

**MORPHOLOGICAL AND BIOCHEMICAL
CHARACTERIZATION OF AROMATIC RICE
(*Oryza sativa* L.) CULTIVARS OF
WAYANAD DISTRICT OF KERALA**

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree of

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2010

DECLARATION

I hereby declare that the thesis entitled **Morphological and biochemical characterization of aromatic rice (*Oryza sativa* L) cultivars of Wayanad district of Kerala** 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 Soc ety

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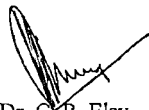
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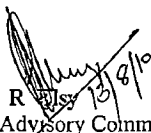
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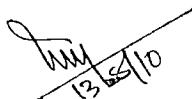
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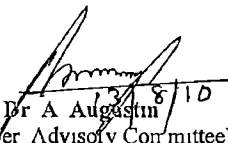
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Introduction

1 INTRODUCTION

The rice plant *Oryza sativa L* is a member of the grass family *Gramineae*. It is one of the leading cereal crops of the world which is a major source of nutrition. It forms the staple food of more than half of the world's population.

Among different groups of rice cultivars, aromatic rice constitutes a small but an important sub group and has a special place in rice market. These are rated as the best in quality due to their flavour and aroma and fetch much higher price than non scented varieties.

India is bestowed with many aromatic rice cultivars which have been grown for 2500 years at different parts. In India aromatic rice cultivars are commonly categorized as Basmati and Non Basmati. The cultivation of Basmati is concentrated in the states of Uttar Pradesh, Himachal Pradesh, Kashmir, Haryana and Punjab. Basmati is accepted as the best scented, long and slender grain rice in world markets and fetches high prices.

The small and medium grained non Basmati aromatic cultivars are widely distributed in different parts of the country including Kerala. The area under aromatic rice in Kerala is very low. *Gandhakasala*, *Jeerakasala*, *Velumbala*, *Chomala*, *Kayama*, *Kothenpalarikkayama* and *Pookkilathari* are some of the non Basmati rice cultivars of Kerala. These traditional cultivars have excellent aroma and cooking qualities and are of great demand in the domestic market. In Kerala, Wayanad district ranks first in cultivation of traditional aromatic rice.

Wayanad is a hilly district situated in the southern region of the Western Ghats. The hill of the district is lofty and has deep valleys. The region is bio geographically rich with significant landscape complexity and biological diversity. The main crop is paddy and is cultivated in the broad and extensive valley bottoms in Wayanad. The low temperature regime (19°C to 30°C) prevailing in this area encourages the cultivation of

aromatic rice cultivars. The traditional aromatic rice cultivars, mainly *Gandhakasala* and *Jeerakasala*, are cultivated mostly by tribal (Kuruma and Kuruchva) communities. These are cultivated during *Nancha (Kharif)* season (May/June to Oct/Nov) in Wayanad district.

Once the district Wayanad (*vayal* paddy field *nadu* territory) was endowed with many land races of rice. At present, there is a fast depletion of area under paddy, leading to the loss of genetic diversity in rice. Cultivation of high yielding varieties adds to the problem of genetic depletion. Even though *Jeerakasala* and *Gandhakasala* are two unique rice cultivars of the area, the research works on these cultivars are very scanty. This reveals the need for their collection, conservation, characterization, and documentation. The importance of this attempt has also to be seen in the context of extension of Intellectual Property Rights (IPR) to agricultural sector. Being a signatory to General Agreement on Tariffs and Trade (GATT), conservation, characterization, and registration of indigenous bio-resources are gaining importance in our country to protect our sovereign rights on biodiversity. Farmers' right over these cultivars are to be protected for which characterization is the prerequisite. Moreover, Geographical Indications (GIs) is a viable Intellectual Property tool to safeguard the rights of local communities and their registration as GIs also demand their characterization and documentation.

Hence, this investigation is aimed to collect the different genotypes of *Gandhakasala* and *Jeerakasala* from Wayanad district and to characterize the types based on

- 1) Morphological studies
- 2) Nutritional studies and
- 3) Biochemical studies

Review of literature

2 REVIEW OF LITERATURE

2.1 Morphological characters

Morphological characterization is essential for effective and efficient crop breeding programme and for setting up the distinctiveness of genotypes. Morphological traits are the oldest and widely used markers because of their simplicity, rapidity and cost effectiveness.

2.1.1 Seedling height

Seedling vigour and growth rate varies in rice varieties and there is no precise definition or means to measure seedling vigour. Prompt emergence and rapid growth of seedlings are generally desired in direct seeding of commercial varieties (Chang and Bardenas 1965).

According to Chang and Tagumpay (1970) there was a positive correlation between seedling height and plant height. Tall seedlings grew into tall plants and short to short plants. He also reported that heritability of seedling height ranged from 0 to 40.4 per cent. Yoshida (1977) reported that rapid seedling growth was a desirable trait of tropical rice varieties which enabled the young plants to become fully established before weeds became a problem.

Yoshida (1981) reported that seedling growth and development were affected by environmental factors and temperature range of 25°C-30°C and 5-6 ppm of oxygen was best for good growth of seedlings. He also reported that upland grown seedlings were shorter with smaller leaves, highly branched roots and they contained more nitrogen and starch than the lowland grown seedlings.

Roy *et al* (1995) found linear relationship of germination rate and seedling vigour index values with seed size. According to them selection of larger seeds resulted in good stand establishment.

Seedling height showed moderate heritability in the range of 60-80 per cent (Privanka *et al* 2000). Reddy (2000) reported a significant positive association of seedling height with days to 50 per cent heading and days from seeding to maturity.

Kumar and Reddy (2003) observed a positive correlation between minimum temperature and root length and also between seedling height and seedling weight. Reduced growth of seedlings at low temperature was also noted.

According to Hui *et al* (2004) increased sowing density led to reduction in seedling quality, root developing ability, ratio of dry weight to seedling height, above ground dry weight and base stem thickness. Rice cultivars performed differently with respect to seedling height, seedling strength, panicle number per unit area, grains per panicle, sterility percentage and grain yield (Roy and Hossain 2004).

Through genetic component analysis Akram *et al* (2007) reported that both additive and non-additive gene action influenced rate of germination index and seedling shoot length. However, the additive component was found to be more pronounced in the inheritance of these two traits.

2.1.2 Leaf

Nanda (1997) reported that leaf sheath contributed very little to photosynthesis but it provided mechanical support to the whole plant. It served as a temporary storage site for starch and sugar before initiation of heading. The leaf characters which a plant breeder endeavours to manipulate in a breeding programme were leaf shape and size (narrow/broad, erect/droopy, leaf angle, leaf colour, toughness, senescence and pubescence). The upper most leaf below the panicle is the flag or boot leaf. Flag leaf supplied photosynthates to panicle and also played an important role in grain filling and protection of panicles from bird damage. The flag leaf generally differed from the other leaves in shape, size and angle.

Katsura *et al* (2008) suggested Leaf Area Index (LAI) as one of the important factors in determining biomass accumulation in rice cultivars

2.1.2.1 Leaf length According to Chang and Bardenas (1965) the leaf length showed variation in varieties Yoshida (1981) reported that the leaf length increased with advancement in leaf number

Elsy *et al* (1992) reported that *Njavara* genotypes exhibited narrow and long leaves Reddy (2000) found that leaf length had positive significant correlation with ligule length grain width panicle length culm diameter 1000 grain weight culm length and grain yield He also reported a negative correlation between leaf length and protein content

Nanda and Agarwal (2006) reported that the traditional tall varieties had long droopy leaves and the semi dwarf modern high yielding varieties had short small and erect leaves Wiangasamut *et al* (2006) reported that less erect leaves during the early vegetative stage was good to cover the land area so less light penetrated to the ground This resulted in lower growth of weeds to compete with the crop According to Babar *et al* (2007) flag leaf length showed a positive correlation with days to heading

2.1.2.2 Leaf width According to Chang and Bardenas (1965) leaf width varied with varieties Reddy (2000) observed that leaf width of *Njavara* genotypes ranged from 1.487 cm to 0.737 cm Leaf width showed a significant positive correlation with ligule length days to 50 per cent heading culm diameter panicle length 1000 grain weight grain length and width and days from seeding to maturity in *Njavara* genotypes Negative correlation of leaf width with culm number and protein content was also reported Khush (2000) reported droopy leaves with medium breadth in Basmati 370 and relatively erect leaves in Pusa Basmati 1

Kumar (2005) reported a positive correlation between leaf width and grain yield Babar *et al* (2007) reported that flag leaf width had a significant positive correlation with grain yield panicle length days to heading and plant height

2.1.2.3 Leaf blade pubescence Chang and Bardenas (1965) reported that leaf blade pubescence differed with varieties According to IRRI (1996) leaf blade pubescence could be glabrous/intermediate/pubescent Toughness of leaf blade was desirable to protect leaves against wind damage where glabrousness was desirable in highly mechanized rice cultivation The mode of inheritance of glabrousness was simple but it was very difficult to combine with good plant and grain types (Nanda 1997) According to Mehla *et al* (2008) leaf hairiness did not help in distinguishing rice cultivars

2.1.2.4 Leaf blade colour According to Chang and Bardenas (1965) leaf blade colour differed with varieties Yoshida (1981) observed broad and light green leaves in *indica* group and narrow and dark green leaves in *japonica* group The colour of leaf blade varied from various shades of green to purple (IRRI 1996) Kumar (2005) reported that leaves of *Njavara* genotypes ranged from light green to dark green in colour Mehla *et al* (2008) found that leaf blade colour was an important leaf character in morphological identification of rice varieties They also suggested that pigmentation of leaf did not help in distinguishing rice cultivars IRRI (2010) reported that the traditional *indica* rice varieties had light green coloured leaves

2.1.2.5 Basal leaf sheath colour IRRI (1996) reported that the colour of basal leaf blade could be classified as green/purple green/light purple/purple Reddy (2000) reported that *Njavara* genotypes showed green basal leaf sheaths Mehla *et al* (2008) found that basal leaf sheath colour was an important leaf character in morphological identification of rice varieties

2.1.3 Ligule

The presence or absence of ligule involved differences in one allele and presence of it was denoted as *Lg* Ligule length colour and shape varied between varieties Length

of the ligule was one of the important traits which were used in identification of *Oryza glaberrima* L from *Oryza sativa* L. Pubescence of ligule might be glabrous or ciliate (Chang and Bardenas 1965)

Ligule shape varied as acute/acuminate/2 cleft or truncate. Degrees of development of the ligule had some relation to the variety and soil humidity (IRRI 1980)

Yoshida (1981) described ligule as a small white membranous and triangular structure located on inside at the junction of leaf blade and sheath. He also reported that some varieties lack the ligule and are called as liguleless rice.

According to Reddy (2000) the average ligule length of *Njavara* genotypes ranged from 1.24 cm to 2.40 cm with white colour.

2.1.4 Collar Chang and Bardenas (1965) described collar or junctura as junction of leaf sheath and blade. Pigmentation of collar differed within the same plant and when pigmented the dorsal, ventral and lateral portions showed different colours. He also reported that the collar colour usually differed from the colour of leaf sheath and leaf blade.

2.1.5 Auricle colour Auricle is one of the plant parts which might be pigmented with anthocyanin and may be present in older leaves (Chang and Bardenas 1965). Yoshida (1981) reported that the well developed auricles could be used as guidance for differentiating rice from barnyard grass which lack auricles. According to IRRI (1996) the auricle colour could be classified as light green or purple. Narada (1997) described the auricles as small/paired/ear like or sickle shaped appendages borne on either side of the base of the leaf blade. Reddy (2000) reported that auricle of *Njavara* genotypes was light green in colour.

2 1 6 Days to heading

Days from sowing to five per cent emergence of all panicles in a plot are called days to first heading. Middle heading and full heading were described for days from sowing to 60 per cent emergence of all panicles and more than 90 per cent emergence of all panicles respectively. It takes 10-14 days for a rice crop to complete heading (Chang and Bardenas 1965).

Yoshida (1981) described that date of anthesis is same as date to heading in rice. The date of anthesis of individual spikelets differed with position of spikelets within the same panicle. Spikelets which were located at upper branches of panicle opened earlier than those on the lower branches. It takes seven to ten days for complete anthesis of all spikelets within the same panicle and 15 to 20 days in case of a crop. Inverse relationship between days to heading and temperature was also reported. According to Elsy *et al* (1992) in *Njavara* genotypes date of heading coincided with date of anthesis. Days to flowering varied from 45 to 150 days in rice varieties of Kerala (Kumari and Nayar 1996). Days to 50 per cent heading was used by Reddy (2000) in identification of *Njavara* genotypes along with other quantitative characters. He also reported a positive correlation of the above trait with leaf width, ligule length, culm diameter, panicle length, 1000 grain weight, grain length, grain width and days from seeding to maturity. Ramakrishnan *et al* (2006) reported that days to flowering showed a positive correlation with plant height, flag leaf area, kernel length, length to breadth ratio and grain weight.

According to Babar *et al* (2007) days to heading had weak significant association with grain yield per plant. However, positive correlation of days to heading with flag leaf length and flag leaf rolling was also reported. Date of heading differed among tillers of the same plant and plants in the same field (Anon 2010).

2 1 7 Culm

The stem of rice is known as culm.

2.1.7.1 Culm length Based on culm length rice plant could be classified into dwarf, short, tall and very tall types (Richharia and Govindaswami 1990). Culm length varied from 75–180 cm in rice varieties of Kerala (Kumari and Nayar 1996). According to Nanda (1997), culm length is a simply inherited character and had a significant effect on grain yield. In rice varieties, culm length ranged from 20 cm to 5 m as in some deep water varieties. He also stated that traditional tall *indica* varieties were non responsive to nitrogen and showed characteristics of lodging and low grain yield.

Thirumeni and Subramanian (1999) concluded that plant height showed a positive correlation with flag leaf area and grain weight. Negative correlation of plant height with number of panicles per plant, grains per panicle and spikelet fertility was also reported.

Reddy (2000) reported a positive correlation of culm length with leaf length, grain width and amylase activity. Negative correlation between culm length and amylose content was also reported. The plant height ranged from 160–175 cm and 90–110 cm in Basmati 370 and Pusa Basmati 1 respectively (Singh 2000).

Kumari *et al.* (2002) reported that *Gandhakasala* and *Jeerakasala* were tall varieties with heavy lodging habit. According to George *et al.* (2005), the average plant height of *Gardhakasala* and *Jeerakasala* was 151.90 cm and 135.90 cm respectively.

Babar *et al.* (2007) reported a positive association of plant height with panicle length and flag leaf length.

Krishna *et al.* (2009) used genetic diversity analysis to report that the plant height (33.51%) contributed maximum to the diversity followed by panicle length (19.73%), days to flowering (16.13%) and 1000 grain weight (11.17%).

2.1.7.2 Culm number Based on culm number, rice could be classified into low, medium and high tillering varieties (Richharia and Govindaswami 1990). Nanda (1997) reported that tiller number is a quantitatively inherited character highly influenced by

environmental factors. According to Kumari *et al* (2002) *Jeevakasala* showed higher effective tiller number (7.6) than *Gandhakasala* (6.8). A negative correlation of culm number with leaf width, culm diameter, panicle length, 1000 grain weight, grain length, grain width, days from seeding to maturity and a positive correlation of culm number with protein content was reported by Reddy (2000). Zhong *et al* (2003) studied the relation of tiller number of rice with Leaf Area Index (LAI) and Leaf Nitrogen Concentration. Relative Tillering Rate (RTR) increased linearly as Leaf Nitrogen Concentration increased in rice plants. LAI had a negative effect on the emergence and survival of tillers.

Natarajan *et al* (2005) reported that under saline conditions the number of productive tillers per hill emerged as the main component of rice grain yield and had a large direct effect on grain yield. Number of grains per panicle and 1000 grain weight did not show any direct effect on yield but they influenced yield via plant height.

Nanda and Agarwal (2006) mentioned that ability of tillering varied with varieties and was affected by various environmental factors such as spacing, light, nutrient supply and cultural practices. Modern high yielding varieties had high tillering ability than tall traditional varieties and spreading types. However, all tillers of high tillering varieties were not productive. Awan *et al* (2007) reported that mother tiller had high grain yield and 1000 grain weight when compared to primary, secondary and tertiary tillers. Feng *et al* (2007) reported that excessive tillers within a plant reduced the quality of rice and late emerged tillers showed inferior quality than old tillers. He also found a close relation of tillering ability with panicle length and yield and concluded that excessive or insufficient tillering was unfavourable for high yield.

Mohanan and Mini (2008) observed that tertiary tillers were produced only in high tillering varieties and showed reduced height, less number of seeds per panicle and panicle density. He also reported that tertiary tillers did not contribute to economic yield. Mohapatra and Kariali (2008) observed that late tillers had small sized panicles compared to early tillers.

2 1 7 3 Culm diameter According to Reddy (2000) culm diameter had a positive correlation with panicle length 1000 grain weight grain length grain width days from seeding to maturity and grain size. However, negative association of culm diameter with protein content was also reported.

2 1 7 4 Internode and septum colour According to Chang and Bardenas (1965) internode and septum come under plant parts which may be pigmented with anthocyanin and variations in colour were mentioned as colourless/ green/ pink and red.

According to Riechharia and Govindaswami (1990) internode colour could be green or light gold or purple lines or purple and same description was also accepted by IRRI (1996).

Reddy (2000) reported that the culm internode of *Njavara* genotypes was light gold in colour whereas Ptb 10 showed green colour for culm internode.

Maximum node production rate had a significant negative direct effect on days to heading and had positive direct effects on maximum tiller density, mass of panicles at harvest, panicle density and leaf mass at heading (Samonte *et al* 2005).

Mehla *et al* (2008) suggested that the anthocyanin pigmentation of stemnodes and internodes did not play an important role in identification of rice cultivars.

2 1 8 Panicle

The duration of panicle development ranged from 27–46 days and it varied with variety and weather. Early maturing varieties showed shorter duration than late maturing varieties (Yoshida 1981).

2 1 8 1 Panicle length According to Chang and Bardenas (1965) varieties differed greatly in the panicle length, shape, angle of the primary branches, weight and density.

(number of spikelets per unit of length) Racemose type of branching in rice panicles was also reported

Panicle length showed a positive correlation with 1000 grain weight, grain length, grain width, days from seeding to maturity, fat content and amino acid content. However, negative correlation of panicle length with protein content was also reported (Reddy 2000)

Singh (2000) reported that Basmati 370 and Pusa Basmati 1 had long panicles. According to Kumari *et al* (2002) and George *et al* (2005) the average panicle length of *Gandhakasala* and *Jeejakasla* was 28.4 cm and 27.1 cm respectively and average number of grains per panicle was 104.80 and 110 respectively. Babar *et al* (2007) reported that the taller plants could bear longer panicles.

2.1.8.2 Panicle type Type of panicles could be classified as compact/intermediate and open (IRRI 1996). Nanda (1997) suggested semi compact panicles as desirable types than open panicles that had few spikelets.

Wang *et al* (2007) reported that cultivars with compact panicles showed lower grain weight and larger variation in brown rice length, brown rice width, length to width ratio, chalky grain percentage and amylose content among grains within a panicle than the loose panicle cultivars.

2.1.8.3 Secondary branching Chang and Bardenas (1965) reported that the secondary branching was absent in *Oryza glaberrima* L. species.

Kato (1996) observed that rice spikelets on Primary Branches (PB) of panicles generally produced fully filled grains compared to Secondary Branches (SB). The number of PB per panicle showed positive and strong association with number of spikelets per panicle. Thus, he suggested that indirect selection on the number of spikelets per panicle via selection on the number of primary branches per panicle was more

effective than direct selection on the number of spikelets per panicle. He also reported that number of PB per panicle did not show positive correlation with number of spikelets on SB as well as number of SB per PB.

Sreejayan *et al.* (2006) reported that *Njavara* genotypes showed light and heavy secondary branching pattern in panicles.

According to Mehla *et al.* (2008) secondary branching of panicles did not help in distinguishing rice cultivars.

2.1.8.4 Panicle exertion Chang and Bardenas (1965) described that the extent to which the panicle and a portion of the uppermost internode extend beyond the flag leaf sheath determines the exertion of the panicle.

Degree of exertion of panicle differed with varieties and it ranged from complete exertion to its enclosure in the flag leaf sheath. Complete exertion of panicle was a desirable trait. Spikelets of incompletely exerted panicles partially enclosed in the flag leaf were either sterile or partially filled and resulted in loss of grains. Incomplete panicle exertion led to pathogenic discoloration of flag leaf sheath and grains. The genetics of panicle exertion was simple but was highly influenced by air temperature and shading (Nanda, 1997).

George *et al.* (2004) reported that *Deepthi* variety showed long exerted panicles. Sreejayan *et al.* (2006) reported that panicle exertion of *Njavara* genotypes varied as partly exerted, well exerted and moderately well exerted.

Panicle exertion was a better indicator of cold tolerance. Incomplete panicle exertion was one of the symptoms of cold injury at the reproductive stage of the rice and resulted in loss of grain yield and disease resistance (Cruz *et al.* 2008).

Anon (2010) reported that the exertion of panicle varied within tillers of the same plant and between plants in the same field

2 1 8 5 Panicle shattering The rate of shattering could be classified as tight/intermediate/shattering types. If few or no grains were removed from panicle by pressure it was considered as tight panicle. Intermediate panicle shed 20-50 per cent of grains while shattering panicles shed 50 per cent of grains. Shattering was described as a simple Mendelian character and probably polygenic inheritance. *Indica* varieties had good shattering ability than *japonica* varieties (Chang and Bardenas 1965)

2 1 8 6 Panicle threshability Reddy (2000) observed difficult threshability of panicles in *Njavara* genotypes

2 1 9 Spikelet

2 1 9 1 Awning Chang and Bardenas (1965) described awn as a filiform extension of the keel of the lemma. The length of awn could be classified as long/medium/short/tip awn. They also reported that awn length showed a discontinuous variation when the plants were grown at different sets of environments. The presence or absence of awns generally involved difference in one allele and their presence is denoted by a dominant allele *An*.

Khush (2000) reported that Basmati 370 showed partial tip awning. Mehla *et al* (2008) reported that awning and distribution of awns played a secondary role in distinguishing rice cultivars. Elay *et al* (2010) found *Gadhakasala* as an awnless variety whereas *Jee akasala* showed partial short awns.

2 1 9 2 Awn colour The colour of awn could be classified as straw/gold/brown/red/purple/black (IRRI 1996). According to Reddy (2000) the awn colour of *Njavara* genotypes ranged from straw to gold. Khush (2000) reported that Basmati 370 had straw coloured awns.

2 1 9 3 *Apiculus colour* According to Takahashi (1957) the apiculus colour at anthesis could be classified as straw white/seashell pink/rose red/tyrian rose/pomegranate purple/amaranth purple/pansy purple/blackish red purple. However white/straw white/warm buff/ochraceous buff/tawny (light to dark brown)/russet/faded pink/faded red purple/faded purple colours were reported at maturity stage.

Chang and Bardenas (1965) described apiculus as the extending tip of the lemma or palea and apiculus might be separated as lemmal apiculus and paleal apiculus. According to IRRI (1996) the colour of apiculus varied as white/straw/brown/red and purple. Kumar (2005) noted that the apiculus colour of *Njava a* genotypes varied as straw/brown/black.

2 1 9 4 *Stigma colour* According to Chang and Bardenas (1965) stigma was one of the plant parts which might be pigmented with anthocyanin. Mehla *et al* (2008) observed that the stigma colour did not play an important role in distinguishing rice cultivars.

2 1 10 Lemma and palea

2 1 10 1 *Lemma and palea colour* Colour of fertile lemma and palea at maturity were white/straw tawny (light to dark brown)/gold/brown furrows/brown spots (piebald)/russet/reddish brown/shades of purple and sooty black (Chang and Bardenas 1965).

According to Reddy (2000) lemma and palea colour of *Njava a* genotypes varied as gold, gold furrows on straw background, golden yellow and black and same was reported by Sreejayan *et al* (2006).

2 1 10 2 *Lemma and palea pubescence* *Indica* rice cultivars showed thin and short hairs on lemma and palea whereas dense and long hairs were reported in *japonica* varieties (Chang and Bardenas 1965 and IRRI 2010).

2 1 11 Sterile glumes

2 1 11 1 *Length of sterile glumes*

The pair of bracts above the rudimentary glumes should be designated as sterile lemmas (non flowering glumes or empty glumes or outer glumes) These are generally shorter than the fertile lemma and palea and it might exceed one third length of the fertile glumes but both sterile glumes differed in size and shape (Chang and Bardenas 1965) classified length of sterile glumes as long (more than one-third of fertile lemma) and extra long (longer than lemma)

2 1 11 2 *Sterile glume colour* Colour of sterile glumes could be colorless (white)/straw/gold/brown/red and purple (Chang and Bardenas 1965)

2 1 12 *Spikelet sterility* Injury to the stem low night temperature high day time temperature and rapid desiccation of water from hot winds caused spikelet sterility and resulted in yield loss of about 12.5 per cent (Peterson *et al* 1997)

Rice cultivars with numerous spikelets per panicle (extra heavy panicle types) frequently failed to exhibit their high yield potential due to low grain filling (Kato *et al* 2007) Awan *et al* (2007) found that tertiary tillers showed maximum spikelet sterility and minimum by main culm

According to Ohe *et al* (2007) high temperature led to yield loss because of the reduction in percentage of ripened grains and increase in the percentage of sterile spikelets

Tao *et al* (2008) observed that high temperature reduced the rate of glume opening of spikelets from 40.0 per cent to 23.6 per cent and it delayed heading period by about two hours He also reported that high temperature during grain filling stage reduced the rate of grain shattering by increasing the number of sterile spikelets The early

flowering superior spikelets usually exhibited a faster grain filling rate and leave grain weight than late flowering inferior spikelets (Yang *et al* 2008)

Singh *et al* (2009) reported that rice grain sterility increased with deficiency of water during panicle development stage. It was also observed that barriers to water loss in water stress conditions resulted in decreased sterility of spikelets

2.1.13 Grain

Rice grain is also known as rough rice. The characteristics of grains *i.e.* shape, size, translucency and colour had a direct bearing on marketability (Rendona and Mackill 1997). Duration of grain development in rice ranged from 25 to 40 days.

2.1.13.1 1000 grain weight (TGWT) Evans (1972) reported grain size as a highly important quality trait in rice that determined grain weight. He also mentioned that grain weight was one of the three components (number of panicles per plant, number of grains per panicle and grain weight) of grain yield. According to Yoshida (1981) weight of a single rice grain ranged from 12 to 44 mg at zero per cent moisture content. Reddy (2000) reported that TGWT showed a positive correlation with grain length, grain width and days to seeding to maturity. Negative correlation of TGWT with amylose content and protein content was also reported. Ryu *et al* (2001) classified Korean rice *et al* based on the TGWT, shape of brown rice and amylose content of rice grain. In 97 per cent of 207 varieties TGWT of brown rice came in the range of 17 to 27 g. Mustafa and Elsheikh (2007) suggested that number of filled grains per panicle, number of panicle per culm, panicle length and TGWT were important characteristics in improving the rice yield. Wu *et al* (2008) reported TGWT as an important factor that affected grain yield and quality of the rice grain. He stated that among rice yield components TGWT showed relatively high heritability (80%). According to Elsy *et al* (2010) TGWT of *Gandhakasala* varied from 18.0 g to 23.0 g. However, it ranged between 15.0 g to 18.9 g in *Jeejakasala* genotype.

2.1.13.2 Grain length According to Yoshida (1981) the dimensions of grain *viz.* length, width and thickness widely varied with varieties. He also mentioned the relation of grain

size and shape with cooking and processing characteristics. The grains of *indica* group were slender and flat whereas *japonica* group contained short and round grains. Reddy (2000) reported positive correlation of grain length and width with grain yield. However kernel length had positive significant correlation with length to breadth ratio, kernel length after cooking and gel consistency. Xu *et al.* (2002) reported a positive correlation of grain size with grain length, width and thickness. According to Kumari *et al.* (2002) and George *et al.* (2005) *Gandhakasala* grains were short bold with an average kernel length of 4.07 mm whereas grains of *Jeerakasala* were long and slender with 4.74 mm of kernel length. Traore (2005) reported that the long grained aromatic rice had the greatest demand and was the most expensive rice in local markets. Elsy *et al.* (2010) observed that grains of *Jeerakasala* were slightly longer than *Gandhakasala*. It was also reported that kernel length of *Gandhakasala* ranged from 4.0 mm – 4.8 mm and in *Jeerakasala* it ranged from 4.7 mm – 5.8 mm.

2.1.13.3 Grain breadth Reddy (2000) reported a positive correlation between grain width and days from seeding to maturity. Khatun *et al.* (2003) observed a positive correlation of length to breadth ratio with gel consistency, protein content, water uptake ratio and kernel length after cooking. A positive correlation of head rice with milled rice kernel breadth and gel consistency was also reported. According to George *et al.* (2005) the average kernel breadth of *Gandhakasala* and *Jeerakasala* were 2.40 mm and 2.20 mm respectively. Anon (2010) reported that the kernel breadth of *Gandhakasala* ranged from 1.72 – 2.00 mm where 2.13 – 2.73 mm in *Jeerakasala*.

2.1.13.4 Seed coat colour Kumari *et al.* (2002) reported that both *Gandhakasala* and *Jeerakasala* had white kernel colour. George *et al.* (2004) reported that *Deepthi* variety had red kernel colour.

2.1.13.5 Endosperm type According to IRRI (1996) endosperm could be classified as non-glutinous (non-waxy), glutinous (waxy) and intermediate. Tan *et al.* (2000) reported that grain shape and endosperm opacity were the two main attributes that determined the appearance of the rice grains. Opacity of the endosperm was caused by the loose packing and presence of air spaces between the starch granules. The degree of opacity or

chalkiness in the endosperm varied with cultivars and it consisted of three sub traits i.e. white belly, white back and white center.

2.1.13.6 Milling recovery The weight of hull accounted for about 21 per cent of the total grain weight and proportion of hull to grain was about 20 per cent (Yoshida 1981). According to George *et al.* (2005) milling recovery of *Gandhakasala* and *Jeerakasala* were 72 and 71 per cent respectively. George *et al.* (2004) reported that the milling recovery of *Deepthi* rice variety was 66.02 per cent. In rice milling recovery ranged from 70.4 to 79.2 per cent for total rice and from 23.8 to 74.5 per cent for head rice.

2.1.14 Maturity

Depending on the variety and the environment under which rice is grown, rice plant usually takes three to six months from germination to maturity. Kawano and Tanaka (1968) reported that varieties of very short duration might not produce high yield because of limited vegetative growth and those of very long growth duration might not be high yielding because of excessive vegetative growth that might cause lodging. About 120 days from seeding to maturity appeared to be optimum for maximum yield at high nitrogen levels in the tropics. However, longer growth duration might produce higher yield when fertility was low, presumably because there was more time to extract soil nitrogen.

Most of the tropical traditional rice varieties were sensitive to photoperiod and had long maturity period. Planting most of the photoperiod sensitive varieties under short day length would result in fewer tillers, shorter plants and earlier maturity (Chang and Bardenas 1965). He also stated that maturity was computed in days i.e. from seeding to ripening of more than 80 per cent of the grains on the panicle and applicable ranges of maturity for tropical varieties were 100 or less, 101, 115, 116, 130, 131, 145, 146, 160, 161, 176, 176, 190, 191, 205 and 206 or more. According to him, varieties also differed in duration from anthesis to full maturity and in duration of grain development varying from 25 to 40 days.

Kumari *et al* (2002) reported that maturity days in *Gandhakasala* and *Jeerakasala* ranged from 150-180 days. It was also recorded as 190 days (George *et al* 2005). According to George *et al* (2004) *Deepthi* was a medium duration variety and maturity days ranged from 155 to 160 days.

2.1.15 Yield

Yield is a complex trait controlled by many genes.

2.1.15.1 Grain yield Kaul and Bhan (1974) found that number of Grains per Panicle (GNP) showed a significant positive correlation with yield and GNP as a reliable index for the yielding capacity of rice crop. Positive correlation of number of spikelets per square meter with yield was reported by Evans (1993).

In recent years rice breeders have realized that increasing grain yield is a complex objective. Yield potential could be estimated from a number of component traits under high inputs like panicle number/m², number of filled grains/panicle, grain weight and harvest index. Grain yield in the tropics was higher in the dry season than wet season and this was mainly because of a difference in the intensity of solar radiation (Chang 1997).

Balochi *et al* (2002) reported that number of panicles per hill, grain weight per hill and fertile grains per panicle made a significant contribution to grain yield per unit area. According to Kumari *et al* (2002) *Jeerakasala* had more grain yield than *Gandhakasala*. Correlation and path coefficient studies revealed that number of filled grains per panicle, the number of productive tillers per square meter, biological yield and harvest index had direct positive effect on grain yield (Surek and Beper 2003).

According to Laza *et al* (2004) rice cultivars with intermediate panicles (100-115 spikelets per panicle) produced higher grain yield over cultivars with small panicles (60-80 spikelets per panicle). Duy *et al* (2004) reported that number of spikelets per panicle varied with the tiller positions and it was highest on main stem followed by Primary

Tillers and Secondary Tillers He also reported that number and development of spikelets on a panicle directly affected the grain yield

Vanaja *et al* (2004) reported that in rice hybrids the grain yield increase was due to significant and favourable heterosis in yield components *viz* number of spikelets per panicle panicle length leaf area per plant and number of panicles per m²

The path coefficient analysis indicated that number of productive tillers per hill had the largest direct effect on yield and it was considered as the main component of rice grain yield under saline conditions However other characters such as number of grains per panicle and 1000 grain weight did not show any direct effect on yield whereas they influenced yield via plant height (Natarajan *et al* 2005) Grain yield of *Gandhakasala* and *Jeejakasala* was 2179 kg ha⁻¹ and 2743 kg ha⁻¹ respectively (George *et al* 2005)

Erect leaf was a highly heritable character and was considered as one of the most important traits associated with high grain yield (Nanda and Agarwal 2006) Number of grains per panicle and spikelet fertility had positive correlation with grain yield (Ramakrishnan *et al* 2006) According to Chandra *et al* (2006) grain yield showed significant positive correlation with grain number 1000 grain weight biological yield and harvest index Grain yield showed a positive association with fertile spikelets and negative association with sterility percent

Awan *et al* (2007) reported that yield components were significantly affected by different tillers of the same plant He also reported that grain yield and 1000 grain weight were highest in primary tillers and lowest in tertiary tillers

Babar *et al* (2007) observed a negative correlation between grain yield and flag leaf rolling However flag leaf rolling decreased photosynthetic rate resulting in the reduction of plant growth and grain yield Furuhashi *et al* (2008) reported that a linear relationship occurred between increased number of panicles per unit and grain yield

According to Li *et al* (2009) the higher biomass accumulation along with the intense solar radiation, large diurnal temperature range, greater LAI were the important morphological factors contributing to the high yield in Taoyuan rice variety. Munda *et al* (2009) reported that application of FYM @ 2.5 tonnes per hectare increased the growth and yield attributes as well as grain yield of rice.

2.1.15.2 Straw yield According to Yoshida (1981) the critical sulfur and N:S (nitrogen:sulfur) ratios varied with the growth stage of the rice plant and their presence in the plant tissues was required to obtain maximum dry weight. According to Kumari *et al* (2002) and George *et al* (2005) *Jeerakasala* showed more straw yield than *Gandhakasala*. Straw yield of *Gandhakasala* and *Jeerakasala* were recorded as 4038 kg ha⁻¹ and 4385 kg ha⁻¹ respectively. According to Kumar (2005) the straw yield of *Njavana* genotypes was lower (3314.56 kg/ha) when compared to check variety (7348.65 kg/ha).

2.1.16 Pest and disease incidence

According to Elsy (2002) pest and diseases were very important biotic stresses that led to low yield in rice. Negligible losses due to pest and diseases in *Gandhakasala* and *Jeerakasala* were reported by Kumari *et al* (2002).

2.2 Nutritional characterization

Morphological markers are not very good for the depiction of genotypes. Solitary based on these markers is not reliable. Hence, biochemical characterization along with morphological markers is efficient and essential.

2.2.1 Carbohydrates

Brown rice contains 0.83 to 1.36 per cent total sugars as glucose with reducing sugars ranging from 0.09 to 0.13 per cent. Milled rice contains 0.37 to 0.53 per cent total sugars with 0.05 to 0.08 per cent reducing sugars. Percentage of total sugar varied with

variety and degree of milling. The main non-reducing and reducing sugars were sucrose and glucose respectively. Fructose, galactose, maltose, raffinose and other oligosaccharides had also been reported (Luh 1980). Yu *et al.* (1996) suggested that sugars played important role in regulating metabolic activities in addition to providing essential carbon source for the growth of young seedlings and maintaining turgor pressure for the expression of tissue during germination. Based on soluble carbohydrate content in grain, Reddy (2000) classified *Njavara* genotypes into low (upto 1.5%), medium (1.5 to 2.5%) and high (>2.5%) soluble carbohydrate groups. According to Kumar (2005) soluble carbohydrate content had a negative genotypic correlation with grain yield. The soluble carbohydrate content of *Njavara* genotypes ranged between 1.64 to 2.19 per cent.

2.2.2 Protein

Chang and Bardenas (1965) reported that protein content affected the viscosity of rice grain. Environmental conditions and soil nutritional conditions were affecting the protein content of rice grain (Yoshida 1981). Protein content of *indica* rice ranged between 4.9 to 19.3% whereas in *japonica* rice it ranged between 5.9 to 16.5 per cent (Lin *et al.* 1993).

Protein content of rice had been studied by many researchers and reported as a quantitative trait. Rice protein is one of the most nutritious among cereals and enriched with lysine. The proportion of proteins (6%) in rice grain was relatively less compared to starch (90%). Protein content of milled rice was very low (7% at 14% moisture). Milled rice had around 80 per cent glutelin (alkali soluble), 10 per cent globulin (salt soluble), 5 per cent albumin (water soluble) and less than 5 per cent prolamin (alcohol soluble). Among these albumin had the highest lysine content followed by glutelin, globulin and prolamin. Glutelin constituted the major component of rice endosperm protein. Protein content defined most of the physicochemical properties of cooked rice (Juliano 1993, Hamakar 1994).

Shi *et al* (1996) reported that protein and lysine content of milled rice are determinants of its nutrient value. Nutrient quality traits were controlled by cytoplasmic maternal and seed direct effects. He also concluded that the protein content and protein index were affected by seed direct effects, whereas lysine content, lysine index and the ratio of lysine content to protein content were more affected by cytoplasmic effects than maternal effects.

Khatun *et al* (2003) reported positive correlation between length to breadth ratio and protein content. According to Kumar (2005), protein content showed a negative correlation with grain yield. The content of protein is one of the primary determinants of taste in white rice (Nanda and Agarwal 2006).

Samonte *et al* (2006) reported grain yield had positive correlation with nitrogen use efficiency, nitrogen content and Nitrogen Translocation Ratio (NTR), where NTR also showed correlation with grain protein concentration. He suggested that plant breeders could use these significant correlations to their advantage in breeding for rice cultivars as it not only helped in high yield but also utilized N efficiently and produced grains with a higher protein concentration.

2.2.3 Amylose

Rice grain consists of 90 per cent starch. The amylose content in starch ranged from 15 to 35 per cent. Many of the cooking and eating characteristics of milled rice were influenced by the ratio of two kinds of starches, viz. amylose and amylopectin, in rice grain. The content of amylose and amylopectin varied with varieties and method of processing (Rao *et al* 1952).

On the basis of amylose content, milled rice could be classified as waxy and non-waxy rice. Amylose content was almost absent in the waxy (glutinous/sweet) rice and starch of these grains contained entirely amylopectin (Chang and Bardenas 1965; Kumar and Khush 1986a). Such rice could not expand in volume, were glossy and sticky and

remained firm when cooked. Non waxy (nonglutinous) rice could be subdivided into high (> 25%) low (10-19%) or intermediate (20-25%) amylose containing types. High amylose rice showed high volume expansion (not necessarily elongation) and a high degree of flakiness. They became less tender on cooking and hard upon cooling. Rice with very low amylose content became very sticky, moist and tender on cooking, whereas intermediate amylose content rice became fluffy, soft and moist (Kumar and Khush 1986b, Nanda, 1997, Cruz and Khush 2000). Majority of Indian rice cultivars had high amylose content. But intermediate amylose rice cultivars are being preferred by most of the rice growing areas of India. Therefore, nowadays development of improved germplasm with intermediate amylose content is taken into consideration in the grain quality improvement programme.

Khatun *et al* (2003) reported that amylose content had significant negative correlation with gel consistency and positive correlation with the expanded volume of grain. Zhang *et al* (2003) reported that amylose content varied among grains of the same panicle. It also varied between outer and inner layers of the same grain. Genetic and environmental factors affected amylose content of rice grains. Miyano and Suzuki (2005) reported that amylose content and whiteness of rice endosperm were affected by environmental conditions. He also reported a negative correlation between moisture content of environment and amylose content. Nanda and Agarwal (2006) reported that amylose content varied as much as six per cent depending upon environmental conditions. The higher temperature during ripening stage lowered the amylose content and cooler temperature had the opposite effect. Amylose was one of the primary determinants of taste in white rice. According to Rang *et al* (2006) non aromatic cultivars exhibited low to intermediate amylose content (9-20% to 20-25%) whereas aromatic cultivars had low amylose content.

The amylose content of rice grain determined the texture and appearance of cooked rice. Amylose content of rice grain was determined by waxy gene. In glutinous rice the waxy gene was non functional resulting in very low amylose (<2%) and such

rice cultivars were very sticky when cooked. Non glutinous rice had a fully or partly functional *waxy* gene (Arvan 2007).

Igarashi and Kohara (2008) reported that amylose content differed with location of grains. Grains harvested from the first branches or early flowers tend to have higher amylose content. The primary rachis branches showed higher amylose content than that of secondary rachis branches because the starch content of the grains on the secondary rachis branches was lower than that on the primary rachis branch. He also reported negative correlation of amylose content with seedling age and temperature during grain filling.

2.2.4 Aroma

Aromatic rice cultivars had a characteristic natural aroma and flavour. Aroma of cooked rice adds market value to the product. The volatile aromatic component responsible for aroma had been identified as 2-Acetyl-1-Pyrroline (2-AP). The odour of 2-AP akin to popcorn (Buttery *et al.* 1983). Pleasant aroma of aromatic rice cultivars was not only associated with cooked rice but also emitted a specific flavour in fields (anthesis stage) and at harvesting, storage and milling practices (Yoshida 1981; Efferson 1985).

The content of 2-AP in non-aromatic rice cultivars ranged from 0.004–0.006 ppm. IRRI (1985) reported that aromatic cultivars were rich in 2-AP (15 times more) than non-aromatic rice cultivars and found 2-AP was present in almost all parts of the plant (stems, leaves and grains) except roots.

Weber *et al.* (2000) concluded that the pleasant aroma of raw or cooked aromatic or non-aromatic rice cultivars was controlled by a blend of various volatile compounds. They also stated that most of volatile compounds found in aromatic and non-aromatic rice cultivars were similar but in different proportions.

The genetic and environmental factors influenced the development of aroma in aromatic rice cultivars. Cooler temperature at flowering and grain development stage

encouraged the development of aromatic rice cultivars (Singh *et al* 2000) Khush (2000) reported that Basmati 370 as a strongly scented variety and Pusa Basmati 1 showed mild aroma on cooking George *et al* (2005) reported that *Gandhakasala* and *Jeerakasala* fell into moderately scented group Traore (2005) reported that aromatic rice cultivars possessed a natural flavor that was similar to buttered popcorn in aroma

Hien *et al* (2006) identified the role of original cultivation area in the development of aroma and reported that the strength of aroma of aromatic rice cultivars decreased when grown outside the original cultivation area According to Sukla *et al* (2006) the aroma of rice played an important role in consumer acceptability and had a special place in world rice markets The temperature between 22⁰C and 26⁰C at flowering and dough stage was ideal for the expression of aroma in *Gandhakasala* and *Jeerakasala* (Kumari 2007)

Aroma was one of the most valuable traits in grain quality and it helped to fetch a higher premium in the market (Bourgis *et al* 2008 Sakthivel *et al* 2009)

2.3 Biochemical characterization

2.3.1 Isozyme characterization

Isozymes are generally made up of a number of subunits and it is the varying number of the subunits which give rise to isozymes A number of major biological problems such as evolution of population the transformation the regulation of gene expression and metabolic regulation in differentiated tissues are understood in the light of isozymes

Isozymes are detectable through electrophoresis due to differences in their net electrical charges The term isoenzyme is synonym to isozymes They had common catalytic activity and synthesized under control of different genes Isozymes are active in different tissues and also had different molecular properties Variations in isozymes

might be arising from the allelic segregations at a single locus representing more subtle changes in the enzyme molecules. Molecular forms of isozymes could be separated by several biochemical techniques of which gel electrophoresis was the most widely used and easy to operate method.

A number of researchers used isozymes to determine the genetic divergence among cultivars and their wild relatives (Oka 1958, Chu 1967, Glaszmann 1987, Rutger 1999). Pawar and Gupta (1975) studied variation of peroxidase isozyme pattern in tall and dwarf varieties of rice at different developmental stage. They had taken samples at weekly intervals starting from soaked seeds (12h) until the post panicle stage and observed that some isozymes remained constant throughout the developmental stages and a few disappeared at certain stages. Two specific bands viz A3 and A7 were observed in the tall variety and A4 in the dwarf variety. Fu and Pai (1979) reported that *indica* and *japonica* rice groups possessed different peroxidase alleles.

Glaszmann (1987) applied isozyme polymorphism in the classification of rice germplasm which he divided into six varietal groups.

Tao *et al* (1999) commented that isozymes were the most widely used protein markers in plant breeding and studies on isozymes had been a valuable tool for rice geneticists.

Zeidler (2000) reported that isozymes were powerful tools for creating genetic variability within and between populations of plants and animals. He also concluded that isozymes were able to solve other questions of population biology, conservation biology and ecology as well.

Ishikawa *et al* (2000) found that isozymes served as useful gene markers in genetic studies at the plant and cellular level and were useful for uniting conventional linkage and restriction fragment length polymorphism linkage maps. Reddy (2000) applied peroxidase and esterase polyacrylamide gel electrophoresis technique in

biochemical characterisation of *Njavara* rice genotypes. He reported that germinated seed sample expressed more peroxidase bands than quiescent seed.

Esterase isozymes were useful for distinguishing between *indica* and *japonica* varieties. 11A band was characteristic of *indica* varieties and 10A was characteristic of *japonica* (keng) varieties (Cai and Wang 2000). Bimb *et al* (2004) used esterase isozymes as genetic markers to estimate the genetic diversity of 24 cultivated fine and aromatic rice cultivars.

Kaewmala *et al* (2005) studied esterase isozyme electrophoresis technique to separate out the rice seed mixtures of cv Kaodawkmalı 105 (KDML 105) and Chinat 1 (CN 1). They assayed five enzymes, i.e. esterase (EST), glutamate oxaloacetate transaminase (GOT), leucine amino peptidase (LAP), malic enzyme (ME) and malate dehydrogenase (MDH) and concluded that EST showed a significant difference between varieties.

Peroxidases were widely distributed in plant tissues and had a great physiological importance because of their association with numerous catalytic functions. Nasseer *et al* (2006) applied peroxidase and MDH isozyme analysis to distinguish aromatic and non-aromatic rice cultivars.

Datta *et al* (2008) described that isozyme assay was simple, reliable and it was considered as a valuable tool for assessing the genetic structure and taxonomic ability within and between groups of basmati rice cultivars. Based on the banding pattern of peroxidase and esterase isozymes, they differentiated 61 basmati genotypes. They had utilized both staining intensity (dark, medium, light and very light) and molecular weight of isozymes to differentiate genotypes.

2.3.2 Peroxidase activity

Catalase activity was decreased while peroxidase and polyphenoloxidase activities were increased during senescence of both attached and detached rice leaves (Kar and Mishra 1976). Fang and Kao (2000) reported that FeSO_4 was effective in stimulating peroxidase (POD) activity in detached rice leaves under both light and dark conditions. It was indicated that POD activity induced by Fe^{2+} , CuSO_4 and ZnSO_4 were also observed to induce POD activity in detached rice leaves along with FeSO_4 . Using isoelectric focusing to separate POD, it was found that excess Fe^{2+} , Cu^{2+} or Zn^{2+} induced both quantitative and qualitative metal specific changes in POD isozyme pattern in detached rice leaves.

Materials and methods

3 MATERIALS AND METHODS

The present investigation was carried out at the Department of Plant Breeding and Genetics and Centre for Plant Biotechnology and Molecular Biology College of Horticulture Vellanikkara during 2008-2010. Field experiments (Plates 1 and 2) related to the investigation were laid out at the Regional Agricultural Research Station (RARS) Ambalavayal Wayanad which is located at an altitude of 700 to 2100 m above the Mean Sea Level (MSL) and between North latitude $11^{\circ} 27'$ and $15^{\circ} 46'$ and East longitude of $75^{\circ} 21'$.

3.1 Materials

Gandhakasala and *Jeejakasala* are the most popular traditional aromatic rice cultivars of Wayanad district Kerala. A basic survey was conducted to collect different samples of *Gandhakasala* and *Jeejakasala* from various parts of the district. The approximate area under cultivation in each panchayat was also collected through Krishi bhavans under State Department of Agriculture. 65 samples of *Gandhakasala* and 10 samples of *Jeejakasala* were collected. From these samples based on grain characteristics 10 *Gandhakasala* and two *Jeejakasala* genotypes were selected for this study along with Deepthi (WND 3) as check variety. The details of these selected genotypes are given in Table 1.

3.2 Methods

Field experiments were carried out during *Kharif* season of 2009 following cultural practices recommended in Package of Practices KAU (2007). Chemical fertilizers were not applied during the crop growth and organic manures alone were applied @ 5t/ha as basal application. The 13 genotypes were grown in a Randomized Complete Block Design (RCBD) with three replications in plots of 2.5 m X 2.5 m with 20 cm X 10 cm spacing.

The selected genotypes were characterized based on morphological, nutritional and biochemical studies.

Table 1 Details of the genotypes used in the study

Sl No	Genotypes	Source	
		Panchayat	Name of the Farmer/Institute
	<i>Gandhakasala</i> genotypes		
1	GT1	Thirunelli	Sudhakaran
2	GT2	Thirunelli	Subramannian A D
3	GT3	Thirunelh	Kunhappan
4	GT4	Mananthavady	Narayanan M R
5	GT5	Noolpuzha	Krishnan Kutty
6	GT6	Noolpuzha	Madhavan C T
7	GT7	Vellamunda	Chiramadathu Moide
8	GT8	Nenmeni	Dayanandan
9	GT9	Panamaram	Ashok Kumar
10	GT10	Ambalavayal	RARS KAU
	<i>Jeerakasala</i> genotypes		
11	JT11	Noolpuzha	Velayadhan P
12	JT12	Panamaram	Preetha, P K
	Local check		
13	DT13 Deepthi (WND 3)	Ambalavayal	RARS KAU

3.2.1 Morphological characterization

The traditional aromatic rice genotypes were characterized and evaluated based on morphological characters. Observations on following characters were recorded following the Standard Evaluation System for Rice (IRRI 1996) from 10 randomly selected plants in each replication and the mean worked out.

3.2.1.1 Seedling height (SH) At fifth leaf stage seedling height was recorded in centimeters from the base of the shoot to the tip of the tallest leaf blade.

3 2 1 2 Leaf length (LL) At late vegetative stage leaf length was measured in centimeters from the topmost leaf blade below the flag leaf on the main culm

3 2 1 3 Leaf width (LW) At late vegetative stage leaf width was measured at the widest portion of the blade on the leaf below the flag leaf

3 2 1 4 Leaf blade pubescence (LBP) At late vegetative stage blade surfaces were classified as

Code	Guide
1	Glabrous (Smooth including ciliated margins)
2	Intermediate
3	Pubescent

3 2 1 5 Leaf blade colour (LBC) At late vegetative stage blade colour was classified into seven broad classes as

Code	Guide
1	Light green
2	Green
3	Dark green
4	Purple tips
5	Purple margins
6	Purple blotch (Purple mixed with green)
7	Purple (full)

3 2 1 6 Basal leaf sheath color (BLSC) At late vegetative stage colour of the outer surface of the leaf sheath was classified as

Code	Guide
1	Green
2	Purple lines

3	Light purple
4	Purple

3 2 1 7 Ligule length (LgL) At late vegetative stage ligule length was measured in millimeters from the collar to the tip. Its absence was denoted by a blank

3 2 1 8 Ligule colour (LgC) At late vegetative stage the colour of ligule was classified as

Code	Guide
1	White
2	Purple lines
3	Purple

3 2 1 9 Ligule shape (LS) At late vegetative stage ligule shape was classified as

Code	Guide
1	Acute to acuminate
2	2 Cleft
3	Truncate

3 2 1 10 Collar colour (CC) At late vegetative stage collar colour was classified as

Code	Guide
1	Light green
2	Green
3	Purple

3 2 1 11 Auricle colour (AC) At late vegetative stage auricle colour was classified as

Code	Guide
1	Light green
2	Purple

3 2 1 12 Days to 50 per cent heading Number of days from seeding to flowering of 50 per cent of the population was counted

3 2 1 13 Culm length (CL) At a stage after flowering culm length was measured in centimeters from ground level to the base of the panicle

3 2 1 14 Culm number (CmN) At a stage after flowering culm number was recorded after full heading as the total number of (grain bearing and non bearing) tillers

3 2 1 15 Culm diameter (CmD) At a stage after flowering culm diameter was measured in millimeters

3 2 1 16 Culm internode colour (CmIC) At a stage after flowering the outer surface of the internodes on the culm was recorded as

Code	Guide
1	Green
2	Light gold
3	Purple lines
4	Purple

3 2 1 17 Septum colour The colour of septum was seen by slitting longitudinally the lower portion of the culm and the cut surface was examined and recorded as

Code	Guide
1	Light green
2	Light gold
3	Purple lines
4	Purple

3 2 1 18 Panicle length (PnL) At a stage near to maturity length of panicle was measured in centimeters from the base to the tip of the panicle

3 2 1 19 Panicle type (PnT) At a stage near to maturity panicles were classified according to their mode of branching angle of primary branches and spikelet density as

Code	Guide
1	Compact
5	Intermediate
9	Open

3 2 1 20 Panicles secondary branching (PnBr) At a stage near to maturity based on secondary branches bearing the spikelets panicles were classified as

Code	Guide
0	Absent
1	Light
2	Heavy
3	Clustering

3 2 1 21 Panicle exertion (PnEx) At a stage near to maturity the exertion of the panicle above the flag leaf sheath after anthesis was classified as

Code	Guide
1	Well exerted the panicle base appears above the collar of the flag leaf blade
3	Moderately well exerted the panicle base is above the collar of the flag leaf
5	Just exerted the panicle base coincides with the collar of the flag leaf
7	Partly exerted the panicle base is slightly beneath the collar of the flag leaf
9	Enclosed – the panicle is partly or entirely enclosed within the leaf sheath of the flag leaf

Rating is based on majority of the plants in the plot

3.2.1.22 Panicle threshability (PT) At maturity stage the matured panicle was grasped by the hand and a slight roll or pressure was applied with the palm and the fingers. Based on the extent of grain removal threshability was classified as

Code	Guide
1	Difficult few or no grains removed
2	Intermediate 25 to 50 per cent of grains removed
3	Easy more than 50 per cent of grains removed

3.2.1.23 Panicle shattering (PS) At maturity stage extent to which grains have shattered from the panicle was described as

Code	Guide
1	Very low (less than 1%)
3	Low (1-5%)
5	Moderate (6-25%)
7	Moderately high (26-50%)
9	High (more than 50%)

3.2.1.24 Number of grains per panicle At maturity stage total number of grains per panicle was counted

3.2.1.25 Awn presence (An) At maturity stage the awning character was recorded as

Code	Guide
0	Absent
1	Short and partly awned
5	Short and fully awned
7	Long and partly awned
9	Long and fully awned

3 2 1 26 Awn colour (AnC) At maturity stage the colour of awns was recorded as

Code	Guide
0	Awnless
1	Straw
2	Gold
3	Brown
4	Red
5	Purple
6	Black

3 2 1 27 Apiculus colour (ApC) At maturity stage apiculus colour was classified into seven classes as

Code	Guide
1	White
2	Straw
3	Brown
4	Red
5	Red apex
6	Purple
7	Purple apex

3 2 1 28 Stigma colour (SgC) At flowering stage colour of stigma was classified as

Code	Guide
1	White
2	Light green
3	Yellow
4	Light purple
5	Purple

Sigma colour was determined from blooming spikelets (between 9 am and 2 pm) with the aid of hand lens

3 2 1 29 Lemma and palea color (LmPe) At maturity stage when the terminal spikelets were ripened the colour of lemma and palea was classified into 11 classes as

Code	Guide
0	Straw
1	Gold and/or gold furrows on straw background
2	Brown spots on straw
3	Brown furrows on straw
4	Brown
5	Reddish to light purple
6	Purple spots on straw
7	Purple furrows on straw
8	Purple
9	Black
10	White

3 2 1 30 Lemma and palea pubescence At flowering stage pubescence of the hull was classified as

Code	Guide
1	Glabrous
2	Hairs on lemma keel
3	Hairs on upper portion
4	Short hairs
5	Long hairs (velvety)

3 2 1 31 Sterile lemma colour At maturity stage when the terminal spikelets were approaching maturity the colour of the sterile lemmas was classified in to four classes as

Code	Guide
1	Straw (el or)
2	Gold
3	Red
4	Pu ple

3 2 1 32 Sterile lemma length (Length of sterile glumes) At maturity stage measurement was made on each of the two sterile lemmas and classified as

Code	Gu de
1	Short (not longer than 1 5 mm)
2	Medium (1 6 2 5 mm)
3	Long (longer than 2 5 mm but shorter than lemma)
4	Extra long (Equal to or longer than the lemma)
5	Asymmetrical

3 2 1 33 Spikelet sterility At maturity stage spikelet sterility readings were obtained from counts of well developed spikelets in proportion to total number of spikelets on five pan cles and classified as

Code	Guide
1	Highly fertile (>90%)
3	Fertile (75 90%)
5	Partly sterile (50 74%)
7	Highly sterile (<50% to trace)
9	Completely sterile (0%)

3 2 1 34 1000 gram weight At matur ty stage a random sample of 1000 well developed whole gra ns dried to 13 per cent moisture content was weighed on a precision bala nce and actual measurements were expressed in grams

3 2 1 35 Grain length At maturity stage the length of grains in millimeters was taken from the base of the lower most sterile lemma to the tip of the grain and actual measurements were expressed

3 2 1 36 Grain breadth At maturity stage the distance across the fertile lemma and palea at the widest point of the grain was measured in millimeters and actual measurements were expressed

3 2 1 37 Seed coat (bran) colour At maturity stage brown rice (dehulled grains) was classified as

Code	Guide
1	White
2	Light brown
3	Speckled brown
4	Brown
5	Red
6	Variable purple
7	Purple

3 2 1 38 Milling recovery Total milled rice was calculated by ratio between weight of total milled rice to weight of rough rice and expressed in per cent

3 2 1 39 Maturity Maturity was recorded as the duration in days from seeding to the time when more than 80 per cent of the grains on the panicle were fully ripened

3 2 1 40 Grain yield Weight of grains obtained from each plot was taken after drying and was expressed in Kg/ha

3 2 1 41 Straw yield Dry weight of the straw from each plot was recorded and expressed in Kg/ha

3 2 1 42 Pest and disease incidence Damage from stem borer was scored using the scale as

Code	Grade
0	No damage
1	1-10 per cent
3	11-20 per cent
5	21-30 per cent
7	31-60 per cent
9	60 per cent and above

3 2 2 Biochemical characterization

Gandhakasala and *Jeerakasala* were characterized based on the following quality parameters

3 2 2 1 Total carbohydrates

Total carbohydrates were estimated by Anthrone method suggested by Sadasivam and Manickam (1996). 50 mg of rice powder was hydrolyzed with 5 ml of 2.5 N HCl by keeping in boiling water bath. After three hours cooled to room temperature, made up to 100 ml and centrifuged. Then 0.5 ml of aliquot was pipetted into a test tube and made up to 1.0 ml by adding distilled water. Then 4 ml of anthrone reagent was added followed by heating in a boiling water bath. After eight minutes it was cooled rapidly and the intensity of green colour read against a blank in a spectrophotometer at 630 nm. Blank was set up with 1.0 ml of distilled water.

Stock solution of glucose was prepared by dissolving 100 mg of glucose in 100 ml of distilled water. Working standard of glucose was prepared by making up 10 ml stock to 100 ml with distilled water. Then 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard was pipetted into a series of test tubes and the developed colour was read as in the case of sample. The amount of total carbohydrates was calculated using a standard graph and expressed in per cent.

3 2 2 2 Protein content

Protein content was estimated by Lowry's method suggested by Sadasivam and Manickam (1996). For estimating protein, 500 mg of rice powder was homogenized in 10 ml buffer (pH 7.0) by means of pestle and mortar. The supernatant was collected after centrifugation. Then 0.1 ml and 0.2 ml of aliquot was pipetted into two different test tubes and made up to 1 ml by adding distilled water. A blank was set up with 1.0 ml distilled water. Then 5 ml of alkaline copper sulphate reagent was added to each tube and mixed well. After 10 minutes, 0.5 ml of Folin Ciocalteu reagent was added, mixed well, incubated at room temperature in the dark for 30 minutes. Blue colour developed was read at 660 nm in a spectrophotometer.

Stock solution of protein was prepared by dissolving 50 mg of bovine serum albumin in 50 ml of distilled water. Working standard of bovine serum albumin was prepared by making up 10 ml stock to 50 ml with distilled water. Then 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard was pipetted into a series of test tubes and the intensity of colour was developed and read as in the case of sample. The amount of protein was calculated using a standard graph and expressed in per cent. The genotypes were grouped using following classes:

Class	Protein content
Low	Up to 10 per cent
Medium	10 to 12 per cent
High	> 12 per cent

3 2 2 3 Amylose content

Amylose content was estimated by the method suggested by Sadasivam and Manickam (1996). For estimating amylose content, 100 mg of rice powder was taken in a test tube. One ml of ethanol followed by 10 ml of 1 N NaOH were added to the tube and heated for 10 minutes in a boiling water bath. Then the volume was made up to 100 ml in

a volumetric flask To 2.5 ml of the extract 20 ml of distilled water was added followed by three drops of phenolphthalein Then 0.1 N HCl was added to the solution drop by drop until pink colour just disappeared To this 0.1 ml of iodine reagent was added and made up to 50 ml in an amber coloured volumetric flask The colour was then read at 590 nm in a spectrophotometer 100 mg of amylose was dissolved in 10 ml of 1N NaOH and made up to 100 ml with water Then 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the standard amylose solution was pipetted into a series of test tubes and colour was developed and read as in the case of the sample One ml of iodine reagent was diluted to 50 ml with distilled water for a blank The amount of amylose present in the sample was calculated using the standard graph The genotypes were grouped using following classes

Class	Amylose content
Low	10 to 20 per cent
Intermediate	20 to 25 per cent
High	25 to 30 per cent

3.2.2.4 Aroma/Scent

The presence of aroma in rice was evaluated by a simple laboratory technique suggested by Cruz (2002) and accepted by IRRI (2002) One gram of brown rice was placed in a test tube and added 20 ml of distilled water Then test tubes were covered with aluminum foil and placed in a boiling water bath After 30 minutes based on the odour samples were scored as strongly aromatic, moderately aromatic, slightly aromatic and non aromatic Basmati 370 was used as check for comparison

3.2.3 Isozyme characterization

All the *Gandhakasala* and *Jeevakasala* genotypes along with WND 3 were characterized with respect to peroxidase (PRX) and esterase (EST) the commonly occurring plant enzymes using the following procedures

3 2 3 1 Gel electrophoresis

For the separation of multiple forms of enzymes and soluble proteins polyacrylamide gel electrophoresis (PAGE) was carried out using Hofler's small vertical slab gel electrophoresis unit. Acrylamide monomer ($\text{CH}_2=\text{CHCONH}_2$) was polymerized with bisacrylamide [$\text{CH}_2(\text{NHCONH})_2$ bis] to obtain the gel. Freshly prepared ammonium persulphate (APS) was used as chain initiator and N,N,N,N-tetramethylethylenediamine (TEMED) as catalyst. Polyacrylamide gel is preferred because of its chemical inertness, high resolution, ease in handling and preparation.

Preparation of the gel

Reagents

The separation of isozymes and soluble proteins were carried out in the anionic system. The following stock solutions were used:

Monomer stock solutions

Acrylamide	30.0 g
Bisacrylamide	0.8 g

Volume was made up to 100 ml with distilled water and stored in amber coloured bottles away from light.

4x Resolving gel buffer (1.5M Tris HCl, pH 8.8)

Tris base	18.5 g
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18.5 g of Tris base was dissolved in 70 ml distilled water, the pH adjusted to 8.8 with 1N HCl and the volume made up to 100 ml with distilled water.

4.1 Stacking gel buffer (0.5 M Tris HCl, pH 6.8)

Tris base	6.0 g
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Tris base was dissolved in 70 ml distilled water and the pH was adjusted to 6.8 with HCl and volume was made up to 100 ml

Ammonium per sulphate (APS)

Ammonium per sulphate (10%) solution was freshly prepared each time by dissolving 0.1 g APS in 1.0 ml distilled water

Working gel solution

Working solution was prepared by mixing the stock solutions in the proportions as given in Table 2. Gels having 10 per cent acrylamide concentration were used for both peroxidase and esterase isozyme separation

Stacking gel solution

To prepare 5 ml of stacking gel solution stock solutions were mixed in the following proportions

Monomer	0.67 ml
Stacking gel buffer	1.25 ml
Distilled water	3 ml
10% APS	25 μ l
TEMED	5 μ l

Table 2 Gel recipes for electrophoresis

Reagent	Acrylamide concentration (10%)	
	10m	20ml
Monomer (ml)	3.33	6.66
Resolving buffer(ml)	2.5	5
Distilled water (ml)	4.1	8.2
10% APS (μ l)	50	100
TEMED(μ l)	5	10

After preparing the working solution it was gently poured in between the glass plates kept in Hoefer dual gel casting unit. Polymerization was achieved within 20 to 30 minutes. For peroxidase and esterase isozyme staining gel to a width of 1 to 1.5 cm was also used for better resolution of bands.

Preparation of sample

Quiescent seeds and germinated seeds (7DAS) were used for the isozyme assay. Germinated seeds were pressed with blotting paper to remove water and used for extraction of enzymes.

For extraction of peroxidase 500 mg of the sample was taken and homogenized in a pre-cooled mortar along with 0.5 ml of 50 mM Tris HCl extraction buffer (pH 7.0) containing 50mM of Ascorbic acid, Sucrose, soluble Poly Vinyl Pyrrolidone (PVP) and traces of Cystane HCl. The samples were ground at 4°C by keeping the mortar and pestle in an ice tray. For extraction of esterase 500 mg of the sample was ground in 0.5 ml of Tris HCl buffer (pH 7.0).

The homogenized samples were centrifuged at 14,000 rpm for 10 minutes in a Kubota high speed centrifuge at 4°C. After centrifugation the supernatant was collected.

in eppendorf tubes labelled and used for running the gel. Fresh samples were used for the assay though enzyme extract can be stored at sub zero temperature for one day.

Electrode buffer (0.025M Tris, 0.192 M glycine, pH 8.3)

Tris base 1.5125 g

Glycine 7.2 g

Tris base and glycine were dissolved in 350 ml of distilled water. pH adjusted to 8.3 and the volume was made up to 500 ml with distilled water.

After polymerization the gel plates were clamped in a vertical position to the electrophoretic unit by means of bulldog clips. Then the upper and lower tanks of the unit connected to cathode and anode respectively were filled with electrode buffer. Then 15 μ l of enzyme was loaded in each well. Sample buffer (0.2 ml of bromophenol blue (0.05% solution), 1.0 ml of 0.5 M Tris HCl buffer (pH 6.8), 4 ml of distilled H₂O and 0.3 ml of glycerol) was mixed with enzyme solution and used as trace dye. Electrophoresis was carried out at 4°C. A constant current of 15 mA per plate was maintained throughout the run.

Staining solution for peroxidase (modified from Shaw and Koen, 1968)

100 ml of staining solution contained

0.2 M acetate buffer pH 5.6 100 ml

Benzidine 0.1 g

H₂O₂ (3%) 0.4 ml

Fresh staining solution was prepared each time. Acetate buffer and benzidine were mixed, boiled, cooled, filtered and then H₂O₂ was added to the mixture just before immersing the gel in staining solution. The gels were immersed in the staining solution for about 15 minutes in dark with continuous shaking. After the bands were fully developed the gels were transferred to distilled water. The gels were photographed on

the same day of run and the relative positions of various bands and the dye front were noted as zymogram on a graph paper using a scale

Staining solution for esterase (Sadasivam and Manickam 1996)

200 ml of staining solution contained

Sodium dihydrogen phosphate	2.8 g
Disodium dihydrophosphate	1.1 g
Fast blue RR salt	0.2 g
α Naphthyl acetate	0.03 g
Water	200 ml

After the run was over the gels were taken out and incubated in staining solution for 30 minutes at 37°C in dark. The gels were destained with 7 per cent acetic acid. Gels were photographed on the same day and the relative positions of various bands and the dye front were recorded as a zymogram on a graph paper by means of a scale.

Nomenclature of the isozymes

For the nomenclature of the isozymes the names described by Berg and Wijsman (1982) were followed. The enzymes were referred by the following abbreviations:

1 Peroxidase PRX

2 Esterase EST

The relative mobility (R_m) of each band was calculated as

$$R_m = \frac{\text{Distance of band from origin}}{\text{Total distance run}}$$

Based on relative mobility of each band the isozyme pattern was schematically drawn

Numbering of isozymes

For numbering all the isozymes of an enzyme in the sample studied were pooled. The slowest moving anodal band was numbered 1 (e.g. PRX 1) and faster ones were given the subsequent numbers. The R_m value of each band was indicated in bracket.

Measurement of similarity

The measurement of electrophoretic similarity among the *Gandhakasala* and *Jeerakasala* genotypes and with that of the check variety (WND 3) was calculated by making pair wise comparison of the genotypes using the method of Sockel and Sneath (1963) using the formula

Similarity index (SI)

Number of homologous bands

Number of homologous bands + Number of nonhomologous bands

Average of similarity indices for both the enzymes was computed and data compared.

3.2.2.6 Peroxidase activity

Peroxidase activity was estimated by the method suggested by Sadasivam and Manickam (1997). Germinated seeds (7DAS) were used for finding out peroxidase activity. Germinated seeds were pressed with blotting paper to remove water and used for extraction of enzymes. For extraction of peroxidase, 500 mg of sample was taken and homogenized in a pre-cooled pestle and mortar along with 0.1M phosphate buffer. The homogenized samples were centrifuged at 14,000 rpm for 15 min in a Kubota high speed centrifuge at 5°C. After centrifugation, the supernatant was collected and used as enzyme source within two hours. 3 ml of phosphate buffer, 0.05 ml of 20 mM guaiacol solution and 0.10 ml of enzyme extract was pipetted out in a cuvette. To this, 0.03 ml of H₂O₂ was added, mixed well and placed the cuvette in the spectrophotometer and waited

until the absorbance had reached to 0.05. The time required to increase the photometer reading from 0.05 to 0.1 was noted down by using stop watch. Peroxidase enzyme activity was calculated and expressed in units per ml.

3.2.4 Statistical analysis

The data collected with respect to the quantitative traits and nutritional quality parameters as mentioned above was tabulated and subjected to statistical analysis.

3.2.4.1 Analysis of variance

Analysis of variance was carried out using MSTATC package. Treatments were compared using Duncan's Multiple Range Test (DMRT).

3.2.4.2 Estimation of genetic parameters

Phenotypic and genotypic variances

These were estimated according to the method suggested by Lush (1940)

$$\text{Genotypic variance } (\sigma^2_g) = (Mg - Me)/r$$

$$\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + \sigma^2_e$$

Where

R = Number of replications

Mg = Mean sum of squares for genotypes

Me = Mean sum of squares for error

σ^2_e = Expected mean sum of squares for error

Phenotypic and genotypic coefficients of variation (PCV and GCV) were computed as

$$\text{PCV} = (\sigma_p / \text{Grand mean}) \times 100$$

$$\text{GCV} = (\sigma_g / \text{Grand mean}) \times 100$$

The estimates of PCV and GCV were classified as

Less than 25 per cent	~
25-50 per cent	moderate
>50 per cent	- high

3.2.4.3 Heritability

Heritability in broad sense (H^2) was estimated using the following formula suggested by Lush (1940) and expressed in per cent

$$H^2 = (\sigma^2_g / \sigma^2_p) \times 100$$

The range of heritability was categorized as

0-25 per cent	low
25-50 per cent	- moderate
>50 per cent	- high

3.2.4.4 Genetic advance (GA)

This was estimated using the formula

$$GA = K \times \sigma_P \times H^2$$

Where

$K = 2.06$ selection intensity at 5 per cent

σ_P - phenotypic standard deviation

3.2.4.5 Genetic gain

$$\text{Genetic gain} = (GA / \text{Grand mean}) \times 100$$

Genetic gain was classified according to Johnson *et al* (1955) as given below

1-10 per cent	low
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11-20 per cent	moderate
21 per cent and above	high

3.2.4.6 Phenotypic and genotypic correlation coefficients

Phenotypic and genotypic correlation coefficients between yield and each of the biometrical and nutritional quality parameters as listed earlier and the mutual correlations among themselves were worked out using SPAR 1 package.

3.2.4.7 Path analysis

Path analysis was used to reveal the cause-effect relationship in the system of correlated variables under study. Path analysis for grain yield at the genotypic level was carried out using the 13 quantitative characters.

Thirteen quantitative characters namely seedling height, leaf length, leaf width, culm length, culm number, culm diameter, panicle length, number of grains per panicle, grain length, grain breadth, milling recovery, maturity days and straw yield were selected for path analysis with respect to yield.

3.2.5 GPS (Geographical Positioning System)

GPS is a satellite-based radio navigation system developed and operated by the US Department of Defense (DOD). GPS permits land, sea and airborne users to determine their three-dimensional position, velocity and time with precision and accuracy (DOD, 2010). GPS readings were recorded from five locations (Thirunelly, Mananthavady, Nenmeni, Ambalavayal and Panamaram) by using Garmin Etrex hand-held device.

Results

4 RESULTS

4.1 Sample collection

Gandhakasala is cultivated in an area of 327 ha in Wayanad district and *Jeerakasala* in 22 ha during 2008. Details of area under cultivation for *Gandhakasala* rice is given in Table 3. It is mostly cultivated in Panamaram (78 ha), Thirunelly (60 ha), Noolpuzha (38 ha), Mananthavady (23 ha) and Kanayampetta (20 ha) panchayats. *Jeerakasala* is cultivated in small patches in different panchayats.

From the different localities 65 samples of *Gandhakasala* and 10 samples of *Jeerakasala* were collected. The samples showed high level of variability with respect to grain shape, grain colour and apiculus colour. Samples with uniform small grains and straw coloured apiculus were selected as *Gandhakasala* types for the present study and samples with slightly elongated grains and partial awns were selected as *Jeerakasala* types. And as such 10 *Gandhakasala* samples and two *Jeerakasala* samples were selected for further study.

4.2 Morphological characterization

Ten *Gandhakasala* genotypes and two *Jeerakasala* genotypes along with Deepthi as check variety were morphologically characterized and the data are presented as qualitative characters and quantitative characters.

4.2.1 Qualitative characters

The qualitative characters of 13 genotypes are presented in Table 4. These genotypes revealed low variability with respect to most of the qualitative characters. Least variation was observed among the genotypes with respect to characters like leaf blade pubescence, basal leaf sheath colour, ligule colour, ligule shape, collar colour, auricle colour, culm internode colour, septum colour, panicle type, panicle secondary branching, shattering, threshability, apiculus colour, stigma colour, sterile lemma colour and spikelet sterility.

Table 3 Details of area under cultivation for *Gandhakasala* rice in Wayanad during 2008

Name of panchayat	Area (ha)
Thavinhal	5
Thirunelli	60
Panamaram	78
Mananthavady	23
Thondernadu	5
Edavaka	26
Vellamunda	12
Padinharathara	3
Meppady	3
Kottahara	3
Kaniyambetta	20
Muttill	5
Sulthan Bathery	6
Noolpuzha	38
Poothadi	12
Meenangady	5
Ambalavayal	3
Mullenkollu	3
Pulpally	15
Nenmeni	2
Total	327

Table 4 Qualitative characters (standard scores) of aromatic rice genotypes

SI No	Character	Genotypes												
		GT1	GT2	GT3	GT4	GT5	GT6	GT7	GT8	GT9	GT10	JT11	JT12	DT13
1	Leaf blade pubescence	2	2	2	2	2	2	2	2	2	2	2	2	2
2	Leaf blade colour	2	2	2	2	2	2	2	2	2	2	3	3	3
3	Basal leaf sheath colour	1	1	1	1	1	1	1	1	1	1	1	1	1
4	Ligule colour	1	1	1	1	1	1	1	1	1	1	1	1	1
5	Ligule shape	2	2	2	2	2	2	2	2	2	2	2	2	2
6	Collar colour	1	1	1	1	1	1	1	1	1	1	1	1	1
7	Auricle colour	1	1	1	1	1	1	1	1	1	1	1	1	1
8	Culm internode colour	1	1	1	1	1	1	1	1	1	1	1	1	1
9	Septum colour	1	1	1	1	1	1	1	1	1	1	1	1	1
10	Panicle type	5	5	5	5	5	5	5	5	5	5	5	5	5
11	Panicle secondary branching	2	2	2	2	2	2	2	2	2	2	2	2	2
12	Panicle exertion	1	1	1	1	1	1	1	1	1	1	1	1	3

GT1 to GT10 *Gandhakasala* genotypes
 JT11 & JT12 *Jeerakasala* genotypes
 DT13 Deepthi (check var ety)

SI No	Character	Genotypes													
		GT1	GT2	GT3	GT4	GT5	GT6	GT7	GT8	GT9	GT10	JT11	JT12	DT13	
13	Shattering	3	3	3	3	3	3	3	3	3	3	3	3	3	
14	Threshability	1	1	1	1	1	1	1	1	1	1	1	1	1	
15	Spikelet awning	0	0	0	0	0	0	0	0	0	0	0	1	1	0
16	Awn colour	0	0	0	0	0	0	0	0	0	0	0	2	2	0
17	Apiculus colour	2	2	2	2	2	2	2	2	2	2	2	2	2	2
18	Stigma colour	3	3	3	3	3	3	3	3	3	3	3	3	3	3
19	Lemma and palea colour	1	1	1	1	1	1	1	1	1	1	1	1	1	0
20	Lemma and palea pubescence	3	3	3	3	3	3	3	3	3	3	3	3	3	4
21	Sterile lemma colour	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	Spikelet sterility	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	Seed coat colour	1	1	1	1	1	1	1	1	1	1	1	1	1	5
24	Stem borer damage	1	1	1	1	1	1	1	1	1	1	1	1	1	1

GT1 to GT10
JT11 & JT12
DT13

Gandhakasala genotypes
Jeerakasala genotypes
Deepthi (check variety)

In general *Gandhakasala* *Jeerakasala* and Deepthi have intermediate leaf blade pubescence green basal leaf sheath colour white and 2 cleft ligules light green colour for collar auricle and septum green colour for internode intermediate panicle heavy secondary branching low shattering difficult threshability of panicle straw colour for apiculus and sterile lemma yellow stigmas and highly fertile spikelets. Leaves of *Gandhakasala* genotypes exhibited green colour whereas *Jeerakasala* and Deepthi exhibited dark green colour. The crop exhibited low incidence of stem borer damage (1.10%) and was free from other pests and diseases.

With respect to panicle exertion Deepthi had moderately well exerted panicles while the rest had well exerted panicles. The *Jeerakasala* genotypes showed straw coloured short and partly awned grains whereas *Gandhakasala* and Deepthi were lacking awns. Straw coloured lemma and palea was observed for the genotype Deepthi and golden colour for the aromatic genotypes. Regarding seed coat colour Deepthi was observed to have red colour while the rest showed white coloured seed coat. Grains of *Gandhakasala* and *Jeerakasala* showed hairs on upper portion.

4.2.2 Quantitative characters

The quantitative characters of 13 genotypes were analyzed for significant difference using Duncan's Multiple Range Test and results are presented in Table 5.

4.2.2.1 Seedling height

The seedling height of genotypes at fifth leaf stage was found to vary from 28.93 cm to 40.79 cm. Among these JT12 was the shortest (28.93 cm) followed by GT2 (29.33 cm), GT9 (29.83 cm) and GT1 (30.16 cm). The check variety Deepthi recorded the highest seedling height (40.79 cm) followed by GT3 (38.60 cm) and JT11 (37.83 cm). The mean seedling height of Deepthi was more (40.79 cm) than *Gandhakasala* (33.37 cm) and *Jeerakasala* (33.38 cm).

Table 5 Quantitative characters of aromatic rice genotypes

Sl No	Genotype	Seedling height (cm)	Leaf length (cm)	Leaf width (cm)	Ligule length (mm)	Culm length (cm)	Culm number	Culm diameter (mm)	Days to 50 per cent heading	Panicle length (cm)
1	GT1	30 16 ^D	58 83 ^A	1 23 ^{AB}	2 38 ^{ABC}	120 61 ^A	6 97 ^E	6 58 ^{ABCD}	122 33 ^B	27 97 ^{AB}
2	GT2	29 33 ^D	53 71 ^{AB}	1 19 ^{AB}	2 26 ^{ABC}	106 58 ^B	7 27 ^{DE}	6 11 ^{ABCD}	122 00 ^B	26 07 ^{BC}
3	GT3	38 60 ^A	54 01 ^{AB}	1 07 ^B	2 05 ^C	105 19 ^{BC}	7 43 ^{CDE}	6 44 ^{ABCD}	122 00 ^B	27 63 ^{AB}
4	GT4	33 19 ^C	59 17 ^A	1 12 ^{AB}	1 95 ^C	117 80 ^A	7 53 ^{CDE}	5 97 ^{BCD}	122 67 ^B	28 09 ^{AB}
5	GT5	33 82 ^C	54 89 ^{AB}	1 14 ^{AB}	1 95 ^C	104 38 ^{BC}	7 83 ^{BCDE}	6 32 ^{ABCD}	122 00 ^B	26 85 ^{BC}
6	GT6	34 91 ^{BC}	57 81 ^A	1 16 ^{AB}	2 15 ^{BC}	108 21 ^B	8 80 ^{ABCD}	5 79 ^{CD}	120 67 ^B	26 84 ^{BC}
7	GT7	35 34 ^{BC}	56 33 ^A	1 30 ^A	2 16 ^{BC}	119 79 ^A	7 23 ^{DE}	6 40 ^{ABCD}	121 67 ^B	30 14 ^A
8	GT8	34 42 ^C	54 65 ^{AB}	1 30 ^A	2 36 ^{ABC}	105 85 ^B	8 33 ^{ABCDE}	5 63 ^D	122 00 ^B	27 96 ^{AB}
9	GT9	29 83 ^D	59 13 ^A	1 25 ^{AB}	2 37 ^{ABC}	122 52 ^A	9 03 ^{ABC}	7 18 ^A	121 33 ^B	28 65 ^{AB}
10	GT10	34 09 ^C	59 26 ^A	1 21 ^{AB}	2 37 ^{ABC}	119 30 ^A	8 47 ^{ABCDE}	7 09 ^A	120 67 ^B	28 15 ^{AB}
Mean *		33 37	56 88	1 20	2 20	113 02	7 89	6 35	121 73	27 84
11	JT11	37 83 ^{AB}	49 28 ^{BC}	1 07 ^B	2 64 ^{AB}	95 85 ^{CD}	9 70 ^A	7 10 ^A	125 33 ^A	26 12 ^{BC}
12	JT12	28 93 ^D	54 74 ^{AB}	1 16 ^{AB}	2 73 ^A	107 97 ^B	9 47 ^{AB}	6 74 ^{ABC}	125 67 ^A	29 96 ^A
Mean **		33 38	52 01	1 12	2 68	101 91	9 58	6 92	125 50	28 04
13	DT13	40 79 ^A	45 69 ^C	1 23 ^{AB}	1 48 ^D	90 76 ^D	7 63 ^{CDE}	6 99 ^{AB}	116 00 ^C	24 47 ^C

* Mean for *Gandhakasala* genotypes

** Mean for *Jeerakasala* genotypes

GT1 to GT10

JT11 & JT12

D T13

Gandhakasala genotypes

Jeerakasala genotypes

Deepthi (check variety)

Sl No	Genotype	Number of grains per panicle	Length of sterile glumes (mm)	1000 grain weight (gm)	Grain length (mm)	Grain breadth (mm)	Milling recovery (%)	Maturity days	Straw Yield (kg)	Grain yield (kg)
1	GT1	119 78 ^{ABC}	2 35 ^B	12 27 ^E	5 93 ^F	2 53 ^{DE}	67 33 ^C	167 33 ^B	4000 00 ^{ABC}	2640 00 ^A
2	GT2	106 42 ^{DEF}	2 05 ^{BC}	13 41 ^{DE}	6 29 ^{DE}	2 53 ^{DE}	66 50 ^C	167 0 ^B	4373 33 ^{AB}	2696 00 ^A
3	GT3	126 35 ^A	1 87 ^{CD}	14 98 ^{CD}	6 51 ^{CD}	2 66 ^C	65 75 ^{CD}	167 0 ^B	4426 67 ^{AB}	2607 33 ^A
4	GT4	116 11 ^{ABCD}	1 79 ^{CD}	13 02 ^{DE}	6 10 ^{EF}	2 60 ^{CD}	71 33 ^B	167 67 ^B	4373 33 ^{AB}	2585 33 ^A
5	GT5	116 24 ^{ABCD}	2 21 ^{BC}	13 66 ^{DE}	6 47 ^{CD}	2 60 ^{CD}	64 58 ^D	167 00 ^B	3466 67 ^{BC}	2416 00 ^{AB}
6	GT6	114 18 ^{ABCDE}	2 35 ^B	15 90 ^C	6 65 ^C	2 67 ^C	66 00 ^{CD}	165 67 ^B	4746 67 ^A	2663 33 ^A
7	GT7	112 96 ^{BCDE}	2 11 ^{BC}	14 21 ^{CDE}	6 52 ^{CD}	2 66 ^C	66 17 ^{CD}	166 67 ^B	3626 67 ^{ABC}	2480 00 ^{AB}
8	GT8	102 19 ^{EF}	2 17 ^{BC}	13 70 ^{DE}	6 41 ^{CD}	2 82 ^B	60 33 ^E	167 00 ^B	4426 67 ^{AB}	2624 00 ^A
9	GT9	112 95 ^{BCDF}	1 79 ^{CD}	13 09 ^{DE}	6 12 ^{EF}	2 50 ^E	64 67 ^D	166 33 ^B	2906 67 ^C	2080 00 ^B
10	GT10	108 06 ^{CDEF}	2 01 ^{BCD}	13 33 ^{DE}	6 45 ^{CD}	2 59 ^{CD}	71 17 ^B	165 67 ^B	3360 00 ^{BC}	2456 00 ^{AB}
Mean *		113 52	2 07	13 78	6 34	2 62	66 38	166 73	3970 67	2524 80
11	JT11	123 51 ^{AB}	2 93 ^A	19 61 ^B	8 63 ^A	2 62 ^C	70 33 ^B	170 33 ^A	4106 67 ^{AB}	2504 00 ^{AB}
12	JT12	99 50 ^F	2 96 ^A	20 04 ^B	7 94 ^B	2 49 ^E	71 17 ^B	170 67 ^A	4106 67 ^{AB}	2472 33 ^{AB}
Mean **		111 51	2 96	19 82	8 28	2 56	70 75	170 50	4106 67	2488 16
13	DT13	116 57 ^{ABCD}	1 60 ^D	26 17 ^A	7 87 ^B	3 39 ^A	75 33 ^A	161 00 ^C	3973 33 ^{ABC}	2822 33 ^A

* Mean for *Gandhakasala* genotypes

** Mean for *Jeerakasala* genotypes

GT1 to GT10

JT11 & JT12

DT13

Gandhakasala genotypes

Jeerakasala genotypes

Deepthi (check var ety)

4 2 2 2 Leaf length

Among *Gandhakasala* types leaf length ranged between 53.71 cm to 59.26 cm. The shortest leaves were observed for DT13 (45.69 cm) followed by JT11 (49.28 cm). The check variety Deepthi was having shortest leaves of 45.60 cm followed by *Jeerakasala* types (52.01 cm) and *Gandhakasala* types (56.88 cm).

4 2 2 3 Leaf width

The average leaf width of *Gandhakasala* types was 1.20 cm whereas it was 1.12 cm in *Jeerakasala* and 1.23 cm in Deepthi.

4 2 2 4 Ligule length

The ligule length ranged between 1.48 cm (DT13) and 2.73 cm (JT12). The ligules were small (1.48 cm) in Deepthi when compared to aromatic types.

4 2 2 5 Culm length

The mean culm length was more for *Gandhakasala* types (115.02 cm) and *Jeerakasala* types (101.91 cm) whereas it was only 90.76 cm in Deepthi. The genotypes GT1, GT4, GT7, GT9 and GT10 exhibited significantly high mean value for culm length.

4 2 2 6 Culm number

Culm number ranged between 6.97 (GT1) and 9.70 (JT11). The mean culm number for *Gandhakasala* types was 7.89 and that for Deepthi was 7.63 whereas *Jeerakasala* types exhibited a higher culm number of 9.58.

4 2 2 7 Culm diameter

Culm diameter ranged between 5.63 mm (GT8) and 7.18 mm in GT9

4 2 2 8 Days to 50 per cent heading

The check variety Deepthi was the earliest for days to 50 per cent heading (116.00 days). *Gandhakasala* types took 121.73 days to 50 per cent heading whereas *Jeerakasala* types took 125.50 days.

4 2 2 9 Panicle length

Panicle length ranged between 24.47 cm in Deepthi (Plates 3, 4 and 5) to 30.14 cm in GT7 (Plates 6, 7 and 8). In general the panicles of *Gandhakasala* ranged between 26.07 to 30.14 cm and panicles of *Jeerakasala* ranged from 26.12 in JT11 to 29.96 cm in JT12 (Plates 9, 10 and 11). Deepthi had lesser panicle length of 24.47 cm compared to aromatic types.

4 2 2 10 Number of grains per panicle

The number of grains per panicle ranged from 99.50 to 126.35. *Gandhakasala* types recorded 113.52 grains per panicle whereas *Jeerakasala* types recorded 111.51 grains and Deepthi 16.57 grains.

4 2 2 11 Length of sterile glumes

The check variety showed shortest sterile glumes of 1.60 mm whereas it was 2.07 mm in *Gandhakasala* and 2.96 mm in *Jeerakasala* types. *Jeerakasala* genotypes had significantly high mean value for length of sterile glumes.



Plate 1. Field layout of aromatic rice genotypes



Plate 2. Field view of the *Gandhakasala* genotype (GT8)



Plate 3. Panicles of *Deepthi* (WND.3)



Plate 4. Panicles of *Deepthi* (WND.3)
(enlarged view)



Plate 5. Single panicle of *Deepthi* (WND.3)



Plate 6. Panicles of *Gandhakasala* (GT7)



Plate 7. Panicles of *Gandhakasala* (GT7)
(enlarged view)



Plate 8. Single panicle of *Gandhakasala* (GT7)



Plate 9. Panicles of *Jeerakasala* (JT12)



Plate 10. Panicles of *Jeerakasala* (JT12)
(enlarged view)



Plate 11. Single panicle of *Jeerakasala* (JT12)

4.2.2.12 1000 grain weight

The average 1000 grain weight of genotypes ranged from 12.27 g to 26.17 g. Highest 1000 grain weight was recorded for Deepthi (26.17 g) followed by JT12 (20.04 g) and JT11 (19.61g). In general 1000 grain weight ranged between 12.27 to 15.90 g with a mean value of 13.78 g for *Gandhakasala* types and 19.61 to 20.04 g with a mean value of 19.82 g for *Jeerakasala* types. Deepthi recorded more 1000 grain weight (26.17 g) than aromatic types.

4.2.2.13 Grain length

JT11 had possessed the longest grains (8.63 mm) followed by JT12 (7.94 mm) and Deepthi (7.87 mm). In *Gandhakasala* the grain length (rough rice) ranged between 5.93 to 6.65 mm, with a mean value of 6.34 mm whereas in *Jeerakasala* grain length ranged between 7.94 to 8.63 mm with a mean value of 8.28 mm. All *Gandhakasala* types showed lesser grain length (Plates 12 and 13) than *Jeerakasala* types (Plates 14 and 15) and Deepthi (Plates 16 and 17).

4.2.2.14 Grain breadth

Grain breadth was highest for Deepthi (3.40 mm), followed by GT8 (2.82 mm). Mean grain breadth for *Gandhakasala* types were 2.62 mm whereas it was 2.56 mm in *Jeerakasala* and 3.40 mm in the non aromatic check variety (Deepthi).

4.2.2.15 Milling recovery

Milling recovery ranged from 60.33 per cent (GT8) to 75.33 per cent (DT13). Non aromatic variety recorded the highest milling recovery (75.33%) compared to aromatic types. The genotype GT8 showed lowest value for milling recovery.



Plate 12. Grains (paddy) of *Gandhakasala*(GT7) Plate 13. Grains (milled) of *Gandhakasala* (GT7)



Plate 14. Grains (paddy) of *Jeerakasala* (JT12) Plate 15. Grains (milled) of *Jeerakasala* (JT12)



Plate 16. Grains (paddy) of *Deepthi* (WND.3) Plate 17. Grains (dehulled) of *Deepthi* (WND.3)

4.2.2.16 Days to maturity

Days to maturity ranged from 161.00 days (Deepthi) to 170.67 days (JT12). Aromatic types took more number of days to maturity (166.73 days and 170.50 days for *Gandhakasala* and *Jeerakasala* types respectively) compared to Deepthi (161.00).

4.2.2.17 Straw yield

Straw yield ranged between 2906.67 (GT9) and 4746.67 kg ha⁻¹ (GT6).

4.1.2.18 Grain yield

Grain yield ranged between 2080.00 kg ha⁻¹ (GT9) to 2822.33 kg ha⁻¹ (Deepthi). *Gandhakasala* genotypes showed a mean grain yield of 2524.80 kg ha⁻¹ whereas *Jeerakasala* genotypes showed 2488.16 kg ha⁻¹ and Deepthi, 2822.35 kg ha⁻¹.

4.3 Nutritional characterization

The mean performance of 13 genotypes of aromatic rice genotypes are presented in Table 6.

4.3.1 Total carbohydrates

The genotype JT12 had the lowest total carbohydrates content of 58.40 per cent. The genotypes GT2 and GT1 expressed the highest total carbohydrates content of 81.87 per cent and 76.27 per cent respectively. Mean total carbohydrates content ranged for *Gandhakasala* genotypes was 69.56 per cent and for *Jeerakasala* it was 61.06 per cent. Deepthi exhibited 71.73 per cent of total carbohydrates content.

Table 6. Nutritional and biochemical characters of aromatic rice genotypes

S. No.	Genotypes	Nutritional characters				Biochemical character
		Total carbohydrates (%)	Protein content (%)	Amylose content (%)	Aroma	Peroxidase activity units/ml
1	GT1	76.27 ^B	8.29 ^{CDE}	21.87 ^B	M	0.50 ^A
2	GT2	81.87 ^A	6.88 ^E	23.07 ^A	M	0.31 ^{AB}
3	GT3	71.20 ^{CD}	10.46 ^A	18.27 ^J	M	0.31 ^{AB}
4	GT4	66.67 ^D	7.99 ^{CDE}	17.87 ^L	M	0.60 ^A
5	GT5	72.00 ^C	9.41 ^{ABC}	19.87 ^F	M	0.38 ^A
6	GT6	70.67 ^{CDE}	9.29 ^{ABC}	18.00 ^K	M	0.27 ^{AB}
7	GT7	66.67 ^D	10.20 ^{AB}	18.87 ^I	M	0.49 ^A
8	GT8	69.20 ^{DE}	7.33 ^{DE}	20.40 ^E	M	0.51 ^A
9	GT9	61.20 ^{DEF}	10.32 ^{AB}	18.27 ^J	S	0.28 ^{AB}
10	GT10	59.87 ^{DEF}	8.72 ^{ABCD}	20.47 ^D	M	0.36 ^A
Mean *		69.56	8.16	19.70		0.40
11	JT11	63.73 ^{DE}	8.65 ^{BCD}	19.67 ^G	M	0.27 ^{AB}
12	JT12	58.40 ^G	8.01 ^{CDE}	20.73 ^C	S	0.40 ^A
Mean**		61.06	8.33	20.20		0.34
13	DT13	71.73 ^C	9.73 ^{ABC}	19.40 ^H	NS	0.57 ^A

* Mean for *Gandhakasala* genotypes** Mean for *Jeerakasala* genotypesGT1 to GT10 – *Gandhakasala* genotypesJ11 & J12 – *Jeerakasala* genotypes

DT13 – Deepthi (Check variety)

M – Moderately aromatic

S – Slightly aromatic

NS – Non aromatic

4.3.2 Protein content

Protein content of *Gandhakasala* genotypes ranged between 6.88 to 10.46 per cent with a mean value of 8.16 per cent. In *Jeerakasala* protein content ranged between 8.01 per cent and 8.65 per cent with a mean value of 8.33. Deepthi expressed a protein content of 9.73 per cent.

Based on protein content in grain, selected genotypes were divided into three groups viz., low (up to 10%), medium (10–12%) and high (>12%) protein groups. The *Gandhakasala* genotypes GT3, GT7 and GT9 were found to have intermediate protein content while the rest had low protein content.

4.3.3 Amylose content

Like other Indian rice genotypes *Gandhakasala*, *Jeerakasala* and Deepthi comes under the group non waxy rice. Amylose content was ranging from 17.87 per cent (GT4) to 23.07 per cent (GT2). Deepthi recorded an amylose content of 19.40 per cent. Based on amylose content in grain genotypes were divided into low (10 to 20%), intermediate (20 to 25%) and high (25 to 30%) amylose groups. Accordingly, intermediate amylose content was noticed for the genotypes GT1, GT2, GT8, GT10 and JT12 while rest of the genotypes had low amylose content.

4.3.4 Aroma/Scent

Check variety (Deepthi) did not express aroma. Based on aroma, aromatic rice genotypes were divided into two groups viz., slightly aromatic and moderately aromatic. GT1, GT2, GT3, GT4, GT5, GT6, GT7, GT8, GT10 and JT11 were found to be moderately aromatic, while genotypes GT9 and JT12 were slightly aromatic. Basmati 370 used as check for comparison expressed slight aroma.

4.4 Isozyme characterization

4.4.1 Peroxidase

4.4.1.1 Quiescent seed

No peroxidase band was observed in quiescent seed sample.

4.4.1.2 Germinated seed

Six bands were resolved for germinated seed samples (Fig.1. and Plate 18). The bands PRX -1 (0.050), PRX-2 (0.157) and PRX-3 (0.343) appeared with more intensity in most of the *Gandhakasala* genotypes, while it was less intense in *Jeerakasala* genotypes and Deepthi. The band PRX-4 (0.464) was common for all the genotypes and hence no value in identification of genotypes. The genotype JT11 expressed lighter intensity for PRX-5 (0.480) while rest of the genotypes expressed thick bands. The band PRX-6 (0.514) appeared with less intensity in most of the genotypes and it was absent in GT3 and GT8 genotypes.

Based on similarity index (SI) values, GT3 was having 100 per cent similarity with GT8 and 83.3 per cent with all other genotypes. The genotype GT8 was having 83.3 per cent similarity with all genotypes. All other genotypes showed 100 per cent SI with each other. Based on SI with check variety genotypes GT1, GT2, GT4, GT5, GT6, GT7, GT9, GT10, JT11 and JT12 were grouped into one group whereas GT3 and GT8 formed another group (Table 7).

4.4.2 Esterase

4.4.2.1 Quiescent seed

No esterase band was observed in quiescent seed sample.

Table 7. Similarity indices among aromatic rice genotypes based on peroxidase isozyme pattern in germinated seeds

Genotypes	GT1	GT2	GT3	GT4	GT5	GT6	GT7	GT8	GT9	GT10	JT11	JT12	DT13
GT1	1												
GT2	1	1											
GT3	0.833	0.833	1										
GT4	1	1	0.833	1									
GT5	1	1	0.833	1	1								
GT6	1	1	0.833	1	1	1							
GT7	1	1	0.833	1	1	1	1						
GT8	0.833	0.833	1	0.833	0.833	0.833	0.833	1					
GT9	1	1	0.833	1	1	1	1	0.833	1				
GT10	1	1	0.833	1	1	1	1	0.833	1	1			
JT11	1	1	0.833	1	1	1	1	0.833	1	1	1		
JT12	1	1	0.833	1	1	1	1	0.833	1	1	1	1	
DT13	1	1	0.833	1	1	1	1	0.833	1	1	1	1	1

GT1 to GT10 -- *Gandhakasala* genotypes

JT11 & JT12 -- *Jeerakasala* genotypes

DT13 -- Deepthi (check variety)

4.4.2.2 Germinated seed

Four esterase bands (EST-1 to EST-4) were observed in germinated seed (Fig. 2. and Plate 19). The bands EST -1 (0.026) was common for all genotypes. The bands EST -3 (0.276) and EST-4 (0.449) were less intense in all aromatic genotypes than in Deepthi. It is to be specially mentioned that the band EST-2 (0.103) was present only in Deepthi.

Based on SI all aromatic genotypes were formed into one group whereas the check variety (Deepthi) formed another group (Table 8).

4.4.3 Peroxidase activity

Peroxidase activity in all the genotypes was on par and it ranged from 0.27 units/ml for GT6 and JT11 to 0.60 units/ml for GT4. Peroxidase activity of 0.57 units/ml was recorded for the check variety (Table 6). Peroxidase activity of aromatic rice genotypes is shown in Fig. 3.

4.5 Estimation of genetic parameters

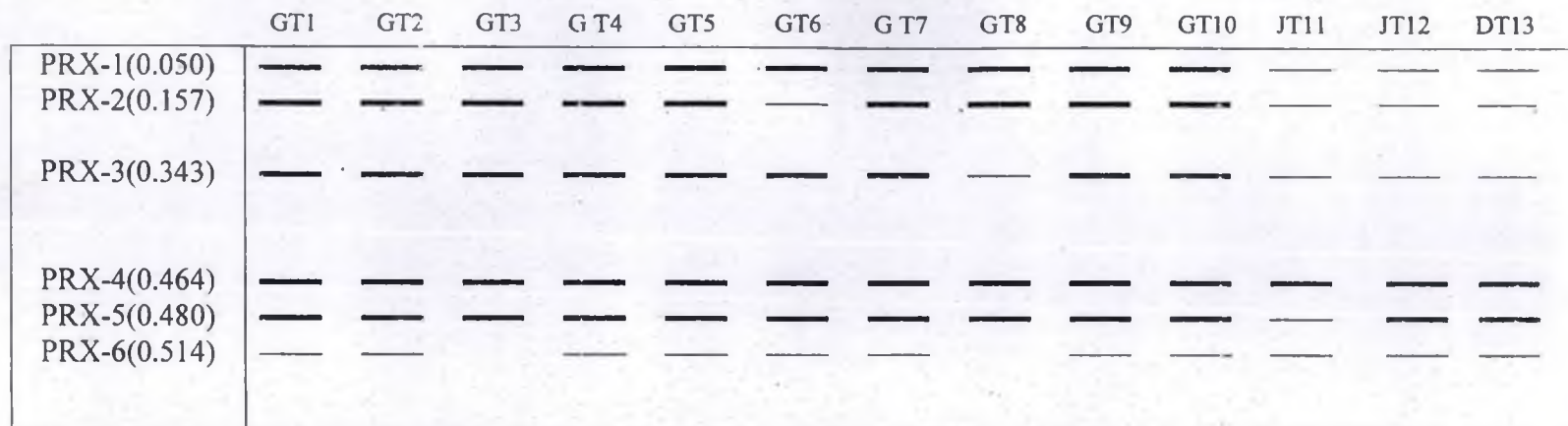
4.5.1 Genetic variability

The extent of genetic variability with respect to 18 quantitative characters, in a set of 13 genotypes, was estimated in the present study.

The abstract of analysis of variance of these characters is given in Table 9. The data on range, mean and estimates of genetic parameters for the above characters are presented in Table 10.

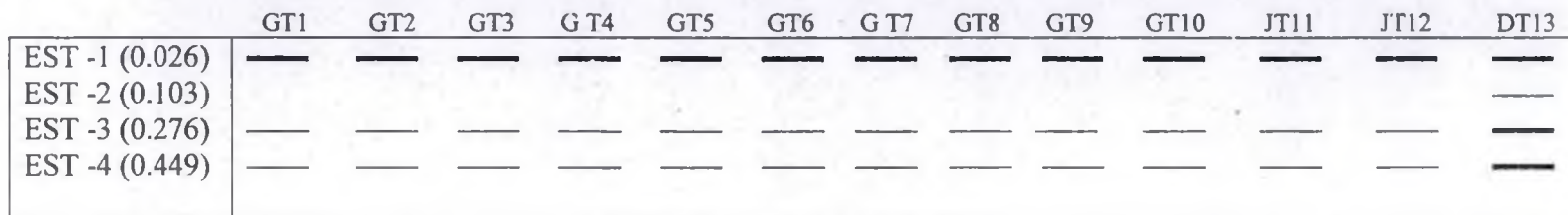
Results from the analysis of variance, revealed significant difference among 13 genotypes for all quantitative and biochemical characters except for leaf width.

Fig. 1. Zymogram of peroxidase in seedlings (seven days after sowing) of selected aromatic rice genotypes

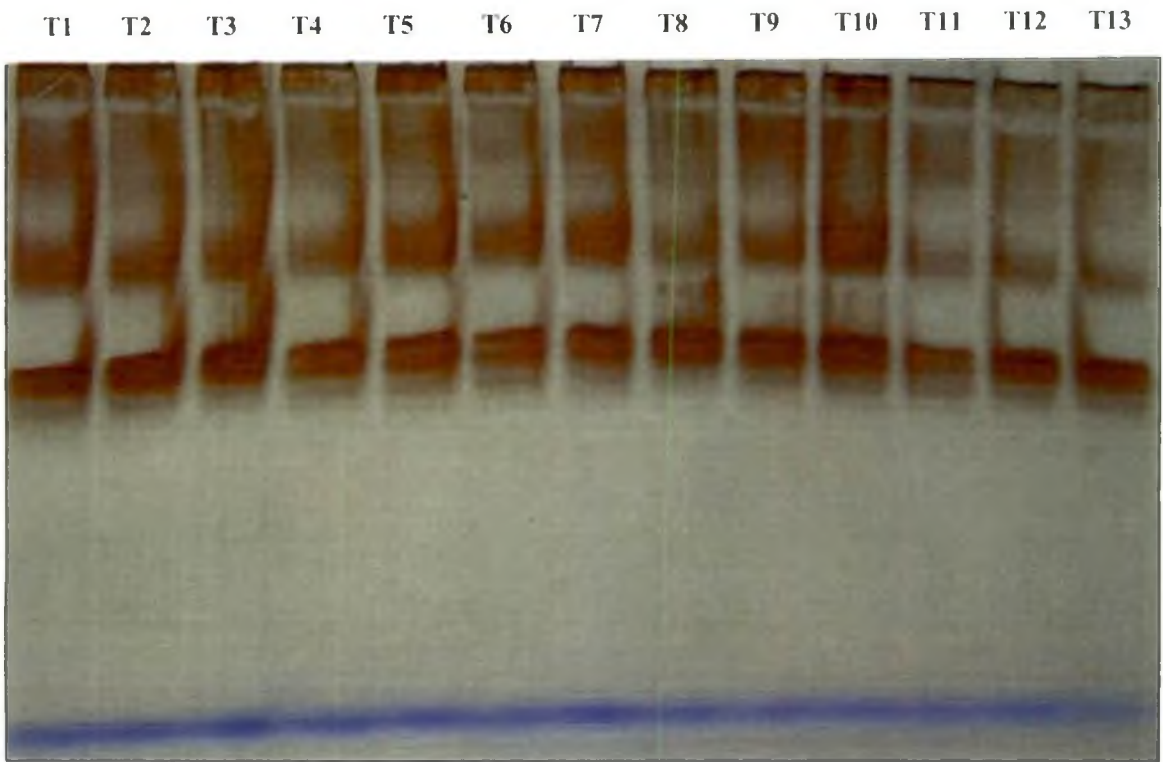


GT1 to GT10 - *Gandhakasala* genotypes, JT11 and JT12 - *Jeerakasala* genotypes, DT13 - Deepthi (check variety)
 — Thick band , — Light band

Fig. 2. Zymogram of esterase in seedlings (seven days after sowing) of selected aromatic rice genotypes

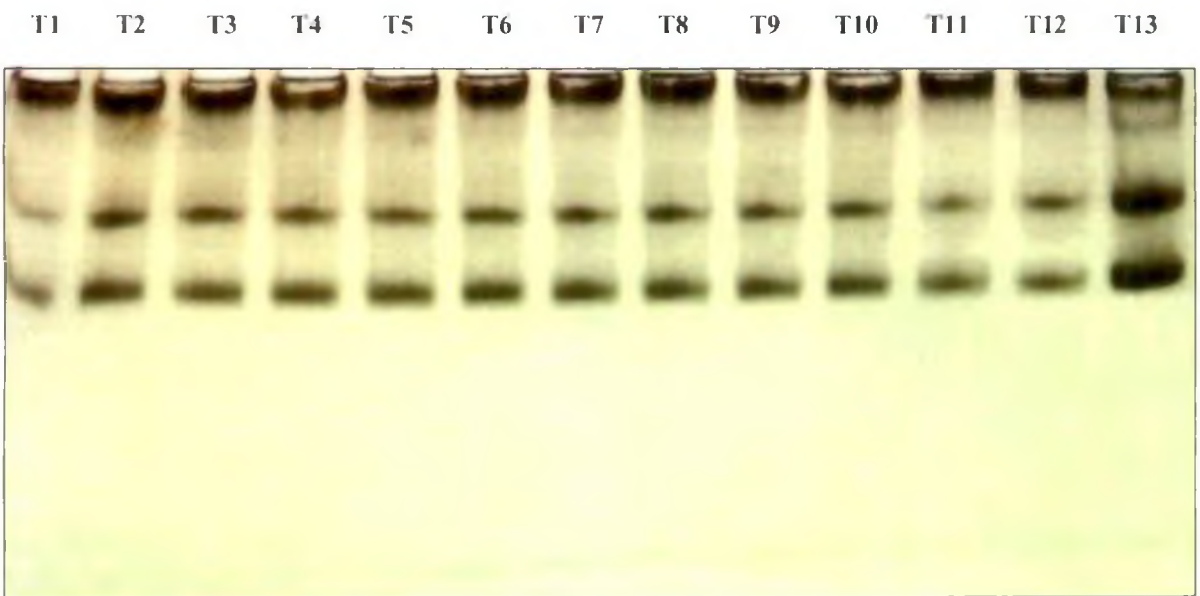


GT1 to GT10 - *Gandhakasala* genotypes, JT11 and JT12 - *Jeerakasala* genotypes, DT13 - Deepthi (check variety)
 — Thick band , — Light band



T1 to T10 -- *Gandhakasala* genotypes, T11 and T12-- *Jeerakasala* genotypes, T13-- Deepthi (check variety)

Plate 18. Peroxidase banding pattern in germinated seeds (seven days after sowing) of aromatic rice genotypes



T1 to T10 -- *Gandhakasala* genotypes, T11 and T12 -- *Jeerakasala* genotypes, T13-- Deepthi (check variety)

Plate 19: Esterase banding pattern in germinated seeds (seven days after sowing) of aromatic rice genotypes

Fig. 3. Peroxidase activity of aromatic rice genotypes

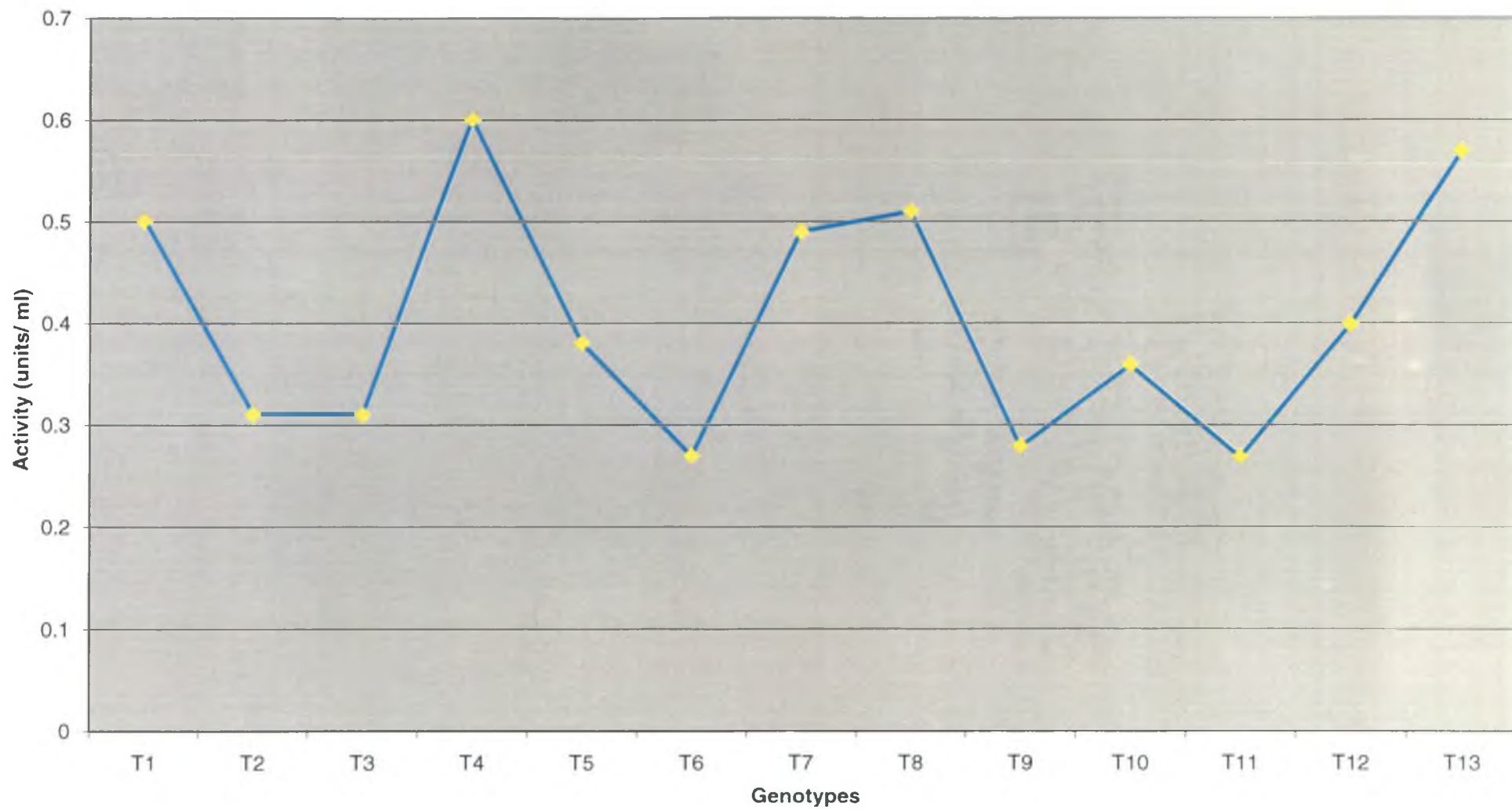


Table 9 Analysis of variance for grain yield and associated quantitative nutritional and biochemical characters of aromatic rice genotypes

Mean sum of squares											
Source of variation	Seedling height	Leaf length	Leaf width	Ligule length	Culm length	Culm number	Culm diameter	Days to 50% heading	Panicle length	Number of grains per panicle	Length of sterile glumes
Replications	2 756	25 125	0 015	0 005	97 640	0 751	2 117	0 188	4 266	1 36	0 126
Treatments	41 214**	49 012**	0 017	0 315**	292 996**	2 438**	0 804*	16 250**	7 361**	183 31**	0 509**
Error	3 133	10 542	0 008	0 0714	29 788	0 772	0 321	1 873	2 208	44 01	0 049

1000 grain weight	Grain length	Grain breadth	Milling recovery	Days to maturity	Straw yield	Grain yield	Total carbo hydrates	Protein content	Amylose content	Peroxidase activity
0 406	0 026	0 007	2 3883	0 188	4295040	318016	1 592	0 346	0 032	0 041
47 803**	2 084**	0 167**	46 554**	16 253**	771006**	96589**	133 341**	4 217**	7 329**	0 003**
1 084	0 017	0 002	0 762	1 873	367236	58570	43 756	2 493	0 858	0 000

* Significant at 5% level

** Significant at 1% level

Table 10 Range mean and estimates of genetic parameters for grain yield and associated quantitative nutritional and biochemical characters of aromatic rice genotypes

Sl No	Character	Range	Mean±SEM	Genotypic variance (Vg)	Phenotypic variance (Vp)	Genotypic coefficient of variation (GCV)	Phenotypic coefficient of variation (PCV)	Heritability in broad sense(H ²)	Genetic Advance (GA)	Genetic gain
1	Seedling height (cm)	28.93(T17) 40.79(T13)	33.24±1.44	12.69	15.82	10.50	11.72	80.20	6.57	19.76
2	Leaf length(cm)	45.69(T13) 59.26 (T10)	55.27±2.65	12.82	23.36	6.51	8.77	55.10	5.50	9.95
3	Leaf width (cm)	1.07(T3&T11) 1.30 (T8)	1.18±0.07	0.00	0.012	4.69	8.89	27.90	0.06	5.04
4	Ligule length(cm)	1.48(T13) 2.73 (T12)	2.22±0.22	0.08	0.153	12.83	17.6	53.20	0.43	19.37
5	Culm length(cm)	90.76(T13) 122.52 (T9)	110±4.46	87.44	117.23	8.55	9.89	74.70	16.67	15.21
6	Culm number	6.97(T1) 9.70 (T11)	8.13±0.72	0.56	1.33	9.16	14.17	41.80	0.99	12.18
7	Culm diameter	5.63(T8) 7.18 (T9)	6.40±0.46	0.16	0.48	6.18	10.7	33.40	0.48	7.40
8	Days to 50% heading	116.00(T13) 125.67 (T12)	121.87±1.12	4.79	6.66	1.80	2.12	71.90	3.82	3.13

Sl No	Character	Range	Mean±SEM	Genotypic variance (Vg)	Phenotypic variance (Vp)	Genotypic coefficient of variation (GCV)	Phenotypic coefficient of variation (PCV)	Heritability in broad sense(H ²)	Genetic advance (GA)	Genetic gain
9	Panicle length(cm)	24.47(T13) 30.14(T7)	27.61±1.21	1.72	3.93	4.75	7.18	43.70	1.79	6.48
10	Number of grains per panicle	99.50(T12) 123.51(T11)	113.4±5.42	46.43	50.59	6.01	8.38	51.30	10.06	8.86
11	Length of sterile glumes(mm)	1.60(T13) 2.98(T12)	2.17±0.18	0.15	0.20	18.05	20.76	75.60	0.70	32.26
12	1000 grain weight(gm)	12.27(T1) 26.17(T13)	15.64±0.85	15.57	16.65	25.22	26.09	93.50	7.86	50.26
13	Grain length (mm)	5.93(T1) 8.63(T11)	6.76±0.11	0.69	0.71	12.28	12.43	97.60	1.69	25.00
14	Grain breadth(mm)	2.49(T12) 3.39(T13)	2.66±0.04	0.056	0.056	8.79	8.95	96.40	0.47	17.67
15	Milling recovery(%)	60.33(T9) 75.33(T13)	67.74±0.71	15.26	16.02	5.77	5.91	95.20	7.85	11.59
16	Days to maturity	161.00(T13) 107.67(T12)	166.87±1.10	4.79	6.66	1.31	1.55	71.90	3.82	2.29

Sl No	Character	Range	Mean±SEM	Genotypic variance (Vg)	Phenotypic variance (Vp)	Genotypic coefficient of variation (GCV)	Phenotypic coefficient of variation (PCV)	Heritability in broad sense(H ²)	Genetic Advance (GA)	Genetic gain
17	Straw yield(kg/ha)	2906.67(T9) 4746.67(T6)	3997.94±4.95	134590	501826	9.18	17.72	26.80	391.39	9.79
18	Grain yield(kg/ha)	2080.00(T9) 2822.33(T13)	2542.05±1.98	12673	71243	4.43	10.50	17.80	97.81	3.85
19	Total carbohydrates(%)	58.40(T12) 81.87(T2)	68.42±5.40	29.86	73.62	7.98	12.54	40.56	7.17	10.47
20	Protein content(%)	6.88(T2) 10.46(T3)	8.84±1.28	0.57	3.06	8.57	19.80	18.70	0.68	7.69
21	Amlylose content (%)	17.87(T4) 23.07(T2)	19.75±1.56	2.157	3.02	7.44	8.79	71.50	2.56	12.96
22	Peroxidase activity(units/ml)	0.27(T6&T11) 0.60(T4)	0.40±1.69	0.014	0.00	28.75	29.20	96.90	0.24	60.00

Among the quantitative characters seedling height varied from 28.93 to 40.79 cm with an average of 33.24 ± 1.44 cm. Length and width of leaf varied from 45.69 to 59.76 cm and 1.07 to 1.30 cm with means 55.27 ± 2.65 cm and 1.18 ± 0.07 cm respectively. Length of ligule and culm varied from 1.48 to 2.73 cm and 90.76 to 122.52 cm with an average of 2.22 ± 0.22 cm and 110.00 ± 4.46 cm respectively. With respect to mean culm number and mean culm diameter the variability ranged from 6.97 to 9.70 and 5.63 to 7.18 mm with an average of 8.13 ± 0.72 and 6.40 ± 0.46 mm respectively. In the case of number of days to 50 per cent heading the range of variation was from 116.00 to 125.67 days with an average of 122.00 ± 1.12 days. Panicle length varied from 24.47 to 30.14 cm average being 27.61 ± 1.21 cm. With respect to number of grains per panicle and length of sterile glumes the range varied from 99.50 to 173.57 and 1.60 to 2.98 mm with an average of 113.40 ± 5.42 and 2.17 ± 0.18 mm respectively. 1000 grain weight ranged from 17.27 to 26.17 gm with a mean of 15.64 ± 0.85 gm. Grain length and grain breadth varied from 5.93 to 8.63 mm and 2.49 to 3.59 mm with an average of 6.76 ± 0.11 mm and 2.66 ± 0.04 mm respectively. Milling recovery and days from seeding to maturity ranged from 60.35 to 75.33 per cent and 161.00 to 170.67 and had a mean of 67.74 ± 0.71 per cent and 166.87 ± 1.10 days respectively. Straw yield and grain yield varied from 2906.67 to 4746.67 kg/ha and 2080.00 to 2822.33 kg/ha with an average of 3997.94 ± 4.95 kg/ha and 2542.05 ± 1.98 kg/ha respectively.

Among the biochemical characters total carbohydrates ranged from 58.40 to 81.87 per cent with mean value of 68.42 ± 5.40 per cent. Protein content varied from 6.88 to 10.46 per cent average being 8.84 ± 1.78 per cent. Amylose content and peroxidase activity varied from 17.87 to 23.07 per cent and 0.27 to 0.60 units/ml with an average of 19.75 ± 7.56 per cent and 0.40 ± 1.69 units/ml respectively.

4.5.2 Phenotypic and Genotypic coefficients of variation

Moderate estimates of GCV and PCV were observed with respect to the 1000 grain weight. Low GCV and PCV was observed with respect to all other characters viz seedling height, leaf length, leaf width, ligule length, culm length, culm number, culm diameter, days to 50 per cent heading, panicle length, number of grains per panicle.

length of sterile glumes grain length grain breadth milling recovery days to maturity straw yield and grain yield

All biochemical characters except peroxidase activity showed low GCV and PCV

4.5.3 Heritability

Among quantitative characters heritability (in broad sense) estimates ranged between 17.80 per cent (grain yield) and 97.60 per cent (grain length). Heritability estimates with respect to quantitative characters namely grain length (97.60%), grain breadth (96.40%), milling recovery (95.20%), 1000 grain weight (93.50%), seedling height (80.20%), length of sterile glumes (75.60%), culm length (74.70%), days to maturity (71.90%), days to 50 percent heading (71.90%), leaf length (55.10%), ligule length (53.20%) and number of grains per panicle (51.30%) were found to be high. Panicle length (43.70%), culm number (41.80%), culm diameter (33.40%), leaf width (27.90%) and straw yield (26.80%) exhibited moderate heritability. Grain yield (17.80%) exhibited low heritability (Table 10).

Among biochemical characters heritability ranged from 18.70 to 96.90 per cent. Heritability was low for protein content (18.70%) and intermediate for total carbohydrates (40.56%). Estimates of heritability values were high for peroxidase activity (96.90%) and amylose content (71.50%).

4.5.4 Genetic gain

Genetic gain among quantitative characters varied from 2.29 per cent for days to maturity to 50.26 per cent for 1000 grain weight. Among quantitative characters genetic gain was high for 1000 grain weight (50.26%), length of sterile glumes (32.26%), grain length (25.00%). Genetic gain was moderate for quantitative characters like seedling height (19.76%), ligule length (19.37%), grain breadth (17.67%), culm length (15.21%), culm number (12.18%), milling recovery (11.53%), while leaf length (9.95%), straw yield (9.79%), number of grains per panicle (8.86%), culm diameter (7.40%), panicle length (6.48%), leaf width (5.04%), grain yield (3.85%), days to 50 percent heading (3.13%) and maturity days (2.29%) showed low genetic gain.

Among biochemical characters genetic gain ranged from 7.69 per cent to 60.00 per cent. Genetic gain was low for protein content (7.69%) while it recorded moderate for total carbohydrates (10.47%) and a mylose content (12.96%) and peroxidase activity exhibited high genetic gain (60.00%).

4.5.5 Correlation coefficients for quantitative characters

Correlation coefficients between grain yield and yield components in aromatic rice genotypes are presented in Table 11.

Genotypic correlation coefficient revealed highly significant positive correlation of grain yield with straw yield (0.945), grain breadth (0.822) and seedling height (0.660) while panicle length (0.988), culm length (0.821), leaf length (0.768), culm number (0.765), culm diameter (0.694) and days to maturity (0.658) had highly significant negative effects.

Seedling height had highly significant positive correlation with grain breadth (0.761) and number of grains per panicle (0.687). It recorded highly significant negative correlation with culm length (0.691), leaf length (0.684) and panicle length (0.631) while it had significant negative correlation with maturity days (0.505).

Leaf length showed highly significant positive correlation with culm length (0.1037) and panicle length (0.865). It recorded highly significant negative correlation with grain length (0.859) and grain breadth (0.803).

Leaf width was found to have highly significant positive association with panicle length (0.607). It recorded highly significant negative correlation with number of grains per panicle (0.775), maturity days (0.615) and straw yield (0.559).

Table 11 Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients between grain yield and yield components in aromatic rice genotypes

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1 000	0 684**	0 311	0 691**	0 096	0 139	0 631**	0 687**	0 436	0 761 *	0 278	-0 505*	0 320	0 660 *
X2	0 490	1 000	0 224	1 037**	0 135	0 215	0 865**	0 139	0 859 *	0 803**	0 446	0 238	0 372	0 768*
X3	0 112	0 009	1 000	0 494	0 194	0 061	0 607*	0 775**	0 440	0 341	0 384	0 615*	0 559*	0 087
X4	0 486	0 764	0 278	1 000	0 163	0 023	0 875**	0 168	0 766**	0 662**	0 277	0 207	0 505*	0 821**
X5	0 002	0 008	0 269	0 234	1 000	0 401	0 013	0 379	0 706**	0 211	0 114	0 471	0 192	0 765**
X6	0 005	0 250	0 150	0 006	0 299	1 000	0 124	0 318	0 559*	0 079	0 688 *	0 024	1 107 *	0 694**
X7	0 005*	0 450	0 005	0 581*	0 170	0 003	1 000	0 427	0 348	0 681**	0 323	0 605*	0 490	0 988**
X8	0 383	0 170	0 210	0 169	0 114	0 135	0 312	1 000	0 062	0 124	0 143	0 106	0 009	0 082
X9	0 399	0 642	0 649**	0 271	0 477	0 325	0 239	0 004	1 000	0 362	0 549*	0 198	0 168	0 241
X10	0 656	0 592	0 132	0 580	0 146	0 003	0 497	0 115	0 352	1 000	0 380	0 811**	0 181	0 822**
X11	0 239	0 298	0 171	0 238	0 005	0 388	0 223	0 121	0 528	0 356	1 000	0 215	0 066	0 440
X12	0 386	0 254	0 241	0 117	0 378	0 006	0 375	0 134	0 160	0 686	0 170	1 000	0 075	0 638 *
X 3	0 006	0 004	0 210	0 321	0 004	0 394	0 112	0 007	0 008	0 114	0 001	0 184	1 000	0 945**
X14	0 251	0 277	0 072	0 343	0 264	0 286	0 279	0 098	0 116	0 414	0 180	0 143	0 661	1 000

* Significant at 5 % level

**Significant at 1 % level

X1 seedling height
 X2 leaf length
 X3 – leaf width
 X4 culm length
 X5 culm number
 X6 culm diameter
 X7 panicle length

X8 number of grams per panicle
 X9 grain length
 X10 gram breadth
 X11 milling recovery
 X12 maturity days
 X13 straw yield
 X14 grain yield

Culm length had highly significant positive correlation with panicle length (0.875) It recorded highly significant negative correlation with grain length (0.766) grain breadth (0.662) and significant negative correlation with straw yield (0.505)

Culm number showed highly significant positive correlation with grain length (0.706)

Culm diameter showed highly significant positive association with milling recovery (0.688) and significant positive association with grain length (0.559) It recorded highly significant negative correlation with straw yield (0.107)

Panicle length was found to have significant positive correlation with maturity days (0.605) while grain breadth (0.681) had highly significant negative effects

Grain length showed significant positive effect with milling recovery (0.549) Grain breadth had highly significant negative correlation with maturity days (0.811)

4.5.6 Direct and indirect effects of quantitative characters on grain yield

Direct and indirect effects of 13 yield components on grain yield are presented in Table 12

It was observed that straw yield showed very high direct positive effect (0.835) on grain yield followed by milling recovery (0.550) and seedling height (0.507) Grain breadth had high negative direct effect (0.937) on grain yield followed by leaf length (0.801) maturity days (0.667) and culm number (0.458) while grain length (0.164) had low negative direct effect on grain yield

Seedling height exhibited high positive indirect effect on grain yield via leaf length (0.393) and it had moderate positive indirect effect on grain yield by maturity days (0.258) while it had low positive indirect effect via panicle length (0.185) followed by

Table 12 Direct and indirect effects of 13 yield components on grain yield of aromatic rice genotypes

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	rg
X1	0 507	0 393	0 010	0 016	0 012	0 028	0 185	0 202	0 065	0 615	0 132	0 258	0 057	0 660**
X2	0 248	0 801	0 008	0 025	0 037	0 138	0 239	0 090	0 106	0 556	0 164	0 170	0 038	0 768**
X3	0 062	0 078	0 084	0 009	0 123	0 083	0 030	0 158	0 044	0 124	0 094	0 161	0 176	0 087
X4	0 246	0 612	0 023	0 034	0 107	0 034	0 309	0 089	0 106	0 544	0 131	0 078	0 268	0 821**
X5	0 013	0 065	0 022	0 008	0 458	0 165	0 090	0 060	0 078	0 139	0 031	0 252	0 039	0 765**
X6	0 026	0 200	0 125	0 002	0 137	0 551	0 021	0 071	0 053	0 031	0 213	0 046	0 329	0 694**
X7	0 176	0 361	0 005	0 019	0 078	0 022	0 532	0 165	0 031	0 466	0 122	0 250	0 092	0 988**
X8	0 194	0 136	0 025	0 006	0 052	0 074	0 166	0 527	0 006	0 107	0 067	0 090	0 062	0 082
X9	0 202	0 520	0 023	0 022	0 218	0 179	0 127	0 021	0 164	0 330	0 291	0 106	0 074	0 241
X10	0 332	0 475	0 011	0 020	0 068	0 018	0 264	0 061	0 058	0 937	0 196	0 458	0 095	0 822**
X11	0 121	0 239	0 014	0 008	0 026	0 214	0 118	0 064	0 086	0 334	0 550	0 114	0 001	0 440
X12	0 196	0 203	0 202	0 004	0 173	0 038	0 200	0 071	0 026	0 643	0 094	0 667	0 154	0 638**
X13	0 035	0 036	0 018	0 011	0 019	0 217	0 059	0 039	0 014	0 106	0 001	0 123	0 835	0 945**

X1 seedling height
 X2 leaf length
 X3 leaf width
 X4 culm length
 X5 – culm number
 X6 – culm diameter
 X7 panicle length

X8 – number of grains per panicle
 X9 grain length
 X10 grain breadth
 X11 – milling recovery
 X12 maturity days
 X13 straw yield

Residual value 0 1687

milling recovery (0.132) It showed a high negative effect through grain breadth (0.615) and moderate negative effect through number of grains per panicle (0.202)

Leaf length showed high positive indirect effect on grain yield via grain breadth (0.556) and it had low positive indirect effect through culm diameter (0.138) and grain length (0.106) It showed a moderate negative effect through seedling height (0.248) followed through panicle length (0.239) and it showed low negative indirect effect through maturity days (0.170) and milling recovery (0.164)

Leaf width exhibited low positive indirect effect on grain yield via maturity days (0.161) followed through culm number (0.123) It recorded low negative indirect effect via straw yield (0.176) and grain breadth (0.124)

Culm length had high indirect positive effect on grain yield via grain breadth (0.544) while it showed low indirect positive effect through culm number (0.107) and grain length (0.106) Culm length recorded high indirect negative effect on grain yield via leaf length (0.612) while it showed moderate indirect negative effect via straw yield (0.268) and seedling height (0.246) It recorded low indirect negative effect through milling recovery (0.151)

Culm number showed low positive indirect effect on grain yield through grain breadth (0.139) It recorded moderate negative indirect effect with days to maturity (0.257) and low negative indirect effect with culm diameter (0.165)

Culm diameter exhibited moderate indirect positive effect on grain yield through milling recovery (0.213) and leaf length (0.200) while it had low positive indirect effect through leaf breadth (0.125) It had a high indirect negative effect via straw yield (0.329) and low indirect negative effect by culm number (0.157)

Panicle length showed high positive indirect effect on grain yield via grain breadth (0.466) and it had low positive indirect effect through number of grains per

panicle (0 165) It had a high negative indirect effect through leaf length (0 361) and moderate negative indirect effect via maturity days (0 250) while it recorded low negative indirect effect via seedling height (0 176) and milling recovery (0 122)

Number of grains per panicle showed low indirect positive effect on grain yield via seedling height (0 194) followed through panicle length (0 166) and leaf length (0 136) while it had low indirect negative effect via grain breadth (0 107)

Grain length showed high positive indirect effect on grain yield via leaf length (0 520) while moderate indirect effect via milling recovery (0 291) and seedling height (0 202) It recorded low indirect effect through panicle length (0 127) It had high negative indirect effect via grain breadth (0 330) and moderate negative effect through culm number (0 218) while low negative effect via culm diameter (0 179) and maturity days (0 106)

Grain breadth showed high positive indirect effect on grain yield via leaf length (0 475) followed by maturity days (0 458) and seedling height (0 332) It had moderate positive effect through panicle length (0 264) while it showed low positive indirect effect through milling recovery (0 196)

Milling recovery showed moderate positive indirect effect on grain yield through leaf length (0 239) while it had low positive indirect effect via seedling height (0 121) followed by panicle length (0 118) and maturity days (0 114) It recorded a high negative indirect effect via grain breadth (0 334) and it showed moderate negative indirect effect through culm diameter (0 214)

Maturity days showed high positive indirect effect on grain yield via grain breadth (0 643) while it had moderate positive indirect effect via leaf breadth (0 202) and low positive indirect effect through straw yield (0 154) It recorded moderate negative indirect effect through leaf length (0 203) and panicle length (0 200) whereas low negative indirect effect through seedling height (0 196) and culm number (0 173)

Straw yield exhibited moderately positive indirect effect via culm diameter (0.217) while it had low negative indirect effect through maturity days (0.123) and grain breadth (0.106)

Residual effect was observed to be 0.1687

4.5.7 GPS (Geographical Positioning System) parameters

During sunny days based on three satellites GPS readings were recorded at five locations using Garmin Etrex hand held device and the data are presented in Table 13

Of the five locations selected for GPS reading Panamaram recorded the lowest altitude of 726 m and Ambalavayal the highest altitude of 909 m. The genotypes GT9 and JT12 were collected from Panamaram and it was interesting to note that these genotypes expressed only slight aroma when grown and evaluated at Ambalavayal whereas all other genotypes under study expressed moderate aroma.

Table 13 GPS readings from five locations in Wayanad district Kerala

Location	Latitude and Longitude	Altitude (m)
Thirunelli	11 ^o 50 N and 76 ^o 04 E	785 m
Mananthavady	11 ^o 48 N and 76 ^o 00 E	755 m
Nenmen	11 ^o 37 N and 76 ^o 16 E	871 m
Ambalavayal	11 ^o 36 N and 76 ^o 12 E	909 m
Panamaram	11 ^o 44 ¹ N and 76 ^o 04 E	726 m

Discussion

5 DISCUSSION

Gandhakasala and *Jeerakasala* are the most popular traditional aromatic rice cultivars of Wayanad district Kerala. These genotypes are known for their characteristic fragrance and hence fetch a premium price in the domestic market (often 3 to 4 times more than the ordinary rice cultivars). According to George *et al* (2005) these popular genotypes also have considerable export potential. Hence it is essential that in-depth studies are to be undertaken to reveal the extent of variability existing in these genotypes so that the best types can be selected for commercial cultivation. Moreover, genotypes with better nutritional qualities can be utilized in rice improvement programmes. Morphological and nutritional characterization of these genotypes is the prerequisite for the protection of IP rights over these cultivars and their products. In this perspective the present study assumes significance.

5.1 Morphological characterization

5.1.1 Qualitative characters

Among the morphological characters studied, qualitative characters like leaf blade pubescence, basal leaf sheath colour, ligule colour, ligule shape, collar colour, auricle colour, culm internode colour, septum colour, panicle type, panicle secondary branching, shattering, threshability, apiculus colour, stigma colour, sterile lemma colour, spikelet sterility and pest and disease incidence showed no variation and hence were of limited use in distinguishing aromatic rice genotypes among themselves and also from Deepthi.

In general, *Gandhakasala*, *Jeerakasala* and Deepthi have intermediate leaf blade pubescence, green basal leaf sheath colour, white and 2-cleft ligules, light green colour for collar, auricle and septum, green colour for internode, intermediate panicle, heavy secondary branching, low shattering, difficult threshability of panicle, straw colour for apiculus and sterile lemma, yellow stigmas and highly fertile spikelets. The crop

exhibited low incidence of stem borer damage (1 10%) and was free from other pests and diseases

Among the qualitative characters leaf blade colour panicle exertion spikelet awn ng awn colour lemma and palea colour lemma and palea pubescence and seed coat colour showed more variability and hence can be considered as morphological markers for the identification of aromatic rice genotypes

Gandhakasala genotypes lack awns have golden coloured lemma and palea well exerted panicles and white seed coat colour whereas *Jeerakasala* genotypes have straw coloured short and partial awns golden lemma and palea colour well exerted panicles and white seed coat colour The check variety Deepthi lack awns have straw colour lemma and palea moderately well exerted panicles and red seed coat colour (Table 14) These results are in agreement with Elsy *et al* (2010) who stated that grains of *Jeerakasala* are short awned while *Gandhakasala* grains are awnless

According to Kumari *et al* (2002) both *Gandhakasala* and *Jeerakasala* had white kernel colour George *et al* (2004) reported that Deepthi (WND 3) had red kernel colour Above statements coincides with reports of present study

5.2 Quantitative characters

Quantitative characters like seedling height leaf length ligule length culm length culm number culm diameter days to 50 per cent heading panicle length number of grains per panicle length of sterile glumes 1000 grain weight grain length grain breadth milling recovery days to maturity straw yield and grain yield showed significant variation and were found to be more useful for the characterization of aromatic rice genotypes According to Chang and Bardenas (1965) the leaf length and leaf width varied with varieties

Table 14 Comparison of qualitative characters of aromatic rice genotypes with Deepthi

S No	Character	Mean		
		Gandhakasala group	Jeerakasala group	Deepthi
	<i>a) Similar characters</i>			
1	Leaf blade pubescence	Intermediate	Intermediate	Intermediate
2	Basal leaf sheath colour	Green	Green	Green
3	Ligule colour	White	White	White
4	Ligule shape	2 cleft	2 cleft	2 cleft
5	Collar colour	Light green	Light green	Light green
6	Auricle colour	Light green	Light green	Light green
7	Culm internode colour	Green	Green	Green
8	Septum colour	Light green	Light green	Light green
9	Panicle type	Intermediate	Intermediate	Intermediate
10	Panicle secondary branching	Heavy	Heavy	Heavy
11	Shattering	Low	Low	Low
12	Threshability	Difficult	Difficult	Difficult
13	Apiculus colour	Straw	Straw	Straw
14	Stigma colour	Yellow	Yellow	Yellow
15	Sterile lemma colour	Straw	Straw	Straw
16	Spikelet sterility	Highly fertile	Highly fertile	Highly fertile
17	Pest and disease incidence	Low incidence	Low incidence	Low incidence
	<i>b) Dissimilar characters</i>			
1	Leaf blade colour	Green	Dark green	Dark green
2	Panicle exertion	Well exerted	Well exerted	Moderately well exerted
3	Spikelet awning and awn colour	Absent	Present (Straw colour short and partial)	Absent
4	Lemma palea colour	Golden	Golden	Straw
5	Lemma and palea pubescence	Hairs on upper portion	Hairs on upper portion	Short hairs
6	Seed coat colour	White	White	Red

With respect to variation in quantitative traits *Gandhakasala* rice genotypes exhibited a mean seedling height of 33.37 cm whereas *Jeerakasala* exhibited 33.38 cm and *Deepthi* exhibited 40.79 cm. This indicated that the aromatic cultivars had less seedling vigour and hence had less competing ability with weeds. On the other hand *Deepthi* with more seedling height can compete with weeds in a better way. *Gandhakasala* group showed 56.88 cm and 1.20 cm for leaf length and leaf breadth respectively while *Jeerakasala* group had 52.01 cm and 1.12 cm. On the other hand ligule length was low in *Deepthi* compared to aromatic genotypes. The mean values for ligule length was 2.20, 2.68 and 1.48 cm in *Gandhakasala*, *Jeerakasala* and *Deepthi* respectively. Aromatic types showed longer and narrower leaves and longer ligules than *Deepthi*. Culm length was more in *Gandhakasala* (113.02 cm) and *Jeerakasala* (101.91 cm) compared to *Deepthi* (90.76 cm). As a result aromatic types showed lodging at the end of maturity. *Gandhakasala* and *Jeerakasala* took more days to 50 per cent heading (121.73 and 125.50 respectively) compared to *Deepthi* (116.00). Length of sterile glumes was more for *Jeerakasala* genotypes. The mean value for 1000 grain weight was less in *Gandhakasala* (13.78 gm) compared to *Jeerakasala* (19.82 gm) and *Deepthi* (26.17 gm). *Jeerakasala* exhibited high grain length (8.28 mm) compared to *Gandhakasala* (6.34 mm) and *Deepthi* (7.87 mm). *Deepthi* showed high grain breadth of 3.40 mm while *Gandhakasala* and *Jeerakasala* groups had slender grains with a grain breadth of 2.62 mm and 2.56 mm respectively. High grain breadth and grain length would have added to high 1000 grain weight in *Deepthi*. In general aromatic genotypes had lesser milling recovery than *Deepthi*, indicating the need for specially designed milling machines for maximum milling recovery (Table 15). Both *Gandhakasala* (166.73) and *Jeerakasala* (170.50) took more days to maturity compared to *Deepthi* (161.00). *Jeerakasala* group exhibited a straw yield of 4106.67 kg/ha whereas *Gandhakasala* group exhibited 3970.67 kg/ha and *Deepthi* 5973.33 kg/ha. *Deepthi* exhibited grain yield of 2822.33 kg/ha while *Gandhakasala* exhibited 2524.80 kg/ha and *Jeerakasala* 2488.16 kg/ha.

According to Kumari *et al* (2002) and George *et al* (2005) the average panicle length of *Gandhakasala* and *Jeerakasala* was 28.4 cm and 27.1 cm respectively. George *et al* (2005) recorded a mean straw yield of 4038 kg/ha and 4385 kg/ha for

Table 15 Comparison of quantitative, nutritional and biochemical characters of aromatic rice genotypes with Deepthi

Sl No	Character	Mean		
		<i>Gandhakasala group</i>	<i>Jeerakasala group</i>	Deepthi
1	Seedling height (cm)	33 37	33 38	40 79
2	Leaf length (cm)	56 88	52 01	45 69
3	Leaf width (cm)	1 20	1 12	1 23
4	Ligule length (cm)	2 20	2 68	1 48
5	Culm length (cm)	113 02	101 91	90 76
6	Culm number	7 89	9 58	7 63
7	Culm diameter (mm)	6 35	6 92	6 99
8	Days to 50 % heading	121 73	125 50	116 00
9	Panicle length (cm)	27 84	28 04	24 47
10	Number of grains per panicle	113 52	111 51	116 57
11	Length of sterile glumes (mm)	2 07	2 96	1 60
12	1000 grain weight (gm)	13 78	19 82	26 17
13	Grain length (mm)	6 34	8 28	7 87
14	Grain breadth (mm)	2 62	2 56	3 40
15	Milling recovery (%)	66 38	70 75	75 33
16	Maturity days	166 73	170 50	161 00
17	Straw yield (Kg/ha)	3970 67	4106 67	3973 33
18	Grain yield (Kg/ha)	2524 80	2488 16	2822 33
19	Total carbohydrates (%)	69 56	61 06	71 73
20	Protein content (%)	8 16	8 33	9 73
21	Amylose content (%)	19 70	20 20	19 40
22	Aroma	Aromatic	Aromatic	Non aromatic
23	Peroxidase activity (umts/ml)	0 40	0 34	0 57

Gandhakasala and *Jeerakasala* respectively. According to Kumari *et al* (2002) maturity days in *Gandhakasala* and *Jeerakasala* ranged from 150 to 180 days.

The genotypes GT2, GT3, GT6, GT8 and GT10 from *Gandhakasala* group and JT12 from *Jeerakasala* group were considered as better performing genotypes based on quantitative characters like seedling height, culm length, culm number, days to 50 per cent heading, panicle length, 1000 grain weight, length and breadth of grains, maturity days, straw yield and grain yield.

5.3 Nutritional characterization of aromatic rice genotypes

The results with respect to various nutritive quality parameters are discussed below.

5.3.1 Total carbohydrates

Rice is nutritious. Rice is high in complex carbohydrates, contains almost no fat, is cholesterol free and is low in sodium. A half cup of cooked white rice provides 82 calories, zero grams fat, 45 grams total carbohydrates, zero grams sugar and three grams protein. Carbohydrates are the most common source of energy in living organisms. In the present study, the genotypes GT2 and GT1 had the highest total carbohydrate content of 81.87 per cent and 76.27 per cent respectively. In general, *Gandhakasala* types and Deepthi had more mean carbohydrate content than *Jeerakasala* types.

On the other hand, nowadays there is an urge to go for low carbohydrate diets or low carb diets. Such diets are dietary programs that restrict carbohydrate consumption, usually for weight control or for the treatment of obesity. Foods high in digestible carbohydrates are replaced with foods containing a higher percentage of proteins and fats and often other foods low in carbohydrates (e.g. green leafy vegetables). Apart from obesity, low carbohydrate diets are often recommended in the treatment of diabetes.

epilepsy and chronic fatigue syndrome. In the present study *Jeerakasala* genotype JT12 had low carbohydrate content of 58.40 per cent.

5.3.2 Protein content

Rice is the single most important source of protein in the diets of tropical Asia because of the amount consumed. Among the cereals the protein of rice is one of the most nutritious and is considered as an indicator of its nutritional quality (Juliano, 1978). Rice varieties having high protein content in grain are good as weaning foods and also as food for the invalids.

Intermediate protein content was expressed by three *Gandhakasala* genotypes viz. GT3 (10.46%), GT9 (10.32%) and GT7 (10.20%) indicating the nutritional superiority than other types under study. Rest of the genotypes showed low protein content.

Since protein content is highly influenced by environmental conditions and soil nutritional conditions (Yoshida, 1981) further studies are needed to confirm the protein content expressed in aromatic rice genotypes which were under study.

5.3.3 Amylose content

Rice grain consists of 90 per cent starch. Many of the cooking and eating characteristics of milled rice are influenced by the ratio of amylose and amylopectin in rice grain. The content of amylose and amylopectin vary with varieties and method of processing. Amylose content was almost absent in the waxy rice (Chang and Bardenas, 1965; Kumar and Khush, 1986). High amylose rice showed high volume expansion and high degree of flakiness. They became less tender on cooking and hard upon cooling. Rice with intermediate amylose content became fluffy, soft and moist, whereas low amylose content rice became very sticky, moist and tender on cooking (Kumar and Khush, 1986; Nanda, 1997; Cruz and Khush, 2000). According to Rao *et al.* (1952) amylose content in starch ranged between 15 to 35 per cent. Amylose content correlated

negatively with taste panel scores for cohesiveness tenderness colour and gloss of boiled rice (IRRI 2002)

In the present study the aromatic rice genotypes GT1 GT2 GT8 GT10 and JT12 had intermediate amylose content of 21.87 per cent 23.07 per cent 20.40 per cent 20.47 per cent and 20.73 per cent respectively. Hence they may become fluffy soft and moist on cooking adding to the rice preference in specialty preparations. Since intermediate amylose rice are preferred in most of the rice growing regions of the world these aromatic rice genotypes will have better consumer preference in market.

5.3.4 Aroma

Bourgis *et al* (2008) and Saktivel *et al* (2009) found aroma is one of the most valuable traits in grain quality and it helps to fetch a higher premium price in the market. The volatile aromatic component 2-Acetyl-1-Pyrroline (2-AP) was responsible for aromatic rice cultivars. Aromatic rice is preferred in some areas of Asia and draws a premium price in certain specialty markets. Middle East consumer prefer rice with a strong aroma. They believe that rice without a distinctive aroma is like food without salt (Graham 2002).

In the present study aromatic rice genotypes came under moderately aromatic and slightly aromatic groups. This is in agreement with the reports of George *et al* (2005) and Elsy *et al* (2010). Most of the genotypes except GT9 and JT12 expressed moderate aroma and hence can be recommended for commercial cultivation as aromatic cultivars. Presence of aroma in these genotypes is a unique feature that makes these rice suitable for registration as Geographical Indication. Deepthi as expected did not express aroma.

Taking into consideration of nutritional characters it was found that all the selected genotypes except GT3 expressed intermediate amylose content a preferred character in cooking quality for domestic market. On the other hand GT3 with desirable characters expressed low amylose content indicating its stickiness on cooking. So this

genotype would be suitable for countries where stickiness is a preferred character. All the above genotypes expressed moderate aroma and hence will fetch premium price in the market.

JT12 from *Jeeerakasala* group performed well with respect to most of the quantitative characters but was only slightly aromatic.

More studies on these genotypes are needed to use them in crop improvement programmes.

5.4 Biochemical characterization

5.4.1 Isozyme analysis

The results of isoenzyme analysis with respect to peroxidase and esterase are discussed below.

5.4.1.1 Peroxidase

5.4.1.1.1 Quiescent seed

Reddy (2000) reported that germinated seed sample expressed more peroxidase bands than quiescent seed and the results of present study coincided with this report. No peroxidase band was observed in quiescent seed. This might be due to the low activity of peroxidase in quiescent seed (Tomas and Gupta, 1981).

5.4.1.1.2 Germinated seed

Pawar and Gupta (1975) found variation in the peroxidase isozyme pattern in tall and dwarf varieties to determine the genetic divergence among cultivars and their wild relatives. In the present study peroxidase polymorphism in germinated seed sample was

mostly observed for intensity of the bands. The bands PRX 1 (0 050), PRX 2 (0 157) and PRX 3 (0 343) appeared with more intensity in most of the *Gandhakasala* genotypes while it was less intense in *Jeerakasala* genotypes and Deepthi. These indicated their use as markers for characterizing *Gandhakasala* genotypes even though more studies are required in this direction. The band PRX 4 (0 464) was common for all the genotypes and hence no value in identification of genotypes. The genotype JT11 expressed lighter band for PRX 5 (0 480) where rest of the genotypes expressed thick bands. The band PRX 6 (0 514) appeared with less intensity in most of the types and it was absent in GT3 and GT8 genotypes.

Based on SI with check variety genotypes GT1, GT2, GT4, GT5, GT6, GT7, GT9, GT10, JT11 and JT12 were grouped into one group whereas GT3 and GT8 formed another group.

5 4 1 2 Esterase

Esterase polymorphism compared to peroxidase polymorphism was low in rice genotypes under study.

5 4 1 2 1 Quiescent seed

No esterase band was observed in quiescent seed sample. This might be due to the very low esterase activity in quiescent seed (Tomas and Gupta, 1981).

5 4 1 2 2 Germinated seed

Bimb *et al.* (2004) used esterase isozyme as genetic markers to estimate the genetic diversity of 24 fine and aromatic rice cultivars. Karwala *et al.* (2005) concluded that ETS showed a significant difference between varieties. In the present study the isozyme band EST 2 (0 103) was specific for Deepthi and the aromatic rice genotypes could be distinguished from Deepthi by the absence of these bands. Similarly EST 3

(0 276) and EST 4 (0 449) were less intense in all aromatic genotypes compared to that in Deepthi indicating their possibility as an isozyme marker

Based on SI all aromatic genotypes formed one group whereas T13 formed another group

5 4 2 Peroxidase activity

Peroxidases are one of the enzymatic potential sources of Reactive Oxygen Intermediates (ROI) in plants whereas ROI are the signaling molecules which are actively produced to control processes such as programmed cell death abiotic stress responses pathogen defense and systematic signaling (Kar and Mishra 1976)

In the present study the peroxidase activity of aromatic rice genotypes ranged from 0 27 units/ml (GT6 and JT11) to 0 60 units/ml (GT4) The check variety had peroxidase activity of 0 57 units/ml Significant difference was not noticed for peroxidase activity among the genotypes

5 5 Phenotypic and genotypic coefficients of variation

High magnitude of GCV and PCV was not observed for any character (Table 10) Moderate level of variability observed for 1000 grain weight and peroxidase activity indicated the usefulness of these characters in rice improvement programme Low variability for quantitative characters namely seedling height leaf length leaf width ligule length culm length culm number culm diameter days to 50 per cent heading panicle length number of grains per panicle length of sterile glumes grain length grain breadth days to maturity milling recovery straw yield grain yield and nutritional characters like total carbohydrates protein content and amylose content reflects little possibility of improving these characters through selection Similar results of low GCV and PCV for seedling height leaf length leaf width ligule length days to 50 per cent

maturity panicle length days to maturity culm length culm number and culm diameter protein content and amylose content were reported by Reddy (2000)

Considerable influence of environmental factors was observed in case of all quantitative and biochemical characters as these characters showed high PCV than GCV. This fully justified the need for registration of Wayanad *Gandhakasala* Rice and Wayanad *Jeerakasala* Rice as Geographical Indications.

5.6 Heritability

In a general sense heritability specified the proportion of the total variability that is due to genetic causes or the ratio of genotypic variance to the total variance. It is a good index of the transmission of characters from parents to their offspring or it is the heritable portion of phenotypic variance (Nadarajan and Gunasekaran 2005). In the present study quantitative characters like seedling height leaf length ligule length culm length days to 50 per cent heading number of grains per panicle length of sterile glumes 1000 grain weight grain length grain breadth milling recovery days to maturity and biochemical characters like amylose content and peroxidase activity exhibited high degree of broad sense heritability. These results revealed that these characters are useful in the selection of elite types from homozygous material. Similar reports were also made by Priyanka *et al* (2000) and Reddy (2000) for seedling height days to 50 per cent heading grain length 1000 grain weight culm length and amylose content. Moderate heritability was observed for quantitative characters like leaf width culm number culm diameter panicle length and straw yield and also for total carbohydrate content. Low heritability was observed for grain yield and protein content. Hence it was assumed that these characters were highly influenced by environmental factors and hence could not be used as indices for selection purpose.

5.7 Genetic gain

The heritability indicates only the effectiveness with which selection of genotype can be based on the phenotypic performance but fails to show the genetic progress (Johnson *et al* 1955). High heritability does not therefore necessarily mean greater genetic gain. Genetic gain was calculated in order to ascertain its relative utility. The difference between the mean phenotypic value of the progeny of selected plants and the base or parental population is known as genetic gain (Singh and Narayanan 1993). High expected genetic gain was observed for quantitative characters like length of sterile glumes, 1000 gram weight, grain length and peroxidase activity. This indicated that considerable level of improvement could be made in the population by selection based on these characters. Similarly moderate estimate of genetic gain was observed for quantitative characters like seedling height, ligule length, culm length, culm number, grain breadth and milling recovery and for nutritional characters like total carbohydrates and amylose content. Expected genetic gain was low for protein content indicating that it is difficult to improve the population by selecting for these characters. Expected high, moderate and low genetic gain were reported by Reddy (2000) for 1000 grain weight, seedling height and panicle length respectively.

According to Panse (1957) a high heritability value does not necessarily lead to a high genetic gain. If the heritability was mainly due to the non additive genetic effects (dominance and epistasis) the expected genetic gain would be low and when it was chiefly due to the additive effects a high genetic gain would be expected. High heritability accompanied by high genetic gain expressed by length of sterile glumes, 1000 gram weight, grain length and peroxidase activity indicated that selection might be effective for these characters. Days to 50 per cent heading, leaf length, number of grains per panicle and maturity days exhibited high heritability accompanied with low genetic gain indicating non additive gene action and hence selection for such traits might not be rewarding.

5.8 Correlation coefficients of quantitative characters with yield

Studies on association of characters gain importance in plant breeding because they aid the plant breeders to know the inter character influence and help to strike economic and reliable balances among various characters. Moreover, genotypic correlations have their own importance because of their stability and reliability namely linkage and pleiotropy. Since yield is a complex character the practice of unilateral selection often results in retrograde or less optimum progress in isolating superior genotypes. Therefore the knowledge of inter relationships of characters plays a vital role in developing appropriate selection criteria for the improvement of complex characters like grain yield.

Among the correlation coefficients of 13 characters with grain yield for most of the characters like seedling height, leaf length, leaf width, culm length, culm number, culm diameter, panicle length, number of grains per panicle, grain length, grain breadth, milling recovery, maturity days and straw yield the phenotypic correlation coefficients were higher than genotypic correlation coefficients indicating the influence of environment on these characters.

The highest significant positive genotypic correlation of grain yield was with straw yield followed by grain breadth and seedling height. This revealed that improvement of grain yield could be achieved by exercising selections simultaneously for increased straw yield, grain breadth and seedling height (Fig. 4).

5.9 Path analysis for quantitative characters and yield

Though the correlation studies were helpful in measuring the association between grain yield and yield components they did not provide the exact picture of the direct and indirect causes of such association which could be obtained through path analysis (Wright 1923). Path analysis is very useful to point out the important yield components which can be utilized for formulating selection parameters.

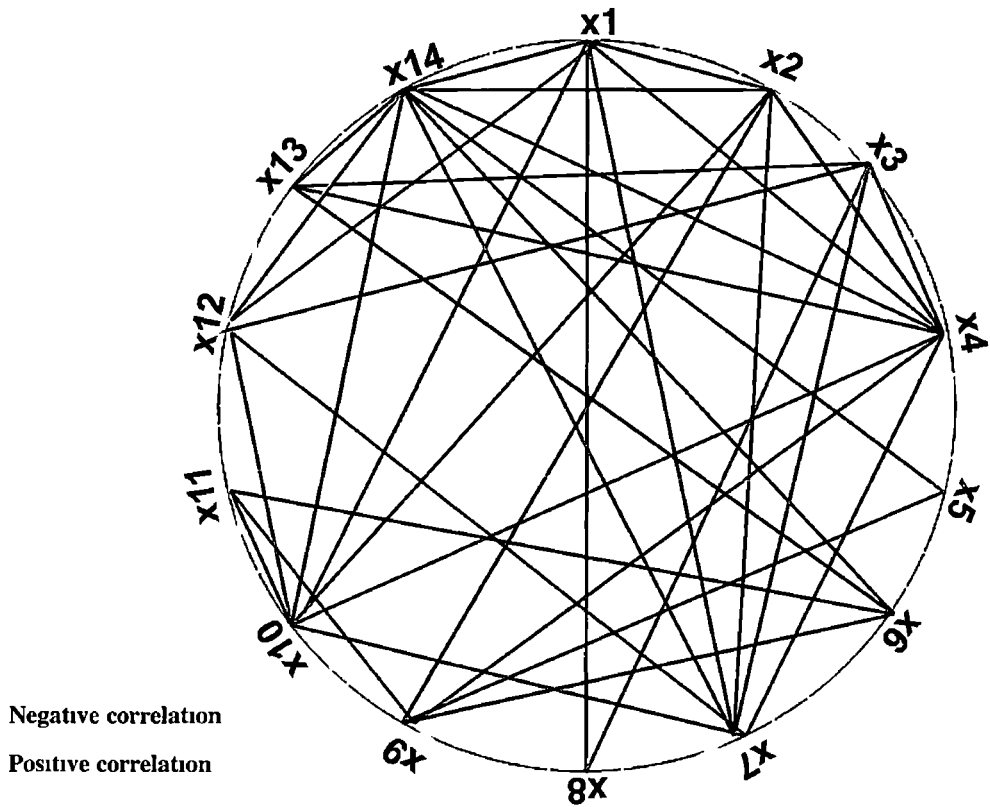


Fig 4 Genotypic correlation between quantitative characters and grain yield of aromatic rice genotypes

Path coefficient analysis performed using 13 quantitative characters showed significant and positive correlation of straw yield, milling recovery and seedling height with grain yield indicating their use in formulating selection parameters.

The highest positive direct effect (0.835) was exhibited by straw yield. This was mainly due to high positive significant correlation between straw yield and grain yield. The positive correlation of straw yield with grain yield was expounded partly by its high positive direct effect and partly by its positive indirect effect through culm diameter. Selection based on this character will be useful in increasing the grain yield in aromatic genotypes.

Second highest positive direct effect on grain yield was contributed by the character milling recovery. The positive correlation of milling recovery with grain yield was expounded partly by its high positive direct effect and partly by its positive indirect effect through leaf length, seedling height, panicle length and maturity days.

Third highest positive direct effect on grain yield was contributed by the character seedling height. The positive correlation of seedling height with grain yield was expounded partly by its high positive direct effect and partly by its positive indirect effect through leaf length, maturity days, panicle length and milling recovery. Selection for this character will also be useful in improving the population (Fig. 5).

The characters like grain breadth, leaf length, maturity days and panicle length had high negative direct effect on grain yield. Selection for optimum grain breadth, shorter leaves, shorter duration and compact panicles will help in improving grain yield of aromatic rice genotypes.

The residual effect obtained in path analysis was 0.1687. This indicated that the 0.8315 per cent variation in grain yield was contributed genotypically by 13 yield components namely seedling height, leaf length, leaf width, culm length, culm number,

culm diameter panicle length number of grains per panicle grain length grain breadth
milling recovery maturity days and straw yield

5.10 GPS (Geographical Positioning System) parameters

Of the five locations selected for GPS reading Panamaram recorded the lowest altitude of 726 m and Ambalavayal the highest altitude of 909 m. The genotypes GT9 and JT12 were collected from Panamaram and it was interesting to note that these genotypes expressed only slight aroma when grown and evaluated at Ambalavayal whereas all other genotypes under study expressed moderate aroma. This indicated the influence of environment on the expression of aroma in rice.

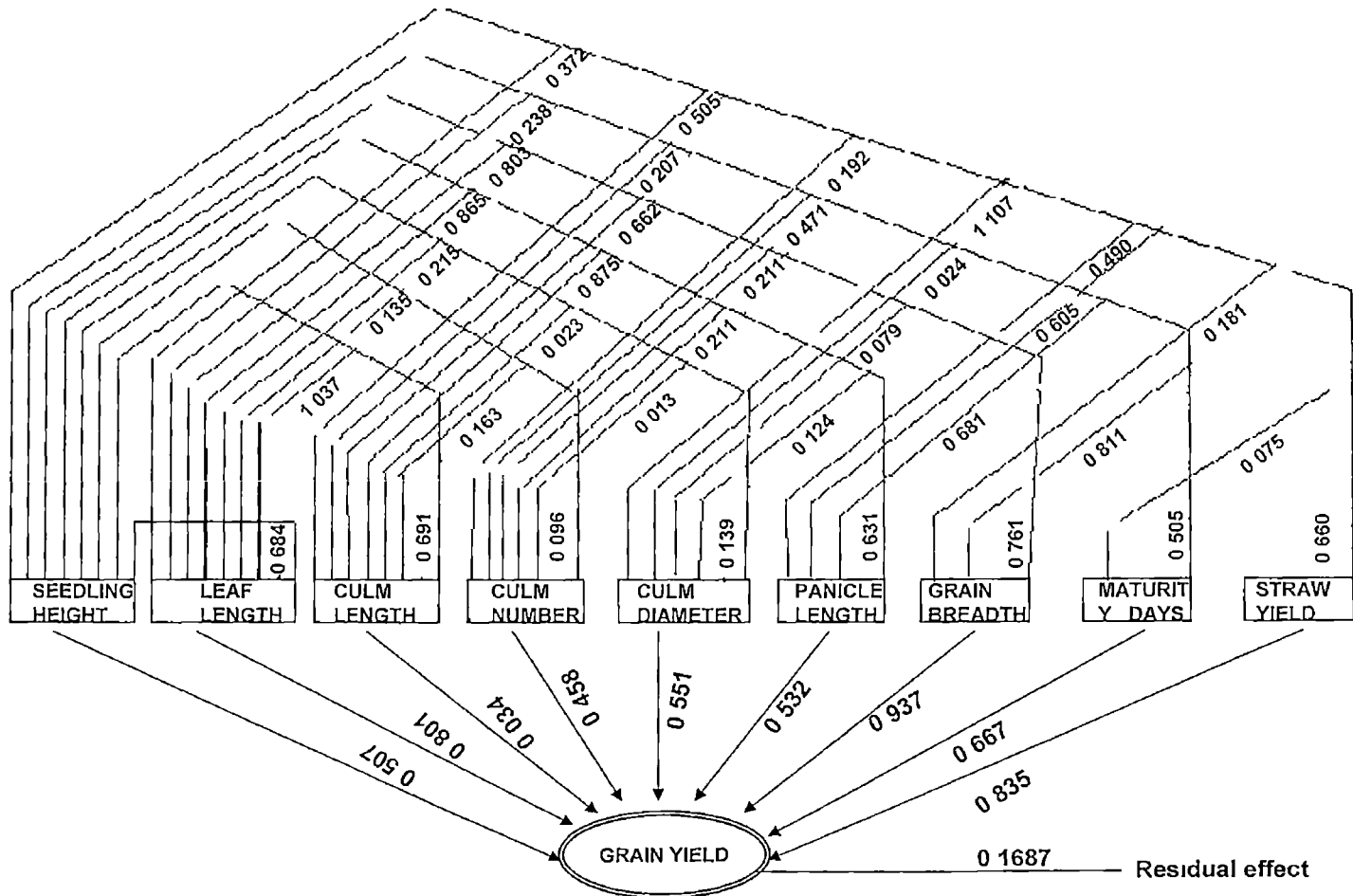


Fig 5 Path diagram constructed using genotypic correlation coefficients among grain yield and nine component characters in aromatic rice genotypes

Summary

6 SUMMARY

Investigations were undertaken in the Department of Plant Breeding and Genetics and in the Centre for Plant Biotechnology and Molecular Biology College of Horticulture Vellanikkara during 2008-2010 to characterize the aromatic rice genotypes (*Gandhakasala* and *Jeerakasala*) based on morphological, nutritional and biochemical analysis. Field experiments related to the investigation were laid out at the Regional Agricultural Research Station (RARS) Ambalavayal, Wayanad.

Ten *Gandhakasala* and two *Jeerakasala* genotypes collected from Wayanad district formed the material for this study along with Deepthi (WND 3) as check variety. The genotypes were laid out in a Randomized Complete Block Design (RCBD) with three replications in plots of 2.5 m X 2.5 m with 20 cm X 10 cm spacing during Kharif season of 2009. The morphological, nutritional and biochemical observations were recorded at different stages of plant growth following standard procedures.

The salient findings could be summarized as follows:

- 1) In general, *Gandhakasala*, *Jeerakasala* and Deepthi have intermediate leaf blade pubescence, green basal leaf sheath colour, white and 2-cleft ligules, light green collar, auricle and septum, green colour internode, intermediate panicle, heavy secondary branching, low shattering, difficult threshability of panicle, straw colour apiculus and sterile lemma, yellow stigmas and highly fertile spikelets. Leaves of *Gandhakasala* genotypes exhibited green colour, whereas *Jeerakasala* exhibited dark green colour. The crop exhibited low incidence of stem borer damage and was free from other major pests and diseases. With respect to panicle exertion, Deepthi had moderately well-exserted panicles, while aromatic genotypes had well-exserted panicles. The *Jeerakasala* genotypes showed straw-coloured, short and partly awned grains, whereas the rest were lacking awns. Straw colour, lemma and palea was observed for Deepthi and golden yellow for the aromatic genotypes. Regarding seed coat colour, Deepthi was observed to have red colour, while aromatic genotypes

showed white coloured seed coat Grains of *Gandhakasala* and *Jeerakasala* showed hairs on upper portion

- 2) Ligule length was low in Deepthi compared to aromatic genotypes Length of sterile glumes was more for *Jeerakasala* genotypes *Gandhakasala* genotypes had small grains with a mean 1000 grain weight of 13.78 gm compared to *Jeerakasala* (19.82 gm) and Deepthi (26.17 gm) *Gandhakasala* genotypes found to have less grain length and grain breadth compared to Deepthi *Jeerakasala* genotypes had more grain length and less grain breadth compared to Deepthi Aromatic genotypes took more days to maturity and expressed lesser milling recovery than Deepthi *Jeerakasala* group exhibited a straw yield of 4106.67 kg/ha whereas *Gandhakasala* group exhibited 3970.67 kg/ha and Deepthi 3973.33 Kg/ha Deepthi exhibited grain yield of 2822.33 kg/ha while *Gandhakasala* exhibited 2524.80 kg/ha and *Jeerakasala* 2488.16 kg/ha
- 3) Evaluation of aromatic rice genotypes with respect to total carbohydrates content revealed that the genotype JT12 had low total carbohydrate content (58.40%) than other genotypes In general *Gandhakasala* genotypes and Deepthi had more mean carbohydrate content than *Jeerakasala* genotypes
- 4) Intermediate protein content was expressed by three *Gandhakasala* genotypes viz GT3 (10.46%) GT9 (10.32%) and GT7 (10.20%) indicating their nutritional superiority than other types under study
- 5) With respect to amylose content the genotypes GT1 GT2 GT8 GT10 and JT12 expressed intermediate amylose content Since intermediate amylose rice is preferred in most of the rice growing regions of world these genotypes will have better consumer preference
- 6) Most of the aromatic rice genotypes under study were moderately aromatic indicating their suitability for commercial cultivation The check Variety Deepthi did not express aroma
- 7) The genotypes GT2 GT3 GT6 GT8 and GT10 from *Gandhakasala* group and JT12 from *Jeerakasala* group were considered as better performing genotypes based on quantitative characters like seedling height culm length culm number days to 50 per cent heading panicle length 1000 grain weight length and breadth of grains

maturity days straw yield and grain yield. With respect to nutritional characters all these selected genotypes except GT3 expressed intermediate amylose content a preferred character in cooking quality for domestic market. GT3 with desirable quantitative characters expressed low amylose content indicating its stickiness on cooking. So this genotype would be suitable for countries where stickiness is a preferred character. All the above genotypes are moderately aromatic and hence will fetch premium price in the market.

- 7) Biochemical characterization indicated the possibility of utilizing peroxidase polymorphism expressed by PRX 1 (0 050) PRX 2 (0 157) and PRX 3 (0 343) to identify *Gandhakasala* genotypes from *Jeejakasala* genotypes and Deepthi. The isozyme band EST 2 (0 103) was specific for Deepthi and the aromatic genotypes could be distinguished from Deepthi by the absence of this band. Similarly EST 3 (0 276) and EST 4 (0 449) were less intense in all aromatic genotypes compared to that in Deepthi indicating their possibility as an isozyme marker.
- 8) Heritability studies indicated that the quantitative characters like seedling height leaf length ligule length culm length days to 50 per cent heading number of grains per panicle length of sterile glumes 1000 grain weight grain length grain breadth milling recovery and maturity days and biochemical characters like peroxidase activity and amylose content exhibited high degree of heritability. This indicated that these characters are useful in the selection of elite genotypes.
- 9) High heritability accompanied by high genetic gain expressed by length of sterile glumes 1000 grain weight grain length and peroxidase activity indicated that selection may be effective for these characters. Days to 50 per cent heading leaf length number of grains per panicle and maturity days exhibited high heritability accompanied with low genetic gain indicating non additive gene action and hence selection for such traits might not be rewarding.
- 10) Correlation studies revealed that highest significant positive genotypic correlation of grain yield was with straw yield followed by grain breadth and seedling height.
- 11) Path analysis to reveal direct and indirect effects of yield components revealed highly direct positive effect of straw yield milling recovery and seedling height on grain yield.

Suggested future line of work

- 1) Germplasm conservation purification and improvement of aromatic genotypes
- 2) Detailed molecular characterization
- 3) In depth biochemical studies to identify aromatic components
- 3) Registration of aromatic cultivars as farmers' variety under PPV & FR (Protection of Plant Varieties and Farmer's Rights) Act 2001 to protect farmers' rights
- 4) Registration of aromatic rice under The Geographical Indications of Goods (Registration and Protection) Act 1999 to enhance market potential

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**MORPHOLOGICAL AND BIOCHEMICAL
CHARACTERIZATION OF AROMATIC RICE
(*Oryza sativa* L.) CULTIVARS OF
WAYANAD DISTRICT OF KERALA**

By

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ABSTRACT OF THE THESIS

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Abstract

ABSTRACT

The present study was undertaken in the Department of Plant Breeding and Genetics and in the Centre for Plant Biotechnology and Molecular Biology College of Horticulture Vellanikkara and at RARS Ambalavayal during 2008-2010 with an aim to characterize the aromatic rice cultivars of Wayanad district based on morphological, nutritional and biochemical studies.

Gandhakasala and *Jeerakasala* are two popular and traditional non-Basmati aromatic rice cultivars of Wayanad district Kerala. In Wayanad *Gandhakasala* is cultivated in an area of 527 ha while *Jeerakasala* in 22 ha. Based on grain characters 10 samples of *Gandhakasala* and two samples of *Jeerakasala* were selected for characterization. Deepthi (WND 3) was used as check variety.

Among morphological studies qualitative characters like leaf blade pubescence, panicle exertion, spikelet awning, awn colour, lemma and palea pubescence and seed coat colour showed variation and hence these can be used as morphological markers to distinguish aromatic genotypes among themselves and with Deepthi. Straw coloured short and partial awns were the characteristic feature of *Jeerakasala* grains while awns were absent in *Gandhakasala* and Deepthi. Aromatic genotypes exhibited well-exserted panicles and white seed coat colour whereas Deepthi showed moderately well-exserted panicles and red seed coat colour.

Mean performance of aromatic genotypes indicated that ligule length, grain length, grain breadth, 1000 grain weight, days to 50 per cent heading, milling recovery and maturity days provided a good base for selection. *Jeerakasala* genotypes took more days to 50 per cent heading and to maturity than *Gandhakasala* genotypes and Deepthi. In general *Gandhakasala* genotypes had lesser 1000 grain weight with a mean value of 13.78 gm compared to *Jeerakasala* (19.82 gm) and Deepthi (26.17 gm). High grain breadth and grain length would have added to high 1000 grain weight in Deepthi. In

general aromatic genotypes have lesser milling recovery than Deepthi indicating the need for specially designed milling machines for maximum milling recovery

The genotype GT2 appeared to have higher total carbohydrate content of 81.87 per cent while it was low for JT12 (58.40%). *Jeerakasala* genotypes exhibited low mean carbohydrate content of 61.06 per cent compared to *Gandhakasala* (69.56%) and Deepthi (71.73%). The three *Gandhakasala* genotypes GT3, GT9 and GT7 exhibited intermediate protein content indicating their nutritional superiority.

The amylose content of aromatic genotypes ranged between 17.87 (GT4) and 23.07 (GT2) percent. Five aromatic genotypes GT1, GT2, GT8, GT10 and JT12 had intermediate amylose content. Since intermediate amylose rice is preferred in most of the rice growing regions of the world, these genotypes will have better preference in market. Most of aromatic genotypes under study were moderately aromatic, indicating their suitability for commercial cultivation.

Biochemical characterization based on isozyme studies revealed the possibility of utilizing peroxidase polymorphism for identifying the aromatic rice genotypes especially *Gandhakasala* genotypes from other cultivars. Studies on esterase polymorphism revealed the presence of EST 2 band only in Deepthi, indicating its use as a biochemical marker to distinguish aromatic rice genotypes from Deepthi.

Heritability and genetic gain studies indicated that selection of characters like length of sterile glumes, 1000 grain weight, grain length and peroxidase activity may be effective in crop improvement programme.

Correlation and path studies revealed that grain yield could be improved by simultaneous selection for high seedling height, grain breadth, milling recovery and straw yield.