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Evaluation of Biochemical properties of Traditional Nutri-cereals of Tamil Nadu

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ABSTRACT: The minor millets or small millets have been renamed as "Nutri-cereals" in India. Nutri-cereals are extraordinarily tolerant to drought and other abiotic stresses, such as high temperatures and poor soils and also require minimum inputs for the growth. This makes them "climate smart" and a good source for genetic traits that can strengthen the resilience of other crops in the face of climate change. In developing countries, lack of dietary diversity is one of the key factors behind malnutrition and the prevalence of non-communicable diseases such as diabetes. Dietary fibre, minerals, carotenoids, flavonoids and polyphenols are abundant in nutri-cereals cultivars and consumption of these traditional nutri-cereals cultivars, on the other hand, benefits human health. Now-a-days, traditional 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 biochemical properties *viz.,*α-amylase activity, dehydrogenase activity and total phenol content. Among the nutri-cereals tested, high α-amylase activity and dehydrogenase activity were recorded by Periyurvathalmalai local (1.80 mg maltose min^{-1}) and Varagu PGR (0.280) in Proso milletand Kodo millet respectively. The total phenol content was maximum in Varagu PGR (Kodomllet)with 17.58 µg /100g. Among the twelve traditional variety, Varagu PGR (Kodomllet) have the high secondary metabolites with improved seed quality. The results of this experiment revealed that the above traditional 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 nutri-cereals, α-amylase activity, dehydrogenase activity, Total phenol content.*

INTRODUCTION

Millets – The Miracle Grains are a set of grass speciesproducing small seeds and are grown for fodder and human food throughout the world.Besides, human consumption, these millets also serve as a raw material for allied agro-industries such as poultry and cattle feed, starch, value added products, alcohol,biofuel etc. Small millets are a group of grassy plants having short slender culm categorized under coarse cereals which belong to the family poaceae. In developing countries, lack of varied diet in the day-to-day life is majorly contributing to malnutrition and the predominance of diabetes. In the context of prevalence of large-scale existence of malnutrition among women and children in the state (nearly 45 %) the role of millets get manifold.

Dietary diversity can be obtained by growing small millets as a complement to the existing crops. Compared to the majorly cultivated cereals such as rice and wheat, small millets are nutritious.These small millets are composed of 65% carbohydrates with a high proportion of non starchy polysaccharides, high dietary fibre and low glycemic index also rich in phytochemicals including phytic acid which helps in prevention of constipation, lowering of blood cholesterol, helps to reduce the incidence of cardio vascular diseases, cancer and prevents diabetes. These are rich in calcium, phosphorus, iron, protein, B complex vitamins such as niacin, thiamine, riboflavin and essential amino acids like methionine and lecithin making them a valuable tool in the fight against malnutrition and can help stave off anaemia and celiac disease. Due to these reasons, small millets are rightly mentioned as "God's own grains and nutri-cereals".

The nutrient content alike other major cereals and the non-nutrient contents with numerous health benefits makes the millets as a potential crop for the future. Several research have shown that nutri-cereals seeds are rich resource on non-nutrient, particularly phenolic compounds that can act as antioxidant which protects body tissues against oxidative stress and reduces the risk of non-communicable diseases such as diabetes. Millets are rich in phenolics, tannins and phytate which as antioxidant (Kumari *et al.,* 2017).

With this background, it was understood that small millets opened up a new opportunity to benefit the farmer status, empowerment and income, conservation, nutrition, environment and eventuallyleading to food and nutrition security.The nutri-cereals very much of resilience to unfavorable climatic circumstances such as floods, droughts, medicinal capabilities and nutritional value due to their inherent high vigour status. The current study has been focused on the biochemical characterization of traditional nutri-cereals types by measuring α-amylase, dehydrogenase and total phenols.

MATERIALS AND METHODS

A. Materials

The nutri-cereals samples selected for this study were obtained from Department of Plant Genetic Resource, Tamil Nadu Agricultural University, Coimbatore (Table 1). Biochemical properties of traditional variety seeds were investigated at the laboratory of Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore.

Table 1: List of traditional nutri-cereals utilized in this study.

Sr. No.	TNAU PGR ID	Vernacular Name
Finger millet		
1.	TNAUF00602307	Vathalmalai local
2.	TNAUF00602309	Karunchurutai local
Foxtail millet		
3.	TNAUF00700782	Singarapettai local
4.	TNAUF00700783	Sekkanampatti local
Little millet		
5.	TNAUF00800173	Pappanur local
6.	TNAUF00800174	Singarapettai local
7.	TNAUF00800175	Sekkanampatti local
8.	TNAUF00800176	Vathalmalai local
Proso millet		
9.	TNAUF00900376	Periyurvathalmalai local
Kodo millet		
10.	TNAUF01100182	Varagu PGR
Barnyard millet		
11.	TNAUF01000233	Kuthiraivali (SGD 079)
12.	TNAUF01000234	Kuthiraivali (SGD 0137)

Methods

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

Three replicates of 500mg of pre-germinated seed samples were weighed 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. For 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 (Paul *et al.,* 1970).

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

C. Determination of Dehydrogenase activity (OD value)

Preconditioned seeds for 7 h in water were taken and were bisected longitudinally into two halves. One half with embryo was soaked in 0.5% 2,3,5-triphenyl tetrazolium chloridesolution and kept in dark for 2h at 40°C for staining. After expiry of the period, the excess solution was decanted 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 (Kittock, 1968).

D. 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 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 µg 100 g^{-1} seed material.

RESULT AND DISCUSSION

A. α- amylase activity

α -amylase is responsible for majority of carbohydrate metabolism in the endosperm of seeds. The overall vigour status of rice seed was improved by this enzyme (Galani*et al.,* 2011). The α-amylase activity will act as a biochemical indication for identifying the capacity for nutri-cereals seed germination, which leads to seed vigour differences (Gowda, 1992). In the present investigation, the highest α-amylase activity was observed in the traditional nutri-cereals variety Periyurvathalmalai local (Proso millet) with 1.80 mg maltose min^{-1} followed byVaragu PGR (Kodomllet) with 1.26 mg maltose min^{-1} which was on par with Singarapettai local (Little millet) with 1.26 mg maltose min^{-1} and Vathalmalai local (Little millet) with 1.04 mg maltose min^{-1} (Table 2). Least activity was recorded in Sekkanampatti local (Foxtail millet)with 0.48 mg maltose min^{-1} and Singarapettai (Foxtail millet) with 0.60 mg maltose min^{-1} (Table 2: Fig. 1). Amylases hydrolyses the starch reserves into simple sugars which are taken up by the developing embryo. High α - amylase could have increased the availability of starch assimilation that promoted early germination besides, significant increase in radicle length triggered by synthesis of auxins (Bharathi *et al.,* 2004; Mishra *et al.,* 2010) and traditional rice varieties.

B. Dehydrogenase activity

The dehydrogenase enzyme activity is a good stable metabolic marker to estimate the degree of vigour in seeds (Saxena *et al.,* 2013) and have positive association with vigour and viability of seeds (Kharlukhi, 1983). The maximum dehydrogenaseactivity was recorded by Varagu PGR (Kodomllet) with 0.280, Sekkanampatti local (Foxtail millet)0.266, Kuthiraivali SGD 0137 (Barnyard millet) with 0.266, Singarapettai local (Little millet) with 0.266 whereas the minimum activity was found in Vathalmalai local (Finger millet) with 0.189 and Karunchurutai (Finger millet) with 0.277 (Table 2, Fig. 1). Verma *et al.* (2003) noted that the dehydrogenase activity was reduced as the ageing progressed and improved the shelf life seeds. Also reported that dehydrogenase activity maintains viability and vigour of the seeds (Bhanuprakash *et al.,* 2010; Prakash *et al.* 2020).

C. 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 seeds (Zhou *et al.,* 2004; Yawadio *et al.,* 2007). The highest total phenolic content was observed in Varagu PGR (Kodomllet) (17.58 µg /100g), and the lowest was recorded by Kuthiraivali SGD 0137 (Barnyard millet) (2.76 µg /100g) (Table 2:Fig. 1). Similarly coloured kala namak rice seeds having higher total phenol content while the non-coloured rice seeds have lower total phenol content (Anuprialakmi *et al.,* 2019). Cereal phenolics has a potential role as protective factors against free radical mediated pathologies, such as cancer and atherosclerosis in human (Kehrer, 1993). Phenolic compound concentrations, variations were also observed in the concentration of these compounds in grains with the identical pericarp colour (Shen *et al.,* 2009).Traditional rice high amount of total phenol content.

Table 2: Evaluation on biochemical parameters of traditional nutri-cereals.

Crops		α- amylase activity (mg maltose min^{-1})	Dehydrogenase activity (OD value)	Total phenol content (µg/100g)
Finger millet	V_1 - Vathalmalai local	0.80	0.189	5.36
	V_2 - Karunchurutai	0.76	0.194	3.32
Proso millet	V_1 - Periyurvathalmalai local	1.80	0.246	4.74
Kodo millet	V_1 -Varagu PGR	1.26	0.280	17.58
Foxtail millet	V_1 - Singarapettai	0.60	0.245	4.44
	V_2 - Sekkanampatti local	0.48	0.266	4.14
Barnyard millet	V_1 -Barnyard millet (SGD 079)	0.65	0.218	3.48
	V_2 - Barnyard millet (SGD 0137)	0.72	0.266	2.76
Little millet	V_1 - Pappanur local	0.88	0.246	5.72
	V_2 - Sekkanampatti local	0.71	0.253	5.14
	V_3 –Singarapettai local	1.26	0.266	5.60
	V_4 - Vathalmalai local	1.04	0.246	5.30
Mean		0.91	0.242	5.63

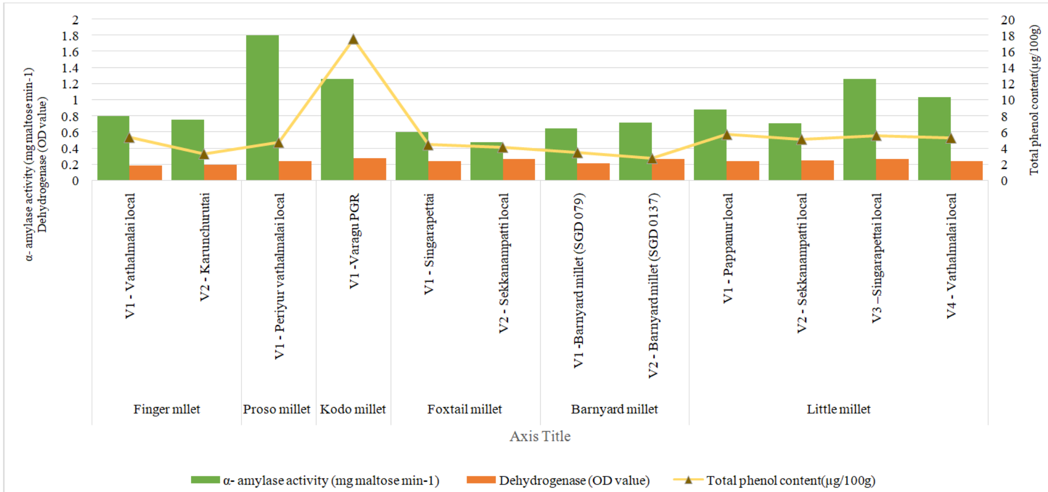

Fig. 1. α- amylase activity, Dehydrogenase activity and Total phenol in the twelve traditional nutri-cereals of Tamil Nadu.

CONCLUSION

The present study emphasized on α-amylase activity, dehydrogenase activity and total phenol content of traditional nutri-cereals of Tamil Nadu. The α-amylase and dehydrogenase activity in traditional nutri-cereals seeds are important biochemical traits responsible for improving seed germination, vigour and viability, respectively. Total phenol content is the secondary metabolites which is responsible for playing a key role in pigmentations, plant growth and resistance to pathogens and antioxidant activity. Regarding the significance of consumer preference of traditional nutri-cereals, the information about these enzyme activities could aid in the conservation and multiplication of seeds and ultimately leads to increase in consumption. Among the twelve varieties, Varagu PGR (Kodomllet) have the high secondary metabolites with improved seed quality can be considered for future use in breeding programme.

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Conflict of Interest. None.

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