Research Paper

Assessment of Advanced Breeding Lines of Rice (*Oryza sativa* L.) for Nutritional and Nutraceutical Grain Quality Characteristics

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ARTICLE INFO

Article history:
Received: 14 June 2024
Revised version received: 16 July 2024
Accepted: 09 September 2024

Citation:
Rebeira, S.P., Jayatilake, D.V., Prasantha, B.D.R., Senarathne Menike, B.M.K., Piyasiri, C.H., Weerasinghe, W.D.P., Samarasinghe, W.L.G. (2024). Assessment of advanced breeding lines of rice (*Oryza sativa* L.) for nutritional and nutraceutical grain quality characteristic. *Tropical Agriculturist, 172*(3), 16-30.

Abstract

The current rice breeding programs concurrently aim to meet the demand for high-quality and nutritionally enriched rice, targeting both local and international markets. The objective of this study was to develop advanced breeding lines (ABL) of rice with better nutritional and nutraceutical properties. Nine ABLs were developed by a cross of At 405/*Pokkali* following modified bulk method. Selected nine ABLs at F_{10} generation and parents were analyzed for grain quality characteristics. Considering crude protein content (CPC), total dietary fiber (TDF), total polyphenol content (TPC), proanthocyanidin content (PAC), and radical scavenging activity (RSA), the accession *Pokkali* was significantly better performing than the variety At 405. However, crude fat content (CFC) and crude fiber content (CFBC) of both *Pokkali* and At 405 performed similarly. Therefore, considering the nutritional and nutraceutical aspects, the results indicated that *Pokkali* can be considered as an elite accession, compared to At 405. The pigmented ABL GQ13 reported a higher CFC, TDF, TPC, PAC and RSA compared to other ABLs. Similarly, pigmented ABL GQ10 reported a higher CFC, CFBC, TDF, TPC, PAC and RSA while non-pigmented ABL GQ09 reported a higher CPC, CFC and TDF among the evaluated ABLs. The ABLs GQ13, GQ10, and GQ09 either outperformed or comparable to the better parent, *Pokkali*. Therefore, the results suggested that these ABLs GQ13, GQ10 and GQ09 could be strongly recommended for further evaluation to apply for the rice breeding programs targeting improvements of nutritional and nutraceutical grain quality.

Keywords: Antioxidant, Breeding lines, Crude fat, Crude protein, Rice, Total dietary fiber

Introduction

Being a staple cereal food, rice is considered to be the main dietary source of carbohydrates and provides a considerable portion of the daily requirement of proteins, minerals, fats and fiber, especially in developing countries (Kennedy & Burlingame, 2003). Although rice consists of minute quantities of fat, and comparatively lesser content of protein, the quality of the rice fat and proteins is superior to other cereals, such as wheat, corn and millets, and is with high digestibility (Xu *et al.,* 2001; Samaranayake *et al.,* 2017; Liyanaarachchi *et al.,* 2021). Moreover, the nutraceutical properties of rice are important in reducing the risk of common dietary disorders. Hence, introducing rice varieties with higher nutritional value including, higher contents of proteins, beneficial fats, fiber, and minerals along with nutraceuticals such as antioxidants and dietary fiber is considered a timely requirement.

The rice breeding programs of Sri Lanka had been mainly focused on achieving the production goals through developing high yielding varieties, with tolerance/resistance to abiotic/biotic stress factors. Few studies have reported attempts on improving the physical and physicochemical qualities (Abeywickrama *et al.,* 2010; Abhayagunasekara *et al.,* 2018) and the nutritional and nutraceutical qualities of rice (Abeysiriwardena & Gunasekara, 2020), targeting improvement of the grain quality, catering to the local and international consumer demands.

The rice germplasm of Sri Lanka, comprises a large collection of heirloom traditional accessions and newly improved rice varieties, which are diverse in its genetic makeup (Wasala *et al.,* 2017). Among them several rice accessions have been reported as carrying importance with respect to nutritional and nutraceutical properties (Rajapakse *et al.,* 2011; Gunaratne *et al.,* 2013; Abeysekera *et al.,* 2017a; Abeysekera *et al.,* 2017b; Rebeira *et al.,* 2022). In addition, based on indigenous knowledge several traditional rice accessions have been recognized as carrying medicinal properties (Wickramasinghe & Noda, 2008), however; the undesirable plant architecture and low yield have acted as the main restrictions to popularize traditional accessions for wider commercial cultivation. Through breeding programs, the desirable nutritional and nutraceutical properties identified in a rice accession can be introgressed to a popular variety which carry desirable plant architecture for field cultivation, high yield and convey resistance/tolerance to biotic and abiotic stresses, yet, needs improvement in its grain quality attributes (Abeysekera *et al.,* 2017b; Abeysiriwardena & Gunasekara, 2020).

In Sri Lankan, as a developing country, the improvement of nutritional and nutraceutical attributes of commercial rice varieties can lead to a remarkable positive influence on the health and wellbeing of the population, especially the children and adults who are prone to malnutrition due to economic constraints limiting their purchasing power. Furthermore, the common health issues arising due to the consumption of a high caloric staple food can be resolved by improving the nutraceutical and functional properties of rice, which could lead to lesser prevalence of dietary disorders associated with starch digestibility (Zhao *et al.*, 2018). Therefore, the objective of current study was carried out to develop advanced rice breeding lines carrying elite grain quality characteristics through making crosses between selected parents and to assess them with respect to nutritional and nutraceutical properties. The developed advanced lines and the data generated will further facilitate to identify the potential lines to be used as candidates in yield trials, national coordinating rice varietal trials and in future rice breeding programs targeting grain quality improvements.

Materials and Methods

Plant material and experimental stations

The seeds of rice variety At 405 and traditional cultivar *Pokkali* (accession number: RRDI 809) were sourced from breeder seed stocks maintained at the Rice Research and Development Institute (RRDI), Batalagoda, Sri Lanka. Field experiments involving the hybridization, selection and advanced rice breeding line development was conducted at the RRDI and grain quality characterization experiments were conducted at the Grain Quality Laboratory of RRDI, Functional Genomics Laboratory of the Department of Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka and Food Research Unit, Department of Agriculture, Peradeniya, Sri Lanka.

Development of advanced rice breeding lines

Parent lines, At 405 and *Pokkali* for the bi-parental cross were selected based on their differences in the grain quality characteristics (Rebeira *et al.,* 2022) and the results of previous preliminary tests. Accordingly, the two parental rice accessions were planted as three-rowed progeny lines at two different planting times to synchronize flowering. The cross was made between the parental accessions, adopting a hot water treatment method for emasculation. Seeds of the cross were maintained as a bulked population until up to F_4 generation. At F_4 generation, individual plant selections were carried out based on desired new plant type characteristics, *i.e.* desirable panicle characteristics, semi-dwarf type plants, low tillering (9-10 at transplant conditions), absence of lodging and seed shattering, and resistance to pests and diseases in the field, as evaluated at the RRDI, Batalagoda in rice breeding trials through visual observations and assessments. From F_5 generation, three-rowed progeny lines were established and subsequent generation advancements were carried out up to F_9 generation. At F_{10} mature seed samples were harvested and dehulled following the methods adopted by Cruz and Khush (2000) for the evaluation of grain quality characteristics, such as pericarp colour, grain dimensions, proximate composition, total dietary fiber content, and antioxidant properties.

Evaluation of grain physical characteristics

Grain pericarp colour of the selected advanced breeding lines and their parents were determined using a colourimeter (CS-10, Company name, City name, China) and was clustered into pigmented and non-pigmented categories based on the range of *L**, *a**, and *b** values in order to unbiasedly categorize the grains according to the colour. The length, width, and thickness of triplicated grain samples were measured using a micrometer screw gauge (Mitutoya Co., City name, Japan) and the size categories were determined based on the length and the shape categories derived from the length-width ratio, referring to the reference values of IRRI standards (Rice Knowledge Bank, 2023). The grain surface area and volume were calculated based on Varnamkhasti *et al.* (2008). Flour samples of brown rice were prepared following the method described by Rebeira *et al.* (2022).

Evaluation of nutritional composition and nutraceutical properties

Proximate composition of the brown rice samples was determined according to the methods described by AOAC standards (2000) and total moisture content (TMC), crude protein content (CPC), crude fat content (CFC), crude fiber content (CFBC), and carbohydrate contents (CHOC) were expressed as a percentage on a wet basis. Total dietary fiber (TDF) content was determined according to Tuncel *et al.,* (2014), while total antioxidant properties were determined in terms of total phenolic compounds (TPC), proanthocyanidin content (PAC) and radical scavenging activity (RSA) following the methods described by Somaratne *et al.* (2017) and Gunaratne *et al.* (2013).

Statistical analysis

The data for physical, nutritional and nutraceutical characteristics were tested for normality using Shapiro-Wilk test (p=0.01) and those which did not follow a normal distribution were transformed using the Johnson transformation function to reach normality in Minitab (Minitab LLC., City name, State name, USA; https://www.minitab.com/en-us). One–way analysis of variance (ANOVA) was followed by mean separation using the Tukey method to determine the significance at p<0.05.

Results and Discussion

Evaluation of selected advanced breeding lines for grain quality

At F_{10}, nine advanced breeding lines (GQ 01, GQ 02, GQ 05, GQ 07, GQ 09, GQ 10, GQ 12, GQ 13, and GQ 16) of At 405/*Pokkali* cross were selected for further evaluation with respect to physical, nutritional and nutraceutical grain quality characteristics.

Grain physical characteristics

The 3D scatter diagram of colourimeter readings for *L**, *a**, and *b** values clustered the advanced breeding lines into pigmented and non-pigmented groups (Figure 1) (pigmented range *L**: 30.55±2.35 - 37.76±1.35, *a**: 16.00±0.45 - 24.38±2.72, *b**: 15.09±1.14 - 27.67±1.24 and non-pigmented range *L**: 55.53±9.61 - 68.46±3.12, *a**: 1.77±0.69 - 4.13±0.82, *b**: 19.62±2.46 - 23.15±1.23). Accordingly, the lines GQ 01, GQ 05, GQ 07, GQ 10, and GQ 13 were clustered into red pigmented group along with *Pokkali*, while GQ 02, GQ 09, GQ 12, and GQ 16 were clustered into white non-pigmented group along with At 405.

Physical grain properties, grain dimensions, grain surface area and volume of the selected advanced breeding lines and the parents *Pokkali* and At 405 are presented in Table 1. The highest and the lowest grain lengths were reported in the two parents At 405 (6.55±0.02mm) and in *Pokkali* (5.28±0.03 mm), respectively. The grain length of At 405 was significantly higher compared to *Pokkali* and all nine advanced breeding lines. Among the evaluated At 405 was the only to be categorized as a long grained rice variety (>6.5 mm, Rice Knowledge Bank, 2023). Based on the grain length, the advanced breeding line GQ 01 and GQ 09 were not significantly different to *Pokkali* and were categorized as short grains (<5.5 mm, Rice Knowledge Bank, 2023). The rest of the advanced breeding lines GQ 10, GQ 16, GQ7, GQ 05, GQ 12, and GQ 13 (5.51±0.12 - 5.98±0.19 mm) were categorized as medium grains.

The highest and the lowest grain width were reported in *Pokkali* (2.27±0.01 mm) and At 405 (1.61±0.02 mm), respectively. Grain width of GQ 09, *Pokkali,* GQ 01, GQ 16, GQ 02, GQ 13, and GQ 12 were not significantly different from each other. Similarly, the grain width of GQ, 10, GQ 05, GQ 07, and At 405 was not significantly different from each other.

The highest and lowest grain thickness were recorded in *Pokkali* (2.03±0.01 mm) and GQ 07 (1.35±0.07 mm). The grain thickness of *Pokkali* was highly different to all nine GQ lines as well as the other parent At 405. However, GQ 07, At 405, and GQ 09 were not significantly different from each other.

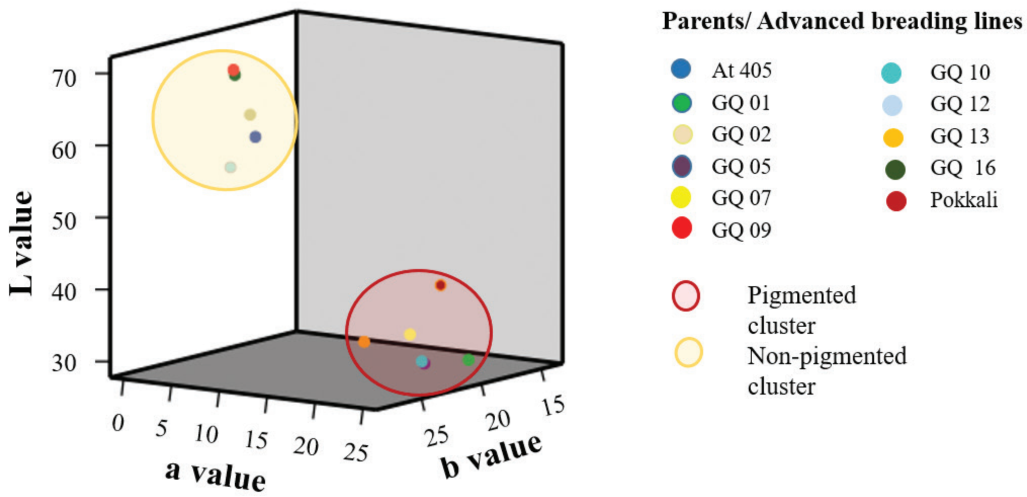

Figure 1. Clustering of the nine selected progeny lines and their parents into pigmented and non-pigmented clusters based on the results of their *L, *a**, and *b** values**

Table 1. Grain dimensions, size and shape classification of the selected advanced breeding lines and the parents *Pokkali* and At 405

Advanced breeding line/ Parent	Grain length (mm)#	Grain width (mm)	Grain thickness (mm)	Length to width ratio	Grain surface area (mm^2)	Grain volume (mm^3)
GQ 01*	5.29±0.04^{f}	2.25±0.04^{abc}	1.72±0.02^{bcd}	2.34±0.03	20.08±0.19^{cd}	10.92±0.17^{de}
GQ 02**	5.51±0.12^{de}	2.24±0.02^{abc}	1.70±0.03^{cd}	2.46±0.03	20.50±0.53^{bc}	11.18±0.41^{cd}
GQ 05**	5.74±0.04^{c}	2.14±0.03^{d}	1.76±0.02^{b}	2.69±0.03	21.03±0.34^{bc}	11.40±0.28^{bcd}
GQ 07**	5.75±0.08^{c}	1.63±0.03^{e}	1.35±0.07^{f}	3.52±0.01	15.42±0.40^{e}	6.72±0.23^{f}
GQ 09*	5.43±0.03^{ef}	2.27±0.04^{ab}	1.65±0.03^{def}	2.39±0.04	20.15±0.08^{cd}	10.97±0.09^{de}
GQ 10**	5.98±0.19^{b}	2.15±0.02^{d}	1.74±0.02^{bc}	2.78±0.08	21.66±0.72^{ab}	11.83±0.53^{abc}
GQ 12**	5.69±0.07^{cd}	2.22±0.02^{bc}	1.72±0.01^{bcd}	2.54±0.02	20.90±0.25^{bc}	11.43±0.20^{bcd}
GQ 13**	5.58±0.13^{de}	2.21±0.05^{c}	1.67±0.04^{de}	2.52±0.0	20.36±0.85^{c}	11.02±0.68^{cd}
GQ 16**	5.79±0.08^{c}	2.24±0.02^{abc}	1.74±0.035^{b}	2.59±0.05	21.64±0.33^{ab}	12.00±0.28^{ab}
*Pokkali**	5.28±0.03^{f}	2.27±0.01^{a}	2.03±0.01^{a}	2.33±0.02	22.34±0.13^{a}	12.76±0.11^{a}
At 405***	6.55±0.02^{a}	1.61±0.02^{e}	1.48±0.02^{e}	4.08±0.06	17.99±0.0^{f}	8.17±0.10^{ef}

*Notes: #Values were expressed as mean ± SE (n=3 for each variety). Mean values in a column superscripted by different letters are significantly different at p< 0.05. Size category: * short; ** medium; *** long according to IRRI standards.*

The derived length: width ratio of the advanced breeding line GQ 07 and the parent At 405 was 3.52±0.01 mm and 4.08±0.06 mm, respectively. Thus, they were both categorized as having a slender shape (length: width ratio>3; Rice Knowledge Bank, 2023). All remaining advanced breeding lines and the parent *Pokkali* reported a length: width ratio between 2 and 3 and hence, was categorized as having an intermediate shape (2< length to width ratio<3; Rice Knowledge Bank, 2023). No round or bold shaped grain types were reported among the selected advanced breeding lines.

The highest grain surface area and the volume were reported in *Pokkali* (22.34±0.13 mm^2 and 12.76±0.11 mm^3), while the lowest were reported in GQ 07 (15.42±0.40 mm^2 and 6.72±0.23 mm^3). The surface area and the volume of advanced breeding lines GQ 10 and GQ 16 did not show a significant difference to *Pokkali*. Additionally, GQ 07 and At 405 were not significantly different from each other with respect to gain surface area and volume.

Consumer preferences for the grain physical quality attributes depend on the geographical location, traditions or personal preferences (Fan *et al.,* 2006; Danbaba *et al.,* 2011). Long-slender shaped rice has high demand in European markets, while consumers in Sri Lanka are said to prefer short-intermediate rice (Wickramasinghe & Noda, 2008). Considering grain physical properties, the parent At 405 bears a non-pigmented, long-slender shaped grain, with a low surface area and a volume. In the local markets, At 405 is referred to as "*Lanka basmathi*", however, it is not comparable to the basmati varieties produced in India and Pakistan, which carry the characteristic long-slender shape, high elongation ratio, unique aroma and a taste (Ritika *et al.,* 2016; Ojha *et al.,* 2018). However, in the local market, it is one of the available long-slender types of rice catering to the niche market. At 405 is a sticky type grain due to low amylose content (Wickramasinghe & Noda, 2008) and therefore, receives a low consumer preference in the local market.

The other parent *Pokkali*, can be described as a red pigmented, short-intermediate shaped grain with a high surface area and volume. The preferred shape of rice by Sri Lankans is generally a short-intermediate type (Abeysekera *et al.,* 2017a). Hence, *Pokkali* could be preferred by Sri Lankan consumers for their everyday consumption. Yet, this landrace carries poor prospects for commercial cultivation due to its undesirable agronomic characteristics. While none of the advanced rice breeding lines developed by crossing *Pokkali* and AT 405 resulted into a long grain, GQ 01 and GQ 09 were categorized as short and intermediate shaped, similar to *Pokkali*. Moreover, considering the low width, thickness, surface area and volume reported by the GQ 07, it could well cater to a potential local niche market who prefers medium size grains with slender shape.

Nutritional composition

The moisture content of *Pokkali*, At 405, and the nine advanced breeding lines ranged from 13.44±0.25 to 14.27±0.06%. Nutritional composition of the selected advanced breeding lines and the parents were assessed in terms of CPC, CFC, CFBC and CHOC of the brown rice and is presented in Table 2.

With respect to CPC, the two parents *Pokkali* and At 405 were greatly different from each other. The lowest and highest CPC were reported in At 405 and GQ 09, as 8.50±0.15% and 11.18±0.50%, respectively. Kennedy and Burlingame (2003) reported that CPC of rice varieties ranged from 4.5-15.9% by analyzing 2,674 rice varieties collected from IRRI, Philippines. The ranges reported in the current study are comparable. The advanced breeding lines GQ01, GQ16, GQ07, and GQ05 were not significantly different to that of GQ 09 and were identified as the highest CPC group. However, of these only the CPC of GQ 09 was reported as significantly higher than *Pokkali* and hence, outperformed both the parents *Pokkali* and At 405. Rice protein is an important dietary source of essential amino acids which are needed in various physiological functions in the human body (Kawakatsu & Takaiwa, 2019). In developing countries, such as Sri Lanka, where low income earning demographics depend heavily on rice for fulfilling their daily dietary protein requirements (Gunaratne *et al.,* 2013; Abeysekera *et al.,* 2017a), advanced breeding lines, such as GQ 09 carry importance in terms of breeding value.

With respect to CFC, the parents *Pokkali* and At 405 did not significantly differ from each other.

Table 2. Moisture content, nutritional and nutraceutical composition of brown rice of the selected advanced breeding lines and their parents *Pokkali* and At 405

Advanced breeding line/ Parent	Total moisture content (%) #	Crude protein content (%)	Crude fat content (%)	Crude fiber content (%)	Carbohydrates (%)	Total Dietary fiber (%)	Total phenolic compounds (mg GAE/ 100 g)	Proanthocyanidin content (mg CTE/ 100 g)	Radical scavenging activity (% inhibition)
GQ 01*	13.44 ± 0.25^{c}	10.68 ± 0.32^{ab}	2.78 ± 0.15^{ab}	2.41 ± 0.21^{de}	68.72 ± 0.17^{bc}	2.68 ± 0.26^{bcd}	159.22 ± 3.83^{c}	165.96 ± 11.08^{cde}	68.59 ± 1.66^{ab}
GQ 02	14.00 ± 0.09^{abc}	9.88 ± 0.55^{bcd}	2.68 ± 0.19^{abc}	3.12 ± 0.09^{a}	$69\ .14 \pm 0.55^{b}$	2.39 ± 0.09^{cd}	81.47 ± 3.68^{g}	108.98 ± 5.65^{g}	57.98 ± 3.13^{cd}
GQ 05*	13.72 ± 0.25^{abc}	10.38 ± 0.19^{ab}	2.72 ± 0.07^{abc}	2.95 ± 0.06^{b}	68.86 ± 0.15^{b}	3.17 ± 0.50^{abc}	110.91 ± 1.93^{d}	146.96 ± 15.01^{def}	72.14 ± 2.71^{a}
GQ 07*	14.27 ± 0.06^{a}	10.46 ± 0.44^{ab}	2.16 ± 0.01^{d}	2.91 ± 0.04^{bc}	68.83 ± 0.39^{b}	3.01 ± 0.40^{bc}	110.49 ± 1.71^{d}	175.91 ± 3.99^{bcd}	67.48 ± 1.83^{bc}
GQ 09	14.02 ± 0.35^{abc}	11.18 ± 0.50^{a}	2.59 ± 0.18^{abc}	2.89 ± 0.06^{bc}	67.91 ± 0.48^{c}	4.11 ± 0.12^{a}	82.39 ± 1.21^{fg}	144.25 ± 2.21^{def}	58.68 ± 2.63^{cd}
GQ 10*	14.15 ± 0.08^{ab}	9.02 ± 0.14^{de}	2.80 ± 0.07^{ab}	2.97 ± 0.04^{ab}	69.81 ± 0.28^{ab}	3.34 ± 0.45^{ab}	239.23 ± 4.36^{b}	193.09 ± 12.33^{abc}	71.21 ± 0.40^{ab}
GQ 12	13.96 ± 0.09^{abc}	10.01 ± 0.07^{bc}	2.69 ± 0.07^{abc}	2.90 ± 0.08^{bc}	69.07 ± 0.07^{b}	3.38 ± 0.28^{ab}	95.64 ± 5.67^{de}	138.82 ± 1.56^{efg}	57.58 ± 1.48^{cd}
GQ 13*	13.82 ± 0.48^{abc}	9.13 ± 0.17^{cde}	2.94 ± 0.12^{a}	2.71 ± 0.01^{cd}	69.80 ± 0.55^{ab}	3.25 ± 0.58^{abc}	546.78 ± 10.73^{a}	212.99 ± 8.79^{a}	70.98 ± 1.60^{ab}
GQ 16	13.85 ± 0.15^{abc}	10.58 ± 0.41^{ab}	2.47 ± 0.10^{bcd}	1.67 ± 0.14^{e}	68.79 ± 0.41^{bc}	2.77 ± 0.14^{bcd}	90.57 ± 2.32^{ef}	122.55 ± 6.8^{fg}	63.46 ± 1.09^{cd}
Pokkali*	13.47 ± 0.81^{c}	9.98 ± 0.14^{bc}	2.41 ± 0.21^{bcd}	2.65 ± 0.11^{cd}	69.77 ± 0.57^{bc}	4.10 ± 0.10^{a}	164.23 ± 2.97^{bc}	202.10 ± 0.70^{ab}	67.15 ± 0.68^{bc}
At 405	13.52 ± 0.12^{bc}	8.50 ± 0.15^{e}	2.34 ± 0.32^{cd}	2.41 ± 0.04^{de}	71.76 ± 0.28^{a}	2.00 ± 0.10^{d}	73.36 ± 2.09^{h}	67.23 ± 0.51^{h}	31.91 ± 4.11^{d}

*Notes: #Values were expressed as mean ± SE (n=3 for each variety). Mean values in a column superscripted by different letters are significantly different at $p<0.05$. *Red pigmented accessions/advanced breeding lines*

However, the CFC among the advanced breeding lines ranged from 2.16±0.01% in GQ 07 to 2.94±0.12% in GQ 13. The advanced breeding lines GQ 10, GQ 01, GQ 05, GQ 12, GQ 02, and GQ 09 were not significantly different from GQ 13 and together they were identified as the highest CFC group. In general, the CFC reported in the current study is comparable with the previously reported CFC for brown rice; 1.9-2.9% w/w (Resurreccion *et al.,* 1979; Gunaratne *et al.,* 2013). The fat in rice is reported to be of higher quality containing bioactive compounds, such as gamma oryzanol and vitamin E, including tocopherols and tocotrienols (Ali & Devarajan, 2017). Therefore, it is expected to carry health benefits. Given the fact that GQ 13 outperforms both the parents and reported highest CFC among the advanced breeding lines, it carries the potential importance with respect to better nutrition in terms of fat content and beneficial substances that gets carried forward with the fat component.

The highest CFBC was GQ 02 (3.12±0.09%) but the lowest was GQ 16 (1.67±0.14%). With respect to CFBC, the two parents *Pokkali* and At 405 did not show a significant difference. In addition, the CFBC of GQ 02 was not significantly different from GQ 10 and those advanced breeding lines formed the highest CFBC carrying group. With respect to CFBC, advanced breeding lines GQ 02, GQ 10, and GQ 05 highly outperformed both parents *Pokkali* and At 405. Fiber plays an important role in healthy functioning of the human gut. The CFBC reported in the current study is higher compared to Gunaratne *et al.* (2013), which reported a CFBC of 0.2-0.9% for a selection of 11 Sri Lankan varieties, in which the two parents *Pokkali* and At 405 were not included. Considering CFBC, the three advanced breeding lines GQ 02, GQ 10, and GQ 05 carry potential for future rice breeding efforts to increase the much needed fiber content in rice as part of a healthy meal.

The CHOC of the parents *Pokkali* and At 405 were highly different from each other. While the highest CHOC was reported in At 405 (71.76 ±0.28%), the lowest was reported in GQ 09 (67.91±0.48%). The parental variety At 405 with higher CHOC showed no significant difference to advanced breeding lines GQ10 and GQ13 with respect to CHOC and represents the lines carrying higher CHOC. The range reported for CHOC in other studies, such as in Oko *et al.* (2012) (53.52-86.82% range) was comparable to the current study (67.91-71.76% range).

Lines carrying high CHOC could be potentially important to feed children with higher energy demand, especially those dealing with malnutrition. The advance breeding line GQ 09, which carries the lowest CHOC, is not significantly different to GQ05, GQ07, *Pokkali*, GQ16, and GQ01. These advanced breeding lines carrying low CHOC could be further developed to feed those with impaired glucose metabolism leading to diabetics and other related disorders.

Nutraceutical properties

The Table 2 reports the nutraceutical properties of the two parents *Pokkali* and

At 405, along with the nine GQ advanced breeding lines in terms of TDF, TPC, PAC, and RSA. With respect to TDF, the parents *Pokkali* and At 405 were highly different from each other. The highest TDF was in GQ 09 (4.11±0.12%) but the lowest was in At 405 (2.00±0.10%). The GQ 09 was not significantly different from *Pokkali*, GQ 12, GQ 10, GQ 13, and GQ 05 and these formed the high TDF group. Therefore, GQ 09 was similar to *Pokkali* but out-performed At 405 with respect to TDF. The TDF is the indigestible part of the plant-based foods, and includes both insoluble and soluble components, while CFBC includes mainly insoluble fibers (Zhao *et al.,* 2018). The CFBC of the advanced breeding line GQ 09 was at an intermediate level, while it reported the best in terms of TDF.

Antioxidant properties can be measured in terms of TPC, PAC, RSA and total flavonoid content (Gunaratne *et al.,* 2013). In the study, TPC, PAC and RSA were used to predict the performance of the parents and the advanced breeding lines in terms of total antioxidant activity. For all three parameters, TPC, PAC, and RSA of the two parents *Pokkali* and At 405 were greatly different from each other, and in all three parameters *Pokkali* reported the higher values corresponding to higher total antioxidant potential.

For the TPC, advanced breeding line GQ 13 (546.78 ±10.73 mg GAE/100 g) reported the highest value but the parent At 405 showed the lowest TPC (74.36±1.48 mg GAE/100 g). The TPC of GQ 13 was greatly higher compared to both parents and the remaining advanced breeding lines. Following right behind was GQ10 and *Pokkali* which were not significantly different from each other. Similarly, the highest PAC was reported in GQ 13 (212.99±8.79 mg CTE/ 100 g) but lowest in At 405 (67.23±0.51 mg CTE/ 100 g). The parent *Pokkali* and advanced breeding line GQ10 was not significantly different to that of GQ 13 and formed the higher TPC group. With respect to RSA, the advanced breeding line GQ 05 reported the highest activity (72.14±2.71%) but the lowest was reported by At 405 (32.91±2.90%). The RSA of GQ 05 was not significantly different to advanced breeding lines GQ10, GQ13, and GQ01 which formed the higher RSA group. GQ 05 highly outperformed both the parents *Pokkali* and At 405. The TPC and PAC values reported in the current study are somewhat comparable with the previous reports (Gunaratne *et al.,* 2013). Although RSA is reported for polished rice in a range of 26.7-81.6%, such is not available for brown rice (Somaratne *et al.,* 2017).

Considering all parameters TPC, PAC and RSA, the parent At 405 reported the lowest values indicating the need to improve. While the advanced breeding line GQ 13 reported best performance in terms of TPC and PAC, GQ 05 reported the best performance for RSA. However, for RSA, it is noteworthy that no significant difference was detected between GQ 05 and GQ 13. This study indicates the potential use of red pigmented advanced breeding lines, such as GQ 13 and GQ 05 for improving the nutraceutical properties of rice.

Considering the nutritionally important grain quality parameters, including CPC, TDF, TPC, PAC, and RSA, the parent At 405 reported lower values compared to the parent *Pokkali*. However, for TFC and CFBC, both parents reported values that are not significantly different from each other (Figure 2). Therefore, although the agronomical characteristics of *Pokkali* are not as improved as At 405, *Pokkali* carries potential for use in breeding programs as a donor for improvement of nutritional and nutraceutical properties.

With respect to the developed advanced breeding lines, the red pigmented GQ 13 was a standout with respect to nutritional and nutraceutical characteristics, such as CFC, TDF, TPC, PAC and RSA (Table 2), where it either tops or is placed not significantly different to the highest performer reported for each of these characters. In comparison to the better parent *Pokkali*, the advanced breeding line GQ 13 highly outperforms the better parent *Pokkali* for CFC, and RSA and is not significantly different to *Pokkali* for parameters, such as CPC, CFBC, TDF, TPC, PAC, and RSA (Figure 2).

The non-pigmented advanced breeding line GQ 09 was placed among the highest for CPC and TDF but was not significantly different to the advanced breeding line in the highest category for CFC (Table 2). Furthermore, the advanced breeding line GQ 09 highly outperforms the better parent *Pokkali* for CPC and CFC characteristics and is not significantly different to *Pokkali* for parameters CFBC, and RSA (Figure 2). Though not found to be carrying the highest reported value for any of the studied characters, the advanced breeding line GQ 10 was reported not to be significantly different to the top performers for TFC, CFBC, TDF, TPC, PAC,and RSA (Table 2).

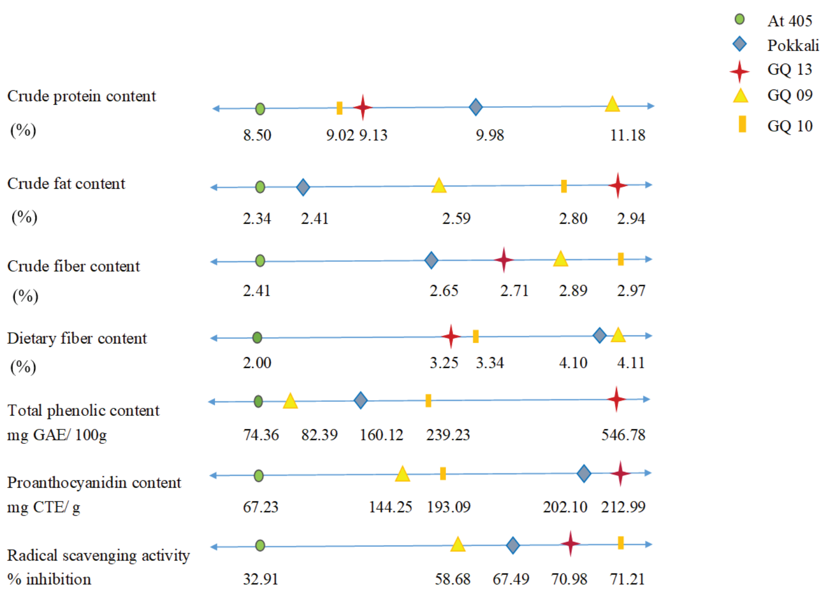

Figure 2. Graphical representation of the nutritional and nutraceutical grain quality characteristics of advanced breeding lines GQ09, GQ 10, and GQ 13, and their parents *Pokkali* and At 405

The advanced breeding line GQ 10 was only found not to carry a high CPC and had a CPC less than the better parent *Pokkali* (Figure 2). Except for CPC, for other parameters (CFC, CFBC, TDF, TPC, PAC and RSA), the GQ 10 either outperformed the better parent *Pokkali* or was not significantly different to *Pokkali* (Figure 2).

Accordingly, of all nine advanced breeding lines developed in the current study, the lines GQ 13, GQ 10 and GQ 09 are the most promising with respect to the assessed nutritional and nutraceutical properties (Figure 2) and can be recommended for further evaluation to use in the national rice breeding program as advanced breeding lines carrying elite grain quality characteristics with respect to nutritional and nutraceutical properties.

Conclusion

The accession *Pokkali* highly outperformed the variety At 405 in nutritional and nutraceutical parameters CPC, TDF, TPC, PAC, and RSA, except for CFC in which they were not significantly different from each other. Hence, considering the nutritional and nutraceutical aspects of the two parents crossed in the current study, *Pokkali* can be considered as the better parent. Of the advanced breeding lines resulted from the cross between At 405 and *Pokkali*, the red pigmented advanced breeding line GQ 13 and GQ 10 reported a higher or comparable CFC, TDF, TPC, PAC and RSA values compared to *Pokkali*. The non-pigmented advanced breeding line GQ 09 reported higher or comparable CPC, CFC, CFBC and TDF values compared to *Pokkali*. Therefore, it is herewith recommended to explore the possibility of using the advanced rice breeding lines GQ 13, GQ 10 and GQ 13 in the national rice breeding program to improve nutritional and nutraceutical qualities of rice.

Acknowledgements

The authors acknowledge that this work was carried out with the aid of a grant from National Research Council of Sri Lanka (NRC/11/86). The RRDI, Bathalagoda and RRRC, Bombuwala are thanked for facilitating the field research work. The authors wish to acknowledge the contribution of late Dr. H.A.M. Wickramasinghe of the Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya in conceptualizing the research work and Ms. T. Kariyawasam at Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya for assisting in statistical analysis.

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