Original Research Article

Biochemical properties of Traditional rice varieties of Tamil Nadu

ABSTRACT

Rice is a reliable dietary source in most of the underdeveloped nations, where the majority of people are deficient in minerals and vitamins. Hence, it is necessary to focus on the development of nutrient rich rice for value addition in order to alleviate malnutrition. Dietary fibre, minerals, carotenoids, flavonoids and polyphenols are abundant in traditional rice cultivars and consumption of these traditional rice cultivars, on the other hand, benefits human health. Now-a-days, traditional rice varieties are gaining importance and it is cultivated by a large number of farmers. These varieties play a major role in the history of crop improvement and seed production. Hence, an experiment has been conducted to analyse the bio chemical properties *viz.,* α-amylase activity, dehydrogenase activity and total phenol content in sixteen traditional rice varieties *viz.,* Mappillai samba, Kichadi samba, Vellaikavuni, Rajamannar, Sembuli samba, Kattuyanam, Karunkuruvai, Norungan, Poongar, Kuzhiyadichan, Chithiraikar, Paalthondi, Maranel, Salem sanna, Kallurundai kar and Illuppai poo samba which were collected from the gene pool of Department of Plant Genetic Resource, Tamil Nadu Agricultural University, Coimbatore. Among the varieties tested, the high α-amylase activity and dehydrogenase activity were recorded by Sembuli samba, Poongar, Maranel and Karunkuruvai and Maranel, Salem sanna and Vellaikavuni, respectively. The total phenol content was maximum in Sembuli samba, Kattuyanam, Mappillai samba and Kallurundai kar. The results of this experiment revealed that the above traditional rice varieties have to be conserved to preserve its precious genome information due to their superior performance of α-amylase activity and dehydrogenase activity as well as maximum phenol content.

Keywords: *Traditional rice, α-amylase activity, dehydrogenase activity, Total phenol content*

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is an important agricultural crop and the world's most important food crop. Rice is also a staple food for more than half of the world's population, rely on it as their main source of calories and protein use of traditional varieties have become recent trend among consumers because of their numerous health benefits such as low sugar content which are highly preferred by diabetic and overweight patients [1]. Seeds are a rich source of natural bioactive compounds such as secondary metabolites and antioxidants [2]. Phenolic compounds are the most

abundant secondary metabolites in plants, playing a key role in pigmentation, growth and reproduction of the plant, together with resistance to pathogens and predators. Coloured rice is considered as a functional food in many Asian countries [3] due to their several health promoting benefits associated with anthocyanins [4], anti-carcinogenic, anti-inflammatory and anti-oxidative properties [5]. These varieties have high amount of glutamic acid, vitamins, fiber and bioactive components like polyphenols, tocopherols, oryzenols, tocotrienols and flavonoids which play an important role in preventing chronic diseases. They also aid in the protection against cytotoxicity [6], anti-neurodegenerative activity and possess antioxidant and scavenging activity which are higher than the white rice varieties [7]. Moreover, pigmented rice varieties contain many pigmented compounds like Cyanidin-3-O-β-D-glucopyranoside [8]. The richness of anthocyanin pigment in the grain gives the rice a black colour in the variety Karuppu kavuni. It helps against asthma, atherosclerosis, diabetes, hypertension and acts as an excellent detox for liver. Similarly, Mappillai samba controls sugar level and also increases haemoglobin content [9].

Traditional rice varieties have gained a fresh appreciation for their rich sources of agronomic features like medicinal qualities, fragrance and stress resistance. Due to the introduction of high yielding, semi-dwarf rice varieties during the first green revolution [10], these ancient rice types were overlooked and planted only infrequently. Until now, the goal has been to bring back historic rice types for a variety of reasons, including their resilience to unfavorable climatic circumstances such as floods, droughts, medicinal capabilities and nutritional value. The role of phenolic compounds as antioxidants includes maintaining the quality and nutritional values of seed by preventing or delaying its deterioration and protecting body tissues from oxidative damage [11, 12]. The current study has been focused on the biochemical characterization of traditional rice types by measuring α-amylase, dehydrogenase and total phenols.

2. MATERIALS AND METHODS

2.1 Rice material

The rice samples used in this study were obtained from Department of Plant Genetic Resource, Tamil Nadu Agricultural University, Coimbatore. The list of rice sample used in this study is given in Table1. Figure 4 represents the traditional rice varieties used in this study. The present investigation of biochemical properties of the seeds of traditional varieties were conducted at Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore.

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Table 1. List of traditional rice varieties used in this study

S. No	TNAU PGR ID	Vernacular Name
1.	TNAUF00103038	Mappillai samba
2.	TNAUF00103126	Kichadi samba
3.	TNAUF00103046	Vellaikavuni
4.	TNAUF00102989	Rajamannar
5.	TNAUF00103036	Sembuli samba
6.	TNAUF00103006	Kattuyanam

7.	TNAUF00103065	Karunkuruvai
8.	TNAUF00100247	Norungan
9.	TNAUF00103047	Poongar
10.	TNAUF00103030	Kuzhiyadichan
11.	TNAUF00103065	Chithiraikar
12.	TNAUF00103083	Paalthondi
13.	TNAUF00103053	Maranel
14.	TNAUF00103040	Salem sanna
15.	TNAUF00100243	Kallurundaikar
16.	TNAUF00103051	Illuppai poo samba

2.2 Determination of α- amylase activity (mg maltose min^{-1})

The pre-germinated seed samples were weighed for 500mg in three replicates and were homogenised in 1.8ml of cold 0.02M sodium phosphate buffer (pH 6.0) and centrifuged at 20,000 rpm for 20 min for extracting the enzymes. To 0.1ml of enzyme extract, 1ml of 0.067 per cent starch solution was added. The reaction was stopped after 10 min. of incubation at 25°C by addition of 1ml of iodine HCl solution (60mg KI and 6mg I_2 in 100ml of 0.05N HCl). The colour change was measured at 620 nm using a UV-Double beam Spectrophotometer 2205. The α-amylase activity was computed and expressed as mg maltose min^{-1} adopting the following formula [13].

$$\alpha - \text{amylase activity (mg maltose min}^{-1}) = \frac{\text{OD value}}{\text{Volume of sample pipetted out}} \times \frac{1000}{500}$$

2.3 Determination of Dehydrogenase activity (OD value)

For estimating the dehydrogenase activity, 0.5% 2, 3, 5-triphenyl tetrazolium chloride solution was dissolved in Sorenson's buffer. Representative seeds from each treatment were taken and preconditioned by soaking in water for 7 h. Seeds were bisected longitudinally into two halves, one half with embryo was steeped in tetrazolium solution and kept in dark for 2h at 40°C for staining. After staining, the excess solution was drained and the seeds were washed thoroughly with distilled water and transferred to a test tube containing 10 ml of 2-methoxy ethanol (methyl cellosolve). The test tube was closed air tight and allowed to remain in the incubator in darkness overnight for extracting the red colour formazon. The coloured solution was decanted and the colour intensity was measured in an ELICO UV-VIS spectrophotometer (Model SP- 2205) using blue filter (470 nm) and methyl cellosolve as the blank. The OD value obtained was reported as dehydrogenase activity [14].

2.4 Determination of Total phenol content

The seeds were dried in hot air oven and powdered with the help of pestle and mortar. Dried powder of about 50 mg was boiled in a water bath with 10 ml of 80 % ethyl alcohol. The homogenate was first cooled and then centrifuged at 600 rpm for 15 min. The supernatant was saved and made upto 20 ml with 80 % ethyl alcohol. 1 ml of alcoholic extract was taken in a test tube then 1 ml of Folin-Ciocalteau reagent (Commercial Folin-Ciocalteau was diluted with distilled water in 1:2 ratio) and 2 ml of 20 % sodium carbonate was added and it was shaken well. The mixture was heated in a boiling water bath for 1 min and cooled under running tap water. The blue solution was diluted to 25

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ml with distilled water and read at 650 nm in a UV-VIS spectrophotometer (Model Systronics 2205). The standard curve was prepared using different concentrations of gallic acid and the phenol content in the sample was expressed in mg 100 g^{-1} seed material.

3. RESULT AND DISCUSSION

3.1. α- amylase activity

α -amylase is responsible for majority of carbohydrate metabolism in the endosperm of rice seeds. The overall vigour status of rice seeds are improved by this enzyme [15]. The α-amylase activity will act as a biochemical indication for identifying the capacity for rice seed germination, which leads to seed vigour differences [16]. In the present investigation, the highest α-amylase activity was observed in the traditional rice variety Sembuli samba (4.88 mg maltose min^{-1}) followed by Poongar (3.40 mg maltose min^{-1}) which was on par with Maranel (3.40 mg maltose min^{-1}) and Karunkuruvai (2.28 mg maltose min^{-1}) (Table 2). The lowest activity was recorded in Kattuyanam (1.50 mg maltose min^{-1}) and Mappillai samba (0.85 mg maltose min^{-1}) (Figure 1). The α -amylase activity recorded was superior in the traditional rice varieties *i.e.,* Sembuli samba, Poongar and Maranel when compared with the recent varieties viz., CO 47 and CO 51. The major site for α -amylase expression has always been thought to be in the aleurone layer in germinating rice seed [17]. Higher α-amylase activity was associated with higher germination of rice seeds. Higher amylase activity in the whole seeds (with hull) rather than hull removed one were registered [18].

3.2 Dehydrogenase activity

The dehydrogenase enzyme activity is a good stable metabolic marker to estimate the degree of vigour in seeds [19] and have positive association with vigour and viability of seeds [20]. The maximum dehydrogenase activity was recorded by Maranel (0.609), Salem sanna (0.596), Vellaikavuni (0.562), Karunkuruvai (0.522), Poongar (0.522) and the mimimum activity was found in Kichadi samba (0.312) and Mappillai samba (0.277) (Figure 2). When compared with recent varities *viz.,* CO 47 and CO 51 the above traditional rice varieties registered less dehydrogenase enzyme activity.

3.3 Total phenol content

Phenols are a category of natural antioxidants, which leads to pharmacological actions. Ferulic and coumaric acids are abundant in grains with light brown pericarp, but the anthocyanins *i.e.,* cyanidin-3-O-d-glucoside and peonidin-3-O-d-glucoside are predominant in red and black pericarp rice [21],[22]. The highest total phenolic content was observed in Sembuli samba (45.36 mg/100g), Kattuyanam (42.34 mg/100g), Mappillai samba (38.84 mg/100g), Kallurundai kar (38.24 mg/100g) and the lowest was recorded by Vellaikavuni (13.9 mg/100g) (Figure 3). Similarly coloured kala namak rice seeds having higher total phenol content while the non-coloured rice seeds have lower total phenol content [23]. The red rice variety contain the highest quantity of phenolic acids than the black, brown and the white rice variety. The higher content of polyphenols in the dark pigmented rice grains (red and black) can be attributed to the difference in pericarp colour. In addition to the

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variations in phenolic compound concentrations, variations were also observed in the concentration of these compounds in grains with the identical pericarp colour [24].

4. Conclusion

The present study emphasized on α-amylase activity, dehydrogenase activity and total phenol content of traditional rice varieties of Tamil Nadu. The α-amylase and dehydrogenase activity in traditional rice seeds are important biochemical traits responsible for improving seed germination, vigour and viability, respectively. Total phenol content is the secondary metabolites playing a key role in pigmentation, plant growth and resistance to pathogens. Regarding the significance of consumer preference of traditional rice varieties, the information about these enzyme activities could aid in the conservation and multiplication of seeds.

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Table 2. Evaluation on biochemical parameters of traditional rice varieties

Traditional rice varieties	Alpha- amylase (mg maltose min^{-1})	Dehydrogenase (OD value)	Total phenol (mg/100g)
Mappillai samba	0.85	0.277	38.84
Kichadi samba	0.96	0.312	24.34
Vellaikavuni	1.38	0.562	13.9
Rajamannar	0.92	0.419	22.76
Sembuli samba	4.88	0.431	45.36
Kattuyanam	0.84	0.344	42.34
Karunkuruvai	2.28	0.522	26.92
Norungan	0.96	0.404	27.34
Poongar	3.40	0.522	15.18
Kuzhiyadichan	1.50	0.421	17.46
Chithiraikar	0.98	0.416	16.44
Paalthondi	1.44	0.411	22.16
Maranel	3.40	0.609	12.46
Salem sanna	1.90	0.596	20.78
Kallurundai kar	1.60	0.384	38.24
Illuppai poo samba	1.08	0.485	31.34
Mean	**1.80**	**0.445**	**26.00**

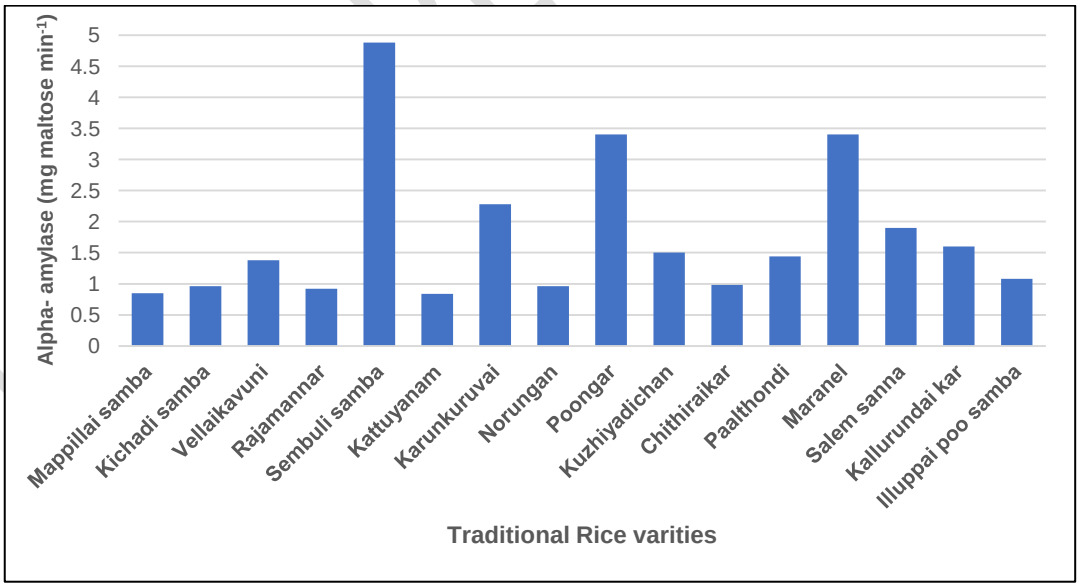

Fig. 1. α- amylase activity in the sixteen traditional rice varieties of Tamil Nadu

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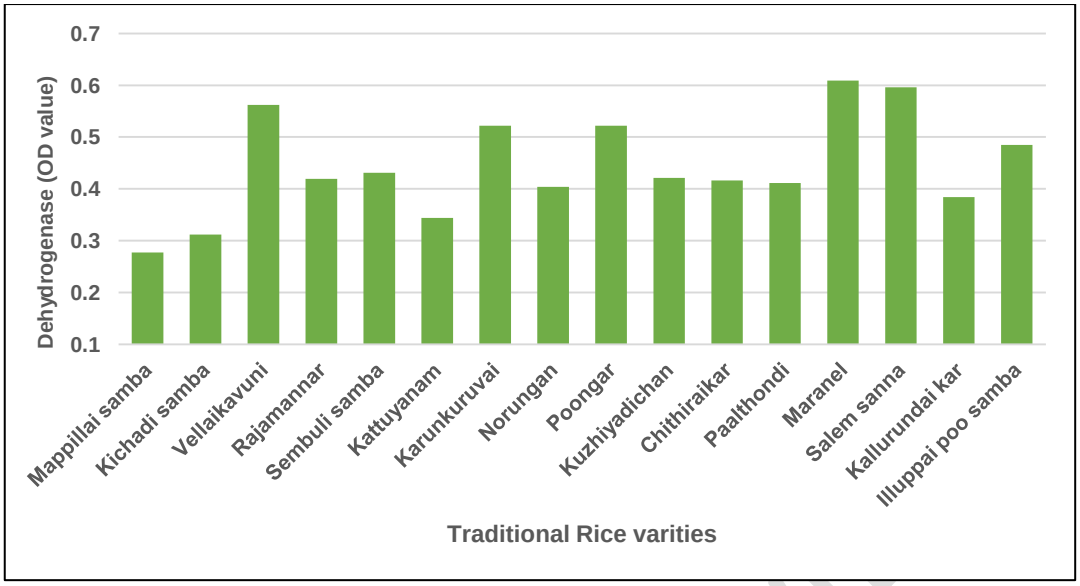

Fig. 2. Dehydrogenase activity in the sixteen traditional rice varieties of Tamil Nadu

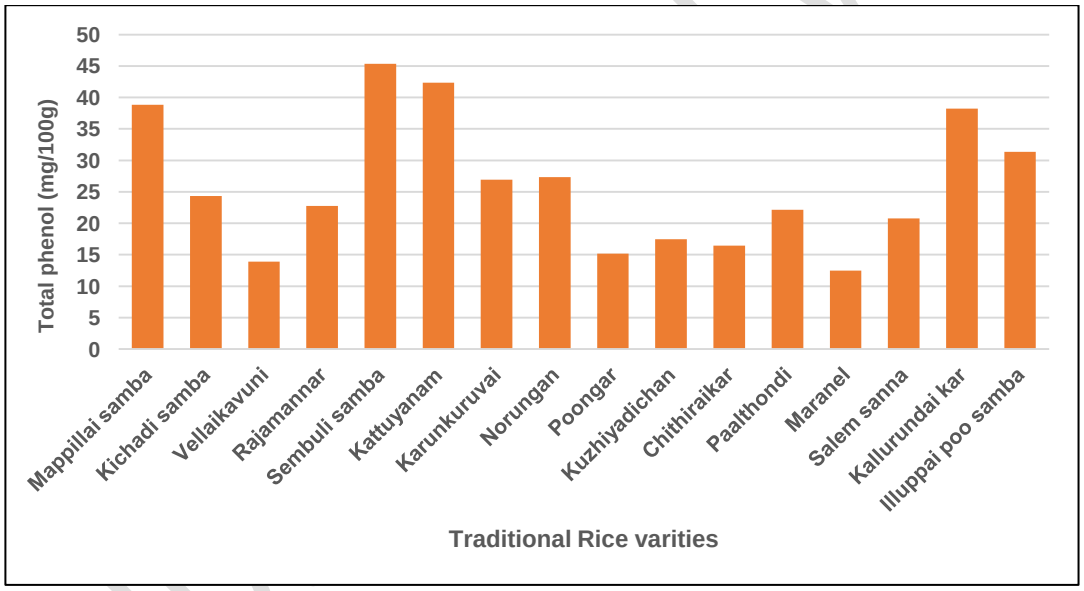

Fig. 3. Dehydrogenase activity in the sixteen traditional rice varieties of Tamil Nadu

1.Mappillai samba 2. Kichadi samba 3. Vellaikavuni 4. Rajamannar 5. Sembuli samba 6. Kattuyanam 7. Karunkuruvai 8. Norungan

9. Poongar 10.Kuzhiyadichan 11. Chithiraikar 12. Paalthondi 13. Maranel 14. Salem sanna 15. Kallurundaikar 16. Illuppai poo samba

Fig. 4. Traditional rice varieties used in this study 1. Mappillai samba, 2. Kichadi samba, 3. Vellaikavuni, 4. Rajamannar, 5. Sembuli samba, 6. Kattuyanam, 7. Karunkuruvai, 8. Norungan, 9. Poongar, 10. Kuzhiyadichan, 11. Chithiraikar, 12. Paalthondi, 13. Maranel, 14. Salem sanna, 15. Kallurundaikar and 16. Illuppai poo samba

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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