	69.52	60.32	11.23	1.77	6.33	1.91	3.31	3.45	198	6	22.46
CR-7-17	77.32	68.94	64.52	9.33	1.52	6.14	2.09	2.94	3.50	220	7	19.45
CR-7-22	76.27	67.77	60.23	9.28	1.52	6.09	2.06	2.96	3.50	232	6	23.66
CR-7-25	77.55	62.53	57.34	9.01	1.60	5.64	1.76	3.20	3.40	245	6	23.25
CR-7-27	52.89	45.58	33.51	9.39	1.49	6.29	2.07	3.04	3.40	165	5	22.46
CR-7-40	78.58	67.73	60.27	9.31	1.57	5.93	1.91	3.10	3.35	190	7	23.64
CR-7-41	76.34	66.79	58.54	11.01	1.72	6.39	2.02	3.16	3.50	210	7	23.51
CR-7-46	74.82	64.85	51.67	9.13	1.54	5.94	2.15	2.76	3.50	255	7	24.11
CR-7-48	78.66	59.53	54.65	8.89	1.47	6.04	2.16	2.80	3.45	238	6	22.72
CR-7-52	76.39	59.27	53.49	9.17	1.51	6.08	2.24	2.71	3.45	243	7	24.63
CR-7-55	73.54	63.61	47.37	8.66	1.45	5.99	1.95	3.07	3.50	145	4	24.37
CR-7-56	76.48	67.54	58.62	9.03	1.55	5.84	1.95	2.99	3.50	242	5	22.31
CR-7-57	78.65	70.29	62.38	9.43	1.57	5.99	1.74	3.44	3.50	210	7	22.46
CR-7-58	76.11	67.83	60.48	9.48	1.40	6.79	1.82	3.73	3.40	199	6	21.82
CR-7-59	73.45	62.35	56.38	8.64	1.44	6.02	2.01	3.00	3.35	220	6	24.36
CR-7-62	72.29	63.44	56.77	10.23	1.66	6.16	2.05	3.00	3.35	245	7	23.88
CR-7-68	76.57	65.51	55.32	9.24	1.60	5.77	2.27	2.54	3.45	211	4	24.07
CRHR-7 (P)	77.21	64.29	54.64	10.25	1.48	6.88	2.21	3.11	3.50	235	7	26.35
Lalat	77.00	65.50	52.20	10.20	1.53	6.66	2.11	3.15	3.60	227	5	26.60
SE	±0.1	±0.5	±0.04	±0.05	±0.03	±0.06	±0.6	±0.4	±0.1	±4.3	±0.1	±0.1
LSD (5%)	0.3	1.5	0.1	0.1	0.9	0.1	1.8	0.1	0.3	12.7	0.3	0.5

Hull-Hulling(%); Mill-Milling(%); HRR- Head rice recovery(%); KL-Kernel length; KB-Kernel breadth; ASV-Alkali spreading value; WU-Water uptake (ml); KLAC- Kernel length after cooking (mm); ER- Elongation ratio; VER- Volume expansion ratio; AC- Amylose content (%).

**Table 3.** Analysis of variance of grain quality characteristics of the doubled haploids derived from Rajalaxmi

Character	Source of variation	Degree of freedom	Mean Sum of Square	CV (%)
Hulling	Variety	21	6.72**	0.1
	Replication	1	0.0008	
	Error	21	0.006	
Milling	Variety	21	17.05**	0.4
	Replication	1	0.06	
	Error	21	0.07	
HRR	Variety	21	56.80**	0.1
	Replication	1	0.02	
	Error	21	0.04	
KLAC	Variety	21	0.645**	1.0
	Replication	1	0.01	
	Error	21	0.09	
ER	Variety	21	0.286**	4.0
	Replication	1	0.0007	
	Error	21	0.003	
KL	Variety	21	0.235**	1.3
	Replication	1	0.002	
	Error	21	0.006	
KB	Variety	21	0.627**	3.1
	Replication	1	0.0001	
	Error	21	0.004	

Table 3 Contd....

Character	Source of variation	Degree of freedom	Mean Sum of Square	CV (%)
L/B	Variety	21	0.140**	2.1
	Replication	1	0.01	
	Error	21	0.004	
VE	Variety	21	0.02	5.3
	Replication	1	0.02	
	Error	21	0.03	
WU	Variety	21	2707.38**	3.0
	Replication	1	245.81	
	Error	21	40.53	
ASV	Variety	21	1.523	25.7
	Replication	1	0.09	
	Error	21	2.37	
AMY	Variety	21	2.936**	0.3
	Replication	1	0.01	
	Error	21	0.004	

\*\* Significant at P=0.0.1 level

**Table 4.** Analysis of variance of grain quality characteristics of the doubled haploids derived from Ajay

Character	Source of variation	Degree of freedom	Mean Sum of Square	CV (%)
Hulling	Variety	21	56.93**	0.2
	Replication	1	0.001	
	Error	21	0.02	
Milling	Variety	21	54.89**	1.2
	Replication	1	0.8	
	Error	21	0.5	
HRR	Variety	21	88.76**	0.1
	Replication	1	0.02	
	Error	21	0.003	
KLAC	Variety	21	1.229**	0.8
	Replication	1	0.03	
	Error	21	0.005	
ER	Variety	21	.195**	2.9
	Replication	1	0.001	
	Error	21	0.002	
KL	Variety	21	.294**	1.4
	Replication	1	0.02	
	Error	21	0.07	
KB	Variety	21	2977.66**	8.8
	Replication	1	1.08	
	Error	21	0.8	
L/B	Variety	21	.124**	1.9
	Replication	1	0.001	
	Error	21	0.003	
VE	Variety	21	0.011	5.1
	Replication	1	0.17	
	Error	21	0.03	
WU	Variety	21	6702.95**	3.0
	Replication	1	3.8	
	Error	21	37.4	
ASV	Variety	21	1.780**	2.4
	Replication	1	0.02	
	Error	21	0.02	
AMY	Variety	21	4.64**	1.2
	Replication	1	0.04	
	Error	21	0.07	

\*\* Significant at P=0.01 level

**Table 5.** Segregation pattern of the grain type in the doubled haploids derived from Rajalaxmi and Ajay

DHs from	Long Slender	Medium Slender	Medium Bold
Rajalaxmi	43	185	15
Ajay	18	160	8

parents(hybrids) in terms of uniformity and other traits. This study demonstrates the utility of anther culture in generating highly uniform grains with good grain quality traits from elite hybrid rice varieties. The DH lines with high yield and good grain quality characteristics can be an ideal substitution to the hybrid rice varieties.

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