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Research Article

Study on combining ability of therapeutic traits in rice grains (*Oryza sativa* L.)

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Abstract
Traditional rice varieties having high flavonoids and anthocyanin traits were hybridized to high-yielding quality cultivars to assess their combining ability. Significant variation was observed among parents and hybrids for the traits studied. The predominance of non-additive gene action was observed for single plant yield, total flavonoid content, amylose content, whereas, trait anthocyanin registered additive gene action in the study. The genotypes ADT 37 and ADT (R) 45 were found to be good general combiners for flavonoids and anthocyanin content. The hybrid ADT (R) 45 x IG6 exhibited significant *sca* for flavonoids, anthocyanin and amylose per cent. The hybrid ADT (R) 45 x *Mikkuruvai* exhibited significant *per se* and *sca* for single plant yield and flavonoid content.

Keywords: Rice, Gene action, Amylose, Flavonoids, Anthocyanins, Combining ability

INTRODUCTION

Rice is a major staple food feeding half of the world's population (IRRI, 2019). Rice besides being an excellent source of complex carbohydrates is also rich in nutrients, vitamins and minerals thus popularly called as 'Grain of Life' (Ricepedia, 2020). It has an influential role in food security and is also recognized for its nutritional and medicinal qualities. The therapeutic value of rice has been recognized for ages as it has been mentioned in ancient literatures. Ayurveda also describes the therapeutic potential of this grain. It is considered as oleaginous, aphrodisiac, tonic, diuretic and fattening (Caius, 1986) agent in ayurvedic medicines. India is blessed with a large number of traditional varieties (Priya *et al.,* 2019). The presence of bioactive compounds in landraces elucidates the therapeutic potential of rice landraces (Pengkumsri *et al.,* 2015; Raghuvanshi *et al.,* 2017; Reshmi and Nandini, 2018 and Latiff *et al.,* 2019). The traditional varieties from different regions possess a varied nutritional and therapeutic profiles.

Collection, conservation and evaluation of these diverse rice accessions will benefit the breeding programmes focused on improving the health benefits of rice upon human consumption. Pigmented rice are reported to possess remarkable nutritional and therapeutic bioactive compounds *viz*., phenols, flavonoids and anthocyanins (Mbanjo *et al.,* 2020). The antioxidant property of pigmented rice helps in minimizing the occurrence of non communicable diseases thereby reducing the oxidative stress (Kiay *et al.,* 2019). Therefore, the present study aims at identifying parents with good general combining ability for therapeutic traits as well as to develop hybrids/ varieties with a remarkable therapeutic profile without compromising on yield.

MATERIALS AND METHODS

A hybridization programme was carried out following a Line x Tester mating design, involving six lines and four testers at the Department of Rice, Centre for Plant Breeding and Genetics, Coimbatore, during 2019 to

2020. Twenty-four hybrids were planted in randomized block design in two replications along with parents at a spacing of 20 cm x 20 cm during summer, 2020. Recommended package of practices was followed for the establishment and growth of the crop. Single plant yield (g) was recorded at maturity. Grain quality traits *viz.,* total flavonoids, anthocyanin and amylose content were estimated in the grains. In the parental genotypes and 24 crosses, good combiners and good cross combinations were deduced using *gca* and *sca* effects (Sprague and Tatum, 1942).

The grains of 24 hybrids and ten parents were harvested and dried to optimum moisture and were powdered to fine flour after subjecting to hulling processes. The quantification of total flavonoids, anthocyanin and amylose content was carried out using 250 mg of fine processed rice flour. Estimation of total flavonoid content was carried out by diluted methanol extract (Jia *et al.,* 1999). The total flavonoid was calibrated with a standard curve of quercetine and expressed as quercetine equivalent (mg of QE/100 g) per gram of rice. Total anthocyanin content in rice grains was estimated as per the method of pH differential suggested by Fuleki and Francis (1968). Amylose content was estimated as per the Juliano *et al.* (1981) and expressed as per cent.

RESULTS AND DISCUSSION

The genetic material exhibiting high *per se* performance and good combining ability for the trait of interest will be useful for trait based rice improvement programmes. The mean values of ten parents and 24 hybrids for yield, total flavonoid content, anthocyanin content and amylose content are depicted in **Tables 1 and 2**. The *per se* performance and *gca* effects are considered the most important and base criteria for the selection of parents by many breeders. The minimum and maximum values for single plant yield were observed in ADT 43 (29.30 g) and CO 51 (48.13 g) among lines and in *Nootripathu* (28.97 g) and *Mikkuruvai* (44.81g) among tester,s respectively. Lines and testers recorded an overall mean value of 37.59 g and 39.15 g, respectively for single plant yield. Among the parents, lines *viz.,* CO51 and IWP and testers namely *Mikkuruvai* and *Kavuni* recorded significantly higher mean values for single plant yield.

For total flavonoid content, the line ADT (R) 45 and testers, IG 6 and *Kavuni* were found to have significantly higher per *se* performance (**Table 1**). The genotype *Kavuni* exhibited a high mean value for anthocyanin content (**Table 1**). The antioxidant and anti- diabetic potential of *Karupu Kavuni* was reported by Reddy (2018) and highlighted that reduced level of total soluble sugars, low fat content and increased protein content in *Kavuni* make it a valuable landrace for exploitation in breeding programmes. The genotypes ADT (R) 45, ADT (R) 48, *Nootripathu*, IG 6 and *Mikkurvai* recorded significant *per se* performance for amylose content (**Table 1**). The testers IG6, *Kavuni*,

Table 1. Mean performance of parents for grain quality and therapeutic traits

Parents	Single plant yield (g)	Total flavonoid content (mg QE100/g)	Anthocyanin content (µg CGE/100g)	Amylose content (%)
LINES				
ADT 37	37.50	103.09	0.19	18.95
ADT 43	29.30	90.08	0.28**	21.01
ADT (R) 45	37.00	151.15**	0.18	21.88*
ADT (R) 48	31.50	100.49	0.20	22.51**
CO 51	48.13**	40.25	0.09	21.16
IWP	42.15**	104.83	0.20	19.17
Mean (Lines)	37.59	98.316	0.18	20.77
TESTERS				
Nootripathu	28.97	231.69	0.14	25.27**
IG6	39.86	405.87**	0.28	28.30**
Mikkuruvai	44.81**	243.22	0.16	27.06**
Kavuni	42.40**	389.80**	0.63**	8.97
Mean (Testers)	39.15	317.64	0.30	22.23
Mean (Parents)	38.26	186.05	0.23	21.36
SEd	0.904	6.173	0.015	0.523
CD at 5 %	1.890	12.40	0.0302	1.051
CD at 1%	2.520	16.544	0.0403	1.402

** Significant at 1% level *Significant at 5% level

Table 2. Mean performance of hybrids for grain quality and therapeutic traits

Hybrids	Single plant yield (g)	Total flavonoid content (mg QE100/g)	Anthocyanin content (µg CGE/100g)	Amylose content (%)
ADT 37 x Nootripathu	39.83**	328.05**	0.10	23.14**
ADT 37 x IG6	35.04	306.43**	0.25**	20.98
ADT 37 x Mikkuruvai	80.03**	274.04	0.09	24.25**
ADT 37 x Kavuni	8.45	303.98**	0.42**	18.68
ADT 43 x Nootripathu	35.47	247.11	0.03	24.97**
ADT 43 x IG6	86.59**	322.93**	0.14	22.23
ADT 43 x Mikkuruvai	36.09	313.22**	0.22*	20.90
ADT 43 x Kavuni	9.96	315.46**	0.38**	22.38
ADT (R) 45 x Nootripathu	35.32	258.66	0.09	23.83**
ADT (R) 45 x IG6	20.84	335.26**	0.28**	23.23**
ADT (R) 45 x Mikkuruvai	50.70**	307.48**	0.06	21.85
ADT (R) 45 x Kavuni	17.69	289.78*	0.40**	18.95
ADT (R) 48 x Nootripathu	47.45**	176.8	0.04	19.48
ADT (R) 48 x IG6	46.66*	221.58	0.20	20.93
ADT (R) 48 x Mikkuruvai	9.55	163.98	0.11	21.72
ADT (R) 48 x Kavuni	10.99	337.1**	0.40**	19.67
CO 51 x Nootripathu	47.60**	286.81	0.12	23.05*
CO 51 x IG6	18.59	277.52	0.20	22.44
CO 51 x Mikkuruvai	29.00	249.37	0.05	23.49**
CO 51 x Kavuni	24.00	187.25	0.37**	21.05
IWP x Nootripathu	67.59**	258.38	0.07	23.09*
IWP x IG6	41.50**	291.79**	0.18	22.06
IWP x Mikkuruvai	44.30**	270.26	0.09	18.51
IWP x Kavuni	25.50	278.38	0.34**	20.36
Mean (Hybrids)	36.20	275.07	0.19	21.71
SEd	0.940	6.1732	0.015	0.523
CD at 5 %	1.890	12.4082	0.0302	1.051
CD at 1%	2.520	16.5442	0.0403	1.402

Mikkuruvai exhibited significantly higher mean values for one or other therapeutic traits studied. *Kavuni* recorded significantly higher flavonoid and anthocyanin content and single plant yield with low amylose content. Hence, the breeding material derived using these testers would help in realizing good recombinants with elevated therapeutic value and also good grain yield.

Among the 24 hybrids, single plant yield ranged from 8.40 g (ADT 37 x *Kavuni*) to 86.59 g (ADT 43 x IG 6) (**Table 2**) with an overall mean value of 36.20 g. Ten cross combinations were reported to be highly significant for the trait single plant yield. Eleven cross combinations namely, ADT 37 x *Nootripathu*, ADT 37 x IG 6, ADT 37 x *Kavuni*, ADT 43 x IG 6, ADT 43 x *Mikkurvai* , ADT 43 x *Kavuni*, ADT (R) 45 x IG 6 , ADT (R) 45 x *Mikkurvai*, ADT (R) 48 x *Kavuni*, IWP x IG 6 and ADT (R) 45 x *Kavuni*, exhibited significantly higher mean values for total flavonoid content and nine hybrids *viz*., ADT 37 x IG 6, ADT 37 x *Kavuni*, ADT 43 x *Mikkuruvai*, ADT 43 x *Kavuni*, ADT (R) 45 x IG 6, ADT (R) 45 x *Kavuni*, ADT (R) 48 x *Kavuni*, CO 51 x *Kavuni* and Improved White Ponni / *Kavuni* recorded significant *per se* performance for the trait anthocyanin content. The amylose content of eight cross combinations was found to be positively significant. Unlike testers none of the hybrids possessed high amylase content. Hybrids recorded either low or intermediate amylose.

Kavuni based hybrids also recorded significant anthocyanin content as that of *Kavuni* making it a potential donor for anthocyanin improvement. Based on *per se* performance, ADT (R) 45 x IG 6 was considered as a superior hybrid for all the therapeutic traits assessed.

Analysis of variance for combining ability pointed out the existence of significant differences among parents, hybrids and parent *vs* hybrid for all the traits studied (**Table 3**). The GCA / SCA variance was found to be less than unity for total flavonoid content, anthocyanin content, amylose percentage and single plant yield indicating the predominance of non-additive gene action due to dominance, epistasis and various other interaction effects with non-fixable genetic variation. Non-additive gene action of amylose content observed in the study was in consonance with the results of Gahtyari *et al.* (2017) and Santha *et al.* (2017). Abhishek *et al.* (2017) reported non-additive gene action for total phenols and flavonoid content in *Brassica oleracea* var. *capitata* L. Epistatic interaction (non additive) for the inheritance of anthocyanin in pigmented rice was reported by Rahman *et al*. (2016) and Basunanda and Murti (2019).

The information on gene action helps in the selection of suitable breeding methods for flavonoid and anthocyanin content improvement in rice. The non-additive gene action of flavonoids, anthocyanin and amylose shows the presence of non-heritable and non-fixable gene action. Therefore these traits can be improved through recombination breeding by exercising selection at later generations or by exploitation of heterosis. Multi-parents crossing programmes can be advocated for combining therapeutic traits and yield superiority. The cross combination identified superior for single plant yield when it is crossed with good general combiners for therapeutic traits would increase the chances of developing elite genotypes with increased therapeutic properties.

The *gca* effects assume greater significance as it indicates the breeding value of genotypes which can be used in devising breeding programmes. Combining ability is important for the identification of superior parents among the genetic material for utilization in crop improvement programmes so as to obtain good cross combinations with desirable traits from them. Among parents, Improved White Ponni, IG6, *Mikkuruvai* and *Nootripathu* recorded significant positive *gca* effect for single plant yield. The lines ADT 37, ADT 43 and ADT (R) 45 exhibited a significant positive *gca* effect for flavonoid content. The genotypes ADT 37, ADT (R) 45, IG 6 and *Kavuni* showed significant *gca* effect for anthocyanin content. For amylose content, IG 6 and *Nootripathu* exhibited significant positive *gca* effect and high *per se* performance. Good general combiners were reported based on *per se* performance and *gca* effect for respective grain quality and therapeutic traits. Among the parental genotypes, IG 6 is a good combiner for yield, anthocyanin and amylose content (**Table 4**).

The specific combining ability effects of 24 hybrids were estimated for single plant yield, total flavonoid content anthocyanin content and amylose content (**Table 5**). Ten out of 24 hybrids evaluated *viz.,* ADT 37 x *Mikkuruvai* and ADT 43 x IG6, ADT (R) 45 x *Mikkuruvai*, ADT (R) 45 x *Kavuni*, ADT (R) 48 x *Nootripathu*, ADT (R) 48 x IG6, ADT (R) 48 x *Kavuni*, CO51 x *Nootripathu,* CO 51 x *Kavuni* and Improved White Ponni x *Nootripathu*, exhibited significant positive *sca* effect for single plant yield.

ADT 37 x *Nootripathu*, ADT 43 x *Mikkuruvai*, ADT (R) 45 with IG6 and *Mikkuruvai*, ADT (R) 48 x *Kavuni,* CO51 x *Nootripathu* and CO51 x *Mikkuruvai* showed significant positive *sca* effects for flavonoid content. The hybrids ADT 43 x *Mikkuruvai*, ADT (R) 45/IG6, CO51 x *Nootripathu* and ADT 37 x IG6 showed significant positive *sca* effects for anthocyanin content. For amylose content, ten hybrids exhibited a positive significant *sca* effect. Gahtyari *et al.* (2017), reported hybrids with a high significant *sca* effect for amylose content.

Among 24 hybrids evaluated based on *per se* performance and *sca* effect, six hybrids were found to be good specific combiners for amylose content. Good specific combiners for amylose content were also reported by Menaka

Table 3. Analysis of variance for combining ability for grain quality and therapeutic traits

Sources of variation	Single plant yield	Total flavonoid content	Anthocyanin content	Amylose content
Replication	0.0021	11.5268	0.0004	0.0188
Hybrids	880.17**	4674.9094**	0.0339	7.0887**
Lines	402.92**	8074.4113**	0.0019	6.4440**
Testers	2223.17**	3219.4139**	0.2330	16.45**
L x T interaction	770.64**	3832.84.12**	0.0047	5.430**
Error	0.8210	47.6577	0.0002	0.298
GCA	4.1431	31.8546	0.0011	0.0627
SCA	384.91	1892.59	0.0022	2.5663
GCA / SCA	0.0107	0.01683	0.50	0.0244

** Significant at 1% level

Table 4. General combining ability effects of parents for grain quality and therapeutic traits

Parents	Single plant yield	Total flavonoid content	Anthocyanin content	Amylose content
LINES				
ADT 37	4.63	28.06**	0.09**	0.15
ADT 43	5.83	24.61**	-0.04	0.76**
ADT (R) 45	-5.06	22.73**	0.07**	0.25
ADT (R) 48	-7.53*	-50.20**	-0.01	-1.36**
CO 51	-6.40*	-24.20**	-0.01	0.97**
IWP	8.53**	-0.36	-0.12**	-0.76**
SE	2.91	2.4407	0.004	0.193
TESTERS				
Nootripathu	9.35**	-15.77	-0.12**	1.27**
IG6	5.34*	17.52	0.07**	0.38*
Mikkuruvai	5.42*	-12.01	-0.09**	-0.10
Kavuni	-20.10**	10.25	0.19**	-1.54**
SE	2.38	1.9929	0.009	0.157

** Significant at 1% level, *Significant at 5% level

Table 5. Specific combining ability effects of hybrids for grain quality and therapeutic traits

Hybrids	Single plant yield	Total flavonoid content	Anthocyanin content	Amylose content
ADT 37 x Nootripathu	-10.67 **	40.69 **	0.01	0.13
ADT 37 x IG6	-10.63 **	-14.22 **	0.02 *	-1.20 **
ADT 37 x Mikkuruvai	33.61 **	-17.07 **	-0.04 **	2.46 **
ADT 37 x Kavuni	-12.32 **	-9.40	0.01	-1.38 **
ADT 43 x Nootripathu	-15.72 **	-36.80 **	-0.04 **	0.95 *
ADT 43 x IG6	38.46 **	5.73	-0.07 **	-0.61
ADT 43 x Mikkuruvai	-10.97 **	25.55 **	0.11 **	-1.88 **
ADT 43 x Kavuni	-11.77 **	5.52	-0.01	1.54 **
ADT (R) 45 x Nootripathu	-5.25 **	-23.37 **	-0.01	1.01 *
ADT (R) 45 x IG6	-15.67 **	19.95 **	0.06 **	0.85 *
ADT (R) 45 x Mikkuruvai	14.40 **	21.70 **	-0.06 **	-0.20
ADT (R) 45 x Kavuni	6.52 **	-18.27 **	0.01	-1.65 **
ADT (R) 48 x Nootripathu	9.51 **	-32.30 **	-0.03 **	-2.32 **
ADT (R) 48 x IG6	12.78 **	-20.80 **	-0.01	0.05
ADT (R) 48 x Mikkuruvai	-24.71 **	-48.88 **	0.01	1.45 **
ADT (R) 48 x Kavuni	2.43 **	101.98 **	0.02	0.82 *
CO 51 x Nootripathu	8.03 **	52.34 **	0.05 **	-0.58
CO 51 x IG6	-16.27 **	9.77	-0.01	-0.49
CO 51 x Mikkuruvai	-6.35 **	11.14 *	-0.04 **	0.87 *
CO 51 x Kavuni	14.59 **	-73.25 **	-0.01	0.20
IWP x Nootripathu	14.11 **	-0.56	0.02	0.82 *
IWP x IG6	-8.68 **	-0.43	-0.01	1.40 **
IWP x Mikkuruvai	-5.98 **	7.57	0.01	-2.70 **
IWP x Kavuni	0.55	-6.58	-0.02	0.48
SE	0.6407	4.8815	0.0095	0.3861

** Significant at 1% level, *Significant at 5% level and Ibrahim (2016) and Kitara *et al.*(2019). The cross combination ADT (R) 48 x *Kavuni* was obtained as a good specific combiner for flavonoid and anthocyanin content. Based on *per se* and *sca* effect, the cross combinations ADT 43 x *Mikkurvai* and ADT (R) 45 x IG 6 were identified as elite specific combiners for both flavonoid and anthocyanin content. The present study might be the first to report the general combiners for the flavonoids (IG 6 and *Kavuni*) and anthocyanin content (*Kavuni*) in rice. The hybrid ADT (R) 45 x IG6 exhibited good *per se* performance and significant *sca* effect for the traits *viz.,* flavonoids, anthocyanin and amylose percentage. Since ADT (R) 45 had a significant *gca* effect for flavonoid and amylose content, the cross ADT (R) 45 x IG6 can be promoted for recombination breeding.

Hybrids with high *sca* effect could be a combination of parents with high/high, high/low, low/high or low/low *gca* effect. Both parents with high *gca*, result in additive/additive gene action which is heritable fixable epistasis. And one parent with a high *gca* effect will have an additive effect which overcomes the epistatic effects of poor general combiner (Fasahat *et al.,* 2016). Kannan and Ganesh (2016), Vadivel *et al.* (2018) and Bano and Singh (2019) also reported good specific combiners with low/high *gca* effect. In the present study, hybrids with high *sca* and similar *gca* combinations were obtained. The elite cross combination ADT (R) 48 x *Kavuni*, is a combination of low/high *gca* combination for flavonoid and anthocyanin content. This indicates the presence of dominance/additive type of gene action. Similarly the parents with high *gca* effects but with non significant *per se* performance are advocated for recombination breeding as reported by Suvathipriya and Kalaimagal (2018) and Deepika *et al.* (2019). It was suggested that high *per se* performance of hybrids with non significant *sca* effect may be due to the positive or complementary interaction of the alleles. Therefore, selection in advanced generations may be fruitful.

Recombination breeding helps to isolate superior segregants which possess additive genetic variance which is fixable in a later generation. Desirable *gca* effect was obtained in the parents (lines) ADT 37, ADT 43, ADT (R) 45 for total flavonoid content and parental lines IWP, *Nootripathu*, IG6, *Mikkuruvai* for single plant yield. Even though the testers did not record significant *gca* for flavonoids, hybrids recorded significant *sca* showing non-additive gene action. The exploitation of segregants from ADT37 x *Nootripathu*, ADT 43 x *Mikkuruvai*, ADT (R) 45 crossed with IG6 and *Mikkuruvai* will yield a superior line with enhanced flavonoids, anthocyanin and amylose among which ADT 43 x *Mikkuruvai* and ADT (R) 45 x *Mikkuruvai* showed significant *gca* effects for single plant yield. ADT 37 x *Mikkuruvai*, ADT (R) 45 x *Mikkuruvai*, ADT (R) 48 x *Kavuni* and CO 51 x *Nootripathu* showed significant *gca* effect for single plant yield and any one of the therapeutic traits. ADT 37 x IG 6, ADT 37 x *Kavuni*, ADT 43 x IG 6, ADT 43 x *Kavuni*, ADT (R) 45 x *Kavuni* hybrids with non significant *sca* effects can be exploited for total flavonoid content improvement through recombination breeding.

In the current era, people are facing several lifestyle related chronic diseases and therefore started following diet guidelines to boost their immunity through their daily intakes. The donor parents *Mikkurvai,* IG 6 and *Kavuni* manifesting high *gca* effect for various therapeutic traits studied could be exploited *via* hybridization programmes for the improvement of therapeutic value in staple food rice. The hybrid ADT (R) 48 x *Kavuni* exhibited a significant *sca* effect for all the three therapeutic traits whereas ADT 43 x *Nootripathu* for amylose content widens the opportunity to exploit these traits through heterosis breeding. The hybrid ADT 45 x *Mikkurvai* besides showing yield superiority was found to be the best for total flavonoid content. Hybrids ADT 37/ *Mikkurvai* and IWP x *Nootripathu* were found best for amylose content with significant single plant yield. Hence these hybrids can be exploited for sustaining food security as well as for ensuring health benefits which is the present day requirement of the consumers and stakeholders and may be commercially exploited after large scale evaluation. Even though there were many reports on landraces/cultivars with therapeutic value, attempts on combining yield potential with that of therapeutic value using these landraces in rice are very less and therefore the present study assumes greater significance.

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