

RESEARCH ARTICLE II

Characterizes the Antioxidant Activity of Recombinant Inbred Rice Lines Derived from Traditional *Kavuni* and CO 50

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ABSTRACT

The present study aimed at investigating the antioxidant activity of traditional *black Kavuni*, CO 50, and their Recombined Inbred Lines. The total antioxidant assay was carried out in twenty-five rice samples. Hydroxyl radical scavenging activity, Superoxide radical scavenging activity, Nitric oxide radical scavenging activity, and total antioxidant activity were assessed by traditional *black Kavuni*, CO 50 and 145-3 Recombined Inbred Line by using standard procedure. The highest percentage of inhibition was observed in black color rice samples, which ranged from 51 to 83 percent. The 145-3 Recombined Inbred Line and *kavuni* had the highest antioxidant activity. In Hydroxyl radical scavenging activity, Superoxide radical scavenging activity, Nitric oxide radical scavenging activity, and total antioxidant activity, the highest inhibition was observed in methanolic extract of *Kavuni* parent and selected 145-3 Recombined Inbred Line.

Keywords: *Black Kavuni*; CO 50; Anti-oxidant activity; Recombined Inbred Line

INTRODUCTION

In India, the traditional rice varieties or pigmented rice varieties were reported to harbor a vast amount of genetic diversity, including nutritional qualities, medicinal properties, aroma (Roy *et al.*, 2015), and an un-adulterated gene pool for valuable characters (Das and Das, 2014). The color of the rice is imparted mainly due to the presence of anthocyanin or proanthocyanin production on the outermost layer of rice which imparts the color (Chaudhary, 2003).

Traditional rice varieties are rich in minerals, bioactive components, and antioxidants (Vichapong *et al.*, 2014). Black rice is one of the pigmented rice varieties and it is considered traditional rice in China, Thailand, and India (Rajeswari *et al.*, 2018). It belongs to *Oryza sativa* species, scientifically called *Zizania aquatica*. The anthocyanin pigment of black rice varieties is promoted by allele (alternate form of gene) of OsB2 also called *Kala4* gene.

All over the world, more than 200 varieties of black rice are available (Zhang *et al.*, 2020). It is

mainly cultivated in Southeast Asian countries like China, Thailand, and India. In India black rice is cultivated in North-eastern states such as Manipur, Mizoram, Meghalaya, Assam and (Saha, 2016). In Odisha it was named Kalabati (kala means 'black and bati means 'rice').

In South India, this rice was cultivated only in limited areas like Karaikudi district of Tamil Nadu and is popularly called '*black Kavuni* rice' (Kumar and Murali, 2020). Black *Kavuni* is a blackish purple traditional pigmented variety in Tamil Nadu. It is called emperor rice of Tamil Nadu. The *Kavuni* black pericarp contains higher levels of phytochemicals, iron, zinc, calcium, copper, sodium, potassium, and magnesium. These therapeutic and nutritional properties of *Kavuni* rice attract the researcher and consumers.

Among the farmers, the cultivation of *Kavuni* is not successful- because of low yield agronomic features (Kumar and Murali, 2020). In order to increase the production of *Kavuni* rice, the twenty-three RILs were developed through bio-fortification

strategy. Therefore, the study aimed at studying the antioxidant properties of Recombinant Inbred Lines and their parent.

MATERIAL AND METHODS

Paddy varieties such as black Kavuni (traditional variety), CO 50 (high yielding variety) and Recombinant inbred lines (RILs) viz., 13-2, 13-3, 13-5, 31-3, 31-6, 32-2, 35-2, 35-3, 39, 40, 131-4, 143-1, 144, 144-1, 144-2, 144-3, 144-5, 145-2, 145-3, 145-6, 148-2, 163-5 and 271-2 were received from the Department of Plant Biotechnology, CPMB, TNAU, Coimbatore. The DPPH assay was carried out based on the method suggested by Lim *et al.*, (2007). The hydroxyl radical scavenging activity was measured by the procedure given by Halliwell *et al.*, (1987). The superoxide anion scavenging activity and Nitric oxide scavenging activity were assessed by the method of Nishikimi *et al.*, (1972) and Marocci *et al.* (1994). The total antioxidant activity was measured using ABTS assay (Gulcin *et al.*, 2007).

RESULTS AND DISCUSSION

Antioxidant activity of RILs and parent rice varieties

The DPPH assay indicated the total antioxidant activity of twenty five rice samples, and mean values ranged from 0.16 to 83.04 percent per 1000 $\mu\text{g}/\text{mL}$ (Figure 1). In twenty three recombinant inbred lines, the antioxidant activity varied from 0.16 to 93.04 percent per 1000 $\mu\text{g}/\text{mL}$. The highest percent of antioxidant activity was observed in 145-3 (82.03%) RIL followed by 145-6 (145-6%) RIL, which was on par with Kavuni parent (83.04%). Reddy *et al.*, (2015) who have observed that the Manipur pigmented rice varieties (Chak-hao Amubi, Chak-hao poireiton and Cha-ha Angangha) had antioxidant activity ranging from 89.28 to 92.67 percent (DPPH). The black Kavuni rice had 95.48 percent of antioxidant activity (Meera *et al.*, 2019). Raghuvanshi *et al.*, 2017 reported that white rice had 20 percent of DPPH scavenging activity and red rice had 25 percent of scavenging activity. Saikia *et al.*, (2012) showed that the pigmented rice (Poreton chakhao and Chak-ha amubi) and non-pigmented rice (Bakul joha and keteki Joha) had antioxidant activity ranging from 94.19 to 96.43 and 30 to 35 percent respectively.

Hydroxyl radical scavenging activity of parent rice varieties and selected RIL (145-3)

The IC₅₀ value of methanol extract in rice samples ranged from 0.68 mg/mL to 174 mg/mL (Table 1). The selected RIL had IC₅₀ value of 0.651 $\mu\text{g}/\text{mL}$ and

73.52 percent of radical scavenging activity on 1 $\mu\text{g}/\text{mL}$. Krishnanunni *et al.*, (2015) reported that black Kavuni (IC₅₀-0.45mg/mL) had ability to scavenge the hydroxyl radical. Compared to kuzhiyadichan rice, it had a reduced ability to increase the concentration of Kavuni extract. And also showed that the phenolic compounds contain a positive correlation with antioxidant activity. Cho *et al.*, (2012) reported that the methanolic extract of black rice showed the strongest scavenging ability against hydroxyl radicals. Similarly in this study, the highest percent of hydroxyl radical scavenging activity was observed in Kavuni parent and 145-3 RIL, which might be a higher content of total anthocyanin.

Superoxide radical scavenging activity of parent rice varieties and selected RIL (145-3)

The superoxide radical scavenging activity percentage of three rice samples ranged from 0.106 to 19.230 percent (Table 2). The highest percent of inhibition was observed in Kavuni parent and selected RIL 145-3. Based on the IC₅₀ values the order of rice samples is as follow: CO 50 > 145-3 = Kavuni. Park *et al.*, (2008) reported that the methanolic extract of black rice contains the highest super scavenging activity than alpha-tocopherol due to the presence of anthocyanin. Pramai and Jiamyangyuen, (2016) reported that, when compared to red and white rice, black rice contains the highest amount of total phenolic and flavanoids compounds. The L* (lightness) value of pericarp revealed a negative correlation with antioxidant activities. Similarly the present study shows that the Kavuni and 145-3 RIL had the lowest L* (lightness) value due to the presence of black pericarp or anthocyanin. It was increased inhibition percent against superoxide radical.

Nitric oxide radical scavenging activity of parent rice varieties and selected RIL (145-3)

Among parents, the Kavuni parent had the highest percent of inhibition and it was 12.5 percent, while the CO 50 parent had the lowest percent of inhibition, which was 0.155 percent (Table 3). The selected RIL 145-3 had a higher percent of inhibition which was 14.70 percent. Reddy, (2018) reported that the karuppu Kavuni had the ability to inhibit the nitric oxide radicals. A higher percentage of inhibition was observed (83%) in 1 mg/mL. Same way in this study the Kavuni parent and 145-3 RIL had a higher percentage of inhibition against nitric oxide due to the presence of total phenolic content.



ABTS radical scavenging activity of parent rice varieties and selected RIL (145-3)

Among parent rice varieties, the highest inhibition observed in methanol extract of *Kavuni* rice was 23.80 percent. It had IC₅₀ value of 2.1 µg/mL (Table 4). The selected RIL had IC₅₀ value of 2.2 µg/mL and 22.72 percent of radical scavenging activity at 1µg/mL. Pramai and Jiamyangyuen, (2016) reported that the Thailand black rice varieties contain higher total antioxidant activity than the red and white rice varieties. Among black rice varieties, the total antioxidant activity differed in the range between 5 to 12 mmol of Trolox equivalent Sompong et al., (2011). In similar way to the present study, higher total antioxidant activity was observed in *Kavuni*,

and 145-3 RIL may be the presence of phenolic compounds

Figure 1. Total antioxidant activity (DPPH) of RILs and parent rice varieties

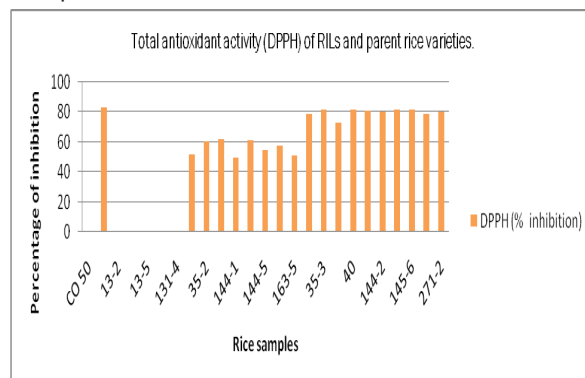


Table 1. Hydroxyl radical scavenging activity of parent and selected RIL (145-3)

S.No	Parent /RILs	Extracts	Concentration (mg/mL)	Inhibition (%)	IC ₅₀ (mg/mL)
1.	Kavuni	Methanol	0.2	15.38	0.65
			0.4	30.76	
			0.6	46.15	
			0.8	61.53	
			1.0	79.92	
2.	CO 50	Methanol	0.2	0.057	174
			0.4	0.115	
			0.6	0.172	
			0.8	0.229	
			1.0	0.287	
3.	145-3 (RIL)	Methanol	0.2	14.71	0.68
			0.4	29.42	
			0.6	44.12	
			0.8	58.83	
			1.0	73.52	

Table 3. Superoxide radical scavenging activity of parent and selected RIL (145-3)

S.No	Parent /RILs	Extracts	Concentration (mg /mL)	Inhibition (%)	IC ₅₀ (mg/mL)
1.	Kavuni	Methanol	0.2	3.846	2.6
			0.4	7.692	
			0.6	11.538	
			0.8	15.384	
			1.0	19.230	
2.	CO 50	Methanol	0.2	0.021	470
			0.4	0.042	
			0.6	0.063	
			0.8	0.085	
			1.0	0.106	



3.	145-3 (RIL)	Methanol	0.2	3.448	2.9
			0.4	6.896	
			0.6	10.344	
			0.8	13.793	
			1.0	17.241	

Table 4. Nitric oxide radical scavenging activity of parent and selected RIL (145-3)

S.No	Parent /RILs	Extracts	Concentration (mg /mL)	Inhibition (%)	IC50 (mg/mL)
1.	Kavuni	Methanol	0.2	3.125	3.2
			0.4	6.25	
			0.6	9.375	
			0.8	12.5	
			1.0	15.635	
2.	CO 50	Methanol	0.2	0.031	322
			0.4	0.062	
			0.6	0.093	
			0.8	0.124	
			1.0	0.155	
3.	145-3 (RIL)	Methanol	0.2	2.94	3.4
			0.4	5.88	
			0.6	8.82	
			0.8	11.76	
			1.0	14.70	

Table 5. ABTS radical scavenging activity of parent and selected RIL (145-3)

S.No	Parent /RILs	Extracts	Concentration (mg mL)	Inhibition (%)	IC50 (mg/mL)
1.	Kavuni	Methanol	0.2	4.76	2.1
			0.4	9.52	
			0.6	14.28	
			0.8	19.04	
			1.0	23.80	
2.	CO 50	Methanol	0.2	0.022	445
			0.4	0.044	
			0.6	0.067	
			0.8	0.089	
			1.0	0.11	
3.	145-3 (RIL)	Methanol	0.2	4.54	2.2
			0.4	9.09	
			0.6	13.63	
			0.8	18.18	
			1.0	22.72	

CONCLUSION

From the above study, it was completed that the recombinant inbred line 145-3 was similar to that of Kavuni parent in the context of antioxidant activity in DPPH assay, Hydroxyl radical scavenging activity, Superoxide radical scavenging activity, Nitric oxide radical scavenging activity and ABTS radical scavenging activity. Therefore the yielding character of Kavuni rice variety is without modification of therapeutic and nutrition properties. In view of the polished rice based foods and rice, consumption turned out to be the incidence of obesity and cardiovascular diseases. The 145-3 RIL owned high antioxidant properties than the white rice varieties. In this connection, the 145-3 RIL rice to be best functional food among diseased consumers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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