RESEARCH NOTE

Genetics of wide compatible gene and variability studies in rice (*Oryza sativa* L.)

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[Revathi S., Sakthivel K., Manonmani S., Umadevi M., Ushakumari R. and Robin S. 2016 Genetics of wide compatible gene and variability studies in rice (*Oryza sativa* L.). *J. Genet.* **95**, 463–467]

Introduction

Hybrid rice technology offers great potential to increase rice production and productivity on a sustainable basis. Exploitation of heterosis in rice has resulted in release of several hybrids for commercial cultivation in India. One of the current aspects of hybrid rice breeding is exploitation of higher degree of heterosis in intervarietal crosses (*indica*/*japonica*) over the intravarietal crosses (*indica* × *indica, japonica* × *japonica* and *javanica* × *javanica*). Wide compatibility (WC) is one of the most important traits in rice which can overcome the fertility barrier in the *indica*/*japonica* hybrids. The *S5n* gene located on chromosome 6 is responsible for the WC in rice (Yang *et al.* 2009). Hence, understanding the inheritance pattern of WC gene is critical to choose the suitable breeding method to be followed for breaking the sterility barrier between *indica* and *japonica*. Quantum jump in yield improvement has been achieved in rice with the development of high yield heterotic hybrids under commercial cultivation. However, rice being the staple food of Indians, improving its productivity has become a critically important issue (Subbaiah *et al.* 2011). Knowledge on the nature and magnitude of genetic variation governing the inheritance of quantitative characters, such as yield and its components are essential for effecting genetic improvement. A critical analysis of genetic variability is necessary for initiating any crop improvement programme and for adopting appropriate selection techniques. To develop high yielding genotypes coupled with good grain quality, and resistance to pest and diseases, population with high genetic variability serves as prime source for effective selection, particularly the F_2 population. F_2 generations are the critical stage in any rice breeding and they determine the eventual success or failure of hybridization programme (Jennings *et al.* 1979). Here, two types of study was carried out in F_1 and F_2 generations of the two crosses: (i) to study genetic basis of inheritance of WC gene and its utilization in plant breeding; (ii) to assess the variability, heritability and genetic advance of grain yield and its 12 attributing traits besides identifying desirable segregants of high grain yield.

Materials and methods

CB174R and CB203R are the restorers of popular high yielding rice hybrids, namely CORH4 and TNRH203 developed from Department of Rice, Tamil Nadu, were crossed with Akshaydhan (donor of WC gene and a medium duration variety released by Directorate of Rice Research, Hyderabad) for generating F_1 and F_2 populations. The study was conducted at Department of Rice, Centre for Plant Breeding Genetics, Tamil Nadu Agricultural University, Coimbatore, during kharif 2013 and rabi 2014. The material for this experiment comprised of 310 F_2 plants of each crosses of rice, namely, CB174R × Akshaydhan and CB203R × Akshaydhan along with their F_1 and parents raised with row spacing of 20 cm and plant to plant spacing of 20 cm. The plants with WC gene were identified using gene specific marker (S5n InDel). The primer sequence used for amplification of S5 locus was 5′-CCTACGTTTGACTGCCTGCCTG-′3 (forward) and 5′-CTACACGCGGCTTCGGGAAAGC-′3 (reverse). Observations were made on plant height, total tillers per plant, total number of productive tillers, days to 50 per cent flowering, panicle length, flag leaf length and width, number of primary and secondary branches per panicle, number of spikelet per panicle, number of filled grains per panicle, 100-grain weight and single plant yield in both the F_2 populations.

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Keywords. wide compatible; F_2 generation; variability; heritability.

Molecular analysis

DNA was extracted from fresh leaf tissue by CTAB protocol. The quantity of DNA was checked on 0.8% agarose gel electrophoresis. Leaf samples were collected from 30 days old seedlings and the leaf tissues were ground extracted with 600 μL of CTAB buffer and incubated for 30 min at 65°C in water bath. The tubes were removed from the water bath and 400 μL of chloroform : isoamyl alcohol mixture (24 : 1 v/v) was added. It was then centrifuged at 12,000 rpm for 15 min at room temperature. The clear aqueous phase was transferred to a new sterile Eppendorf tube. Equal volume of ice cold isopropanol was added and mixed gently by inversion and then kept in the freezer for overnight. These supernatant was centrifuged at 10,000 rpm for 12 min. Finally, DNA pellets were observed at the bottom of the Eppendorf tube. After ethanol wash, the DNA pellets were dissolved in double distilled sterilized water. The PCR was carried out at initial denaturation temperature of 94°C for 5 min followed by amplification for 32 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min and final extension at 72°C for 5 min. Polymerase chain reaction amplifications were performed on the My Thermal Cycler. The amplified products were separated on 3% agarose gel stained with ethidium bromide. The gel was run in 1×TBE buffer. The size of the amplified products was determined by using 100-bp ladder. The gel was documented and banding pattern scored.

Statistical analysis

The data were analysed for WC gene to determine the fitness with diverse segregation ratios to determine mode of inheritance by χ^2 (chi-square) test as suggested by Fisher (1936). According to Goulden (1952), the variance existing in F_2 progenies is considered as phenotypic variance, whereas the average of variance of the parents involved in a particular cross was taken as environmental variance (Empig *et al.* 1970). Therefore, genotypic variance is calculated by subtracting the environmental variance from phenotypic variance. The GCV and PCV values were computed as per Burton and De vane (1953). Heritability and genetic advance as per cent of mean was estimated following the method of Johnson *et al.* (1955).

Results and discussion

In both the crosses, the true F_1s were identified using gene specific marker S5n InDel in the WC gene. These true F_1s were selfed and F_2 populations were generated. In F_2 population of each cross, 310 individual plants were evaluated for segregation for spikelet tip colour and purple base of the leaf sheath along with segregation for the InDel marker S5n. The primer was expected to amplify a fragment size of 281 bp in the WC positive plants. In F_2 of CB174R × Akshaydhan of 310 plants, 72 plants, were homozygous as S5n/S5n; 170 plants were heterozygous (S5i/S5n) and the remaining 68 plants were homozygous for S5i/S5i. All positive plants (S5n/S5−) expressed pink colour sheath and spikelet tip whereas the plants with S5i/S5i produced green leaf sheath and spikelet tip colour (table 1). In the F_2 population of CB203R × Akshaydhan, 78 plants were identified as homozygous for *indica* allele (S5i/S5i) and 232 plants were identified positive for WC gene (table 1). In that, 73 plants were homozygous (S5n/S5n) and 159 were heterozygous (S5i/S5n) (figure 1).

There are three alleles at the S5 locus: a neutral allele (WC allele, S5n), an *indica* allele (S5i) and a *japonica* allele (S5j). It has been shown that different allelic interactions at the S5 locus on chromosome 6 were the main genetic reason for *indica*/*japonica* hybrid sterility. Plants with genotypes S5n S5i and S5n S5j were fertile, but S5i S5j plants were partially or fully sterile because of partial abortion of gametes carrying the S5j allele. The incorporation of the S5n gene from WC varieties (WCV) could overcome embryo-sac sterility of intersubspecific hybrids (Ikehashi and Araki 1986). Therefore, whether the genotype containing S5n S5n is crossed with *indica* (S5i S5i) or *japonica* (S5j S5j), the hybrid would be fertile. This finding made it possible to mine new rice germplasm with the S5n gene using molecular markers and showed the importance of exploring new germplasm to overcome intervarietal hybrid sterility in rice. Proportion of WC gene in F_2 population in both crosses showed 1 : 2 : 1 segregation pattern. This indicated that WCV is governed by single dominant gene. Similar type of result was reported by Ikehashi and Araki (1984) and Vijaya Kumar and Virmani (1992). Simple and dominant nature of inheritance for WC would enable easy incorporation into any genotypes or varieties. Incorporation of diverse neutral alleles into a breeding line should help to solve the problem of hybrid sterility in the wide crosses (Dwivedi *et al.* 1999). Vijayakumar *et al.* (1999) reported that WC gene in male parent give desired higher expression of traits in hybrids and accordant with Yuan (2004) who reported that it was desirable to have both male and female parents of hybrids with WC gene to realize higher heterosis.

The variability and the genetic parameters estimation, which include phenotypic and genotypic coefficient of variations (GCV and PCV), heritability and genetic advance as per cent of mean are provided in tables 2 and 3. The PCV values were relatively higher than GCV values in F_2 generation of both the crosses coupled with narrow differences indicating less environmental influence on the expression of all the traits except primary branches per panicle.

Among the estimates of genetic parameters, heritability serves as a good index for transmission of character from one generation to the next generation, and it should be considered in terms of selection concept (Hanson 1959). Productive tillers exhibited wide variability and little influence of environment as well as higher values of PCV (35.55 and 28.03%) and GCV (34.23 and 27.34%) in CB174R × Akshaydhan and CB203R × Akshaydhan F_2 populations,

Table 1. Phenotypic and genotypic segregation for WC gene.

Crosses	F_1	F_2 observation		χ^2 value
CB 174R × Akshaydhan (green × purple)	Phenotype[#] Purple	Green	68	1.711***
		Purple	242	
		Total	310	
(S5i/S5i × S5n/S5n)	Genotype S5i/S5n	S5i/S5i	68	3.006***
		S5i/S5n	170	
		S5n/S5n	72	
		Total	310	
CB 203R × Akshaydhan (green × purple)	Phenotype[#] Purple	Green	78	0.004***
		Purple	232	
		Total	310	
(S5i/S5i × S5n/S5n)	Genotype S5i/S5n	S5i/S5i	78	0.367***
		S5i/S5n	159	
		S5n/S5n	73	
		Total	310	

[#]Basal leaf sheath and spikelet tip colour. *** Significance at $P = 0.001$.

respectively. High heritability (97.61 and 71.49%) coupled with high genetic advance (95.09 and 54.92%) as per cent of mean in CB174R × Akshaydhan and CB203R × Akshaydhan F_2 populations revealed that the major role of additive gene action in the genetic control of this trait. Similar results were also reported by Vaithiyalingan and Nandarajan (2006), Nayak (2008) and Nandeshwar *et al.* (2010).

Very low variability was noticed for the trait, days to 50 per cent flowering as evidenced by lower values of PCV (2.11 and 1.79%) and GCV (1.72 and 1.47%), high heritability (66.94 and 67.04%) with low genetic advance as per cent of mean (2.90 and 2.48%) in CB174R × Akshaydhan and CB203R × Akshaydhan F_2 populations, respectively, which indicated role of nonadditive with respect to this trait. Kannan Bapu and Soundrapandian (1993) also reported similar results with respect to this trait.

The F_2 population of CB203R × Akshaydhan had registered moderate PCV and GCV (10.67 and 10.49%) values for plant height. Results of the present study are on par with the earlier reports of Kannan Bapu and Soundrapandian (1993), Singh and Choudhary (1996). Contrary to this, low variability was observed in CB174R × Akshaydhan F_2 population. This trait exhibited high heritability coupled with moderate to high genetic advance expressed as per cent of mean in both the cross combinations indicating role of additive gene action in its genetic control.

Panicle length in both the crosses showed low to moderate values of PCV and GCV. This is in accordance with the earlier observations made by Paramasivan (1986). However, high heritability (99.51 and 94.65%), but medium to high genetic advance expressed as per cent of mean.

In case of flag, leaf length and breadth in both the crosses recorded low variability, it indicated that low PCV and GCV values for these traits. But both the crosses of these traits showed high heritability and moderate to high genetic advance as per cent of mean revealed major role of additive

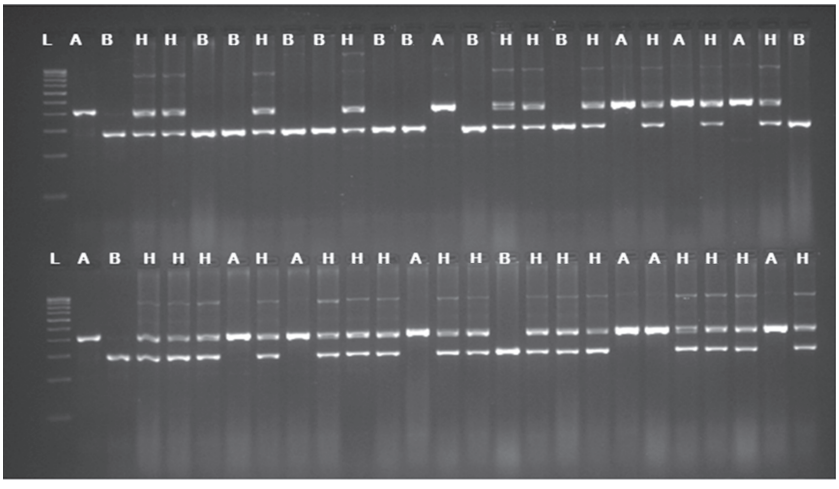

Figure 1. Segregation pattern of WC gene in F_2 population. L, 100bp; A, CB174R; B, Akshaydhan; H, heterozygote.

Table 2. Variability parameter of CB174R × Akshaydhan crosses.

S. no.	Character	Generation	Mean	Range	PV	PCV	GCV	H%	GA	GA%
1	Days to 50% flowering	F_1	107	105–110	4	1.87	1.43	58.33	2.40	2.25
		F_2	106.1	102–112	5.04	2.11	1.72	66.94	3.10	2.90
2	Plant height (cm)	F_1	115	111–120	13.14	3.15	1.94	37.86	2.83	2.46
		F_2	120.09	82–163	129.33	9.47	9.17	93.69	21.95	18.28
3	Total number of tillers (nos.)	F_1	21.25	18–25	7.64	13.01	12.43	91.28	5.20	24.46
		F_2	11.51	4–31	19.49	38.37	37.70	96.58	8.78	76.33
4	Number of productive tillers (nos.)	F_1	20.5	18–24	6	11.95	11.61	94.44	4.77	23.25
		F_2	10.50	3–28	13.94	35.55	35.13	97.61	7.51	71.49
5	Flag leaf length (cm)	F_1	38	36–41	4	5.26	5.10	93.96	3.87	10.19
		F_2	31.80	20–47	26.56	16.21	16.14	99.09	10.52	33.09
6	Flag leaf width (cm)	F_1	1.75	1.5–2	0.04	11.01	9.98	82.05	0.33	18.61
		F_2	1.31	1–2	0.04	16.19	14.94	85.11	0.37	28.39
7	Panicle length (cm)	F_1	31.75	28.5–33	4.07	6.36	6.31	98.69	4.10	12.92
		F_2	29.31	21.5–40.40	10.94	11.28	11.25	99.51	6.78	23.13
8	Primary branches per panicle	F_1	18.5	15–21	4	10.81	6.62	37.50	1.55	8.35
		F_2	12.08	6–17	2.64	13.44	3.05	5.15	0.17	1.43
9	Secondary branches per panicle	F_1	38.63	29–43	25.12	12.98	5.36	17.08	1.76	4.57
		F_2	32.33	13–77	51.49	22.19	17.12	59.54	8.80	27.22
10	Total number of spikelets per panicle	F_1	167.75	143–190	234.5	9.13	7.82	73.42	23.16	13.81
		F_2	193.63	103–331	1618.74	20.78	20.37	96.15	79.69	41.16
11	Number of filled grains per panicle (nos.)	F_1	148.00	138–157	50.86	4.82	2.92	36.75	5.40	3.65
		F_2	166.13	85–303	1371.56	22.29	22.03	97.65	74.50	44.85
12	100 grains weight (g)	F_1	2.09	1.97–2.30	0.014	5.72	2.04	12.73	0.03	1.50
		F_2	1.97	1.20–2.31	0.03	8.25	6.00	52.93	0.18	9.00
13	Single plant yield (g)	F_1	37.35	32.1–42.16	10.5	8.68	4.09	22.26	1.49	3.98
		F_2	23.06	8.5–43.00	46.45	29.56	26.84	82.43	11.57	50.19

Table 3. Variability parameter of CB203R × Akshaydhan crosses.

S. no.	Characters	Generations	Mean	Range	PV	PCV	GCV	H%	GA	GA%
1	Days to 50% flowering	F_1	103	101–105	2	1.38	0.89	41.67	1.21	1.18
		F_2	104.9	101–112	3.54	1.79	1.47	67.04	2.60	2.48
2	Plant height (cm)	F_1	82.78	74–86.5	22.74	5.76	5.14	79.48	7.81	9.43
		F_2	111.34	57–136	141.06	10.67	10.49	96.69	23.66	21.25
3	Total number of tillers (nos.)	F_1	18.67	17–21	3.47	9.97	8.96	80.77	3.10	16.60
		F_2	9.86	2–20	7.93	28.55	27.32	91.59	5.31	53.87
4	Number of productive tillers (nos.)	F_1	16.17	15–17	0.57	4.66	2.99	41.18	0.64	3.95
		F_2	9.30	2–16	6.79	28.03	27.34	95.09	5.11	54.92
5	Flag leaf length (cm)	F_1	37.32	35–39.5	2.47	4.21	3.86	84.05	2.72	7.29
		F_2	31.55	15–49	27.68	16.68	16.56	98.58	10.68	33.86
6	Flag leaf width (cm)	F_1	1.55	1.4–1.8	0.02	8.89	6.12	47.37	0.13	8.68
		F_2	1.24	0.2–1.9	0.04	16.68	14.62	76.75	0.33	26.38
7	Panicle length (cm)	F_1	30.12	28–31.5	1.70	4.33	3.93	82.47	2.22	7.36
		F_2	28.99	22.6–36	5.60	8.17	7.95	94.68	4.62	15.93
8	Primary branches per panicle	F_1	17	15–20	3.20	10.63	4.34	16.67	0.61	3.65
		F_2	12.50	8–18	3.34	6.56	14.62	20.13	0.76	6.06
9	Secondary branches per panicle	F_1	36.33	28–45	44.27	17.36	10.47	36.37	4.98	13
		F_2	31.61	14–49	43.46	20.86	12.37	35.19	4.78	15.12
10	Total number of spikelets per panicle	F_1	176.5	169–190	63.10	4.55	0.95	4.38	0.72	0.41
		F_2	195.08	102–390	1823.10	21.89	21.52	96.69	85.05	43.60
11	Number of filled grains per panicle (nos.)	F_1	160.33	148–173	65.07	5.10	1.92	14.19	2.36	1.49
		F_2	170.29	95–350	1717.91	24.34	23.94	96.75	82.61	48.51
12	100 grains weight (g)	F_1	2.13	2–2.3	0.01	4.72	1.61	11.59	0.02	1.13
		F_2	2.00	1–2.5	0.04	10.56	9.49	80.73	0.35	17.57
13	Single plant yield (g)	F_1	34.89	29.8–38.5	10.37	8.79	4.66	28.11	1.87	5.09
		F_2	29.48	10–54.5	60.98	26.49	24.82	87.77	14.12	47.90

gene action in the genetic control of these traits. Very low variability was noticed for primary, secondary branches per panicle and 100-grain weight as evidenced by lower values of PCV and GCV in CB174R × Akshaydhan and CB203R × Akshaydhan F_2 population. Also having low heritability and genetic advance indicated that high environmental influence, selection of the genotypes based on these traits would result in misleading.

High PCV and GCV values in CB174R × Akshaydhan and CB203R × Akshaydhan F_2 populations for total number of spikelets and number of filled grains per panicle indicated wide variability for this trait. Estimates of heritability and genetic advance as per cent of mean was also high in both the populations, respectively, revealing that most likely the heritability is due to additive gene effects and selection may be effective. This is in accordance with the earlier observations made by Nayak (2008). Wide variability was noticed for grain yield per plant as evidenced by relatively higher values of PCV (29.56 and 26.49%) and GCV (26.84 and 24.82%) in CB174R × Akshaydhan and CB203R × Akshaydhan populations, respectively. Estimates of heritability (82.43 and 87.77%) and genetic advance expressed as per cent of mean (50.19 and 47.90%) were relatively high in CB174R × Akshaydhan and CB203R × Akshaydhan, respectively, indicating major role of additive gene action in the genetic control of this trait in both the populations. These results are in conformity with the reports of Kumar *et al.* (2005), Vaithiyalingan and Nandarajan (2006) and Nayak (2008). It was suggested that total number of tillers and number of productive tillers per plant, flag leaf length and width, panicle length, total number of spikelets per panicle, number of filled grains per panicle and single plant yield must be an important selection criteria for high yielding genotypes while handling early generations in rice.

The present study reveals that inheritance of WC gene is monogenic dominant nature, thus, it can be easily transferred to desirable genotypes or restorer lines through backcross method, to further the development of high heterotic hybrids.

Acknowledgement

We thank the Department of Biotechnology (DBT), Ministry of Science and Technology, New Delhi for their financial assistance to carry out this research (no: BT/PR6091/ 254 GBD/27/376/2012 dated 10 September 2012.

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Received 1 January 2015, in final revised form 20 September 2015; accepted 24 September 2015
Unedited version published online: 1 October 2015
Final version published online: 16 May 2016