PHYSICO-CHEMICAL AND MOLECULAR CHARACTERIZATION OF GRAIN QUALITY OF TRADITIONAL RICE VARIETIES

by

JEENA GEORGE

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DECLARATION

I, hereby declare that this thesis entitled **"PHYSICO-CHEMICAL AND MOLECULAR CHARACTERIZATION OF GRAIN QUALITY OF TRADITIONAL RICE VARIETIES"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Jeena

Vellayani,

Date: 22-08-2016

Jeena George

(2014 -11-128)

CERTIFICATE

Certified that this thesis entitled **"PHYSICO-CHEMICAL AND MOLECULAR CHARACTERIZATION OF GRAIN QUALITY OF TRADITIONAL RICE VARIETIES"** is a record of research work done independently by Ms. Jeena George under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Jayalekshmy. V.G

Dr. V.G. Jayalekshmy
(Major Advisor, Advisory Committee)
Professor (Plant Breeding and Genetics)
College of Agriculture
Vellayani.

Vellayani,
Date: 02/8/16

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Jeena George, a candidate for the degree of **Master of Science in Agriculture** with major in Plant Breeding and Genetics, agree that the thesis entitled **"PHYSICO-CHEMICAL AND MOLECULAR CHARACTERIZATION OF GRAIN QUALITY OF TRADITIONAL RICE VARIETIES"** may be submitted by Ms. Jeena George, in partial fulfilment of the requirement for the degree.

Jayalekshmy. V.G
22.8.16

Dr. V.G. Jayalekshmy
(Chairman, Advisory Committee)
Professor
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani.

Dr. P. Manju
(Member, Advisory Committee)
Professor & Head
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani.

22.8.16

Dr. Usha Mathew
(Member, Advisory Committee)
Professor
Department of Soil Science and Agricultural Chemistry
College of Agriculture, Vellayani.

22/8/16

Dr. Mareen Abraham
(Member, Advisory Committee)
Professor and Head (AICRP on Forages)
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani

22/08/2016

EXTERNAL EXAMINER

Dr. (Mrs.) SHEELA M. N.
Principal Scientist & Head
Division of Crop Improvement
ICAR-Central Tuber Crops Research Institute
Sreekariyam, Thiruvananthapuram-695 017

V.

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Jeena George

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	-	per cent
mm	-	Milli meter
µl	-	Micro litre
µM	-	Micro molar
^{0}C	-	Degree Celsius
ANOVA	-	Analysis of Variance
µg	-	Micro gram
bp	-	base pairs
kg	-	Kilogram
PCR	-	Polymerase Chain Reaction
SSR	-	Simple Sequence Repeats
m^3	-	Cubic meter
EDTA	-	Ethyl diamino tetra acetic acid
et al.	-	and co-workers/co-authors
ml		Milli litre
Fig.	-	Figure
g	-	gram
ppm	-	Parts per million
µg	-	Microgram
RM	-	Rice microsatellite
QTL	-	Quantitative Trait Loci
N	-	Normal
NaoH	-	Sodium hydroxide
mg	-	milligram
min	-	minutes
mM	-	Milli molar
NaCl	-	Sodium chloride

ng	-	Nanogram
HNO_3	-	Nitric acid
$HClO_4$	-	Per chloric acid
H_2SO_4	-	Sulphuric acid
rpm	-	revolutions per minute
S.E(m)	-	Standard Error Mean
RNA	-	Ribo nucleic acid
DNA	-	Deoxyribo nucleic acid
RILs		Recombinant Inbred Lines
OD	-	Optical density
TE	-	Tris EDTA
TAE	-	Tris Acetate EDTA
v/v	-	Volume/ volume
viz.	-	namely
w/v	-	weight/volume
A	-	Absorbance
sec	-	seconds
β	-	Beta
$MgCl_2$	-	Magnesium Chloride
KCl	-	Potassium Chloride
dNTP	-	Dinucleotide triphosphate

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population. Rice provides 20 percent of world's dietary energy supply. Unmilled rice contains greater number of nutrients compared to that of polished rice. In Asia, rice contributes about 40-80% of the calories (FAOSTAT, 2010).

Kerala encompasses a total of 38.90 lakh hectares of gross cropped area. Currently, rice is grown in a gross area of 162.10 thousand hectares producing 697.30 thousand tonnes with productivity of 2565 kg/ha (INDIASTAT, 2015). The prosperity of the people of Kerala largely depends on the performance of rice.

Grain quality of rice includes both physical and chemical attributes (Sreenivasulu *et al.*, 2015). Preferences for the quality factors varies across nations and regions (Calingacion *et al.*, 2014), however buyers lean toward rice with uniform shape and translucent endosperm, consequently appearance quality specifically impacts customer acknowledgment (Zhao and Fitzgerald, 2013).

Breeding for superior genotypes having intermediate amylose content and high yield potential should be the strategy for a successful breeding programme suitable for South and South East Asia. As intermediate amylose content is having more preference in India, genotypes with intermediate amylose content should be utilized for quality improvement in rice. In Kerala, since red rice is preferred to white rice, red kernelled rice with intermediate amylose content should be used for quality breeding (Vanaja and Babu, 2006).

The high genetic variation present in traditional varieties of rice can be exploited for rice improvement. *Indica* varieties constitute 80 per cent of the varieties cultivated all round India. Kerala contributes more than three hundred traditional rice varieties having aroma and therapeutic value, which adds variation to India's bowl of rice diversity (Rekha *et al.*, 2011). The variety Njavara, is an upland traditional variety of rice found in Kerala, which is considered as "God's valuable blessing" in light of its unimaginable ayurvedic use ensuing to mid many years.

During the last twenty years, new research discoveries created by the nutritionists have conveyed to light the role of micronutrients, proteins and vitamins in keeping up great wellbeing, acceptable advancement and even commendable levels of psychological capacity, apart from the problem of protein energy malnutrition (Nagesh *et al.,* 2012). To tackle hidden hunger, efforts have begun to offset the deficiencies caused by Iron, Zinc and Vitamin A. Rice is an excellent vehicle for biofortification through conventional and by deployment of biotechnological tools. Once it is biofortified with key supplements, the farmer is able to cultivate and produce nutritious rice grains indefinitely in a sustainable way, so that the produce reaches the malnourished rural population of India. Hence it is the need of the hour to develop rice varieties with high yield and improved quality.

New developments in genomics have provided tool for tagging novel alleles and offer great opportunity to understand the inheritance of grain quality traits and crop improvement through their use in marker assisted selection (Xu and Crouch, 2008). The determination of target traits can be accomplished by using molecular markers developed from the exact gene sequences or markers that are closely linked to genes.

Among PCR based markers, microsatellites or SSR markers are excellent markers because of their locus identity, high polymorphism information content (PIC) value and multiallelism. Microsatellite marker analysis is found promising to identify major gene locus for grain quality traits that can be useful for plant breeders to recognize new cultivar which can be utilized as donor parent for future breeding programme (Jewel *et al.*, 2011).

On the above grounds, this project was carried out on the following objectives

1. To study the physical and chemical characteristics as well as cooking quality of traditional rice varieties of Kerala

2. Molecular characterization with markers specific to quality traits.

Review of Literature

2. REVIEW OF LITERATURE

The present study aimed at assessing the land races of rice for physical and chemical parameters associated with grain quality and molecular characterization with SSR markers specific to quality traits. The relevant literatures in consonance with the objectives were briefly reviewed in this chapter.

2.1 RICE FEEDS HALF OF WORLD

Rice is one of the most important cereal crop consumed by more than 50 percent of World populace. (Itani *et al.*, 2002). Asia is the land where more than 90 per cent of rice is developed (Tyagi *et al.*, 2004). Rice have a significant position in India's food as well as livelihood security system by contributing 75 per cent of normal calories and 55 per cent protein to the normal day by day eating regimen of the population (Anonymous, 2002).

Quality of rice grains dependent on attributes like appearance of grain, nutritional value, cooking and eating quality (Juliano *et al.*, 1990). Improvement in plant breeding techniques has greatly increased the yield in rice cultivation, but grain quality of developed varieties to be improved further to meet various needs. Improving grain quality is thus essential to rice consumers (Tian *et al.*, 2009).

Rice landraces, kept up by conventional farming practices, have high genetic diversity and specific traits such as disease resistance, environmental constraint tolerance and nourishing quality, which can be utilized in crop improvement programme (Camacho-Villa *et al.*, 2005).

Kerala is well known for the rich genetic diversity of the traditional rice varieties developed in different seasons and in various agro-climatic conditions which incorporates numerous therapeutic rices moreover. Enormous variation exists for grain characters viz., grain colour, grain shape, grain size, kernel colour, aroma, processing, cooking and eating qualities. (Kumari, 2012).

2.2 PHYSICAL CHARACTERISATION

Consumer acceptance for size and shape of grain differs from one group to another (Khush *et al.*, 1979).

The grain size of traditional varieties of Kerala mostly comes under the categories of round , short bold , long bold and long slender .Among that the long bold red kernelled grains were preferred by the local farmers (Kumari, 2012).

According to length and breadth, kernel shape and size were classified in to various categories (SES, 2002). Based on kernel length, the kernels are classified as Extra-long (7.50mm), Long (6.61-7.50mm), Medium (5.51-6.60mm) and short(<5.50mm).

Dipti *et al.* (2002) conducted experiment on the physical, chemical and cooking qualities of six fine varieties of rice. The length and breadth of grains of variesties were found between 3.6 - 6.5 mm and 1.7-3.7 mm.

Subudhi *et al.* (2012) reported that the kernel length of forty one elite rice varieties in eastern India varied from 3.88 (Nuadhusara) to 7.54 mm (Geetanjali) and the kernel breadth ranged between 1.74 (Satyakrishana) to 2.68 (Hanseswari).

Bhonsle and Sellappan (2010) reported that length by breadth ratio of 22 traditional varieties of Goa ranged from 1.5 -3.5 .Out of twenty two varieties studied, eight varieties belonged to short bold, four varieties as long slender, eight varieties as long bold and five varieties as medium slender.

Compared to varieties that are imported having 1.96mm grain breadth , the locally grown varieties tend to have bolder grains with 2.21 to 2.26 mm breadth. (Diako *et al.*, 2011)

Chukwuemeka *et al.* (2015) found that shape of the grain influences its volume and weight. Slender varieties of rice occupy more volume than round varieties.

Vanaja and Babu (2003) reported a positive significant correlation between length and length/breadth ratio of grain as well as a negative significant correlation with breadth which indicated that, an increase in grain size results in increased shape but reduced boldness.

Balakrishnan *et al.* (2013) reported highly significant correlation between kernel length and breadth with length by breadth ratio.

Determination of bulk density, true density, and porosity is helpful in sizing grain hoppers and storage facilities. During aeration and drying processes it can also affect the rate of heat and mass transfer of moisture (Varnamkhastia *et al.*, 2008).

Kanchana *et al.* (2012) reported that, bulk density of rice varieties collected from Tirunelveli, Madurai and Virudhunagar districts of Tamil Nadu showed a range of 0.701 to 0.868 (g/ml).

2.3 COOKING QUALITIES

Cooking and consumption quality is one of the most important components of grain quality. Cultivars with different grain quality are also required for medicinal, ceremonial, or special production purposes (Tian *et al.*, 2009).

Cooking time is vital as it determines the delicacy as well as stickiness of cooked rice (Anonymous, 1997).

Compared to polished rice, brown rice requires longer cooking time as it possess a thick aleurone layer and a pericarp which delay water penetration in to grains, while cooking (Mahadevappa and Desikachar, 1968). Oyegbayo *et al.* (2001) observed that two local rice varieties from Nigeria took longer cooking time of 52 and 56 minutes.

. Deepa *et al.* (2008) reported that the dehusked grains of the varieties Jyothi and IR 64 took 30 min for cooking and the cooked grains were found to be slender as well as flaky due to increased amylose content. Whereas, dehusked grains of variety njavara required a longer cooking time of 38 min.

Linear elongation of rice on cooking is one of the important characteristics that determine the quality of rice. Increase in length without increase in breadth is viewed as a very attractive characteristic of good quality rice (Juliano, *et al.,* 1990).

Shobha (2003) reported that in India, among the nine released hybrid rice varieties, the kernel elongation ratio ranged between 1.70 to 2.00. While evaluating twenty new inter sub –specific rice hybrids, their kernel elongation ratio ranged from 1.51 to 1.82 (Hossain *et al.*,2009).

Subudhi *et al.* (2012) studied the quality characters of forty one elite rice cultivars in eastern India and observed highest elongation ratio in Nuadhusara (2.07) and Nuakalajeera (2.0) and lowest in Chandan (1.44).

Volume expansion ratio is a beneficial character for lower income group , as quantity is the key factor. However, when volume expansion ratio is more, energy content per unit volume will be less (Subudhi *et al.*, 2012).

Vanaja and Babu (2006) reported a wide variation in volume expansion ratio from 1.87 to 3.17 while working with 56 high yielding rice varieties of diverse origin.

Sonowal and Barooah (2015) recorded moderate degree of volume expansion between 2.43 to 3.12 for the varieties like Mulagabharu ,Chakralahi, Dithalu, Manaah and TTB-303142 collected from North east.

Water uptake of rice is an important economic quality, as it gives an estimate of increase in volume, while cooking (Sood and Siddiq, 1986).

Danbaba *et al.* (2011) conducted experiment to study the cooking as well as eating quality of *Ofada* rice. They have observed that the water uptake of *Ofada* rice ranged from 174.00 to 211.00 %. The mean water uptake was found to be 189.7 indicating moderate quality of this character.

Oko *et al.* (2012) reported that increase in water absorption ratio negatively affects the taste of the cooked rice. Hossain *et al* (2009) observed that at higher water uptake (300.00 to 570.00 %), most of the rice shows pasty appearance which is unsuitable for cooking and eating.

2.4 CHEMICAL CHARACTERIZATION

2.4.1 Amylose and Amylopectin Content

Rice starch is made up of glucose polymers like highly branched amylose and relatively unbranched amylopectin (Juliano, 1985). The starch content of rice grains contain 17 to 30% amylose, which is the key element behind the taste and processing qualities (Zhou *et al.*, 2003).

The amylose content of rice is the prime factor for cooking and eating qualities (Juliano, 1972). It determines the delicacy, hardness as well as colour of cooked rice. When the amylose content is high, cooked rice gives non sticky soft or hard appearance. Rice varieties with intermediate amylose content gives soft as well as flaky rice, during cooking. Amylose content is a factor that determines the increase in volume and water uptake while cooking (Deyner *et al.,* 2001)

Low amylose content is generally associated with tender, cohesive and glossy cooked rice and high amylose content results in hard texture and low viscosity with good swelling capacity (Juliano, 1971).

Amylose content (AC) plays an important role in different rice cooking, sensory and processing properties (Bergman *et al.,* 2001). Amylose molecule is a factor that determines the gelatinization temperature, pasting as well as viscoelastic properties of grain (Tavares *et al.*, 2010).

On the basis of amylose content, rice varieties are classified in to waxy (0-2%), very low (3-9%), low (10-19%), intermediate (20-25%) and high (>25%). In that rice with intermediate amylose content are the preferred in most of the rice growing areas (Shobha rani *et al.*, 2012).

Dipti *et al.* (2002) reported that amylose content of six fine rice varieties ranged from 18.60 to 28.00%. BRRIdhan 28 showed highest amylose (28%) followed by Superfast (24.20%), whereas Khazar and Basmati PNR given the lowest (18.60%).

Vinodhana *et al.* (2012) reported that amylose content of 60 genotypes recorded a range of 16.00% (Tephanh) to 33.20% (Vanakkar). Intermediate amylose content was observed in 35 rice genotypes.

Subudhi *et al.* (2012) reported intermediate amylose content in the tested high yielding varieties of eastern India, excluding Vanaprava and Heera .The amylose content of the varieties varied between 22.10 (Utkalprava) to 26.10 (Vanaprava).

The starch of waxy rice varieties constitutes amylopectin alone and they show less water uptake during cooking and gives a sticky texture (Frei and Becker, 2003).

Frei and Becker (2003) reported that compared to those foods with low levels of amylose, consumption of starchy foods having high amylose levels lowers blood glucose level and results in slower emptying of the human gastrointestinal tract.

Cristiane *et al.* (2007) reported that following a diet rich in amylose than a diet rich in amylopectins, results in significant reduction of serum triglyceride and cholesterol levels.

Consumption of cooked rice with high amylose content helps to control serum blood glucose and lipid levels effectively (Magdy *et al.*, 2010).

Chukwuemeka *et al.* (2015) observed that the amylopectin values of rice ranged from 62.80 and 79.80 % respectively, where IRR8 has the highest percentage of amylopectin and Caprice has the lowest percentage of amylopectin, Rice varieties high in amylose would invariably be low in amylopectin content.

Vanaja and Babu (2003) reported a positive correlation of amylose content between water uptake and volume expansion ratio. It indicated that rice varieties with high amylose content will absorb more water and produce a greater volume of cooked material.

Hussain *et al.* (1987) reported a positive and significant relation among amylose content and water absorption, whereas Chauhan *et al.* (1995) observed a negative correlation among two traits.

Madan and Bhat (1984) reported a significant positive correlation of amylose content with volume of cooked rice and water absorption.

Oko *et al.* (2012) observed negative value of correlation between grain elongation during cooking and amylose content indicates that cultivars that elongate more during cooking would likely have a de-creased content of amylose.

Vanaja and Babu (2003) reported that when amylose content of varieties increases, cooking time also increases.

Bhattacharya and Sowbhagya (1971) observed a significant association between water absorption and optimum cooking time.

Oko *et al.* (2012) obtained significant but negative correlation values between amylose content with amylopectin, suggesting that rice cultivars selected for high amylose content may be invariably low in amylopectin content

The positive significant correlation among water absorption, volume expansion and amylose content is an indicator of rice quality and buyers preference in Kerala (Vanaja and Babu, 2003).

2.4.2 Protein Content

The seed-storage protein content determines the nutritional quality of rice grain which can make up to 5-12% (Villareal and Juliano, 1978). Rice bran constitutes 11.3% -to 14.9% of proteins (Zhu *et al.*, 2010).

Dipti *et al.* (2002) conducted experiment in six fine rice varieties .They have observed that protein content of the fine varieties ranged from 6.9-8.6%. The variety BRRI dhan28 recorded maximum protein content of 8.6% which was followed by Badshabhog.

Deepa *et al.* (2008) evaluated and compared physical, chemical and cooking properties as well as nutritive components of dehusked rice of Njavara with two commonly consumed popular rice varieties – Jyothi and IR 64. They have observed that compared to Jyothi and IR 64, Njavara dehusked rice had greater protein, minerals, vitamins and fibre. The njavara paddy collected from Padma, Ayurveda, Mannar recorded 9.5% protein content.

Samak *et al.* (2011) reported that in F5 RILs of rice derived from BPT5204 X HPR 14, total grain protein content was found between 7.39 to 12.81%

Sindhumole (2012) reported that landraces tend to have higher protein content than high yielding varieties, which were developed mainly to optimise the need, and not the yield.

2.4.3 Iron and Zinc Content

Brar *et al* (2011) suggested that utilization of high genetic variation for iron and zinc existing in cereal germplasm is an effective way to reduce the extent of iron and zinc inadequacies in developing world.

Banerjee *et al.* (2010) evaluated forty six rice lines including cultivated and wild accessions and observed that wild rice accessions had greater grain iron content. Jahan *et al*. (2014) reported that among the 52 genotypes studied, Lal Gotal recorded highest iron concentration (100.45 ppm) and Jota Balam recorded the lowest (1.3ppm).

Anandan *et al*. (2011) studied the Iron and Zinc content of 202 genotypes including traditional and improved cultivars of Tamil Nadu and reported that compared to improved cultivars, traditional cultivars were significantly higher in iron and zinc content.

Roy and Sharma (2014) have screened 84 landraces for Iron and Zinc content. Iron content ranged from 0.25 µg/g to 34.8 µg/g whereas zinc content recorded 0.85 µg/g to 195.3 µg/g.

Sellappan *et al* (2009) reported that polishing removes about 70% of micronutrients from rice grain, as almost the entire aleurone layer as well as major part of embryo are lost during the process.

Iron and Zinc content in unpolished rice of 168 F7 RILs derived from Madhukar× Swarna grains ranged from 0.2 to 224 ppm and 0.4 to 104 ppm respectively (Anuradha *et al*., 2012).

In the trial conducted on screening of rice germ plasm for Zinc and Iron contents, the variety Revathy released by Moncombu reported highest Iron content of 219.56 mg/kg and the variety Gouri released by Moncombu reported highest Zinc content of 29.84 mg/kg (RRS, AICRIP Report, 2008), whereas the Iron and Zinc content reported in the cultivated varieties ranged from 23.50-233.70 mg/kg and 49.80- 178.10 mg/kg, respectively (DRR, Annual report 2011-2012).

Nachimuthu *et al*. (2014) observed a positive correlation between Fe and Zn contents in brown rice of 192 genotypes, which indicated the possibility of simultaneous effective selection for both the micronutrients.

2.4.4 Carotene Content

Renuka *et al.* (2016) reported that the beta carotene contents of 39 aromatic *indica* rice varieties ranged from, 1.23 to 9.9 1 μg/g in brown rice and 0.08 to 1.99 μg/g in milled rice.It was observed that milling resulted in the reduction of beta carotene content to a great extent.

Valarmathi *et al.* (2012) conducted biochemical characterization of traditional brown rice "Kavuni". Total carotenoids and beta-carotene content in Kavuni (61-78% and 26-65%) was found to be greater than the popularly eaten varieties like Co 50, White Ponni and IR 64.

Lamberts and Delcour (2008) observed comparative reduction in beta carotene after milling in five rice cultivars (0.020 to 0.085 μg/g).

2.5 MOLECULAR CHARACTERIZATION

Microsatellites, being very powerful molecular markers due to their high genetic variation are utilized in various areas of study (Tautz, 1989; Weber and May, 1989)

Microsatellites have ended up being a greatly important tool for genome mapping in many organisms (Schuler *et al.*, 1996; Knapik *et al.*, 1998) their applications span over different areas ranging from family relationship examination, to population genetics and conservation/management of biological resources (Jarne and Lagoda, 1996). Microsatellites are intense for a variety of uses as a result of their reproducibility, multiallelic nature, codominant inheritance, relative abundance and good genome coverage (Liu and Cordes, 2004).

Amylose synthesis is mainly controlled by Waxy (*Wx*) gene locus in chromosome 6 which encode Granule Bound Starch Synthase enzyme (Smith *et al.*, 1997). Polymorphic microsatellite marker RM190 with $(CT)_n$ dinucleotide repeats at exon 1 in *Wx* gene locus was proved to give significant association with amylose levels (Jayamani *et al.*, 2007).

Cheng *et al.* (2015) found that the amplified products for *Wx*-SSR ranged from 110 to 130 bp in length.

Balakrishnan *et al.* (2013) employed SSR marker RM 190 to assess the genetic diversity among 82 rice genotypes for the genetic polymorphism of the *Wx* gene and the primer showed 48.95% correlation with phenotypical variation

Sabouri (2009) identified that RM4955-RM152 interval have the QTLs that clarified the most variation in amylose content. qAc-8a is identified as major gene and explained 22.10% phenotypic variance.

.Molecular markers can be used to identify linkage to QTL for total grain protein content and these can be selected more easily in a breeding programme than the trait themselves (Samak *et al.*, 2011)

Samak *et al.* (2011) reported that in F5 generation, 7 primers given significant association with total grain protein content. Among that RM520 gave 9.1 per cent phenotypic variability (R2) followed by RM206.

New research discoveries have conveyed the significance of micronutrients in keeping up great wellbeing and rice is an excellent vehicle for biofortification through conventional and deployment of biotechnological tools (Shobharani *et al.*, 2012).

Anuradha *et al.* (2012) genotyped 168 RILs derived from the cross Madhukar x Swarna using 110 SSR markers, covering all the 12 chromosomes. QTLs were identified for Iron concentration on chromosomes 1, 5, 7 and 12 and for Zinc concentration on chromosomes 7 and 12. Four markers essentially connected to Iron and 14 to Zinc concentration were detected. RM535 on chromosome 2 was normal to both Iron and Zinc concentrations.

Materials and Methods

3. MATERIALS AND METHODS

The experimental study entitled "Physico-chemical and molecular characterization of grain quality of traditional rice varieties" was carried out to assess land races of rice for physical and chemical parameters associated with grain quality and molecular characterization with markers specific to quality traits. The materials utilized and the methodologies adopted in the present investigation are described below.

3.1 PLANT MATERIAL

Twenty traditional rice varieties including medicinal rice njavara obtained from germplasms maintained in various research stations of KAU, collected from different parts of Kerala was used for the study. Three samples were drawn from the collected lot of 250 grams of each variety. These germplasm samples were dehusked manually and the raw rice was taken for evaluation. The varieties selected for study are given in (Table 1)(Plate1)

Table 1. List of traditional varieties used for evaluation

SI.No	Variety
1	Gandhakasala
2	Ittikkandappan
3	Chennellu
4	Chootupokkali
5	Cheruvirippu
6	Mullan kayama
7	Cheradi
8	Orumundakan
9	Njavara veluthath
10	Njavara Kunnathur

Sl.No	Variety
11	Njavara Palliyara
12	Jeerakasala
13	Marathondi
14	Veliyan
15	Chettadi
16	Thondi
17	Karimbalan
18	Adukkan
19	Chennelthondi
20	Kannichennellu

3.2 PHYSICAL CHARACTERIZATION

3.2.1 Kernel Length and Breadth

Kernel length and breadth were recorded with the help of Dial Vernier calipers on ten randomly selected fully healthy grains (Khan *et al.*, 2003). Based on length, grains are classified into three classes according to (SES, IRRI, 2002)

Extra long (>7.50mm)

Long (6.61-7.50mm)

Medium (5.51-6.60mm)

Short (<5.50mm)

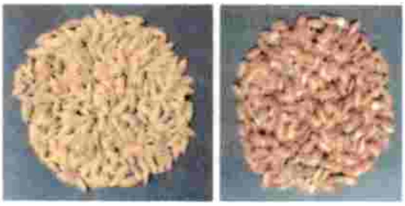
3.2.2 Kernel Length / Breadth Ratio

This was determined by taking the ratio of kernel length and breadth calculated as above. Shapes of rice was determined in terms of length / breadth ratio as slender (more than 3.00mm), medium (2.10 to 3.00mm) bold (1.10 to 2.00mm) and round less 1.00mm) (SES, IRRI,2002).

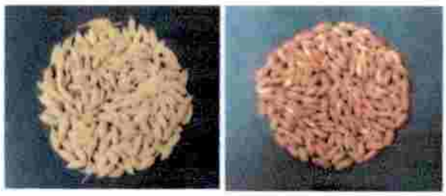
Plate 1.Twenty Traditional varieties collected for study

1.Gandhakasala

2. Ittikkandappan



3. Chennellu

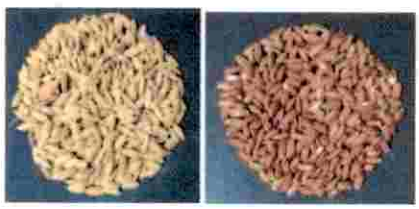


4. Chootupokkali

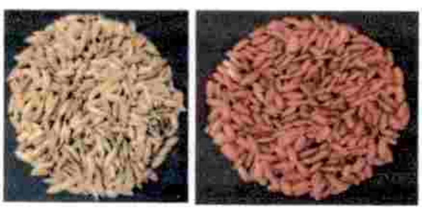
5.Cheruvirippu

6. Mullan kayama

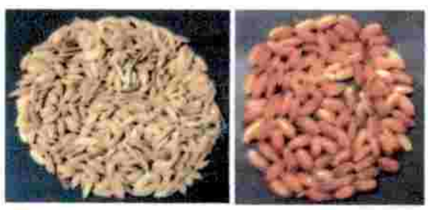
7. Cheradi



8. Orumundakan



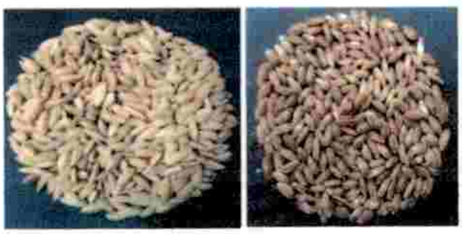
9. Njavara Veluthath



10. Njavara Kunnathur

11. Njavara Palliyara

12. Jeerakasala



13. Marathondi

14. Veliyan

15. Chettadi

16. Thondi

17. Karimbalan

18. Adukkan

19. Chennelthondi

20. Kannichennellu

3.2.3 Bulk Density

It was calculated by using mass/volume relationship. After taring the weight of an empty cylinder of known volume, grains are poured to it from a constant height. Then the top level is striked off and weight is taken (Fraser *et al*., 1978).

3.3 COOKING QUALITIES

3.3.1 Optimum Cooking Time

Optimum cooking time was determined by parallel plates method given by Bhattacharya and Sowbhagya (1971). In this method, about ten grains were taken at two minutes interval from the test tubes placed in water bath and pressed between two glass plates until atleast 90 per cent of rice have no longer opaque centre which is considered as the optimum cooking time

3.3.2 Linear Elongation Ratio

Initial length of unbroken rice kernels were measured and averaged. The length of ten unbroken cooked rice were measured using graph paper and the average was recorded. The linear elongation ratio is recorded as

LER= (Average length of whole cooked grain) / (Average length of whole uncooked grain)

3.3.3 Breadth Wise Elongation Ratio

Average breadth of unbroken rice kernels and average breadth of unbroken cooked rice kernels were calculated as described above. The breadth wise elongation ratio is recorded as

BER= (Average breadth of cooked rice) / (Average breadth of raw rice)

3.3.4 Volume Expansion

The volume of initial uncooked rice was measured by water displacement method in graduated measuring cylinder. Then the rice is cooked in a boiling water bath until its optimum cooking time. The cooked rice is blotted for free water without loss of solid .The cooked rice volume is measured again by ware displacement method (Juliano, 1971). Then volume expansion was calculated as

Volume expansion= Volume of cooked rice/Volume of raw rice

3.3.5 Water Absorption

This was determined by cooking 2.0 g of whole rice kernels in a boiling water bath until its optimum cooking time. The cooked rice is blotted in a paper to drain the water without losing the solids. The cooked samples were then weighed and the water uptake ratio was calculated (Oko *et al.*, 2012).

Water uptake ratio = (weight of cooked rice) / (weight of raw rice)

3.4 CHEMICAL CHARACTERIZATION

3.4.1 Amylose and Amylopectin Content

Weigh 100 mg of sample and add 1 ml of distilled ethanol. After that add 9 ml of 1N NaOH and heated in a boiling water bath for 10 minutes. Make up the volume to 100ml with distilled water. Later, take 5 ml of sample suspension ,add about 50 ml of distilled water and 1 ml of acetic acid (57.75 ml in one liter water) along with 1.5 ml of iodine solution (0.2 percent w/v iodine in 2% potassium iodide). After that make up the volume to 100ml and kept for 20 minutes. The samples were measured at 620 nm using spectrophotometer and NaOH solution was used as blank for calibration. The amylose content of different varieties was calculated in comparison with standard graph made by using Sigma, Potato amylose (Perez and Juliano, 1978) .The amylopectin content was calculated by the difference method.

% Amylopectin = 100 - % Amylose

Based on amylose content, the genotypes were classified into different amylose groups (SES, IRRI, 2002) as low amylose (10-20% amylose), intermediate amylose (20-24% amylose) and high amylose (>25% amylose)

3.4.2 Protein Content

Total crude protein content was determined by micro kjeldahl method using Elite EX, Kjelplus automatic nitrogen analyzer. The rice kernels were ground into fine powder and used for analysis. The digest was titrated with 0.1 N HCL .The nitrogen content was calculated from the titre value and was multiplied by a factor 6.25 to convert into crude protein content (Thongbam *et al.*, 2010)

3.4.3 Iron and Zinc Content

Iron and zinc content of grain samples were estimated by using Atomic Absorption Spectrophotometer (Lindsay and Novell, 1978). One gram of seed was taken and powdered in the grinder. Powdered seed sample was digested in tri-acids (HNO_3+$HClO_4$+H_2SO_4) mixture (10:4:1) in sand bath. The digested sample was cooled for 30 minutes and made up to 50 ml with double distilled water. Then a known quantity of solution was used for subsequent analysis. A suitable blank was run simultaneously to account for the contamination from the reagents.

3.4.4 Carotene Content

10g of powdered rice sample was dispersed in 50 ml water saturated n-butanol to make a homogenous suspension. The suspension was shaken gently and allowed to stand overnight (16 hours) at room temperature at dark. After that these were filtered through Whatman filter paper No.14 and volume of filterate was made up to 100ml. The absorbance (A) of the clear filterate was measured at 440 nm in spectrophotometer against water saturated n-butanol as blank.

Beta carotene content (ppm) = 0.0105 + 23.5366*A

(Joy *et al.*, 2015)

3.5 STATISTICAL ANALYSIS

The data recorded on different traits were subjected to the following statistical analysis.

1. Analysis of variance
2. Simple correlation analysis

The observations were made from three samples and the mean value was subjected to one way analysis of variance using the software package WASP.2. Simple correlation studies were also carried out to identify the relationship between the treatments.

3.6 MOLECULAR CHARACTERIZATION

3.6.1 Molecular markers

Previously reported SSR markers RM190, RM520, RM535 associated to amylose content, protein content and Iron and Zinc content respectively were used for the characterization. Primers for the markers, product size and reference is given in Table 2.

Table 2. Details of SSR markers used for evaluation

MARKER	FORWARD PRIMER	REVERSE PRIMER	PRODUCT SIZE
RM 535	actacatacacggcccttgc	ctacgtggacaccgtcacac	138bp
RM190	ctttgtctatctcaagacac	ttgcagatgttcttcctgatg	124bp
RM 520	aggagcaagaaaagttcccc	gccaatgtgtgacgcaatag	247bp

(www.gramene.org)

3.6.2 Isolation of genomic DNA

Genomic DNA from twenty traditional varieties were isolated by using QIAGEN DNA easy plant mini kit.

- Samples were disrupted (≤100mg wet weight or ≤ ≥20mg lyophilized tissue) using the mortar and pestle with liquid nitrogen. Add 400µl buffer AP_1 and 4µl RNAse A. Vortexed and incubated for 10min at 65°C.Inverted the tube 2-3 times during incubation.
- Added 130µlBuffer P3.Mix and incubated for 5 minutes on ice. Centrifuged the lysate for 5 min at 20,000 x g (14000rpm). Pipetted the lysate into a QIA shredder spin column placed in a 2ml collection tube. Centrifuged for 2 minutes at 20,000 x g.
- Transferred the flow –through into a new tube without disturbing the pellet if present .Added 1.5 volumes of buffer AW1, and mixed by pipetting. Transferred 650 µl of the mixture into a DNA easy mini spin column placed in a 2 ml collection tube. Centrifuged for 1 minute at ≥ 6000 x g (≥8000rpm).Discarded the flow –through.

- Repeated this step with the remaining sample. Placed the spin column into a new 2 ml collection tube. Added 500 μl Buffer AW2, and centrifuged for 1minute at ≥6000 x g. Discard the flow through. Added another 500 μl Buffer AW_2, and centrifuged for 2 minutes at ≥20000 x g. Transferred the spin column to a new 1.5ml or 2ml micro centrifuge tube.
- Added 100 μl Buffer AE for elution. Incubated for 5 minutes at room temperature (15-25° C). Centrifuged for 1 min at ≥6000 x g. Then DNA stored at -20°C.

3.6.3 Agarose Gel Electrophoresis

3.6.3.1 Stock solutions

50X TAE Buffer

Tris base	240g
Acetic acid	57.1ml
0.5M EDTA (pH-8.0)	186.12g
Final volume (Distilled H2O)	1000ml

6X loading dye

Sucrose	4.0g
Bromophenol blue	0.025g
Volume (Distilled H2O)	10ml

(Loading dye solution was stored at 4°C)

Agarose gel electrophoresis was carried out in a BIO-SYS, horizontal gel electrophoresis Unit by using the following procedure.

- For the detection of DNA 0.8% of agarose was prepared and melted in 1x TAE buffer.
- After cooling the solution to 42-45°C, ethidium bromide was added at the rate of 5μl for 100ml.
- The solution was then poured on to a preset, sealed gel casting tray with a comb fixed in position, to a height of 3mm-5mm.
- The gel was allowed to solidify for 15-20 minutes.

- The comb and sealing tapes were then removed and tray was submerged in electrophoresis tank filled with 1x TAE buffer ensuring that the buffer covered the gel to a height of 1mm.
- Required volume of DNA sample and loading dye [glycerol 30% + bromophenol blue] were mixed in the ratio 5:1 and loaded into the slots of gel using a micropipette near the negative terminal.
- The cathode and anode of the electrophoresis unit were attached to the power supply and constant voltage of 60V was used for the run. The power supply was turned off when the loading dye moved about 3/4th of the gel.
- The gel was documented using SYNGENE gel documentation system.

3.6.4 Quantification of DNA

Spectrophotometer reading was relied on to assess the quality and quantity of DNA samples used for the study. 5 µl of sample DNA was dissolved in 0.1x TE buffer was added to 3 ml of distilled water and absorbance at 260 and 280 nm was read against distilled water as blank, using UV spectrophotometer. The concentration of DNA in sample was calculated using formula

$$\textbf{Amount of DNA } (\mu g/ml) = \frac{A_{260} \times 50 \times \text{Dilution factor}}{1000}$$

Where, A_{260} = absorption at 260 nm.

Ratio of absorbance values at 260 nm and 280 nm gives the quality of DNA. A ratio of 1.8 - 2.0 indicates best quality DNA.

3.6.5 Validations of microsatellite markers for grain quality traits

The PCR mixture contained 50 ng template DNA, 10 pmoles of each primer, 10 mM dNTPs, 10X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1 U of Taq DNA polymerase in a reaction volume of 25 µl. Amplifications were carried out in an Eppendorf master cycler nexus gradient. The temperature specifications for the denaturation of DNA strand, annealing of primers and extension steps are given in Table 3. The above steps are repeated for 35 cycles for amplification of DNA as in Fig 1. After completion of amplification, PCR products were stored at –20°C. The amplified product was electrophoretically resolved on a 4% agarose gel containing 0.5 µg/ml of ethidium bromide in 1x TAE buffer and visualized under SYNGENE G-Box F3gel documentation unit.

Table 3.Thermal profile of PCR reaction

Sl.No	Steps	Temperature	Time
1.	Initial denaturation	94 ^{0}C	5 min
2.	Denaturation	94^{0}C	30 sec-1min
3.	Annealing	RM 190 -55^{0}C	30 sec
		RM 520 -55^{0}C	30sec
		RM 535 -56^{0}C	1min
4.	Extension	72^{0}C	2min
5.	Final extension	72^{0}C	5min

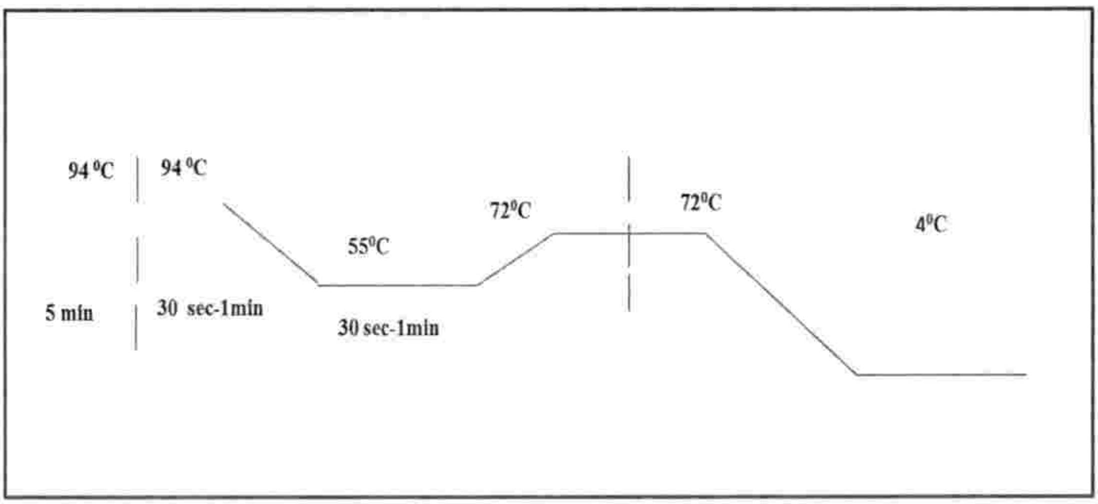


Fig 1.Thermal profile of PCR reaction

Results

4. RESULT

This chapter presents the results obtained for various parameters analysed in the investigation entitled "Physico-chemical and molecular characterization of grain quality of traditional rice varieties". The study was conducted to determine and compare the physico chemical characters of twenty traditional rice varieties including medicinal rice njavara. The study also identified the presence or absence of SSR markers linked to important quality traits.

The analysis was done in triplicate and the mean values were subjected to one way analysis of variance and the values are compared at 1% and 5% levels of significance using the software package WASP.2. The treatments showed significant difference for all the characters at both levels. Simple correlation studies were also carried out to identify the relationship between the treatments. The results obtained for each character are given below.

4.1 PHYSICAL CHARACTERISATION

4.1.1 Kernel Length

The kernel length of the varieties ranged from 4.00 to 6.91 mm. The variety Chennellu showed the highest value and it differed quite significantly from other varieties. Gandhakasala variety showed the lowest value compared to others.

Based on kernel length varieties were grouped in to long (6.61 to 7.50 mm), medium (5.51 to 6.60 mm) and short (less than 5.50 mm) grains. The variety Chennellu was classified as long grain and the varieties Gandhakasala, Cheruvirippu, Mullan Kayama and Njavara Kunnathur were grouped as short grains. The rest of the varieties were medium type grains.

4.1.2 Kernel Breadth

The varieties showed a range between 2.19 to 4.00 mm for kernel breadth. The variety Chennellu showed the highest and Chootupokkali showed the lowest mean value. The statistical analysis showed that the kernel breadth of Chennellu is significantly higher than other varieties .The variety Chootupokkali showed the lowest value and was found to be on par with Njavara Palliyara and Njavara Veluthath.

The results obtained for kernel length and breadth was shown in Table 4.

4.1.3 Kernel Length/Breadth Ratio

The kernel length/breadth ratio varied from 1.60 to 2.91. The highest value was recorded for the variety Chootupokkali, and was found to be on par with the variety Jeerakasala with the ratio 2.66. The variety Mullan Kayama showed the lowest ratio 1.6 (Table 5).

Depending up on the length/breadth ratio, the varieties were classified in to 3 groups, slender (more than 3.00mm), medium (2.10 to 3.00mm) and bold (1.10 to 2.00mm). The varieties Njavara Palliyara, Jeerakasala, Marathondi, Kannichennellu, Ittikandappan, Cheradi, Njavara Veluthath, Chettadi, Adukkan, and Chootupokkali were classified as medium type grains and the rest of the varieties as bold type grains.

4.1.4 Bulk Density

Bulk density showed significant difference among the varieties at both 1% and 5% levels of significance. The bulk densities varied from 681.63 to 787.00kg/m^3. The maximum value was shown by the variety Ittikkandappan (787.00kg/m^3) and it was found to be on par with the varieties Chettadi and Chennellu. The variety Njavara Veluthath showed the minimum value and recorded lowest significant difference from other varieties. The mean values obtained for bulk densities of twenty varieties are shown in Table 6.

Table 4. Mean values for kernel length and breadth of twenty genotypes

SI.No	Variety	Kernel length(mm)	Kernel breadth(mm)
1	Gandhakasala	4.00	2.32
2	Ittikkandappan	5.71	2.66
3	Chennellu	6.91	4.00
4	Chootupokkali	6.36	2.19
5	Cheruvirippu	5.40	2.71
6	Mullan kayama	4.25	2.66
7	Cheradi	6.14	2.73
8	Orumundakan	6.03	2.80
9	Njavara Veluthath	5.56	2.26
10	Njavara Kunnathur	5.12	2.63
11	Njavara Palliyara	5.66	2.28
12	Jeerakasala	6.42	2.42
13	Marathondi	6.00	2.84
14	Veliyan	5.45	2.92
15	Chettadi	6.05	2.83
16	Thondi	5.74	3.03
17	Karimbalan	5.99	2.88
18	Adukkan	6.43	2.80
19	Chennelthondi	5.79	2.95
20	Kannichennellu	5.78	2.75
	MEAN	5.74	2.73
	SE(m)	0.07	0.02
	CD	0.17	0.14

Table 5. Mean values for kernel length / breadth ratio of twenty genotypes

SI.No	Variety	Kernel length/breadth ratio
1	Gandhakasala	1.72
2	Ittikkandappan	2.15
3	Chennellu	1.73
4	Chootupokkali	2.91
5	Cheruvirippu	1.99
6	Mullan kayama	1.60
7	Cheradi	2.25
8	Orumundakan	1.82
9	Njavara Veluthath	2.45
10	Njavara Kunnathur	1.96
11	Njavara Palliyara	2.49
12	Jeerakasala	2.66
13	Marathondi	2.11
14	Veliyan	1.87
15	Chettadi	2.13
16	Thondi	1.91
17	Karimbalan	2.08
18	Adukkan	2.29
19	Chennelthondi	1.96
20	Kannichennellu	2.10
	MEAN	2.11
	SE(m)	0.08
	CD	0.25

Table 6. Mean values for bulk density of twenty genotypes

Sl.No	Variety	Bulk density(kg/m^3)
1	Gandhakasala	751.33
2	Ittikkandappan	787.00
3	Chennellu	767.33
4	Chootupokkali	727.00
5	Cheruvirippu	756.00
6	Mullan kayama	749.00
7	Cheradi	731.33
8	Orumundakan	710.63
9	Njavara Veluthath	681.63
10	Njavara Kunnathur	724.00
11	Njavara Palliyara	754.00
12	Jeerakasala	758.67
13	Marathondi	743.67
14	Veliyan	739.33
15	Chettadi	773.33
16	Thondi	742.67
17	Karimbalan	749.33
18	Adukkan	752.00
19	Chennelthondi	709.00
20	Kannichennellu	750.33
	MEAN	742.88
	SE(m)	8.19
	CD	23.42

4.2 COOKING QUALITIES

4.2.1 Optimum Cooking Time

Optimum cooking time ranged from 31.00 to 52.00 minutes for the varieties. The variety Karimbalan took maximum time for cooking and the variety Jeerakasala took the minimum time of 31.00 minutes (Table 7). Statistical analysis showed that optimum cooking time of Karimbalan variety was on par with Chennellu, Chettadi, Cheruvirippu, and Chootupokkali for the respective trait.

4.2.2 Linear Elongation Ratio

The linear elongation ratio of the varieties ranged from 1.11 to 1.60. The variety Mullan Kayama showed the maximum value and is significantly different from other varieties. The variety Chennellu showed the minimum value and was found to be on par with the variety Cheradi.

4.2.3 Breadth Wise Elongation Ratio

The breadth wise elongation ratio varied from 1.01 to 1.94. The maximum value is for the variety Chootupokkali and minimum value for Chennellu. The variety Chootupokkali showed highest significant difference from other varieties.

The results obtained for linear elongation ratio and breadthwise elongation ratio are shown in Table 8.

4.2.4 Volume Expansion

Volume expansion for the varieties showed a range of 1.98 to 3.43 with the variety Cheradi having the maximum value and the variety Mullan Kayama having the minimum value. Cheradi was found on par with Chennellu and showed highest significant difference between volume expansions of rest of the varieties. The variety Mullan Kayama was found to be on par with the varieties Veliyan, Kannichennellu and Gandhakasala.

Table 7. Mean values for optimum cooking time of twenty genotypes

SI.No	Variety	Optimum cooking time (minutes)
1	Gandhakasala	40
2	Ittikkandappan	37
3	Chennellu	50
4	Chootupokkali	49
5	Cheruvirippu	50
6	Mullan kayama	45
7	Cheradi	49
8	Orumundakan	47
9	Njavara Veluthath	41
10	Njavara Kunnathur	42
11	Njavara Palliyara	45
12	Jeerakasala	31
13	Marathondi	41
14	Veliyan	35
15	Chettadi	50
16	Thondi	40
17	Karimbalan	52
18	Adukkan	48
19	Chennelthondi	47
20	Kannichennellu	43
	MEAN	44.02
	SE(m)	1.13
	CD	3.23

Table 8. Mean values for linear elongation ratio and breadth wise elongation ratio of twenty genotypes

SI.No	Variety	LER	BER
1	Gandhakasala	1.25	1.23
2	Ittikkandappan	1.20	1.43
3	Chennellu	1.11	1.01
4	Chootupokkali	1.20	1.94
5	Cheruvirippu	1.41	1.34
6	Mullan kayama	1.60	1.24
7	Cheradi	1.13	1.74
8	Orumundakan	1.23	1.51
9	Njavara Veluthath	1.29	1.46
10	Njavara Kunnathur	1.40	1.45
11	Njavara Palliyara	1.37	1.51
12	Jeerakasala	1.23	1.23
13	Marathondi	1.21	1.47
14	Veliyan	1.37	1.29
15	Chettadi	1.19	1.27
16	Thondi	1.31	1.30
17	Karimbalan	1.14	1.29
18	Adukkan	1.20	1.31
19	Chennelthondi	1.31	1.33
20	Kannichennellu	1.22	1.48
	MEAN	1.27	1.39
	SE(m)	0.03	0.03
	CD	0.02	0.06

LER-Linear Elongation ratio, **BER-**Breadth wise elongation ratio

Table 9. Mean values for volume expansion and water absorption of twenty genotypes

SI.NO	Variety	Volume expansion	Water absorption
1	Gandhakasala	2.07	1.85
2	Ittikkandappan	2.31	2.11
3	Chennellu	3.34	2.91
4	Chootupokkali	2.50	1.94
5	Cheruvirippu	2.62	2.00
6	Mullan kayama	1.98	1.69
7	Cheradi	3.43	2.62
8	Orumundakan	2.53	2.22
9	Njavara Veluthath	2.50	2.35
10	Njavara Kunnathur	2.50	2.40
11	Njavara Palliyara	3.10	2.68
12	Jeerakasala	2.55	2.32
13	Marathondi	2.50	2.27
14	Veliyan	2.05	1.95
15	Chettadi	2.57	2.17
16	Thondi	2.32	2.13
17	Karimbalan	2.88	2.43
18	Adukkan	3.12	2.40
19	Chennelthondi	2.54	2.45
20	Kannichennellu	2.05	2.08
	MEAN	2.57	2.25
	SE(m)	0.04	0.03
	CD	0.12	0.04

4.2.5 Water Absorption

The range of values for water absorption varied from 1.69 to 2.91. Chennellu had the maximum value and Cheradi showed the minimum (1.69). The variety Chennellu had highest value of water absorption and Cheradi had shown the lowest value.

The mean values obtained for volume expansion and water absorption are shown in Table 9.

4.3 CHEMICAL CHARACTERIZATION

4.3.1 Amylose and Amylopectin Content

Amylose and amylopectin content varied significantly between the varieties. Amylose content of the varieties ranged from 12.32 to 29.82%, whereas amylopectin varied from 70.18 to 87.68%. The variety Cheruvirippu showed maximum value for amylose content and minimum value for amylopectin content. The Jeerakasala variety had minimum amylose content and maximum amylopectin content (Table 10). Statistical analysis showed that Cheruvirippu showed highest significant difference for amylose content and was found on par with that of Veliyan. The lowest significant difference was shown by Jeerakasala which is on par with Kannichennellu.

The variety Jeerakasala showed highest significant difference for amylopectin content and was found on par with that of Kannichennellu.

Based on the amylose content the varieties were grouped in to low amylose (10 to 20%), intermediate amylose (20 to 25%), high amylose (greater than 25%) in accordance with (SES, IRRI, 2002). The varieties Cheruvirippu, Veliyan and Chettadi came under high amylose groups. Intermediate amylose group consisted of the varieties Chennellu, Chootupokkali, Njavara Kunnathur, Njavara Palliyara, Thondi, Adukkan, Chennelthondi, and Orumundakan and the rest of the varieties were classified as low amylose group.

4.3.2 Protein content

The protein content of the varieties ranged from 8.90 to 13.57%. The maximum percentage was recorded for Njavara veluthath and minimum for the varieties Cheradi, Marathondi and Thondi (Table 11). The variety Njavara veluthath and Orumundakan showed high value for protein content and was significantly higher than other varieties.

4.3.3 Iron and Zinc Content

The iron and zinc contents of the varieties ranged from 45.45 to 311.54 mg/kg and 27.88 to 102.41 mg/kg respectively. The maximum iron and zinc content was for the variety Karimbalan with highest significant difference between other varieties. The lowest iron content was for the variety Chettadi (Table 12). Statistical analysis showed that Chettadi had least significant difference from other varieties and was on par with Cheradi. The minimum zinc content was for Gandhakasala which was on par with that of the varieties Kannichennellu, Thondi, Marathondi, Chennellu and Cheruvirippu.

The iron and zinc content of popular rice variety Uma recorded 58.53 mg/kg and 51.63 mg/kg of iron and zinc content respectively. The varieties like Cheradi, Cheruvirippu, Chootupokkali, Njavara veluthath, Thondi, Orumundakan, Gandhakasala, Veliyan and popular rice Uma that were polished, recorded a range of 34.38 to 52.56 mg/kg and 27.02 to 46.98 mg/kg for iron and zinc content respectively (Table 13).

4.3.4 Carotene Content

Carotene content of the varieties ranged between 0.14 to 0.56 mg/100g. The minimum value was recorded for Mullan Kayama and maximum for Njavara veluthath (Table 14). Njavara veluthath showed high carotene content and was found on par with the variety Karimbalan. The variety Mullan Kayama recorded the lowest value for carotene content which was found to be on par with Jeerakasala.

Table 10. Mean values for amylose and amylopectin content of twenty genotypes

SI.NO	Variety	Amylose(%)	Amylopectin(%)
1	Gandhakasala	17.65	82.35
2	Ittikkandappan	17.95	82.05
3	Chennellu	23.06	76.94
4	Chootupokkali	22.35	77.65
5	Cheruvirippu	29.82	70.18
6	Mullan kayama	15.83	84.17
7	Cheradi	19.29	80.71
8	Orumundakan	24.21	75.79
9	Njavara Veluthath	14.78	85.22
10	Njavara Kunnathur	23.99	76.01
11	Njavara Palliyara	21.50	78.50
12	Jeerakasala	12.32	87.68
13	Marathondi	19.13	80.87
14	Veliyan	29.55	70.45
15	Chettadi	28.30	71.70
16	Thondi	21.53	78.47
17	Karimbalan	19.01	80.99
18	Adukkan	20.16	79.84
19	Chennelthondi	20.57	79.43
20	Kannichennellu	13.03	86.97
	MEAN	20.70	79.30
	SE(m)	0.33	0.33
	CD	0.97	0.97

Table 11. Mean values for protein content of twenty genotypes

SI.No	Variety	Crude protein (%) on dry weight basis
1	Gandhakasala	9.68
2	Ittikkandappan	10.90
3	Chennellu	9.01
4	Chootupokkali	10.12
5	Cheruvirippu	9.46
6	Mullan kayama	10.46
7	Cheradi	8.90
8	Orumundakan	13.35
9	Njavara Veluthath	13.57
10	Njavara Kunnathur	11.24
11	Njavara Palliyara	10.68
12	Jeerakasala	9.46
13	Marathondi	8.90
14	Veliyan	10.57
15	Chettadi	10.12
16	Thondi	8.90
17	Karimbalan	9.46
18	Adukkan	9.68
19	Chennelthondi	10.57
20	Kannichennellu	9.68
	MEAN	10.24
	SE(m)	0.20
	CD	0.58

Table 12. Mean values for Iron and Zinc content of twenty genotypes

SI.No	Variety	Iron(mg/kg)	Zinc(mg/kg)
1	Gandhakasala	131.22	27.88
2	Ittikkandappan	106.45	49.50
3	Chennellu	66.83	32.32
4	Chootupokkali	67.78	33.82
5	Cheruvirippu	101.27	33.27
6	Mullan kayama	82.40	54.12
7	Cheradi	46.42	66.03
8	Orumundakan	82.67	50.98
9	Njavara Veluthath	106.72	38.12
10	Njavara Kunnathur	72.50	46.28
11	Njavara Palliyara	92.54	54.23
12	Jeerakasala	81.78	51.00
13	Marathondi	74.42	31.43
14	Veliyan	218.25	56.88
15	Chettadi	45.45	56.13
16	Thondi	73.32	30.38
17	Karimbalan	311.54	102.41
18	Adukkan	156.83	53.92
19	Chennelthondi	62.63	34
20	Kannichennellu	76.1	29.72
	MEAN	102.86	46.62
	SE(m)	3.2	1.94
	CD	9.15	5.56

Table 13. Mean values of Iron and Zinc content in polished and unpolished seeds of selected genotypes including the popular variety Uma

		Unpolished		Polished	
SI.No	Variety	Iron(mg/kg)	Zinc(mg/kg)	Iron (mg/kg)	Zinc(mg/kg)
1	Cheruvirippu	101.27	33.27	43.53	27.24
2	Gandhakasala	131.22	27.88	35.79	31.65
3	Veliyan	218.25	56.88	52.56	46.98
4	Njavara Veluthath	106.72	38.12	46.8	31.2
5	Thondi	73.72	30.38	44.22	27.02
6	Orumundakan	82.67	50.98	45.69	40.2
7	Cheradi	46.42	66.03	44.4	36.57
8	Chootupokkali	67.78	33.82	43.05	31.44
9	Uma	58.53	51.63	34.38	43.95

Table 14. Mean values for carotene content of twenty genotypes

SI.No	Variety	Carotene(mg/100g)
1	Gandhakasala	0.33
2	Ittikkandappan	0.22
3	Chennellu	0.29
4	Chootupokkali	0.30
5	Cheruvirippu	0.32
6	Mullan kayama	0.14
7	Cheradi	0.25
8	Orumundakan	0.29
9	Njavara Veluthath	0.56
10	Njavara Kunnathur	0.31
11	Njavara Palliyara	0.38
12	Jeerakasala	0.15
13	Marathondi	0.37
14	Veliyan	0.23
15	Chettadi	0.16
16	Thondi	0.22
17	Karimbalan	0.55
18	Adukkan	0.24
19	Chennelthondi	0.47
20	Kannichennellu	0.18
	MEAN	0.30
	SE(m)	0.78
	CD	1.86

4.4 CORRELATION ANALYSIS

A simple correlation study was carried out between the physical characters (Table 15), cooking qualities and amylose content (Table 16) and chemical characters (Table 17) separately. Among that significant positive correlation at both 1% and 5% level of significance was shown by the characters like breadth wise elongation ratio with kernel breadth before cooking and kernel length/breadth ratio; volume expansion ratio with water uptake; and iron and zinc content. Kernel length with kernel length/breadth ratio and optimum cooking time with volume expansion ratio showed a significant positive correlation at 5% level. A non- significant positive correlation was observed between the characters like optimum cooking time with amylose content, water absorption, zinc content and carotene content and also kernel length with kernel breadth.

A significant negative correlation at both levels of significance was observed on linear elongation ratio with volume expansion ratio; kernel breadth before cooking with kernel length/breadth ratio. A non-significant negative association was observed between optimum cooking time and amylopectin content.

4.5 MOLECULAR CHARCTERIZATION

4.5.1 Quality and Quantity of Genomic DNA

The genomic DNA isolated from twenty genotypes were checked for quality by using 0.8% agarose gel and by spectrophotometer reading. All the samples showed OD ratio between 1.22 to 2.33 (Table 18).

4.5.2 Validation of SSR Markers Linked to Quality Traits

Previously reported SSR markers like RM190, RM520, RM535 linked with quality traits like amylose content, protein content, iron and zinc content respectively were used for validation

Table 15. Correlation among physical parameters of dehusked grains

	Kernel length	Kernel breadth	Kernel length/breadth ratio	Bulk density
Kernel length	1.0000			
Kernel breadth	0.4089	1.0000		
Kernel length/breadth ratio	*0.4783	*-0.5606	1.0000	
Bulk density	0.0791	0.2692	-0.1171	1.0000

** Significance at 1% and 5% level

* Significance at 5% level

Table 16 .Correlation among cooking qualities and amylose content of dehusked grains

	Linear elongation ratio	Breadth wise elongation ratio	Optimum cooking time	Volume expansion	Water absorption	Amylose
Linear elongation ratio	1.0000					
Breadth wise elongation ratio	-0.1286	1.0000				
Optimum cooking time	-0.2052	0.1524	1.0000			
Volume expansion	*-0.4998	0.0807	*0.5362	1.0000		
Water absorption	*-0.4849	-0.0795	0.2825	**0.8557	1.0000	
Amylose	0.1135	-0.0352	0.3509	0.1318	-0.0133	1.0000

** Significance at 1% and 5% level

* Significance at 5% level

Table 17. Correlation among chemical parameters of dehusked grains

	Amylose	Amylopectin	Protein	Iron	Zinc	Carotene
Amylose	1.0000					
Amylopectin	-1.0000	1.0000				
Protein	0.0061	-0.0061	1.0000			
Iron	0.0562	-0.0562	-0.0189	1.0000		
Zinc	0.0299	-0.0299	0.0103	**0.6419	1.0000	
Carotene	-0.0383	0.0383	0.3085	0.3847	0.1540	1.0000

** Significance at 1% and 5% level

* Significance at 5% level

Table 18. Quality and quantity of DNA of twenty traditional rice varieties used in the study

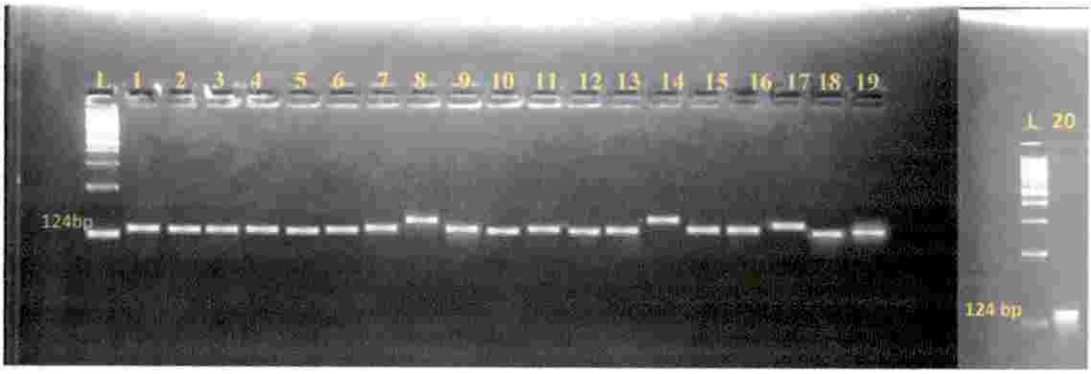
SI.No	Varieties	A 260	A 280	A260/A280	DNA Yield (ng/µl)
1	Gandhakasala	0.011	0.009	1.22	550
2	Ittikkandappan	0.008	0.005	1.60	400
3	Chennellu	0.006	0.004	1.50	300
4	Chootupokkali	0.01	0.007	1.43	500
5	Cheruvirippu	0.014	0.008	1.75	700
6	Mullan kayama	0.008	0.005	1.60	400
7	Cheradi	0.014	0.007	2.00	700
8	Orumundakan	0.011	0.006	1.83	550
9	Njavara Veluthath	0.027	0.015	1.80	1350
10	Njavara Kunnathur	0.013	0.007	1.86	650
11	Njavara Palliyara	0.01	0.006	1.67	500
12	Jeerakasala	0.004	0.003	1.33	200
13	Marathondi	0.013	0.008	1.63	650
14	Veliyan	0.011	0.006	1.83	550
15	Chettadi	0.007	0.004	1.75	350
16	Thondi	0.016	0.010	1.60	800
17	Karimbalan	0.01	0.007	1.43	500
18	Adukkan	0.009	0.006	1.50	450
19	Chennelthondi	0.007	0.003	2.33	350
20	Kannichennellu	0.012	0.007	1.71	600

The SSR marker RM190 was associated with Wx gene locus associated with amylose content. Out of twenty varieties, seventeen varieties (Orumundakan, Chootupokkali, Kannichennellu, Thondi, Njavara veluthath, Veliyan, Marathondi, Karimbalan, Adukkan, Gandhakasala, Chettadi, Chennelthondi, Njavara Kunnathur, Njavara Palliyara , Chennellu, Cheradi, Cheruvirippu) amplified at 124 bp is found to be linked with Wx gene locus and three varieties (Jeerakasala, Ittikkandappan, Mullan Kayama) shown polymorphism (plate 2)

SSR marker RM520 associated with QTL for grain protein content amplified at 247bp for all the genotypes studied (Plate 3). SSR marker RM535 linked with QTL for iron and zinc content amplified at 138 to 380bp. The thirteen varieties (Ittikkandappan, Orumundakan, Cheradi, Mullan Kayama, Njavara Palliyara, Gandhakasala, Karimbalan, Veliyan, Jeerakasala, Njavara Veluthath, Thondi, Adukkan, Njavara Kunnathur) amplified at 138bp were found to be linked with the QTL for iron and zinc content (Plate 4).

Among the twenty genotypes studied, ten varieties (Orumundakan, Thondi, Njavara veluthath, Veliyan, Karimbalan, Adukkan, Gandhakasala, Njavara Kunnathur, Njavara Palliyara, and Cheradi) detected the presence of all the three genes linked with quality traits like amylose content, protein content and Iron and Zinc content.

Plate 2. Validation of SSR marker RM 190 linked to Wx gene locus of amylose content

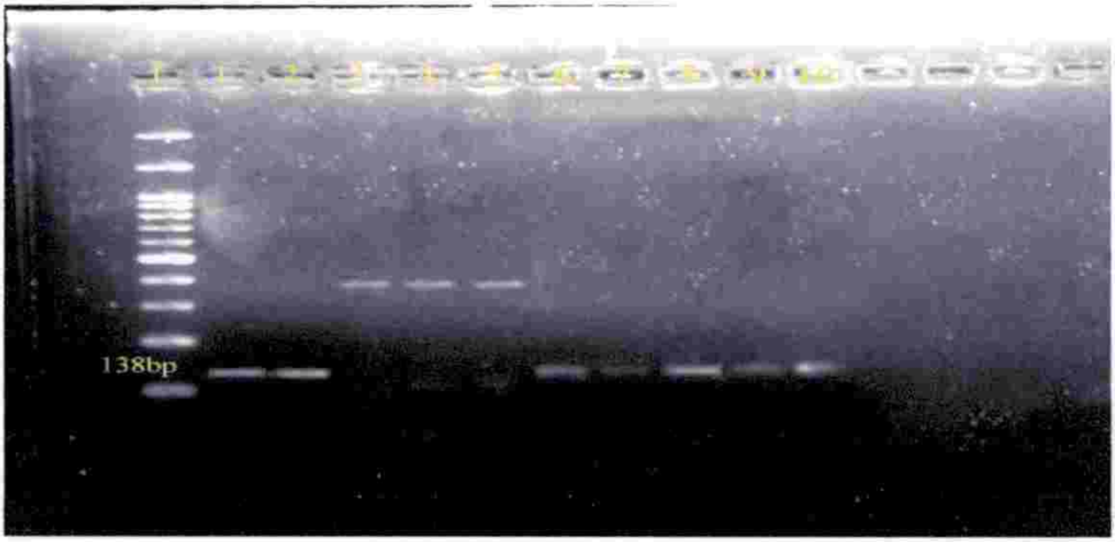


L.100bp ladder ,1.Orumundakan , 2.Chootupokkali ,3. Kannichennellu , 4. Thondi , 5.Njavara veluthath , 6.Veliyan ,7.Marathondi , 8. Jeerakasala, 9.Karimbalan , 10.Adukkan ,11.Gandhakasala ,12.Chettadi ,13. Chennelthondi ,14.Ittikkandappan ,15.Njavara Kunnathur, 16.Njavara Palliyara ,17.Mullan Kayama, 18. Chennellu ,19.Cheradi, 20.Cheruvirippu.

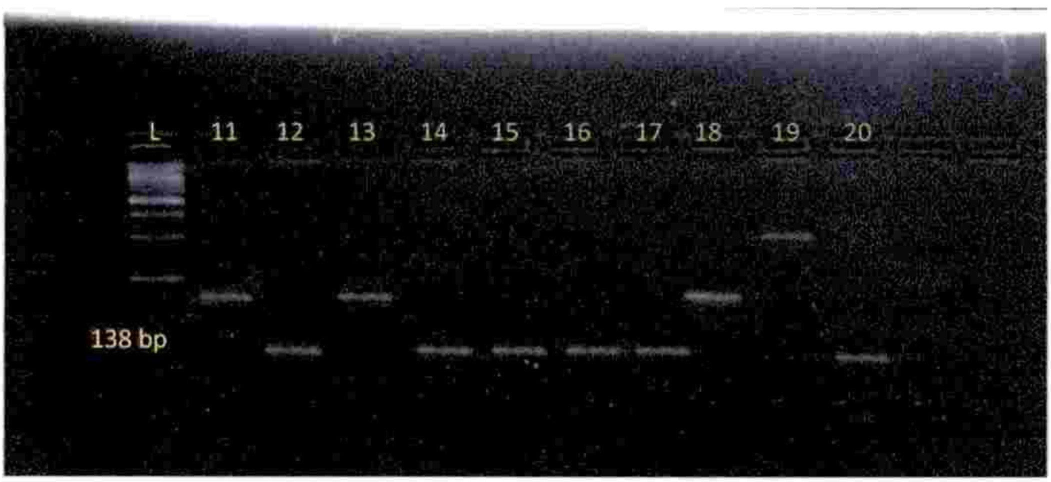
Plate 3. Validation of SSR marker RM 520 linked to QTL for protein content

1. Njavara Kunnathur 2.Njavara Palliyara 3.Chennellu 4.Mullan Kayama 5.Cheradi 6.Cheruvirippu 7.Marathondi 8.Jeerakasala 9.Karimbalan 10.Adukkan 11.Gandhakasala 12.Chettadi 13.Chennelthondi 14.Ittikkandappan 15. Orumundakan, 16.Chootupokkali 17.Kannichennellu 18.Thondi 19.Njavara veluthath 20.Veliyan L.100bp ladder

Plate 4. Validation of SSR marker RM 535 linked to QTL for Iron and Zinc content



L. 100bp Ladder ,1. Ittikkandappan ,2.Orumundakan , 3.Cheruvirippu, 4. Chootupokkali, 5.Chennellu, **6.Njavara Palliyara**, 7. **Cheradi, 8. Mullan kayama , 9. Gandhakasala, 10. Karimbalan**



L. 100bp ladder, 11. Chettadi, **12.Veliyan,** 13. Chennelthondi**, 14. Jeerakasala, 15. Njavara Veluthath, 16. Thondi ,17.Adukkan,**18.Marathondi 19. Kannichennellu, **20. Njavara Kunnathur**

Discussion

5. DISCUSSION

Rice plays an important role in India's food as well as livelihood security system by contributing 75 per cent of normal calories and 55 per cent protein to the normal day by day eating regimen of the population (Anonymous, 2002). Grain quality is an important factor that determines the consumer acceptance. So now, rice breeders are giving importance to quality breeding rather than improvement of yield and resistance.

The micronutrients like iron and zinc are found to be deficit in the diet of underdeveloped countries. As rice is a global grain that feeds millions of mankind, rice supplemented with these micronutrients can substitute to overcome this malnutrition. It was identified that rice covers a vast array of ecological niches and a vast diversity of germplasm of both cultivated and wild rice are available in Kerala (Kumari and Francies, 2002). These traditional varieties may contain favourable genes like genes for resistance to biotic and abiotic stresses as well as genes linked to quality attributes. Hence the conservation and characterization of these varieties are found to be essential for future genetic improvement of rice.

The current investigation was carried out to assess the land races of rice for physical and chemical parameters associated with grain quality and molecular characterization with markers specific to quality traits. The results obtained in this study are critically analysed in this chapter.

5.1 PHYSICAL CHARACTERISATION

According to Khush *et al.* (1979), size and shape of grain are important factors considered by consumers and the acceptance for grain size and shape differ from one group to another. In the present study kernel length showed a significant variation among the varieties. Among the 20 varieties studied, 16 varieties had medium type grains. Dipti *et al.* (2002) conducted studies in six fine rice varieties and observed that grain length and breadth of the varieties varied from 3.60 to 6.50 mm and 1.70 to 3.70 mm respectively. In the present study also kernel length and breadth of the varieties ranged from 4.00 to 6.91 mm and 2.91 to 4.00 mm respectively.

Diako *et al.* (2011) evaluated the physical characteristics of four Ghanaian rice varieties and compared it with two imported rice brands. They have found that the local varieties studied had bolder grains with their width ranging from 2.21 to 2.26 mm, but this study found a still wider range for grain width 2.19 to 4.00 mm. The study conducted by Francis *et al.* (2012) showed that mean kernel length and breadth of njavara genotypes were 5.84 mm and 2.48 mm respectively. Similar trend was observed for the njavara varieties in the current study.

In this study kernel length/breadth ratio varied from 1.60 to 2.91. Based on the ratio, the varieties Njavara Palliyara, Jeerakasala, Marathondi, Kannichennellu, Ittikkandappan, Cheradi, Njavara veluthath, Chettadi, Adukkan were classified as medium grain types and rest of the varieties as bold type grains. No varieties came under slender type. Bhonsle and Sellappan (2010) reported that length/breadth ratio of 22 traditional varieties of Goa ranged from 1.50 -3.50 .Out of twenty two varieties studied, eight varieties belonged to short bold, four varieties as long slender, eight varieties as long bold and five varieties as medium slender.

Balakrishnan *et al.* (2013) reported highly significant correlation between grain length and breadth with length/breadth ratio, while studying 75 local landraces of Tamil Nadu including few popular varieties along with two japonica cultivars. Similar significant association was observed in the present study.

Vanaja and Babu (2003) reported a positive significant correlation of length with length/breadth ratio of grain and negative significant correlation with breadth of grain, while studying the correlation between physicochemical characters of rice grain and cooking qualities in 56 high-yielding genotypes representing different eco geographical conditions in Bangladesh, China, India, Indonesia, Malaysia, Pakistan, Philippines, and Sri Lanka. The study indicated

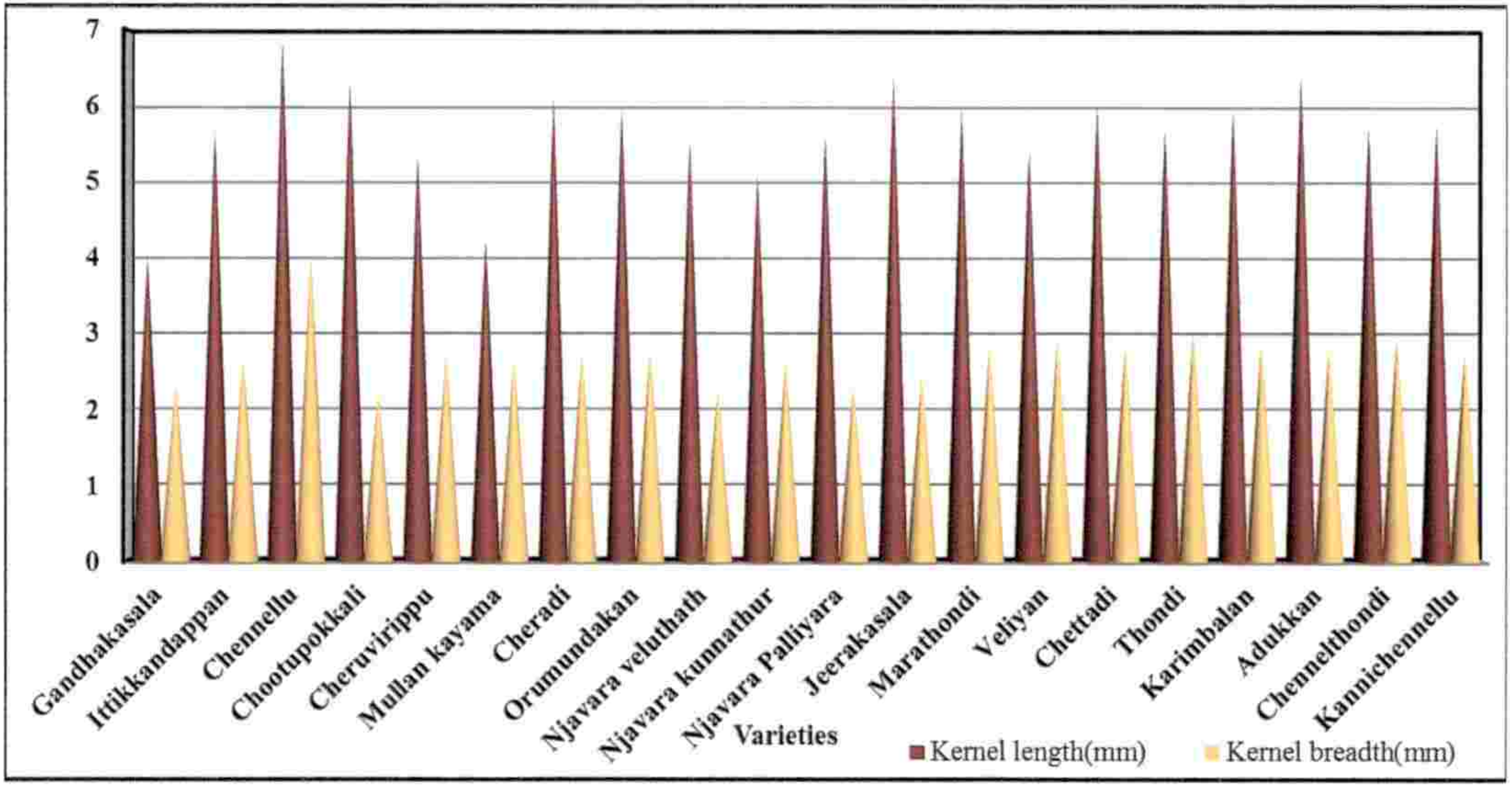


Fig 2. Comparison of kernel length and breadth of twenty genotypes

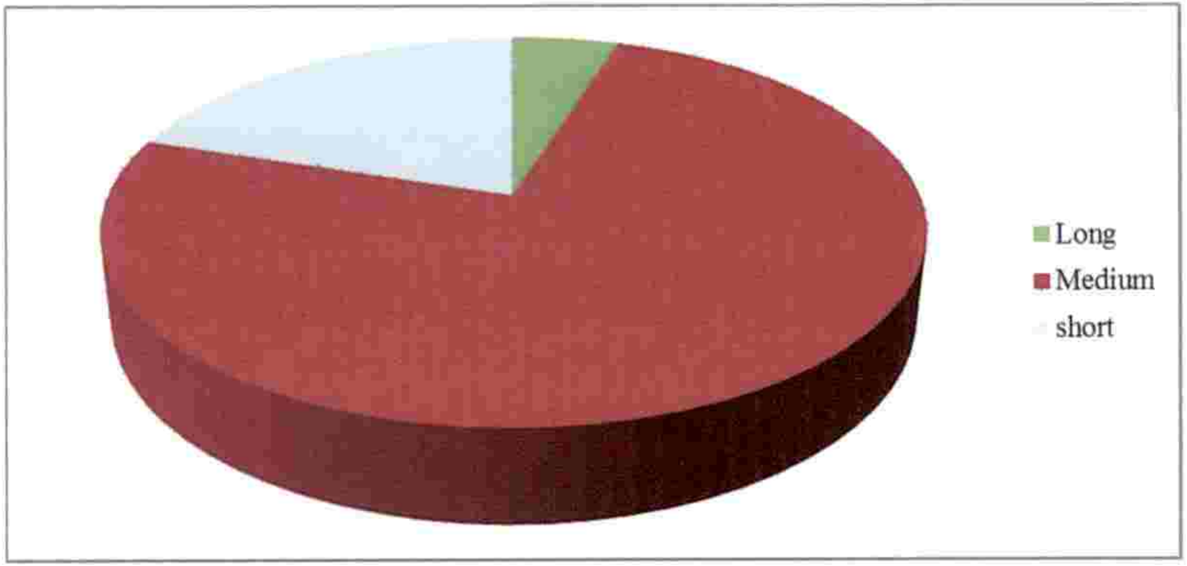


Fig 3. Sectoral diagram representing grain size of twenty genotypes

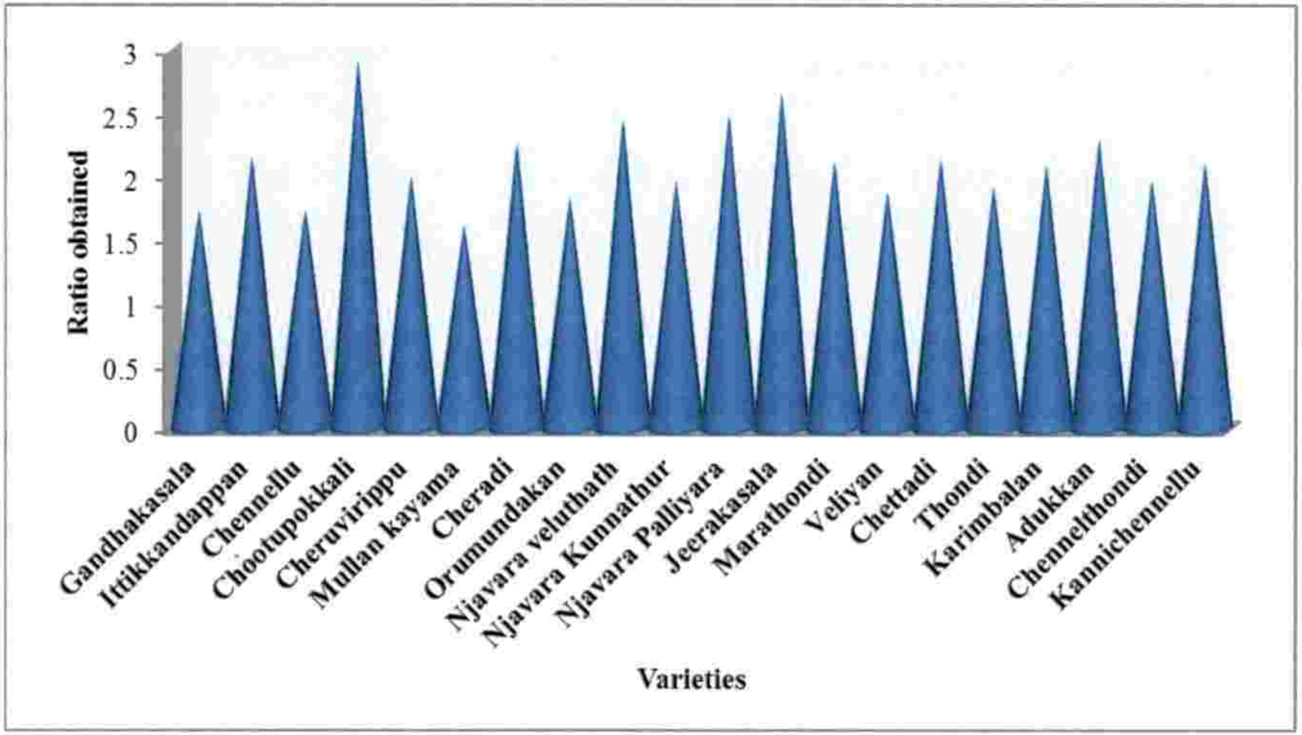


Fig.4. Comparison of kernel length / breadth ratio of twenty genotypes

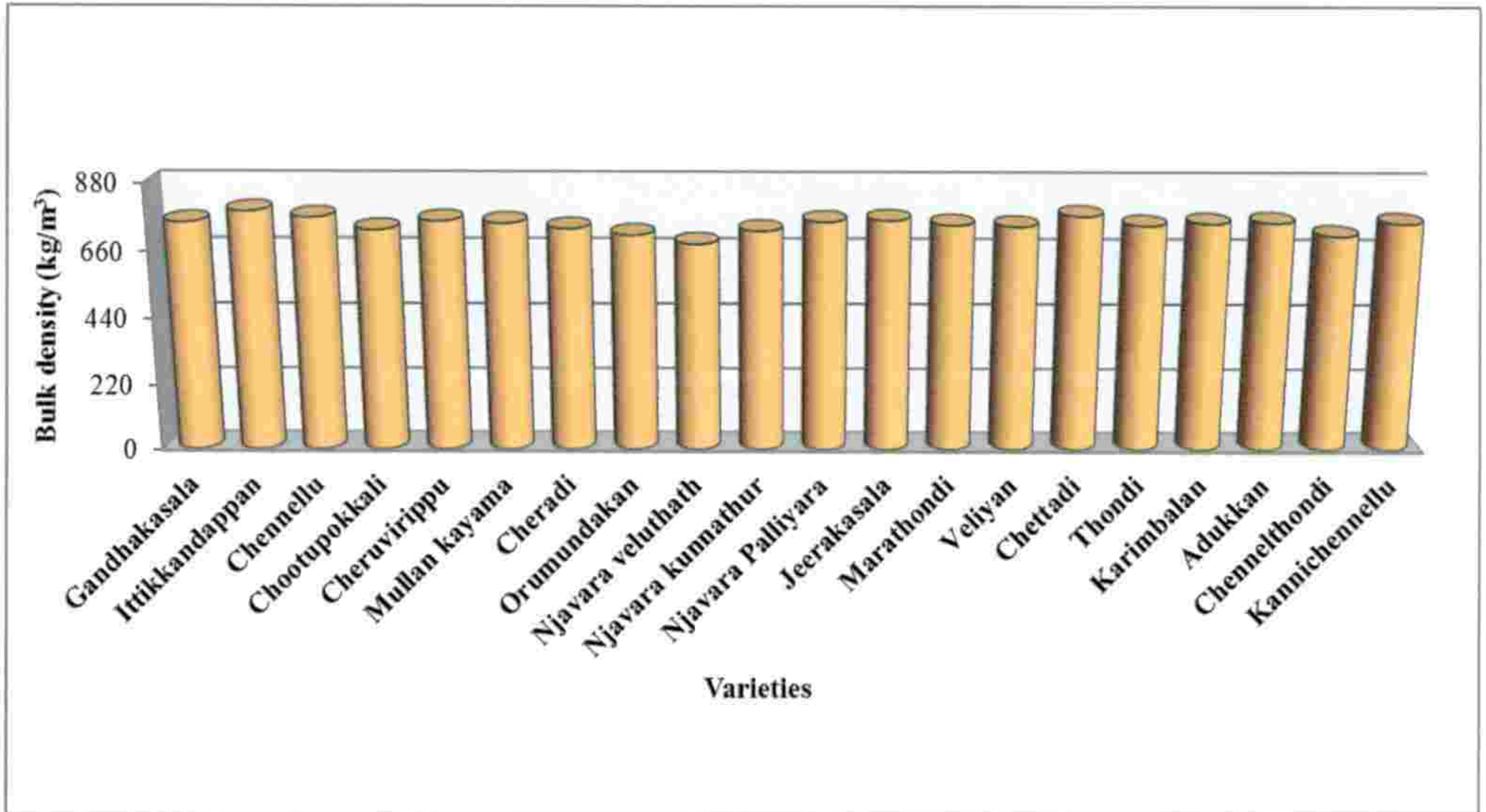


Fig.5. Comparison of bulk density of twenty genotypes

that when grain size increases, its shape also increases, but its boldness is reduced. These findings confirms the results obtained in the present study

Bulk density (BD) is an important physical property of rough and milled rice that is dependent on grain type, moisture content, kernel density and additional physical properties such as kernel shape and dimensional characteristics (Fan *et al.*, 2000). Kanchana *et al.* (2012) reported that bulk density, true density and porosity can affect the rate of heat and mass transfer of moisture during aeration and drying processes and it can also be useful in sizing grain hoppers and storage facilities. According to Nalladulai *et al.* (2002) the bulk density of grains is useful in designing silos and storage bins .In the present investigation, bulk density showed significant difference among the varieties at both 1% and 5% levels. The mean value was observed in the range of 681.63 to 787.00 kg/m^3. Kanchana *et al.* (2012) reported that, bulk density of rice varieties collected from Tirunelveli, Madurai and Virudhunagar district of Tamil Nadu showed a range of 0.701 to 0.868 (g/ml).

5.2 COOKING QUALITIES

Cooking time is an important factor that determines the tenderness as well as stickiness of cooked rice (Anonymous, 1997) .Cooking time of the varieties studied showed a range of 31.00 to 52.00 minutes. A similar value was obtained by Oyegbayo *et al.* (2001). They have observed that cooking time of two local rice varieties in Nigeria was recorded 52 and 56 minutes respectively.

According to Frei and Becker (2003) the difference observed in cooking time may be related to its gelatinization temperature, as gelatinization temperature positively determines the cooking time of rice. It has been found that when gelatinization temperature is higher, the longer time it takes to cook rice and cooking time was observed to be dependent on gelatinization temperature. So the varieties studied may have higher gelatinization temperature.

Grain elongation on cooking is an important quality character that is preferred by traders and consumers (Singh *et al.*, 2006). In the current study linear elongation ratio for the varieties ranged from 1.11-1.60 and breadth wise elongation ratio ranged from 1.01-1.94. Sonowal and Barooah (2015) reported that the kernel length elongation ratio varied from 1.42 to 1.51 for the varieties collected from North eastern part of India.

Sonowal and Barooah (2015) reported that rice kernels absorb water and increase in volume through increase in length or breadth during cooking. It was reported that for high quality rice breadth wise increase is not desirable, whereas, lengthwise increase without increase in girth is a desirable characteristic. They observed that, Northeast varieties taken for study recorded moderate degree of volume expansion as the volume expansion ratio varied between 2.43 to 3.12. In the present study also the volume expansion for the varieties showed a range of 1.98-3.43, which is found to be moderate. Similar trend was reported by Vanaja and Babu (2006), while working with 56 high yielding diverse rice genotypes. When the volume expansion is more, the energy content per unit volume will be less (Subudhi *et al.*, 2012).

The extent of water uptake by rice is taken as an important economic quality as it gives an estimate of increase in volume during cooking (Sood and Saddiq, 1986). In the present study, water absorption varied from 1.69-2.91 and it is similar to the values reported by Danbaba *et al.* (2011) in a study conducted to evaluate the cooking and eating quality of *Ofada* rice. Oko *et al.* (2012) reported that high water absorption ratio has a negative effect on the palatability of the cooked rice. Hossain *et al.* (2009) also reported that majority of rice shows pasty appearance at a higher water uptake (300 to 570%). Hence it is not favorable for cooking and eating quality. In the current study, all varieties showed a ratio less than 300%, which indicates that the varieties have good cooking qualities.

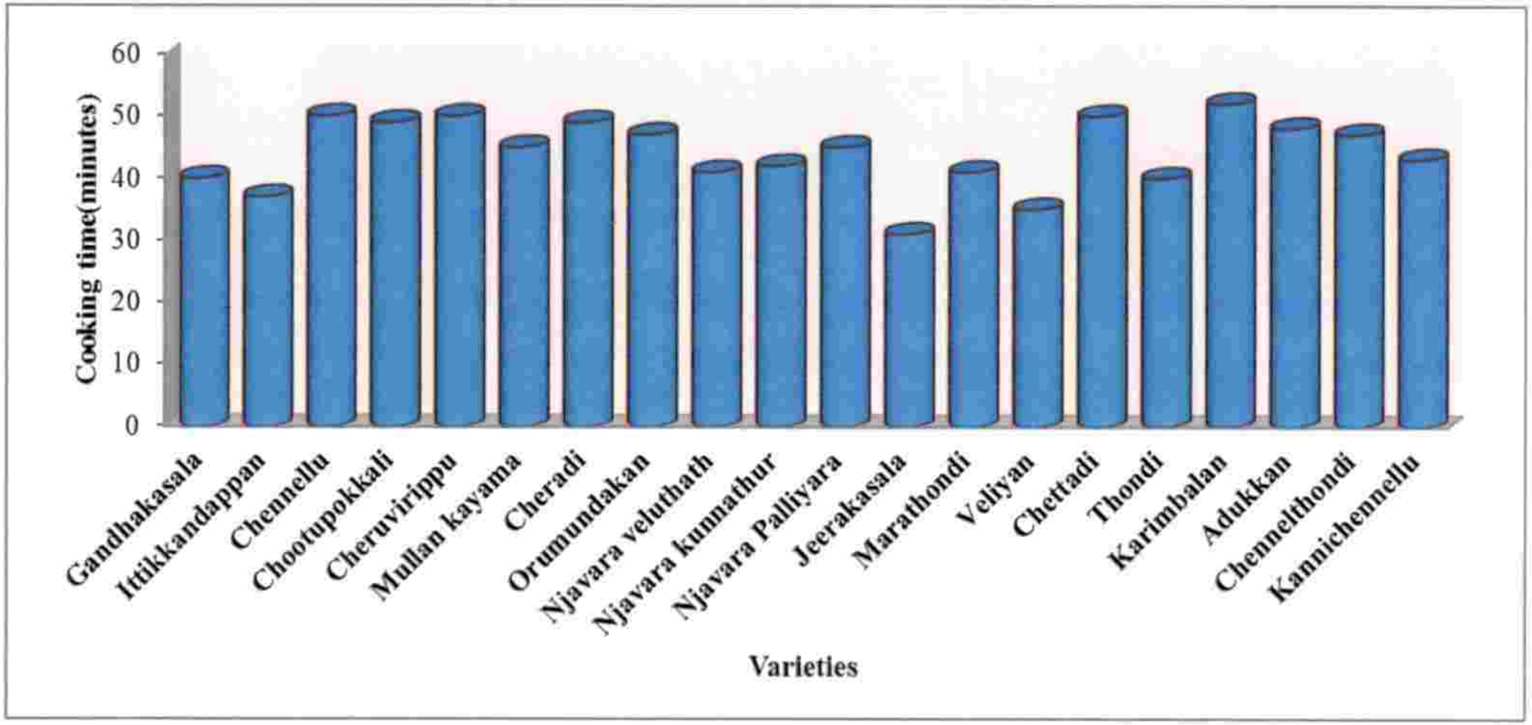


Fig. 6. Comparison of optimum cooking time of twenty genotypes

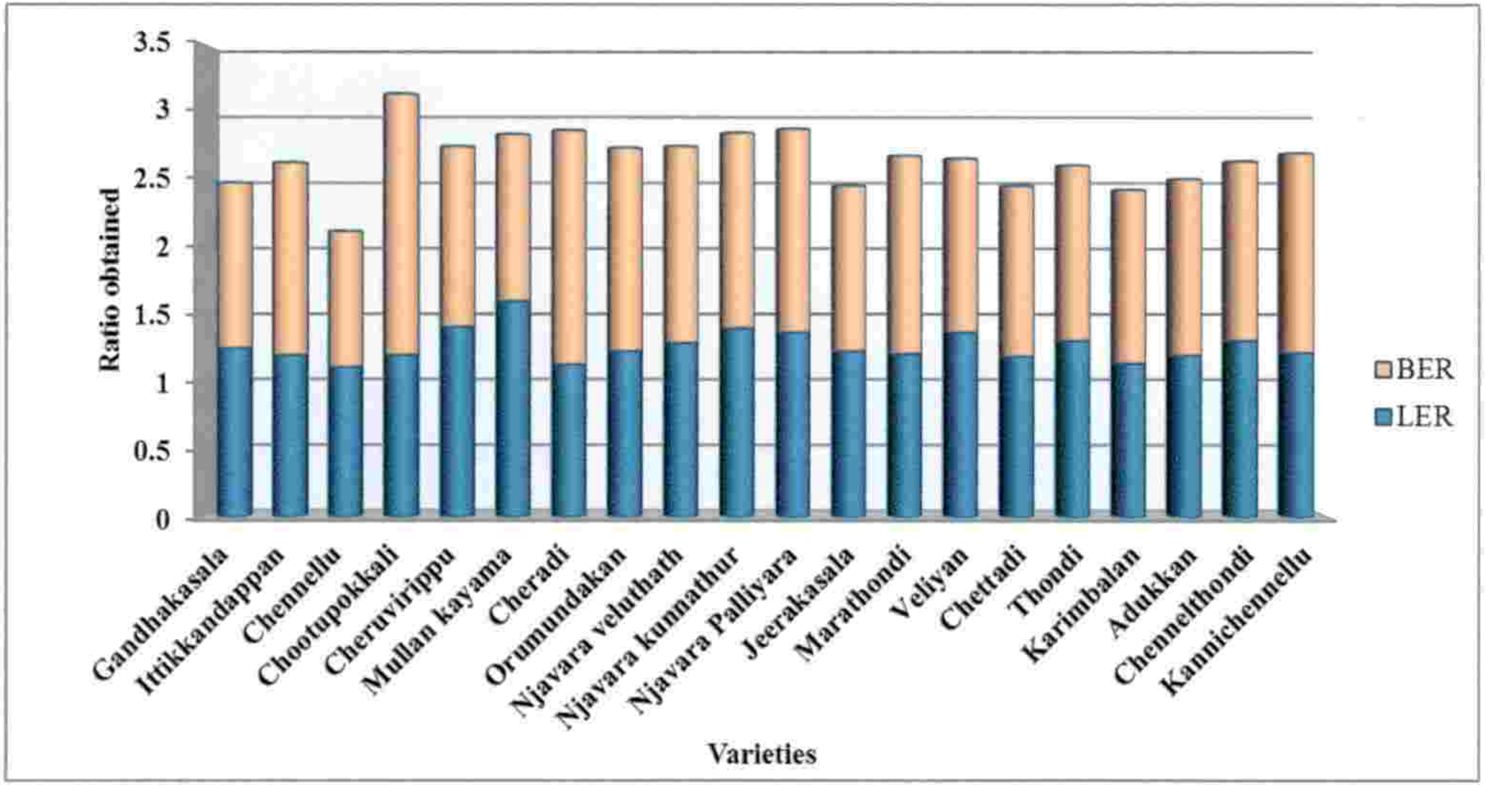


Fig.7. Comparison of linear elongation ratio and breadth wise elongation ratio of twenty genotypes

BER- Breadth wise elongation ratio **LER**-Linear elongation ratio

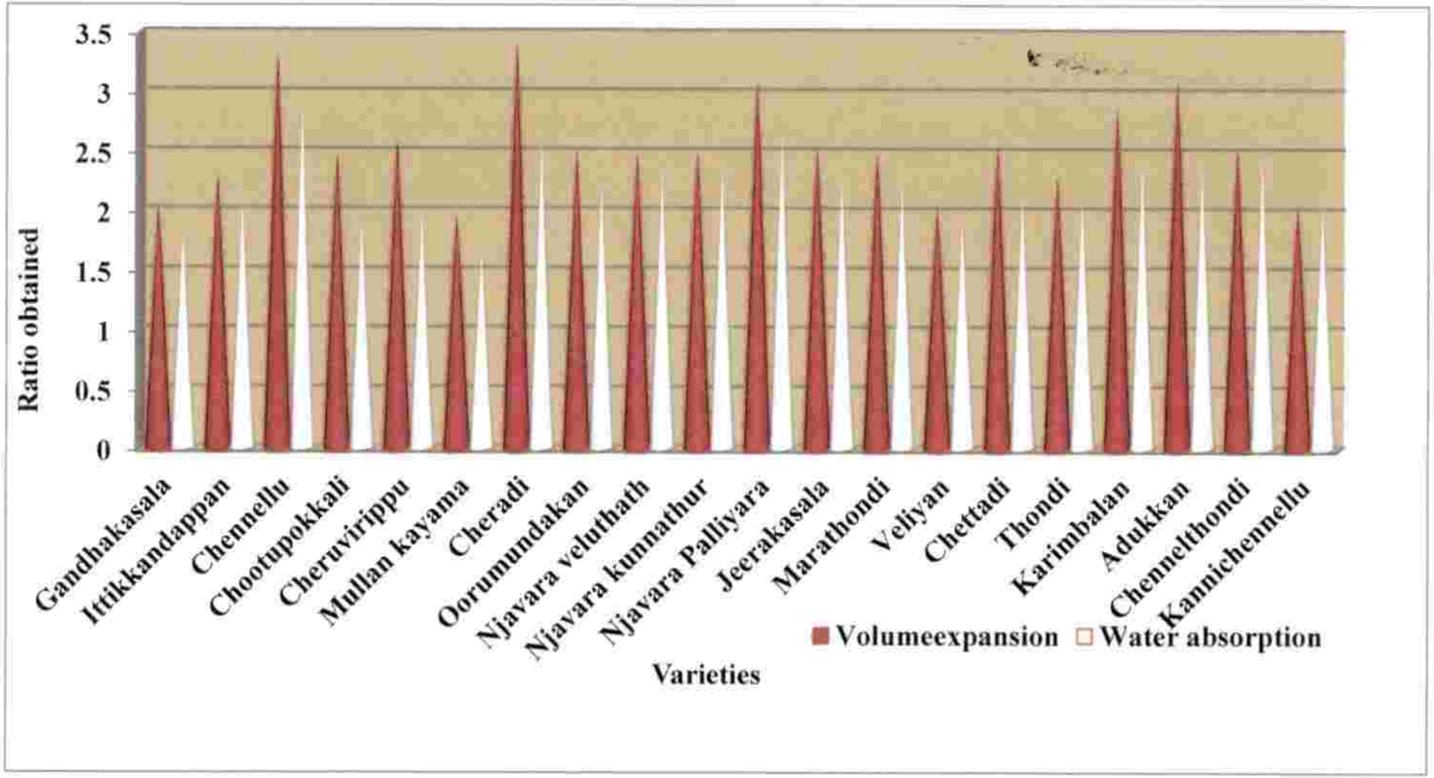


Fig.8. Comparison of volume expansion and water absorption of twenty genotypes

5.3 CHEMICAL CHARACTERIZATION

According to (SES, IRRI, 2002) varieties are classified as low amylose (10 to 20% amylose), intermediate amylose (20 to 24% amylose) and high amylose (greater than 25% amylose). In the present study, the varieties recorded a variation ranging from 12.32% to 29.82% for amylose content. Most of the varieties were classified as low and intermediate amylose groups and three varieties came under high amylose group. Balakrishnan *et al.* (2013) classified eighty two rice genotypes including 75 local landraces of Tamil Nadu and few popular varieties along with two japonica cultivars, into different amylose content groups. It was reported that the lines under the study were under intermediate to high amylose types. The amylose content ranged from 14.22% (*Ganthasala*) to 33.52% (*Vadivel*). Differences observed in amylose content are attributed to characteristics of the varieties and also the differences in the environmental conditions in which the crop is grown, particularly temperature (Hettiarachchy *et al.*, 1997).

Subudhi *et al.* (2012) reported that amylose content is an important factor that determines the stickiness of cooked rice, cohesiveness, tenderness a well as colour of cooked rice. When amylose content is high (greater than 25.0%) cooked rice gives a non-sticky soft or hard texture. Rice varieties with intermediate amylose content (20 to 25%) gives soft and flaky cooked rice and is usually preferred by Indians. In the present study, out of twenty varieties, eight varieties were having intermediate amylose content. It was reported that, compared to those foods with low levels of amylose, consumption of starchy foods having high amylose levels lowers blood glucose level and results in slower emptying of the human gastrointestinal tract (Frei and Becker, 2003). In the present study the varieties like Cheruvirippu, Veliyan and Chettadi recorded high amylose content, hence they are suitable for diabetic patients. Consumption of cooked rice with high amylose content helps to control serum blood glucose and lipid levels effectively (Magdy *et al.*, 2010).

Rice varieties high in amylose would invariably be low in amylopectin content (Cristiane *et al.,* 2007). Subudhi *et al.* (2012) reported that Caprice with highest amylose content recorded the lowest percentage of amylopectin. In the present study also, the variety Cheruviruppu with highest amylose content showed lowest percentage of amylopectin content.

Danbaba *et al.* (2011) reported that correlation studies between the physico chemical characteristics and cooking qualities of *Ofada* rice of Nigeria revealed a positive correlation with amylose content and water absorption, but the present study observed a negative association between the two characters. Vanaja and Babu (2003) studied the correlation of physicochemical character of rice grain with cooking qualities in 56 high yielding diverse genotypes and reported a negative association between those traits.

In the present study the correlation among cooking qualities and amylose content revealed that amylose content was positively correlated with cooking time. So when amylose content is high, varieties took more time to cook. Amylose content was found to have positive correlation with volume expansion, when amylose content was high, greater volume of cooked material was produced. This confirms the finding of Madan and Bhat (1984). Volume expansion and water absorption were found to have positive significant correlation. The positive significant association among water uptake, volume expansion ratio and amylose content indicates rice quality as well as buyer's preference in Kerala (Vanaja and Babu, 2003).

Grain protein content is a polygenic character in rice which plays a very important role in human nutrition. Samak *et al.* (2011) reported that total grain protein content ranged from 7.39 to 12.81 per cent in F5 RILs of rice derived from BPT5204 X HPR14. Similar trend was observed in the present investigation, where the varieties showed a range of 8.90 to 13.57%. Deepa *et al.* (2008) reported that protein content of 9.5% for the njavara paddy collected from Padma, Ayurveda, Mannar, whereas, in the current study, njavara varieties showed a

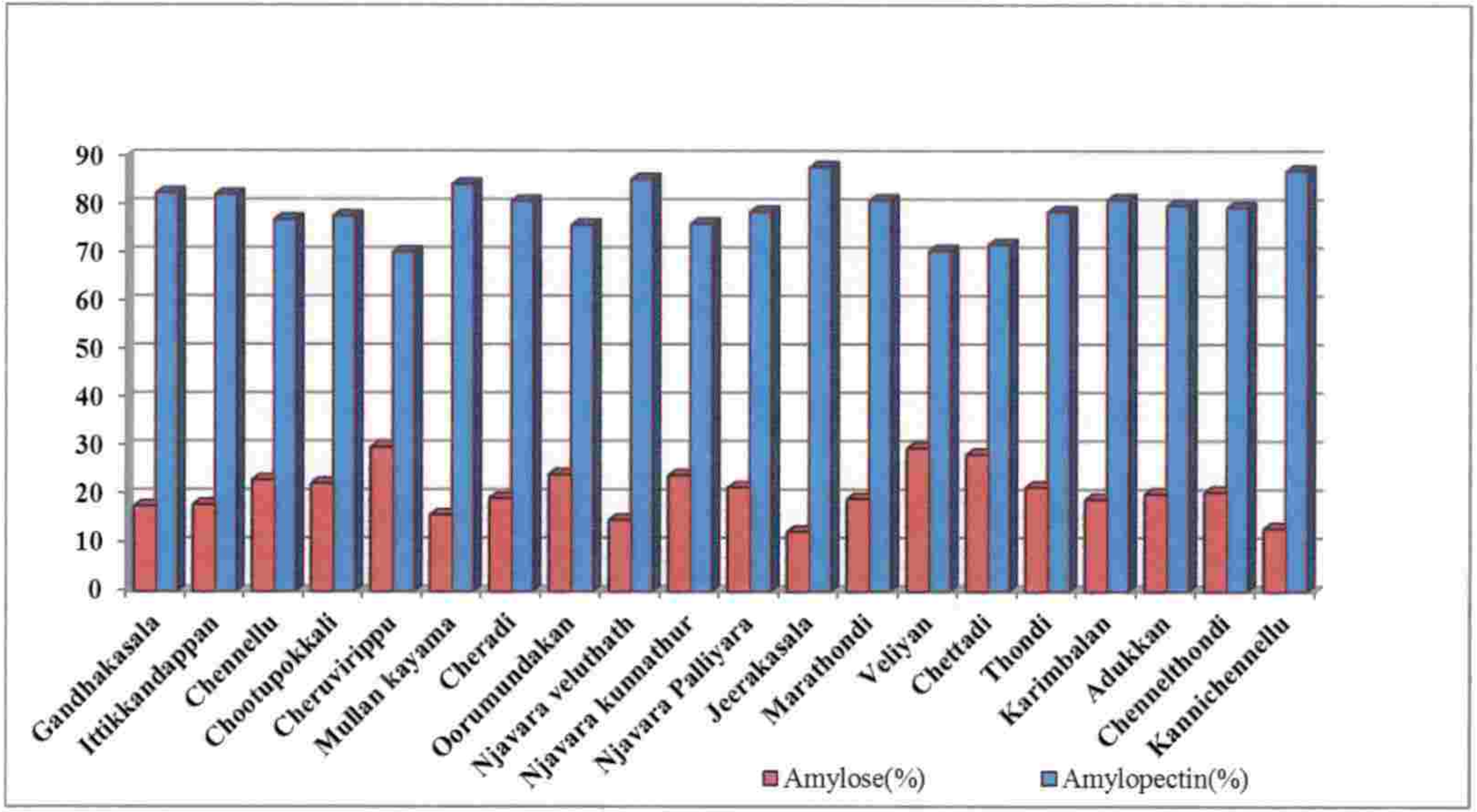


Fig.9. Comparison f amylose and amylopectin content of twenty genotypes

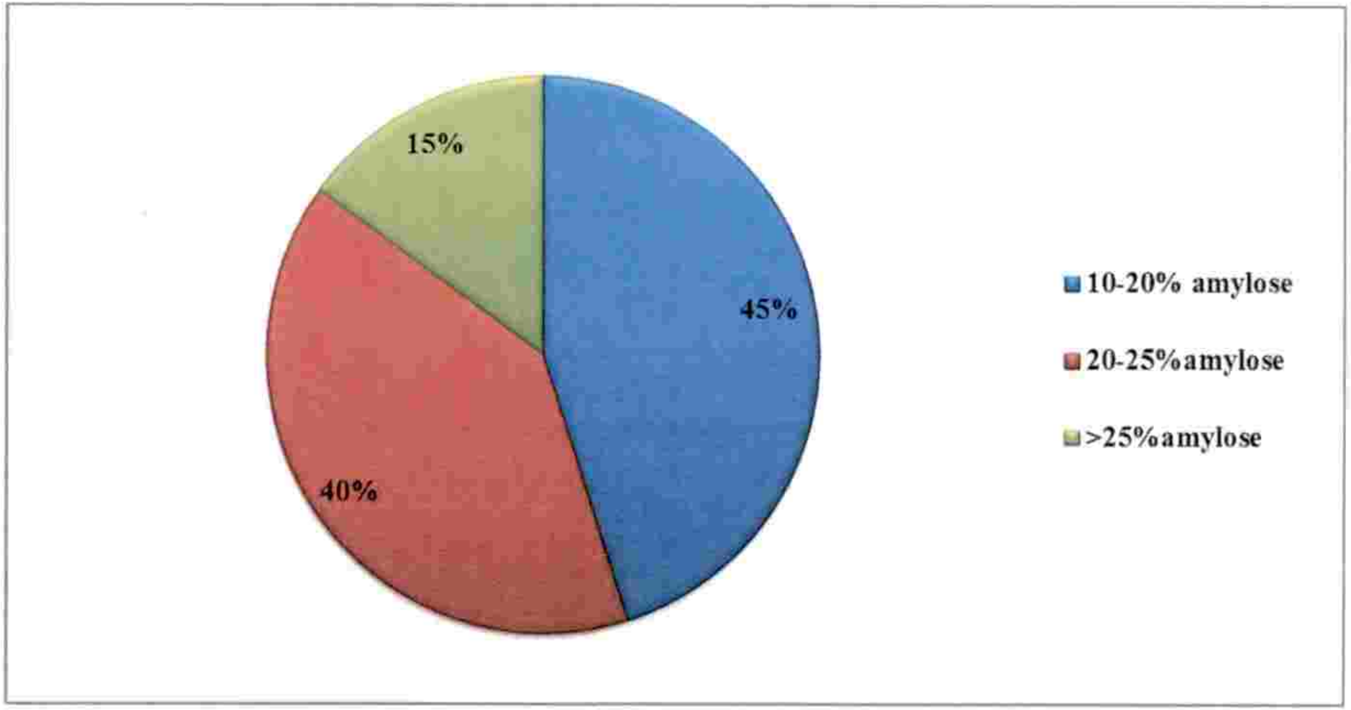


Fig. 10. Sectoral diagram representing amylose content groups

range of 10.68 to 13.57%. Sindhumole (2012) reported that landraces tend to have higher protein content than high yielding varieties, which were developed mainly to optimise the need, and not the yield. Diversity in seed storage protein is important for varietal identification and breeding for improved nutritional quality.

Adequate amounts of essential vitamins and minerals are important in the diet of a healthy and productive population. As staple foods are eaten in large quantities everyday by malnourished poor, addition of even small quantities of micronutrients is beneficial (Mehansho, 2006). Biofortification is an emerging stratergy referred to as the improvement of micronutrient levels of staple crops through biological processes, such as plant breeding and genetic engineering (Bouis and Welch, 2010). Brar *et al.* (2011) suggested that utilization of high genetic variation for iron and zinc existing in cereal germplasm is an effective way to reduce the extent of iron and zinc inadequacies in developing world. In the present study, a higher significant variation was observed for Iron and Zinc content. The Iron and Zinc content of the dehusked grains ranged from 45.45 to 311.4mg/kg and 27.88 to 102.41 mg/kg. The variety Karimbalan showed the highest value for both the traits followed by the variety, Veliyan. The present study also analysed the Iron and Zinc content of popular variety Uma. The dehusked grains of Uma showed 58.53 mg/kg and 51.63 mg/kg of Iron and Zinc content respectively. Among the twenty varieties selected, nineteen varieties had Iron content greater than Uma and seven varieties shown Zinc content higher than Uma. Anandan *et al.* (2011) studied the Iron and Zinc content of 202 genotypes including traditional and improved cultivars of Tamil Nadu and reported that traditional genotypes were significantly higher than that of improved cultivars. In this study, the varieties Karimbalan, Veliyan and Cheradi showed high content of Iron and Zinc. Jahan *et al.* (2014) reported that among the 52 genotypes, local landraces had showed the highest Fe content and they have observed a range of 1.32 to 100.45ppm. Anuradha *et al.* (2012) observed that Iron and Zinc concentration in unpolished rice grains of 168 F7 RILs derived from

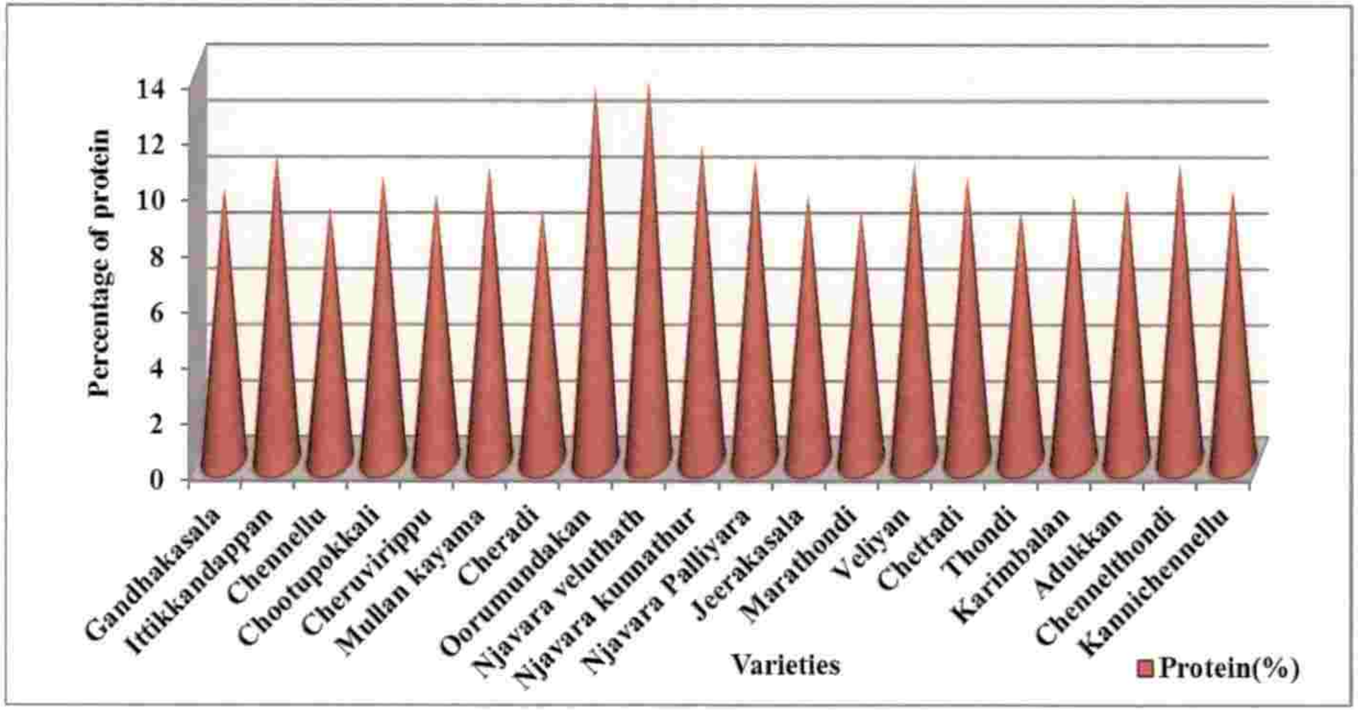


Fig.11. Comparison of protein content of twenty genotypes

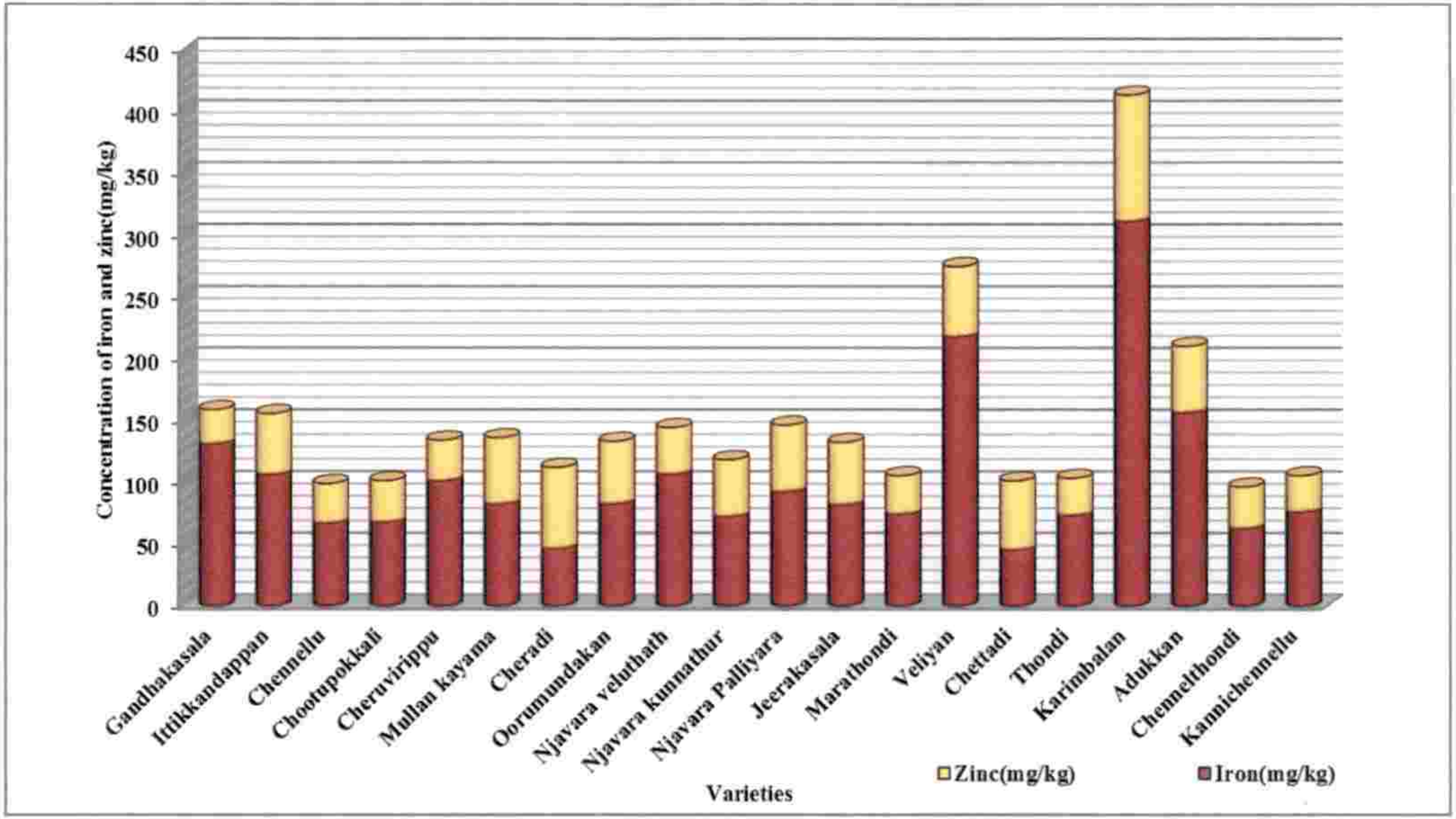


Fig. 12. Comparison of Iron and Zinc content of twenty genotypes

Madhukar×Swarna ranged from 0.2 to 224 ppm and from 0.4 to 104 ppm respectively.

Polishing of rice results in reduction of iron and zinc content of the grain. Babu (2013) studied the variation in Iron and Zinc contents in brown rice as well as polished rice. He has reported that a loss of 10.90 to 82.20% for Iron content and 4.10 to 40.80% occurs for Zinc content at 5% of polishing. Similarly in the present study also a comparative reduction in the nutrients (Iron and Zinc) was occurred after polishing in the selected genotypes including the popular rice Uma. This indicates the importance of rice bran as it constitutes major portion of nutrients. So that mild polishing is recommended for the varieties.

In the correlation studies, a positive significant correlation was observed among iron and zinc content of the varieties. Nachimuthu *et al.* (2014) observed a positive correlation between Iron and Zinc contents in brown rice of 192 genotypes grown in Paddy Breeding Station, TNAU, Coimbatore, which indicated the possibility of simultaneous effective selection for both the micronutrients.

A lack of β carotene is the fundamental driver behind Vitamin A inadequacy (Julie *et al.*, 2009) which results in around 70% of adolescence passings worldwide and visual deficiency in 0.25 to 0.5 million kids each year (Vignesh *et al.*, 2012). Santra *et al.* (1955) observed that beta carotene content in wheat ranged from 2.25 to5.82 μg/g. In the present study carotene content of dehusked rice grains varied from 0.14 to0.56 mg/100g. Renuka *et al.* (2016) reported that the carotene content in unpolished aromatic *indica* rice varieties was in the range of 0.12 to 0.99 mg/100g.

5.4 MOLECULAR CHARACTERIZATION

Microsatellites are considered as powerful tools for a variety of applications (Liu and Cordes, 2004).

Amylose synthesis is mainly controlled by Waxy (*Wx*) gene locus in chromosome 6 which encode Granule Bound Starch Synthase (GBSS) enzyme

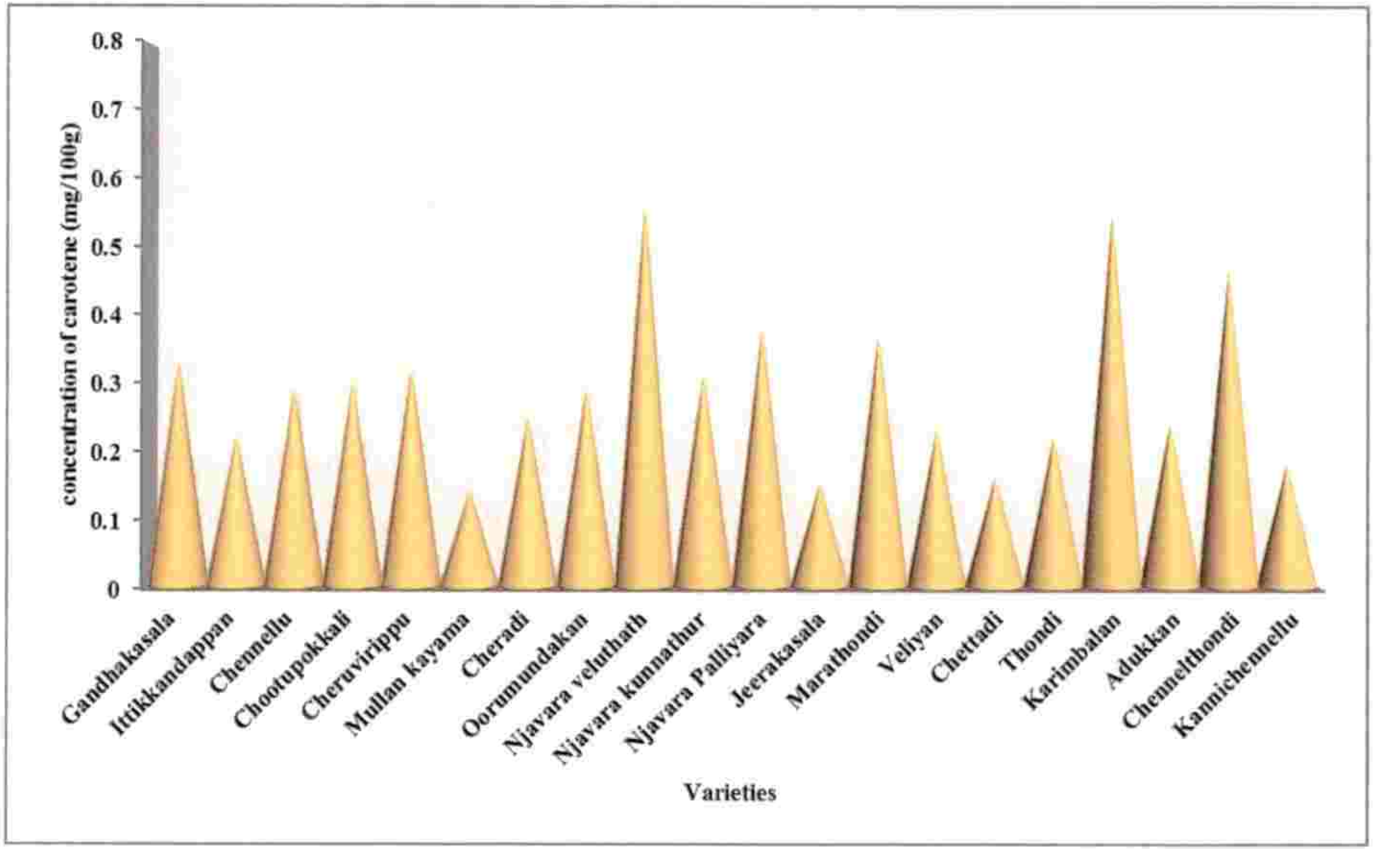


Fig. 13. Comparison of carotene content of twenty genotypes

(Smith *et al.*, 1997). Polymorphic microsatellite marker RM190 with $(CT)_n$ dinucleotide repeats at exon 1 in *Wx* gene locus had significant association with amylose content (Jayamani *et al.*, 2007).

The current study also deployed the marker RM190 to identify the genotypes linked with Wx gene locus. Seventeen varieties amplified at 124bp, whereas three varieties showed polymorphism. The varieties amplified at 124bp had intermediate and high amylose content except Gandhakasala and Marathondi and the three varieties that showed polymorphism had low amylose content (Plate 2) .The difference in amylose content levels of genotypes with same allelic data may be due to environmental effects and due to influence of other genes in the starch synthesis pathway as reported by Juliano and Pascual (1980), while Cheng *et al.* (2015) reported that the amplified products for *Wx*-SSR ranged from 110 to 130 bp in length. Balakrishnan *et al.* (2013) also employed SSR marker RM 190 to assess the genetic diversity among 82 rice genotypes for the genetic polymorphism of the *Wx* gene and the primer showed 48.95% correlation with phenotypical variation

Molecular markers can be used to detect linkage to quantitative trait loci (QTL) for total grain protein content and these can be selected more easily in a breeding programme than the trait themselves (Samak *et al.*, 2011). In the present study, SSR primer RM 520 was used to detect the genotypes linked with grain protein content. All the varieties amplified at 247 bp, indicated the linkage to QTL for grain protein content (Plate 3). It indicated the need of an expression assay to substantiate the variation in protein.

New research discoveries have conveyed the significance of micronutrients in keeping up great wellbeing and rice is an excellent vehicle for biofortification through conventional and deployment of biotechnological tools (Shobharani *et al.*, 2012).

Anuradha *et al.* (2012) genotyped 168 RILs derived from the cross Madhukar x Swarna using 110 SSR markers, including 9 gene specific markers covering all the 12 chromosomes. QTLs were identified for Iron concentration on chromosomes 1, 5, 7 and 12 and for Zinc concentration on chromosomes 7 and 12. Four markers essentially connected to Iron and 14 to Zinc concentration were detected. RM535 on chromosome 2 was normal to both Iron and Zinc concentrations. The current study also deployed the SSR marker RM 535 to detect the presence of QTL linked to Iron and Zinc content. Among twenty genotypes, thirteen varieties amplified at 138bp were found to be associated with QTL for Iron and Zinc content (Plate 4).

Summary

6. SUMMARY

Rice is the staple food that is consumed by almost 50 per cent of world population. Grain quality of rice is an important factor that determines the total economic value of rice. The present study entitled "Physico-chemical and molecular characterization of grain quality of traditional rice varieties" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2014 – 2016, for assessing the land races of rice for physical and chemical parameters associated with grain quality and molecular characterization with SSR (Simple Sequence Repeats) markers specific to quality traits.

Dehusked grains of twenty traditional rice varieties including medicinal rice Njavara were assessed for physical, chemical and cooking quality characteristics. Significant variations were observed among the varieties for all the traits evaluated. In physical characterisation, physical quality parameters like kernel length, kernel breadth, kernel length/breadth ratio and bulk density were analysed. The kernel length of the varieties ranged from 4.00 mm (Gandhakasala) to 6.91 mm (Chennellu) whereas kernel breadth showed a range between 2.19mm (Chootupokkali) to 4.00 mm (Chennellu). Kernel length is a determinant of grain size. It was observed that among the twenty genotypes analysed, the variety Chennellu was classified as long grain and the varieties Gandhakasala, Cheruvirippu, Mullan Kayama and Njavara Kunnathur were grouped as short grains. The rest of the varieties were medium type grains. The kernel length/breadth ratio ranged from 1.60 to 2.91. Based on the kernel length/breadth ratio, the grain shape of selected varieties was classified under medium and bold types. The varieties like Njavara Palliyara, Jeerakasala, Marathondi, Kannichennellu, Ittikkandappan, Cheradi, Njavara veluthath, Chettadi, Adukkan were classified as medium grain types and rest of the varieties as bold type grains, which are the preferred grain types of Keralites.

Cooking quality analysis assessed the parameters like optimum cooking time, linear elongation ratio, breadth wise elongation ratio, volume expansion and water absorption. The variety Karimbalan took maximum time for cooking (52 minutes) and the variety Jeerakasala took the minimum time (31 minutes). The linear elongation ratio for the varieties ranged from 1.11 to 1.60 and breadth wise elongation ratio ranged from 1.01 to 1.94. The volume expansion for the varieties showed a range of 1.98-3.43, which is found to be moderate and all varieties showed a water absorption less than 300%, indicated that the varieties have good cooking qualities.

Chemical characterization showed that, amylose content of the varieties ranged from 12.32% (Jeerakasala) to 29.82% (Cheruvirippu). Most of the varieties are classified as low and intermediate amylose groups and three varieties came under high amylose group. The variety Njavara veluthath recorded maximum protein content (13.57%) and the varieties like Cheradi, Marathondi and Thondi showed the minimum (8.90%). The Iron and Zinc content of the varieties ranged from 45.45 to 311.54 mg/kg and 27.88 to 102.41 mg/kg respectively. The variety Karimbalan recorded maximum Iron and Zinc content. The Iron and Zinc content of popular variety Uma was also analysed and compared with the selected genotypes. The dehusked grains of Uma showed 58.53 mg/kg and 51.63 mg/kg of Iron and Zinc content respectively. Among the twenty varieties selected, nineteen varieties had Iron content greater than Uma and seven varieties shown Zinc content higher than Uma. The variation in Iron and Zinc content due to polishing was analysed in eight traditional varieties and the popular variety, Uma. About fifty percent reduction in Iron and Zinc content was observed in grains after polishing. Consumption of unpolished grains can be recommended to tackle the deficiency of Iron and Zinc. The carotene content of the varieties ranged from 0.14 to 0.56 mg/100g. The minimum value was recorded for Mullan Kayama and maximum for Njavara Veluthath.

A simple correlation study was carried out between the physical characters cooking qualities and amylose content and chemical characters separately. The correlation analysis showed positive significant relationship between Iron and Zinc content which indicated the possibility of simultaneous effective selection for both the micronutrients. The amylose content also showed positive but non-significant relationship with volume expansion and optimum cooking time. A significant negative correlation at both levels of significance was observed on linear elongation ratio with volume expansion ratio; kernel breadth before cooking with kernel length/breadth ratio. A non-significant negative association was observed between optimum cooking time and amylopectin content.

In molecular characterization, SSR marker RM535 linked to QTL (Quantitative Trait Loci) for Iron and Zinc content was detected in thirteen varieties. The marker RM190 linked with Wx gene locus associated with amylose content was detected in seventeen varieties and the marker RM520 linked to QTL for protein content was detected in all the varieties. The varieties identified as rich source of genes linked to quality traits can be used for the transfer of these genes to the cultivated varieties for quality improvement through marker assisted selection.

The present investigation has proven that traditional rice varieties are rich in micronutrients and protein. The varieties like Njavara Veluthath and Orumundakan rich in protein can be utilized as donors for this character in further breeding program. The varieties like Karimbalan and Veliyan which recorded Iron and Zinc content almost double than popular variety Uma can be utilized as lines for biofortification programs which can nourish the growing population and tackle the "hidden hunger".

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PHYSICO-CHEMICAL AND MOLECULAR CHARACTERIZATION OF GRAIN QUALITY OF TRADITIONAL RICE VARIETIES

JEENA GEORGE

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ABSTRACT

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Department of Plant Breeding and Genetics

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ABSTRACT

The present study entitled "Physico-chemical and molecular characterization of grain quality of traditional rice varieties" was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2014 – 2016, for assessing the land races of rice for physical and chemical parameters associated with grain quality and molecular characterization with SSR (Simple Sequence Repeats) markers specific to quality traits.

Dehusked grains of twenty traditional rice varieties including medicinal rice Njavara were assessed for physical, chemical and cooking quality characteristics. Significant variations were observed among the varieties for all the traits evaluated. The kernel length of the varieties ranged from 4.00 mm (Gandhakasala) to 6.91 mm (Chennellu). The kernel length/breadth ratio ranged from 1.60 to 2.91. Based on the kernel length/breadth ratio, the grain shape of selected varieties was classified under medium and bold types. The linear elongation ratio for the varieties ranged from 1.11 to 1.60 and breadth wise elongation ratio ranged from 1.01 to 1.94. The variety Karimbalan took maximum time for cooking (52 minutes) and the variety Jeerakasala took the minimum time (31 minutes).

Chemical characterization showed that, amylose content of the varieties ranged from 12.32% (Jeerakasala) to 29.82% (Cheruvirippu). The variety Njavara Veluthath recorded maximum protein content (13.57%) and the varieties like Cheradi, Marathondi and Thondi showed the minimum (8.90%). The Iron and Zinc content of the varieties ranged from 45.45 to 311.54 mg/kg and 27.88 to 102.41 mg/kg respectively. The variety Karimbalan recorded maximum Iron and Zinc content. The carotene content of the varieties ranged from 0.14 to 0.56 mg/100g. The minimum value was recorded for Mullan Kayama and maximum for Njavara Veluthath. Correlation analysis showed positive significant relationship between Iron and Zinc content. The amylose content also showed

positive but non significant relationship with volume expansion and optimum cooking time.

In molecular characterization, SSR marker RM535 linked to QTL (Quantitative Trait Loci) for Iron and Zinc content was detected in thirteen varieties. The marker RM190 linked with Wx gene locus associated with amylose content was detected in seventeen varieties and the marker RM520 linked to QTL for protein content was detected in all the varieties. The varieties identified as rich source of genes linked to quality traits can be used for the transfer of these genes to the cultivated varieties for quality improvement through marker assisted selection.

The present investigation has proven that traditional rice varieties are rich in micronutrients and protein. The varieties like Njavara Veluthath and Orumundakan rich in protein can be utilized as donors for this character in further breeding program. The varieties which are superior in Iron and Zinc content like Karimbalan and Veliyan can be utilized as lines for biofortification programs which can nourish the growing population and tackle the "hidden hunger".