CHARACTERISATION AND EVALUATION OF THE RICE (*Oryza sativa* L.) CULTIVAR NJAVARA

By

G. SRINIVASA REDDY

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

Department of Plant Breeding and Genetics

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2000

DECLARATION

I hereby declare that this thesis entitled **"Characterization and evaluation of the rice (*Oryza sativa* L.) cultivar Njavara"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

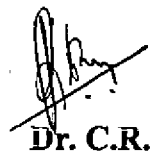
College of Horticulture
Vellanikkara – 680 656
Date: 25-01-2000

G.Reddy.
G.SRINIVASA REDDY

CERTIFICATE

Certified that this thesis entitled **"Characterisation and evaluation of the rice (*Oryza sativa* L.) cultivar Njavara"** is a record of research work done independently by **Sri.G. Srinivasa Reddy**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Place: Vellanikkara
Date: 25-1-2000



Dr. C.R. Elsy
Assistant Professor & Chairperson
Advisory commitee
Department of Plant Breeding & Genetics
College of Horticulture
Vellanikkara – 680 654

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Sri.G. Srinivasa Reddy**, a candidate for the degree of **Master of Science in Agriculture**, agree that the thesis entitled **"Characterisation and evaluation of the rice (*Oryza sativa* L.) cultivar Njavara"** may be submitted by Sri.G.Srinivasa Reddy, in partial fulfilment of the requirement for the degree.

Dr. C.R.Elsy
(Chairperson, Advisory commitee)
Assistant Professor
Dept. of Plant Breeding & Genetics
College of Horticulture
Vellanikkara

Dr.K.Pushkaran
(Member, Advisory committee)
Associate Professor & Head
Dept. of Plant Breeding & Genetics
College of Horticulture
Vellanikkara

Dr.V.V. Radhakrishnan
(Member, Advisory committee)
Associate Professor
Dept. of Plant Breeding & Genetics
College of Horticulture
Vellanikkara

Dr. A.Augustin
(Member, Advisory committee)
Assistant Professor
AICRP (M&AP)
College of Horticulture
Vellanikkara

31/01/2000

EXTERNAL EXAMINER
(M. RANGASWAMY)

ACKNOWLEDGEMENT

I have great pleasure to place on record my deep sense of gratitude and sincere thanks to Dr.C.R.Elsy, Assistant Professor, Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara and chairperson of my advisory committee for assigning this problem for study, for her meticulous and gracious guidance all through the course of my research work as well as in the preparation of the manuscript.

I am extremely grateful to Dr.K.Pushkaran, Associate Professor and Head i/c, Department of Plant Breeding and Genetics for his support and critical scrutiny of the manuscript.

I am very much grateful to Dr.V.V.Radhakrishnan, Associate Professor, Department of Plant Breeding and Genetics and member of my advisory committee for his valuable suggestions during various stages of the study and critical scrutiny of the manuscript.

I am thankful to Dr.A.Augustin, Assistant Professor, AICRP on M & AP and member of my advisory committee for his keen interest and the facilities provided during the course of my research work.

I am thankful to Dr.A.I.Jose, Associate Dean, College of Horticulture, for all the facilities provided for my research work.

I express my sincere gratitude to Dr.Nizar, Scientist, NBPGR Regional Station, Vellanikkara for his help and for sparing the valuable seed material for the study.

It is my pleasant privilege to express my utmost gratitude to Dr.V.K.G.Unnithan, Associate Professor, Department of Agricultural Statistics for his expert advice and everwilling help rendered in the statistical analysis of the data.

I take this opportunity to extend my gratitude to all the teaching and non-teaching staff of the Department of Plant Breeding and Genetics,

Biochemistry Laboratory and the Agricultural Research Station, Mannuthy for their support at different stages of the study.

My heartful thanks are due to Dr.Leenakumari, Associate Professor, Regional Agricultural Research Station, Pattambi for her sincere help and for sparing the seed material for the study.

I am thankful to Mr.Sreekumar, P.K.(CCRP) for the help given to me for photographic works.

My thanks are due to Mr.Achutan, C.R. and Mr.Babu, T.D., Research Associates (Biotech Centre) for their valuable suggestions during the course of isozyme studies.

My cordial thanks are due to my loving friends, Niranjan, Prakash, Govind, Shirish and Vineel.

The award of Junior Research Fellowship by the Indian Council of Agricultural Research is greatly acknowledged.

Reddy.

G.Srinivasa Reddy

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INTRODUCTION

Rice (*Oryza sativa* L.) is grown in over 110 countries of the world totalling 146 million ha and 579 million tonnes of grain. This caters to the needs of more than half of the world's population. Rice is the staple food for the people of East, Southeast and South Asia, where 90 per cent of the world's rice is grown and consumed. In India, rice occupies an area of 43.28 million ha with an annual production of 81.31 million tonnes.

Rice provides more food energy per hectare, more food protein yield per hectare (slightly lower than that of wheat) and a higher net protein utilization value than other cereals. Medicinal uses of rice especially in Ayurvedic system of medicine were well documented.

Njavara is a unique, extra short duration rice cultivar indigenous to Kerala and is widely utilised in the Ayurvedic system of medicine, especially in Panchakarma treatment involving baths and massages for curing paralytic condition (Nadkarni, 1954). Besides, Njavara grain is being utilized in treatment of circulatory, respiratory and assimilatory ailments and in certain parts of the state this peculiar variety is utilized as a starter solid food for infants and as a food grain for the invalids. To a very limited extent it is also used as a food grain crop as a choice variety. Eventhough, this cultivar fetches higher market price due to its increasing demand in Ayurvedic treatment, no serious attempts appear to have been made to collect, catalogue, characterize and evaluate these cultivar types.

Based on glume colour differences, two types of Njavara are recognised, the black and golden yellow glumed types. Ecotypes of this cultivar are present in different localities of the state and hence it is necessary to collect all types under the popular vernacular broad name 'Njavara' for characterisation and evaluation. It is popular as 'Shastika' among Ayurvedic physicians. The importance of this attempt has also to be seen in the context of extension of

Intellectual Property Rights (IPR) to agricultural sector. India now being a signatory to General Agreement on Tariffs and Trade (GATT), conservation, characterisation and registration of indigenous bioresources are attaining significant importance to protect our "Sovereign rights on biodiversity".

Moreover, due to the spread of high yielding and improved varieties, there is every chance that this unique variety of Kerala may become extinct in the near future and therefore collection and conservation of the cultivar deserves serious immediate attention.

Hence this investigation is aimed

1. to collect the different ecotypes of Njavara and to characterize the types based on morphological and biochemical studies.
2. to evaluate the variability available in Njavara and
3. to evaluate the nutritional qualities of the cultivar in comparison with the most prevalent and popular short duration local variety , Ptb-10.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Njavara – a medicinal rice cultivar

Njavara is the unique rice cultivar grown only in Kerala, from time immemorial, bestowed with extra short duration. This is the only cultivar traditionally used effectively in the Ayurvedic system of medicine in certain specific treatments like Panchakarma. It is interesting to note that this treatment is now getting more and more popular, not only in this region of the country but also in other parts of the nation and even in other countries. However informations on the chemical constituents that make the cultivar unique from other varieties are lacking. 'Ashtanga Hridaya' (Vagbhata, 500 B.C.) described two types of Njavara - black and white glumed, of which the latter is superior. However, the yield and quality of Njavara varies with the location. The 'Sushrutha Samhitha' (Sushruthacharya, 2500 B.C.) cited Njavara as a special cereal, having properties to rectify the basic ills affecting the circulatory, respiratory as well as the digestive system. However, it described black glumed Njavara as being the best.

Elsy *et al.* (1992) studied the morpho-physiological characters of black glumed Njavara type exhibiting an extra short growth duration of 69 days with a good source-sink relationship.

Menon and Potty (1996) studied the nutritional uptake of Njavara under different management conditions and systems of cultivation. They suggested the possibility of qualitative variations existing between the black and golden yellow glumed types.

A very early report on the medicinal use of rice was made by Watt (1891) who recorded the use of rice poultice as a substitute for that of linseed meal. Fu *et al.* (1991) reported anthocyanidin, a heart stimulant glucoside, flavanones and steroids as the medicinally active constituents of black glutinous rice.

2.2 Morphological characters

Morphological traits are the oldest and widely used markers because of their simplicity and rapidity. Morphological characterization is essential to establish the distinctness of varieties and also for formulating an efficient and effective crop breeding programme.

2.2.1 Seedling height

Rapid seedling growth is considered as a desirable trait of tropical rice varieties, particularly when combined with early maturity. It enables the young plants to become fully established before weeds become a problem. The relationship between shoot weight and grain weight was less exact and plant height was well correlated with shoot weight. Seedling height ranged from 14 to 30 cm and was not necessarily correlated with plant height at harvest i.e. rapid seedling growth was not necessarily associated with tall, leafy varieties at later stages (Anon., 1968). Faster early growth suppressed weeds and is essential for early maturing varieties (Yoshida, 1977).

2.2.2 Leaf

Tanaka *et al.* (1966) considered that leaf length was the primary factor affecting leaf size though there was quite a deviation. They also considered that leaf spread was correlated with leaf length i.e. the longer the leaves, the more bent they are.

Glabrousness of leaves was associated with glabrousness of lemma and palea (Sharma, 1989).

Rice breeders from experience considered that small, dark green and erect leaves were desirable in varieties to be grown under high nitrogen conditions

(Tsunoda, 1965; Jennings and Beachell, 1965). Matsushima (1966) considered that the upper three leaf blades of the plant should be short, thick and erect to increase the light utilizing efficiency and consequently the percentage of ripened grains. The leaf area index (LAI) should be 5-6. McKenzie *et al.* (1987) reported that rice breeders often select for a favoured plant type. Plant with narrow, erect, dark green leaves and long erect flag leaf was more preferred.

Leaf area index (LAI) at flowering is one of the important factors for high dry matter production. A critical LAI may be obtained by various combination of factors such as number of tillers per unit area, number of leaves per tiller and length and width of leaves. The LAI at flowering was an important way to measure the capacity of a plant community to increase its dry matter weight (Anon., 1964). Many reports indicated that a large leaf area with a narrow leaf angle was closely related to high yielding ability (Yamagata, 1997).

Chang and Tagumpay (1970) reported that erect flag leaves in semidwarf lines and erect leaves below the flag leaf in intermediate and tall lines showed the largest correlation value with grain yield. Thakur (1981) reported leaf angle as erect, horizontal or droopy and was largely influenced by leaf length. The wider the angle, the more the spread of leaves for light interception, especially in the lower leaves. In the ideal plant type, interception of light would be low. One way to achieve this is to develop rice plant types with minimum leaf attachment angles. Plant population per unit area could be increased without adversely affecting photosynthetic rates. The trait was linked with a simply inherited rudimentary juncture condition.

Semi erect flag leaf was considered to enhance photosynthetic efficiency of the rice plant particularly at the grain filling stage (Sharma, 1989).

Beachell (1964) reported that slow leaf senescence is a desirable trait in nitrogen responsive plant types.

2.2.3 Ligule

Ligule colour could be white, purple or with purple lines. Ligule shape varied as acute, acuminate, 2-cleft or truncate (Anon., 1980).

2.2.4 Days to 50 per cent heading

Flowering duration of rice is influenced by several environmental factors, the chief of them being the time of sowing the seeds (Ramiah, 1933). Maturity duration (length of growing season) is an important consideration (Adair *et al.*, 1966).

Ramiah (1954) reported that a very definite and strong association of height and flowering duration exist. The correlation coefficient between these two characters is generally positive and in some cases it is negative.

Days to flowering was positively correlated with plant height (Saini and Gagneja, 1975), panicle length, grains per panicle, 100 grain weight (Reddy and Reddy, 1981) and number of productive tillers (Amirthadevarathinam, 1983). However, days to panicle emergence was found to be negatively correlated with 100 grain weight (Saini and Gagneja, 1975), plant height, number of productive tillers and panicle length (Dhanraj *et al.*, 1987).

Sampath (1984), Rao and Jagadish (1987), Manuel and Palanisamy (1989) observed positive correlation between days to panicle emergence and grain yield. However, negative association between them was reported by Brar and Saini (1976), Dhanraj *et al.* (1987), Kalaimani and Kadambavanasundaram (1988).

Days to flowering varied from 45 to 150 days in rice varieties of Kerala (Leenakumari and Nayar, 1996).

2.2.5 Culm

Alam (1931) suggested that height of plant had some practical utility and stated that there exists . definite correlation between the length of the stem and the number of tillers formed i.e. taller plants formed fewer tillers. Chang and Jodon (1965) reported positive correlation between plant height and panicle length and plant height and percentage of bearing tillers. Tanaka *et al.* (1966) reported that tall stature was associated with long leaves. He also reported that harvest index of rice was negatively correlated with plant height.

Plant height and lodging resistance were negatively correlated at one per cent level of significance. Partial correlation coefficients showed that variation in height alone could control about 33 per cent of lodging resistance (Anon., 1968). Positive correlation between plant height and grain yield was observed by Jangale *et al.* (1987) and Deosarkar *et al.* (1989). However, negative correlation between them was reported by De and Rao (1988).

Semidwarfism in rice may be defined as a distinct genetic reduction in the culm length with little effect on panicle characteristics. Researchers had observed a number of features associated with the semidwarf plant type. Semidwarf plants showed increased tillering ability and responsiveness to nitrogen fertilization, both of which contributed directly to high yield (McKenzie *et al.*, 1987).

An efficient rice cultivar would be one which occupies minimum horizontal space and maximum vertical space which is dependent on the height of the plant (Janoria, 1989). Based on culm length, rice plant could be classified into dwarf, short, medium, tall and very tall plant types (Richharia and Govindaswami, 1990). In the rice varieties of Kerala, culm length varied from 75 cm to 180 cm (Leenakumari and Nayar, 1996).

Ear length and number of tillers contributed mainly to yield and the number of ear bearing tillers was the most potent yield component in rice crop (Kalaimani and Kadambavanasundaram, 1988; De and Rao, 1988). However, Gomathinayagam *et al.* (1988) reported negative correlation between grain yield and tiller number.

Matsushima (1966) considered that rice should have large number of culms which are short in culm height as well as panicle length to protect against lodging and to increase the percentage of ripened grains. Generally 9 to 13 tillers per plant was an ideal range for good varieties (Sharma, 1989).

Based on culm number, rice varieties could be classified into low, medium and high tillering varieties (Richharia and Govindaswami, 1990). Tiller number and panicle number were positively correlated although the ratio of tillers to panicles varied among cultivars. High tillering was adopted at wide range of spacings, capable of compensating for missing hills, permitted faster leaf area development in transplanted rice (Yoshida, 1985).

Semi erect habit of the rice plant is preferable to erect or spreading habit. Erect habit retarded sunlight interception due to intershading. In the case of spreading habit, the leaves of neighbouring plants shade one another and did not allow utilization of solar energy (Sharma, 1989).

High and positive correlations were found between the shoot and panicle characters studied. Panicle length and weight and number of spikelets and grains tended to depend more on stem diameter and shoot length than any of the shoot characters (Enyi, 1956).

The structural strength of rice culms can be partly indicated by the slenderness ratio (l/r) which is quotient between the length of the culm and the

radius of the culm at BI_1 (basal internode 1). Indica varieties with slenderness ratios greater than 600 were liable to bend or lodge (Anon., 1964).

Internode colour in rice cultivars varied from green, yellow (gold) to various shades of purple (Richharia and Govindaswami, 1990).

2.2.6 Panicle

Panicle length showed positive correlation with plant height and growth duration (Chandraratna, 1964; Chang *et al.*, 1973). Sivasubramanyan *et al.* (1968), Saini and Gagneja (1975), Nagesha (1976) and Natarajamoorthy (1979) found significant correlation between panicle length and number of grains per panicle.

Lin (1970) reported negative correlation between panicle length and days to flower in some early varieties of rice. Similar trend was noticed by Yang (1970) in F_2 population of a rice cross and by Majumdar *et al.* (1971) in ten varieties of rice.

Various degrees of positive association between length of the panicle and 1000 grain weight was reported by Reddy and Goud (1970), Lenka and Misra (1973), Sindhu (1973), Natarajamoorthy (1979) and Rao *et al.* (1980). Positive association between panicle length and days to flower was reported by Talwar and Goud (1974), Saini and Gagneja (1975) and Nagesha (1976).

Jangale *et al.* (1987) and Kalaimani and Kadambavanasundaram (1988) reported positive correlation between panicle length and grain yield.

Variability for panicle length expressed in terms of coefficient of variation ranged from 5.64% (Lal *et al.*, 1983), 9.63% (Vinod, 1989) to 11.08% (Singh *et al.*, 1984).

Bhide (1926) and Ramiah (1930) reported that compact panicle was simple recessive to lax panicle. They have also inferred the association of compact panicle with short grain.

Panicle exsertion varied from completely enclosed to partly enclosed and from exserted to fully exserted. The extreme types of poor exsertion was controlled by three recessive genes (Sethie *et al.*, 1937).

Chandraratna (1964) considered poor exsertion of panicle as an undesirable trait as it promoted spikelet sterility and blast infection. Palanisamy (1968) in an attempt to study the relationship between certain plant characters in some rice varieties, found that TKM 6 with fine grain had the longest panicle exsertion, while CO29 possessing medium grain showed lesser exsertion and Ptb-10 a coarse grain variety had the least panicle exsertion.

The shedding or shattering of grain from the ear at the time of harvest is one of the important factors contributing to loss in yield of rice. While a certain amount of mechanical shedding is inevitable and desirable (so as to facilitate threshing), the aim of breeder should be to limit shedding to the minimum (Ghose *et al.*, 1960). Sahu and Sahu (1981) reported that grain shattering in rice varieties ranged from 0-35 per cent. In 68.6 per cent of the varieties, grain shattering was between the range of 1-5 per cent and 6-10 per cent.

The spikelet of a rice plant is attached to the pedicel. At maturity, the region of their articulation becomes very weak in wild rices and the spikelets shatter very easily. In some varieties, the articulation is absent and threshing becomes very difficult. In most cultivated varieties, the articulation is intermediate and ideal; it does not lead to shattering of grains in the field and at the same time, allows easy shattering. This character was influenced by environment (Sharma, 1989).

2.2.7 Spikelet

Rice cultivars are usually awnless or have short-tip awns. This characteristic was controlled by only a few genes, but in some cases, the degree of awning was influenced by environment (Jennings *et al.*, 1979; Chang and Li, 1980). Awning is not a favoured character because it causes inconvenience in seed handling and is eliminated while breeding. Some feel, however, that awns might contribute to photosynthesis and hence to grain filling. Awns were also supposed to afford protection against bird damage. Many traditional upland varieties were awned (Sharma, 1989). The existence of awns had adaptive significance in relation to transpiration from spikelets (Takeda, 1997).

Apiculus colour at heading ranged from pink to dark purple for which several isoalleles had been described. When only the presence or absence of colour was considered, it was controlled simply by three complementary genes (Oka, 1991).

Adair *et al.* (1966) considered smooth hull as a desirable trait in mechanically harvested and processed rice. The length and density of five hairs on palea and lemma were different among cultivars and there were geographical differentiations. The hairiness character controlled by 'gl' (glabrous) gene, was preferred in seeding and harvesting because of small rubbing. It was reported however, that 'gl' gene exerted pleiotropical effects on rice grains toward a smaller size (Takeda, 1997).

It had been recently shown that varieties with long sterile glumes possessed high protein content varying from 9.4 per cent to 11.3 per cent (Richharia and Govindaswami, 1990). The length of an empty glume was in a range of 1/3 to 1/5 of that of a spikelet in ordinary rice cultivars, although there were variants with a long empty glume possessing almost the same length as spikelet (Takeda, 1997).

Variability for chaff percentage expressed in terms of coefficient of variability (C.V.) ranged from 42.72 per cent (Vivekanandan, 1985) to 47.41 per cent (Singh *et al.*, 1980).

2.2.8 Grain

The weight of the grain is of economic importance. Choudhury (1967) found 100 grain weight to be the best indicator in differentiating varieties.

Saini and Gagneja (1975) reported positive correlation between 100 grain weight and days to flower. However, Talwar and Goud (1974) reported negative association between 1000 grain weight and days to flower. Similar trend was reported by Nagesha (1976).

Negative association between earbearing tillers and 1000 grain weight was reported by many workers like Reddy and Goud (1970), and Mishra *et al.* (1973). However, Nagesha (1976) and Satpathy and Nanda (1978) reported positive association between ear bearing tillers and 1000 grain weight.

Kambayashi *et al.* (1984) studied protein content in relation to grain weight and found that endosperm weight was negatively correlated with protein content.

Ghorai and Pande (1982) reported negative association between yield and 1000 grain weight in rice. However, positive correlation between them was reported by Jangale *et al.* (1987), Sampath *et al.* (1989) and Kannanbabu and Soundarapandian (1990). In rice 1000 grain weight ranged from 12.0 g to 47.5 g (Richharia and Govindaswami, 1990).

Grain dimensions have formed the basis of systems of classification mainly on account of their constancy and partly because their mean values decide

commercial grading. In rice varieties, the length of unhulled grain ranged from under 5 mm to over 14 mm. The range in breadth was 1.9-3.7 mm and the range in thickness was 1.5-2.2 mm. In rice, considerable variation in grain size ranging from long bold to short bold was noticed. There was negative association of Kernel length with hulling, milling and head rice recovery (Anon., 1971a).

Small grain size is related to fast grain filling, adapted to areas having drought at later stages of ripening (Yoshida, 1977). Larger grain size was clearly advantageous for early growth as well as for grain filling and yield (Anon., 1979a). Larger grain size was positively correlated with seedling vigour, alpha-amylase activity and seedling respiration rate (Chen *et al.*, 1986; Lee *et al.*, 1986).

The physical properties of the rice grain are determined by the dimensions, shape and weight of the grain and the hardness of the endosperm. Preferences for grain size and shape vary from one group of consumers to another. In general, long grains are preferred in the Indian subcontinent, but in South-East Asia, the demand is for medium to long rices. There was a strong demand for long grain rice in the international market (Anon., 1979b).

Amongst the morphological characters, grain length, length/breadth ratio, grain pubescence and size and colour of sterile lemmas appeared to be quite stable characters and could, therefore, be used as primary diagnostic characters in the classification of paddy varieties. The classification of paddy varieties into different classes on this basis was clear cut and without any ambiguity. Other morphological characters, namely, grain colour, colour of sterile lemma, awns, kernel translucency and abdominal white were also found to be useful and paddy varieties could be grouped into distinct classes on the basis of each of these characteristics. However, they may be altered by external factors. These characters, therefore, should be used as secondary diagnostic characters (Gupta and Agrawal, 1988).

Rice grain is marketed according to its size and shape. Grain type was one of the major quality characteristics where fine and superfine varieties were more preferred (Chaubey *et al.*, 1988). Market quality was determined by the physical appearance of the grain (size and shape; colour of brown rice or milled rice), test weight of the grain, percentage of head rice (whole kernels) and brokens (Chang, 1997).

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 (Buttery *et al.*, 1982). Western Regional Research Center (WRRC) chemists at the USDA reported that 2-acetyl-1-pyrroline ranged from 0.04 to 0.09 ppm in cooked rice by dry basis in the eight aromatic rices, but was only less than 0.01 ppm in the nonaromatic rices (Anon., 1983).

Varieties on the basis of strength of aroma could be classified into non scented, scented and strongly scented types (Richharia and Govindaswami, 1990). Aroma is an important element in the flavour of food and its taste is adversely affected without aroma. Fresh cooked rice of new crop emits a special aroma which sharpens one's appetite. The strength of aroma in aromatic cultivars ranged from 0.25 to 1.50, while that of ordinary rices was zero (Tsuzuki, 1997).

2.2.9 Maturity

Earliness and yield were correlated at 5 per cent level (Anon., 1968). The achievement of high yields by growing early maturing varieties is a desirable goal because it would result in a more efficient daily production of carbohydrates and more efficient utilization of land (Anon., 1971b). Early maturing varieties increased grain production per day, increased water use efficiency, required close spacings to achieve yield potential, while late maturing varieties were adapted to low fertility conditions (Yoshida, 1977).

The early maturing varieties are of special importance for tracts with low and uncertain rainfall or tracts where the growing season is short due to the early setting in of low temperature or tracts which are subject to drought. The early maturing varieties were adapted to a wider range of sowing (Ghose *et al.*, 1960). Rao (1988) reported that early varieties showed higher percentage of high density grains among primary tillers while no difference was observed in late cultures between primary and tertiary tillers. The term high density grain is referred to those grains which are filled to their maximum potential size.

Dhulappanavar and Mensinkai (1967) classified the Indian rice varieties broadly into four maturing groups namely very early (110 days or less), early (110-140 days), late (150-170 days) and very late (180 days). Breeders develop cultivars that have a range of maturity to permit the producer to spread out the planting and harvest seasons. Environmental factors, especially temperature affect the rice flowering dates and maturity. Cultivars varied considerably in maturity when grown in different regions (McKenzie *et al.*, 1987).

The duration (seed to seed) of rice crop varied from 60 to 210 days depending upon genotype, its photosensitivity and day length during the cropping season (Sharma, 1989).

Elsy *et al.* (1992) reported Njavara was an extremely short growth duration rice variety maturing in 69 days. The duration of rice varieties of Kerala ranged from 70-190 days. Hraswa and Ptb-10 are two short duration varieties, maturing in 70-80 days and 95-100 days respectively (Leenakumari and Nayar, 1996).

2.2.10 Grain and straw yield

Chandraratna (1964) reported that the number of grains per plant had highly significant correlation with yield followed by yield of straw. A highly

significant correlation between the number of ears, tillers and yield of straw was also noticed.

Morphological characters associated with high yield potential were short, small, thick and erect leaves, short and thick culms, tight leaf sheaths, upright and compact tillers, high tillering ability, high fertility of spikelets at high nitrogen rates and high harvest index (Yoshida *et al.*, 1972; Chang and Li, 1991). More tillers per plant might have provided a means to increase the number of panicles per unit area. Larger panicles with more florets per panicle, larger grain size and a reduction in floret sterility had a positive effect on yield (McKenzie *et al.*, 1987).

Variability for grain yield in rice, expressed in terms of coefficient of variation, as reported by various workers was 33.07% (Paramasivam, 1981), 55.78% (Vivekanandan, 1985) and 50.29% (Sahu and Sahu, 1990). Elsy *et al.* (1992) reported an average yield of 2.5 t/ha for Njavara while Menon and Potty (1996) reported an average yield of 2.4 t/ha for the same variety. In the rice varieties of Kerala, grain yield ranged from 2 to 7 t/ha with Ptb-10, a short duration variety, yielding 2.5 t/ha (Leenakumari and Nayar, 1996).

In recent years, rice breeders have come to realize that increasing grain yield is a complex objective that in turn entails several factors like yield potential under the most favourable conditions, yield stability across season and location, and crop productivity on a daily field-use basis. Yield potential could be estimated from a number of component traits under high inputs like panicle number/m^2, number of filled grains/panicle, grain weight and harvest index. Grain yield in the tropics was generally markedly higher in the dry season than in the wet season mainly because of a difference in solar radiation intensity (Chang, 1997).

2.2.11 Pest and disease incidence

Rice varieties that are resistant to major diseases and insects would suppress the build up of insect and plant pathogen populations and thus minimize the yield losses (Beachell and Khush, 1969). Rice varieties of Kerala such as Nila, Aathira, Aiswarya, Aruna and Makom are moderately resistant to commonly occuring pest and diseases like Brown Plant Hopper, Gallmidge and Sheath Blight (Leenakumari and Nayar, 1996).

2.3 Biochemical Characterisation

2.3.1 Protein polymorphism

Dickerson (1972) has pointed out that proteins are valuable taxonomic characters because they exhibit conservatism in evolution.

Leaf and seed proteins have a greater specifity than isozyme markers but are complicated to assay (Ladizinsky and Hymowitz, 1979). Wheat workers had used seed proteins to identify the major commercial varieties of the United States (Jones *et al.*, 1982). The protein was used as a marker in studying population structure, phylogenetic relationship between species, the functioning of plant genome, the selection of promising genotypes as parents for hybridization, gene mapping and the construction of genotypes with the required combination of characters (Vodenicharova, 1989).

In an electrophoretic analysis of proteins of caryopses in Brazilian rice cultivars, genetic similarity ranged from 95.6% to 100%. Based on dendrogram of genetic similarity, the cultivars were assigned to various groups with different degrees of similarity. This approach allowed morphologically similar cultivars to be differentiated (Guidolin *et al.*, 1994). In a study by Jun and Xing (1995) leaf proteins were extracted at several growth stages from 3 male-sterile lines and a normal rice cultivar grown under long and short days. The proteins were separated

using SDS-PAGE. The results revealed that at the intermediate stage of glume flower primordium differentiation in the male sterile cultivars, a protein band designated as PPO-1 was present only under long days while in the normal cultivar, the protein was present under both long and short days.

Electrophoretic profiles of seed storage polypeptides were obtained in 58 Brazilian rice varieties from 5 states. Cluster and discriminant function analysis were applied to the electrophoretic data which enabled phenetic distance to be evaluated and 10 affinity groups to be distinguished showed several correlations with geographical distribution. This indicated that the electrophoretic analysis of seed proteins could be used to estimate the genetic relationship among rice varieties and to study the variability even among those of similar genetic background (Montalvan *et al.*, 1995).

2.3.2 Isozyme Analysis

Pawar and Gupta (1975) found variation in the peroxidase isozyme patterns in tall and dwarf varieties of rice. Some isozymes appeared at a particular developmental stage while others disappeared and some remained constant once they were activated. Specific bands A_3 and A_7 were observed in the tall variety and A_4 in the aroma variety.

A number of rice geneticists had used electrophoretic techniques to add isozyme loci to the linkage groups as additional gene-markers (Pai *et al.*, 1975; Ranjhan, 1988). Others have used isozymes to determine the genetic divergence among cultivars and their wild relatives (Nakagahra and Hayashi, 1977; Glaszmann, 1986; Second, 1986).

Dai *et al.* (1978) reported that the male sterile (MS) line of rice had higher peroxidase (PRX) activity than the maintainer line during the tetrad stage and the early uninucleate stage. Li *et al.* (1982) studied the correlation of the PRX

isozyme with heterosis in hybrid rice. In the F_1 of some combinations with good heterosis, a number of complementary bands with high activity were observed.

The peroxidase and acid phosphatase patterns were studied by Phul *et al.* (1987) in the anthers of five male sterile (MS) lines and their maintainer lines. They observed differences in the intensities of bands in the MS and their corresponding maintainers. The better expression of enzymes in the sterile anthers indicated the role they played in breaking down various metabolites that were otherwise important for the formation of fertile anthers.

The study of isozymes had many useful applications which included study of genetically defined variation (genetic analysis, confirmation of hybridity, marking of monogenic traits, analysis of aneuploids, triploids and somaclonal variation), general variation (genetic diversity in germplasm collections and cultivar identification), multilocus analysis (pedigree, heterozygosity, heterosis, ploidy level, quantitative traits, linkage maps and wide crosses) which was assisted by the lack of epistatic or pleiotropic interactions of isozyme markers unlike morphological or physiological characters (Weeden, 1989).

Hen *et al.* (1994) reported that esterase band number and zymogram type were closely related to the morphological characteristics of the cultivars in rice. The zymograms from some plant parts could be used for cultivar identification.

Though isozymes are useful in revealing polymorphism and in distinguishing between a known and an unknown entity the isozymes bear little or no relation to their function in physiological processes and even less in quantitative traits. Genetic affiliation must be studied by crossing and using different methods of progeny analysis. However, intensified activities in isozyme and linkage studies had added a large number of isozyme loci to the linage map which would be helpful in tagging closely related genes (Chang, 1997).

2.4 Nutritional qualities of grain

Having developed the dwarf high yielding varieties of rice, the problem of improvement of quality features such as desirable grain type, high hulling, good cooking behaviour and protein content comes before the agricultural scientists to cater to the demand for quality rice in domestic market as well as for exports for valuable foreign exchange (Pathak and Upadhyay, 1975).

2.4.1 Soluble carbohydrate

Brown rice contains 0.83-1.36 per cent total sugars as glucose with reducing sugars ranging from 0.09-0.13 per cent. Milled rice contains 0.37-0.53 per cent total sugars with 0.05-0.08 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 roles in regulating metabolic activities in addition to providing essential carbon source for the growth of young seedlings and maintaining turgor pressure for the expansion of tissue during germination.

2.4.2 Protein content

High protein content was associated with early heading and light grains (Lambers *et al.*, 1973) and a darker colour of the milled rice (Chang, 1997).

Pathak and Upadhyay (1975) reported negative correlations between the contents of protein and starch, protein and volume expansion and protein per cent and water uptake.

Rice is the single most important source of protein in the diets of tropical Asia, because of the amount consumed. Hence protein of rice was considered as an indicator of its nutritional quality (Juliano, 1978). Among the cereals, the protein of rice is one of the most nutritious. Protein of milled rice was relatively low (about 7% at 14% moisture). A large portion of the total variability in protein was attributable to environment. However, true genetic differences in protein existed.

Protein properties along with starch properties and aroma determined cooking characteristics and nutritive properties of cooked rice (Chang and Somrith, 1979). Nutritional quality was largely determined by protein and lysine contents. Rice varieties generally had 6-8 per cent total protein in the brown rice and one per cent less in milled rice. Lysine contents of rice varieties (3.8-3.9 g/16 g N) were quite high among the major cereals. Rice excelled over other cereals in digestibility when fed to infants (Juliano, 1985).

The brown rice protein content of 17,587 cultivars maintained at International Rice Research Institute (IRRI) ranged from 4.3 to 18.2 per cent with a mean of 9.5 per cent. Mean brown rice protein of *japonica* rices was higher than that of *indica* rices, with means of 11.8 per cent and 9.8 per cent respectively and coefficients of variation of 16 per cent and 21 per cent (Gomez, 1979). Protein content of brown rice varied from 7.1 per cent to 15.4 per cent in weight depending on cultivars (Tanaka, 1997).

2.4.3 Free amino acids

In a study by Zhang and Tang (1986) the free amino acid pool in rice, was dominated by serine, alanine, aspartate and glutamate during the embryo differentiation stage. In the maturation stage, serine, alanine, arginine and lysine were the main components. These amino acids might play an important role in regulating the availability of the whole amino acid pool.

Menon and Potty (1996) reported free amino acid contents of 0.316 mg/g and 0.089 mg/g respectively in black and golden yellow glumed Njavara cultivars grown under wetland conditions. Black glumed Njavara contained the amino acids DL-2-amino-n-butyric acid and DL-iso-leucine while, golden yellow glumed Njavara contained L-Histidine monochloride, L-ornithine monochloride and DL-iso-leucine.

2.4.4 Fat content

Total fat concentrations for 241 milled rices was 0.65 per cent with a range of 0.19 per cent to 2.73 per cent. Fat was unevenly distributed within the endosperm, the highest concentration being in the outer layer and the lowest in the central portion (Luh, 1980).

Lipids were contained in all tissues of the plant and are mainly deposited in the aleurone layer and embryo of the seed. They were not only an essential energy source for germination and growth, but also an important nutritional source for the human beings. The lipid content in the rice grains ranged from 2.3 per cent to 3.9 per cent and differed a great deal among parts of rice plants (Okuno, 1997).

2.4.5 Amylose content

Many of the cooking and eating characteristics of milled rice are influenced by the ratio of two kinds of starches, amylose and amylopectin, in the rice grain. Amylose is almost absent from the waxy (glutinous) rices. Such rices do not expand in volume, are glossy and sticky and remain firm when cooked. Nonwaxy (nonglutinous) rices may have high (25 to 30 per cent), low (10 to 20 per cent) or intermediate (20 to 25 per cent) amylose content. These rices (nonwaxy rices) show high volume expansion (not necessarily elongation) and a high degree of flakiness. They, cook dry, are less tender and become hard upon cooling. Low-amylose rices cook moist and sticky. All of the *japonica* varieties of temperate regions had low amylose

content. Intermediate amylose rices were the preferred types in most of the rice growing areas of the world (Anon., 1979b). Indian varieties were generally high amylose types (Sharma, 1989).

Gomez (1979) grouped rice varieties, on the basis of their milled rice amylose contents, into waxy (0-2 per cent amylose), very low amylose (2-9 per cent), low amylose (9-20 per cent), intermediate amylose (20-25 per cent) and high amylose (25 per cent and greater).

Amylose content was the major determinant of cohesiveness, tenderness, colour and gloss of cooked rice (Singh, 1984). High amylose rice *indica* type, which had amylose amounting to 25 per cent or more is less sticky. The high amylose rice was appropriate for certain cooking styles, for example, fried rice (Amano, 1997).

2.4.6 Starch grain characterisation

Starch accumulates in the form of grains that range in size from 1 to 150 μ, the grains in chloroplasts usually being much smaller than those in the leucoplasts. Starch grains varied in structure as well as in size and were so characteristic of the species that an expert could determine the source of isolated starch grains by microscopic examination (Greulach, 1973). Starch grains were globoid, oval, dumb-bell shaped, flat or multifaceted (Bilgrami *et al.*, 1979).

Starch appears in the form of grains, which commonly stain bluish-black with a solution of iodine in potassium iodide. Starch grains are first formed in chloroplasts. Later the starch is broken down and moves as sugar to storage tissues where it is resynthesized in amyloplasts. Starch grains commonly show layering around a point termed hilum. The position of the hilum, the shape and size of grains and their appearance, solitary or in aggregates (compound starch grains), made it possible to identify the plant species from which the starch was obtained (Fahn, 1990).

2.4.7 Amylase activity

In rice varieties, the activities of alpha-amylase and Beta-amylase were initially low but started to increase within 3-4 days of sowing. Alpha-amylase was the major enzyme of starch degradation in the endosperm of rice (Anon., 1971b).

Starch breakdown during the germination of cereal seeds is the result of the action of hydrolytic enzymes and only through the concerted action of alpha-amylase, Beta-amylase, debranching enzyme and alpha-glucosidase can starch be hydrolysed completely. Among the cereal seeds tested under anoxia, only rice was able to degrade nonboiled, soluble starch, reflecting the ability to degrade the starch granules *in vivo* (Guglielminetti *et al.*, 1995).

2.4.8 Flavonoids

Buttery *et al.* (1983) analysed eight aromatic rices from several countries for aroma and found that they were richer in the principal aroma compound 2 acetyl-1-pyrroline than nonaromatic rices which contain only 0.004-0.006 ppm of this compound.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was carried out at the Department of Plant Breeding and Genetics and Biochemistry Laboratory, College of Horticulture, Vellanikkara during 1997-1999. Field experiments relating to the investigation were laid out at the Agricultural Research Station, Mannuthy, which is located at an altitude of 15 m above the mean sea level (MSL) and is situated at a latitude of 10°32" N and 76°10" E longitude. The soil is a laterite loam.

3.1 Materials

Thirteen Njavara genotypes viz., seven from the National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Vellanikkara and six collected from different locations of Kerala along with Ptb-10 as check variety formed the material for this study. The details of these genotypes are given in Table 1.

3.2 Methods

Field experiments were carried out during *Kharif* season of 1998 and cultural practices as given in Package of Practices, KAU (1996) were followed for raising the crop. No chemical fertilizers were applied during the crop growth and organic manures alone were applied @ 5 t/ha as basal application. The genotypes were grown in a Randomised Complete Block Design (RCBD) with three replications in plots of 2.0 m x 1.0 m with 10 cm x 15 cm spacing.

3.2.1 Morphological characterization

The Njavara types were characterized and evaluated based on morphological characters. Observations on the following characters were recorded following the 'rice descriptor' published by IRRI, Philippines (Anon., 1980) from 10 randomly selected plants in each replication and the mean worked out.

Table 1. Details of the genotypes utilized in the study

Sl. No.	Genotypes	Indigenous Collection (IC) Number	National Indigenous Collection (NIC) Number	Source	
				Village/ Institute	District
	Njavara types				
1	N1*	145236	-	Mulloorkara	Thrissur
2	N2*	-	18430-B	Chittoor	Palakkad
3	N3*	-	18383-A	Chittoor	Palakkad
4	N4*	-	18383-B	Chittoor	Palakkad
5	N5*	203749	-	Edavanna	Malappuram
6	N6*	203767	-	Tellicherry	Kannur
7	N7*	203771	-	Tellichery	Kannur
8	N8	-	-	Alwaye	Alwaye
9	N9	-	-	Amala Cancer Research Institute	Thrissur
10	N10	-	-	Kangikode	Palakkad
11	N11	-	-	Pattambi	Palakkad
12	N12	-	-	Thrissur	Thrissur
13	N13	-	-	Thootha	Palakkad
	Local check				
14	Ptb-10	A short duration traditional variety released from the Regional Agricultural Research Station, Pattambi			

* Njavara genotypes obtained from NBPGR Regional Station, Vellanikkara

3.2.1.1 Seedling height: At 5th leaf stage, 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: 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: 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: At late vegetative stage, blade surfaces are classified as

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

3.2.1.5 Leaf blade colour: At late vegetative stage, blade colour is classified into seven broad classes as

Code	Guide
1	Pale 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 colour: 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 Leaf angle: At prior to heading stage, the angle of openness of the blade tip was measured against the culm on the leaf below the flag leaf as

Code	Guide
1	Erect
5	Horizontal
9	Drooping

3.2.1.8 Flag leaf angle: Stage after heading, it was measured near the collar as the angle of attachment between the flag leaf blade and the panicle axis. Flag leaf angle was classified as

Code	Guide
1	Erect
3	Intermediate
5	Horizontal
7	Descending

3.2.1.9 Leaf senescence: At maturity stage, the leaves below the flag leaf were observed at the time of harvest for their retention of greenness. Leaf senescence was classified as

Code	Guide
1	Late and slow senescence – two or more retain their green colour at maturity
5	Intermediate
9	Early and fast senescence – leaves are dead when the grains become fully ripened

3.2.1.10 Ligule length: At late vegetative stage, ligule length was measured in millimetres from the collar to the tip. Its absence was denoted by a blank.

3.2.1.11 Ligule colour: At late vegetative stage, the colour of ligule was classified as

Code	Guide
1	White
2	Purple lines
3	Purple

3.2.1.12 Ligule shape: At late vegetative stage, ligule shape was classified as

Code	Guide
1	Acute to acuminate
2	2-Cleft
3	Truncate

3.2.1.13 Collar colour: At late vegetative stage, collar colour can be

Code	Guide
1	Pale green
2	Green
3	Purple

3.2.1.14 Auricle colour: At late vegetative stage, auricle colour can be

Code	Guide
1	Pale green
2	Purple

3.2.1.15 Days to 50 per cent heading: Number of days from seeding to flowering of 50 per cent of the population was counted.

3.2.1.16 Culm length: At a stage after flowering, culm length was measured in centimeters from ground level to the base of the panicle.

3.2.1.17 Culm number: At a stage after flowering, culm number was recorded after full heading as the total number of grain bearing and non-bearing tillers.

3.2.1.18 Culm angle: At a stage after flowering, culm angle readings were based on plants grown in the entire plot. It was broadly classified as

Code	Guide
1	Erect – the angle is less than 30° from the perpendicular
3	Intermediate – the angle is about 45°
5	Open – the angle is about 60°
7	Spreading – the angle is more than 60° but the culms do not rest on the ground
9	Procumbent – culm or its lower part rests on ground surface

3.2.1.19 Culm diameter: At a stage after flowering, culm diameter was measured in millimeters

3.2.1.20 Internode colour: 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

3.2.1.21 Panicle length: At a stage near to maturity, length of panicles was measured in centimeters from the base to the tip of the panicle.

3.2.1.22 Panicle type; 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

3.2.1.23 Panicle secondary branching: 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

3.2.1.24 Panicle exsertion: At a stage near to maturity, the exsertion of the panicle above the flag leaf sheath after anthesis was classified as

Code	Guide
1	Well exserted – the panicle base appears way above the collar of the flag leaf blade.
3	Moderately well exserted – the panicle base is above the collar of the flag leaf
5	Just exserted – the panicle base coincides with the collar of the flag leaf
7	Partly exserted – the panicle base is slightly beneath the collar of the flag leaf blade
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.

3.2.1.25 Panicle axis: At maturity stage, the panicle axis can be

Code	Guide
1	Straight
2	Droopy

3.2.1.26 Panicle threshability: At maturity stage, the matured panicle was grasped by the hand and a slight rolling 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 -- 25-50% of grains removed
3	Easy – more than 50% of grains removed

3.2.1.27 Panicle shattering: At maturity stage, the 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%)

3.2.1.28 Awn presence: 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

3.2.1.29 Awn colour: At maturity stage, the colour of awns was recorded as

Code	Guide
1	Straw
2	Gold
3	Brown (Tawny)
4	Red
5	Purple
6	Black

3.2.1.30 Apiculus colour: At maturity stage, apiculus colour was classified into seven classes as

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

3.2.1.31 Stigma colour: At flowering stage, colour of stigma was classified as

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

Stigma colour was determined from blooming spikelets (between 9 a.m and 2 p.m) with the aid of a hand lens.

3.2.1.32 Lemma and palea colour: 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 (tawny)
5	Reddish to light purple
6	Purple spots on straw
7	Purple furrows on straw
8	Purple
9	Black
10	White

3.2.1.33 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)

3.2.1.34 Sterile lemma colour: At maturity stage, when the terminal spikelets were approaching maturity, the colour of the sterile lemmas was classified into four classes as

Code	Guide
1	Straw (yellow)
2	Gold
3	Red
4	Purple

3.2.1.35 Sterile lemma length (Length of sterile glumes): At maturity stage, measurement was made on each of the two sterile lemmas and classified as

Code	Guide
1	Short (not longer than 1.5 mm)
3	Medium (1.6-2.5 mm)
5	Long (longer than 2.5 mm but shorter than lemma)
7	Extra long (equal to or longer than the lemma)
9	Asymmetrical

3.2.1.36 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 panicles 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%)

3.2.1.37 1000 grain weight: At maturity stage, a random sample of 1000 well developed, whole grains dried to 13 per cent moisture content was weighed on a precision balance and actual measurements were expressed in grams.

3.2.1.38 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.

3.2.1.39 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.

3.2.1.40 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

3.2.1.41 Endosperm type: At maturity stage, classification was based on the visual observations of the cut surface of endosperm with weak KI-I solution. Waxy starch stains brown whereas nonwaxy stains blue-black which was coded as 1.

3.2.1.42 Scent (aroma): Scent was detected at field level at flowering time and was classified as

Code	Guide
0	Non scented
1	Lightly scented
2	Scented

3.2.1.43 Maturity: Maturity was recorded as the duration in days from seeding to the time when more than 80% of the grains on the panicle were fully ripened.

3.2.1.44 Grain yield per plot: Weight of grains obtained from each plot was taken after drying and was expressed in kg ha^{-1}.

3.2.1.45 Straw yield: Dry weight of the straw from each plot was recorded and expressed in kg ha^{-1}.

3.2.1.46 Pest and disease incidence: There were no serious pest and disease incidence except leaf folder attack. Damage from this pest was scored using the scale as

Code	Guide
0	No damage
1	1-10%
3	11-20%
5	21-35%
7	36-50%
9	51-100%

3.2.2 Biochemical characterization

Methods

For the separation of multiple forms of enzyme and soluble proteins, poly acrylamide gel electrophoresis (PAGE) was carried out using `Holfer mighty small' vertical slab gel electrophoresis unit.

Acrylamide monomer ($CH = CHCONH_2$) was polymerized with bisacrylamide [$CH_2(NHCONH = NH_2)$bis] to obtain the gel. Freshly prepared ammonium per sulphate (APS) was used as chain initiator and N,N, N',N' tetra methylene diamine (TEMED) as catalyst.

Polyacrylamide gel was preferred because of its chemical inertness, high resolution, ease in handling and preparation.

Preparation of the gel

Reagents

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

Monomer stock solution

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 (1.5 M Tris-HCl, pH 8.8)

Tris base	-	18.5 g

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.

4x stacking gel buffer (0.5 M Tris HCl, pH 6.8)

Tris base	-	6 g

Tris base was dissolved in 70 ml distilled water and the pH was adjusted to 6.8 with HCl and the 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 ml distilled water.

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 while gels of 7.5 per cent acrylamide concentration were used for separating soluble proteins.

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 µl
TEMED	- 5 µl

Table 2. Gel recipes

Reagent	Acrylamide concentration			
	7.5%		10%	
	10 ml	20 ml	10 ml	20 ml
Monomer (ml)	2.49	4.98	3.33	6.66
Resolving buffer (ml)	2.5	5	2.5	5
Distilled water (ml)	4.94	9.88	4.1	8.2
10% APS (µl)	50	100	50	100
TEMED (µl)	5	10	5	10

After preparing the working solution, it was gently poured in between the glass plates kept in 'Hoefer dual gel casting unit'. Polymerisation was achieved within 20 to 30 min. For peroxidase and esterase isozymes and for separation of soluble proteins, stacking gel to a width of 1-1.5 cm was used for better resolution of bands.

3.2.2.1 Protein polymorphism

Poly Acrylamide Gel Electrophoresis was performed to compare the protein banding pattern of 13 Njavara genotypes among themselves and with that of the check variety, Ptb-10.

Gel having 7.5% acrylamide concentration (Table 2) was found to be the best for separation of protein bands. Stacking gel of 1-1.5 cm length was used for better resolution of protein bands.

Electrode buffers (Bernard, 1988)

Lower tank buffer (63 mM Tris, 50 mM HCl, pH 7.47)

Tris	-	22.7 g
1N HCl	-	150 ml
Water to	-	3 lit

Upper tank buffer (37.6 mM Tris, 40 mM glycine, pH 8.89)

Tris	-	4.56 g
Glycine	-	3.0 g
Water to	-	1 lit

Preparation of coomassie staining solution (Anon., 1995)

50% (v/v) Methanol

0.05% (v/v) Coomassie brilliant blue R-250

10% (v/v) Acetic acid

40% H_2O

Coomassie brilliant blue R-250 was dissolved in methanol before adding acetic acid and water.

Destaining solution

7% (v/v) Acetic acid

5% (v/v) Methanol

88% H_2O

Preparation of the sample

Quiscent seeds, germinated seeds (5 days after soaking), tender leaves from 30 day old seedlings were used for protein extraction. Proteins were extracted by grinding 0.5 g each of quiscent seeds, germinated seeds and leaves in 0.5 ml of Tris-HCl buffer (pH 7.0) along with 30 µl of ascorbic acid (50 mM), 30 µl of cystein HCl (50 mM) and 10 µl of mercaptoethanol by means of a mortar and pestle. The homogenized samples were then centrifuged at 15,000 rpm for 15 min at 4°C.

After centrifugation, the supernatant was used for estimating protein content (Lowry, 1951). Then equal quantity of protein was loaded in each well by adjusting the volume of protein extract taken. Bromophenol blue (1% solution) in 0.125 M Tris HCl buffer (pH 6.8) and 20% glycerol was mixed with protein extract and used as tracer dye. Along with samples, 10 µl of bovine serum albumin (1µg/µl) was also loaded in a separate well.

Electrophoresis was performed in 'Hoefer mighty small' unit applying a current of 15 mA per plate and 110 v for 2 hr at room temperature. After the run was over, the gels were stained with Coomassie blue followed by destaining with destaining solution to visualize clear bands.

The gels were photographed and the distances travelled by the protein bands and the dye front were recorded on a graph paper. The relative mobilities of the protein bands were calculated using the formula

$$\text{Relative mobility of proteins (Rm)} = \frac{\text{Distance travelled by protein}}{\text{Distance travelled by solvent}}$$

3.2.2.2 Isozyme analysis

Isozymes are generally made up of a number of subunits and it is the varying combination of the subunits which gives 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.

Enzymes assayed

All the Njavara types along with Ptb-10 were characterized with respect to peroxidase (PRX) and esterase (EST), the commonly occurring plant enzymes.

Preparation of sample

Quiscent seeds, germinated seeds (5 DAS) and tender leaves from 30 day old seedlings were used for the isozyme assay. Germinated seeds were pressed with blotting paper to remove water and used for extraction of enzyme. Tender leaves from 30 day old seedlings were also collected, washed free of dirt, rinsed with distilled water, pressed with blotting paper and used for enzyme extraction.

For extraction of peroxidase, 500 mg of the sample was taken and homogenized in a pre-cooled mortar, along with 30µl of Ascorbic acid (50 mM), 30 µl of soluble Poly Vinyl Pyrrolidone (PVP) (50 mg/50 ml water) and 0.5 ml of Tris-HCl extraction buffer (pH 7.0). 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 15,000 rpm for 15 min in a 'Kubota' high speed centrifuge at 5°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 extracts can be stored at sub-zero temperature for one day.

Electrode buffer (0.025 M 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 µl of enzyme was loaded in each well. Bromophenol blue (1% solution) in 0.125 M Tris HCl buffer (pH 6.8) and 20 per cent glycerol was mixed with enzyme solution and used as tracer dye. Electrophoresis was carried out at 4°C. A constant current of 10 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
H_2O_2 (3%)	- 0.4 ml

Fresh stain was prepared each time. Acetate buffer and benzidine were mixed, boiled, cooled, filtered and then H_2O_2 was added to the mixture just before immersing the gel in staining solution. The gels were immersed in the staining solution for about 15 min. 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, 1992)

200 ml of staining solution contained

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

After the run was over, the gels were taken out and incubated in the staining solution for 30 min 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 norms described by Berg and Wijsman (1982) for peroxidase was followed. The enzymes were referred by the following abbreviations.

1. Peroxidase – PRX
2. Esterase – EST

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

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

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 species studied were pooled. The slowest moving anodal band was numbered 1 (eg. PRX-1) faster ones were given the subsequent numbers.

Measurement of similarity

The measurement of electrophoretic similarity among the Njavara genotypes and with that of the check variety, Ptb-10 was calculated by making pairwise comparison of the genotypes using the method of Sockel and Sneath (1963) using the formula

$$\text{Similarity index (SI)} = \frac{\text{Number of homologous bands}}{\text{Number of homologous bands} + \text{Number of nonhomologous bands}}$$

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

3.2.3 Nutritional qualities of Njavara grain

Njavara genotypes were grouped based on the range of each nutritional quality parameter.

3.2.3.1 Soluble carbohydrate

Soluble carbohydrates in grain was estimated by the method suggested by Dubois *et al.* (1956) and Krishnaveni *et al.* (1984). For estimation of soluble

carbohydrate, 100 mg of brown rice was powdered and homogenized in 5 ml hot methanol (80%) by means of a mortar and pestle. The supernatant was collected after centrifugation. Extraction was repeated until extraction of soluble sugars from the residue was complete (until phenol-H_2SO_4 test gives no orange colour with the supernatant). The supernatants were pooled and volume made up to 100 ml. Then 0.5 ml and 1 ml of the sample solution pipetted into two separate test tubes and the volume was made up to 1 ml in all the tubes. A blank was set up with 1 ml water. Then 1 ml of phenol followed by 5 ml of 98% H_2SO_4 was added to each tube and shaken well. After 20 min, the colour was read at 490 nm in a spectrophotometer. Stock solution of glucose was prepared by dissolving 100 mg glucose in 100 ml of water. Working standard of glucose was prepared by making up 10 ml of stock to 100 ml with distilled water. Then 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard was pipetted into a series of test tubes and colour was developed as in the case of sample. The amount of soluble sugars was calculated using a standard graph and expressed in per cent.

3.2.3.2 Protein content

Protein content was estimated by estimating the total nitrogen content by the method suggested by Fisher (1956). For estimating nitrogen content, 100 mg of brown was digested with 5 ml of H_2SO_4 followed by heating for 10 min. in a waterbath. Then 2 ml of H_2O_2 was added through the sides of the volumetric flask. As the solution retained orange colour, it was heated for 10 min. more and then H_2O_2 was added drop by drop to get a clear solution. The final volume was made up to 100 ml with distilled water.

Then 5 ml of the sample solution was pipetted into a 50 ml volumetric flask. Then 40 ml of 10% NaOH followed by 1 ml of 10% sodium silicate was added and the volume was made up to 50 ml. Then 1.5 ml of Nessler's reagent was added to get orange colour and read in a spectrophotometer at 410 nm.

A standard of 1000 ppm stock solution of ammonium chloride was prepared by dissolving 0.382 g NH_4Cl in 100 ml distilled water. A working standard of 10 ppm concentration was prepared by making up 1 ml stock to 100 ml. Then a series of standards having 50, 60, 70, 80, 90 and 100 ppm were prepared by taking 5, 6, 7, 8, 9 and 19 ml of working standard solutions and colour was developed as in the case of sample by adding 2 ml of 10% NaOH.

Nitrogen present in the sample was calculated using the formula

$$\text{Percentage nitrogen} = \text{factor} \times \frac{\text{Absorbance}}{\text{Volume}} \times \frac{100}{\text{Weight}} \times 100$$

Then the percent Nitrogen was multiplied by the factor 5.95 to get the protein content in rice.

3.2.3.3 Estimation of total free amino acids

Total free amino acids in grain was estimated by the method suggested by Moore and Stein (1948) and Misra *et al.* (1975). For estimating total free amino acids, 500 mg of the brown rice was homogenized in a mortar and pestle with a small quantity of acid-washed sand. To this homogenate, 5 ml of 10% isopropanol was added. After centrifugation, the supernatant was saved. Extraction was repeated again with the residue and the supernatants were pooled. Then 2 ml of the extract was pipetted into a test tube and 2 ml of ninhydrin was added. Then the tubes were heated in a boiling water bath for 20 min. Then 5 ml of the diluent (having equal volumes of distilled water and 10% isopropanol) was added and the contents were mixed. After 15 min. the intensity of the purple colour was read against a reagent blank in a spectrophotometer at 570 nm. The reagent blank was prepared in the same way by taking 2 ml of 10%isopropanolinstead of the extract. A standard with leucine solution, having a concentration range of 10 µg to 100 µg was read in

spectrophotometer. The total free amino acids in the sample was expressed as percentage equivalent of leucine.

3.2.3.4 Chromatography of free amino acids

Free amino acids were separated using paper chromatography by the method suggested by Harborne (1973). Free amino acids were extracted as in 3.2.3.3 procedure and were spotted on a Whatman No.1 filter paper sheet at 3 cm interval on a base line. Then ascending paper chromatography was carried out in an equilibriated chromatographic chamber using the solvent system n-butanol, acetic acid, water in 5:1:4 ratio. When the run was over, the chromatogram was taken out, dried and sprayed with 0.1% ninhydrin (in acetone) reagent. The chromatogram was dried at room temperature for 5 min. and developed the chromatogram by keeping it in an oven at 100°C for 5 min. Amino acids appeared as spots. The position of the spots, on the chromatogram as well as the distance travelled by the solvent front were recorded and the Rf value of each spot was calculated using the formula

$$Rf = \frac{\text{Distance (cm) moved by the solute from the origin}}{\text{Distance (cm) moved by the solvent from the origin}}$$

3.2.3.5 Fat content

Fat content in rice grain was estimated according to procedure of Association of Official Agricultural Chemists (Anon., 1955). For estimating fat content 5 g of the brown rice powder was weighed accurately into a thimble and plugged with cotton. The thimble was then placed in a soxhlet apparatus and extracted with petroleum ether (40-60°C) for about 9 hr. The petroleum ether was transferred into a preweighed beaker. The flask containing the ether extract was washed 4-5 times with small quantities of ether and the washings were also transferred to the beaker. The ether was then evaporated and the beaker with the residue weighed.

Then the fat content in the sample was calculated using the formula

$$\text{Oil in ground sample (\%)} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100$$

3.2.3.6 Amylose content

Amylose content of grain was estimated by the method suggested by McCready *et al.* (1950) and Juliano (1971). For estimating amylose content, 100 mg of brown rice powder was taken in a test tube. One ml of ethanol followed by 10 ml of 1N NaOH were added to the tube and heated for 10 min 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 0.1N HCl was added to the solution drop by drop until pink colour just disappeared. To this, 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 1N NaOH and made up to 100 ml with water. Then 0.2, 0.4, 0.6, 0.8 and 1 ml of the standard amylose solution was pipetted into a series of test tubes and colour was developed as in the case of the sample. One ml of iodine regent 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.

3.2.3.7 Starch grain characterization

A thin transverse section of dehulled rice grain was taken with the help of a sharp blade. Then it was stained with few drops of very dilute iodine reagent and mounted on a slide. When observed under compound microscope, starch grains appear as bluish black grains. The diameter of individual grain was measured with the help of ocular micrometer. The ocular micrometer was later calibrated with

stage micrometer and the diameter of starch grains expressed in microns (Fahn, 1990).

3.2.3.8 Amylase activity

Amylase activity was estimated by the method suggested by Bernfield (1955), Kruger (1972) and Niku-Paavola *et al.* (1972). One gram of germinated seeds(4 DAS)was estracted with 10 ml of ice-cold 10 mM calcium chloride solution for 3 hr at room temperature. The extract was centrifuged at 15,000 rpm at 4°C for 10 min. The supernatant was used as enzyme source.

To quantify the enzyme activity, 50 µl of 1% starch solution was transferred into a test tube and made up to 1 ml. Then 0.3 ml of enzyme solution was diluted to 1 ml and pipetted into the test tube. The test tube was then incubated at 27°C for 15 min. The reaction was stopped by the addition of 2 ml of dinitrosalicylic acid reagent. Then the tubes were heated in a boiling water bath for 5 min. While the tubes were warm, 1 ml of potassium sodium tartrate (40%) solution was added and cooled in running tap water. Then the volume was made up to 10 ml by addition of 6 ml distilled water. The reaction was terminated at zero time in control tubes. Then the absorbance was read at 560 nm. A standard graph was prepared with 0-100 µg maltose. A unit ofα - amylasewas expressed as mg of maltose produced during 5 min incubation with 1% starch.

3.2.3.9 Flavour components

Flavonoids are the major flavour components in rice. For screening of flavour components from the solvent extract, the method suggested by Harborne (1973) was followed. Five grams of brown rice powder was extracted with methanol in a soxhlet apparatus. The extract containing flavonoids was concentrated by evaporating methanol and the same used for spotting. Then thin layer

chromatography (TLC) was performed in an equilibriated chromatographic chamber using hexane and ethyl acetate in 3:1 ratio. After the run was over, the plates were dried and observed under U.V. light for the presence of fluorescent spots.

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 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).

Genotypic variance $(\sigma^2 g) = (Mg-Me)/r$

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

$\sigma^2 e$ = Expected mean sum of squares for error

Phenotypic and genotypic coefficients of variation (PCV and GCV)

These were computed according to Burton (1952).

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

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

The estimates of PCV and GCV were classified as

less than 25 per cent	= low
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 categorised 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 as follows:

$GA = K \times \sigma p \times H^2$

where,

K = 2.06, selection intensity at 5 per cent

σp = phenotypic standard deviation

3.2.4.5 Genetic gain

Genetic gain = (GA/Grand mean) x 100

Genetic gain was classified according to Johnson *et al.* (1955) as follows:

1-10 per cent	= low
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 systems of correlated variables under study. Using SPAR 1 package, path analyses for grain yield, free aminoacid content and protein content at the genotypic level were carried out using the characters which had significant correlations with grain yield, free aminoacid content and protein content.

Four characters viz., leaf length, grain length, grain width and straw yield were selected for path analysis with respect to yield. With regard to free aminoacid content, seven characters namely, seedling height, leaf width, days to 50 per cent heading, culm number, culm diameter, panicle length and days from seeding to maturity are considered for path analysis. With respect to protein content, five characters namely, leaf length, leaf width, days to 50 per cent heading, panicle length and 1000grain weight were selected for path analysis.

RESULTS

RESULTS

4.1 Morphological characterization

Morphological characters are presented as qualitative characters and quantitative characters.

4.1.1 Qualitative characters

The qualitative characters of thirteen genotypes of the rice cultivar, Njavara are presented in Table 3. Njavara genotypes revealed low variability with respect to most of the qualitative characters. Least variation was observed among the Njavara genotypes with respect to characters like leaf blade pubescence, leaf blade colour, basal leaf sheath colour, leaf angle, ligule colour, ligule shape, collar colour, auricle colour, culm angle, internode colour, septum colour, secondary branching of panicle, panicle shattering, panicle threshability, stigma colour, sterile lemma colour, spikelet sterility, awn colour, endosperm type, grain scent and pest and disease incidence. In general, the Njavara genotypes have glabrous and green leaves, green basal leaf sheath, erect leaves, white and 2-cleft ligules, palegreen collar and auricle, erect culms, light gold internode and septum, heavy secondary branching of panicle, very low shattering and difficult threshability of panicle, white stigmas, straw coloured sterile lemmas, highly fertile spikelets, non waxy endosperm, non scented grains and low incidence of pests and diseases.

With respect to flag leaf angle, the genotype N1 alone had erect flag leaf while the rest had intermediate flag leaf. Most of the Njavara genotypes showed early and fast leaf senescence while the genotype N6 and check variety, Ptb-10 exhibited intermediate senescence. Regarding panicle type, the genotype N2 was observed to have compact panicle, while open panicle was observed in the genotypes N6 and N7 and the rest had intermediate panicles. The genotypes N5, N10, N11 and N13 showed partial panicle exsertion whereas the remaining genotypes exhibited well exserted panicles. Regarding panicle axis, the genotypes

Table 3. Qualitative characters of Njavara genotypes (scores)

Character	Njavara genotypes						
	N1	N2	N3	N4	N5	N6	N7
Leaf blade pubescence	1	1	1	1	1	1	1
Leaf blade colour	2	2	2	2	2	2	2
Basal leaf sheath colour	1	1	1	1	1	1	1
Leaf angle	1	1	1	1	1	1	1
Flag leaf angle	1	3	3	3	3	3	3
Leaf senescence	9	9	9	9	9	5	9
Ligule colour	1	1	1	1	1	1	1
Ligule shape	2	2	2	2	2	2	2
Collar colour	1	1	1	1	1	1	1
Auricle colour	1	1	1	1	1	1	1
Culm angle	1	1	1	1	1	1	1
Internode colour	2	2	2	2	2	2	2
Septum colour	2	2	2	2	2	2	2
Panicle type	5	1	5	5	5	9	9
Panicle secondary branching	2	2	2	2	2	2	2
Panicle exsertion	1	1	1	1	7	1	1

Contd..

Table 3. Continued

Character	Njavara genotypes						
	N8	N9	N10	N11	N12	N13	Ptb-10
Leaf blade pubescence	1	1	1	1	1	1	1
Leaf blade colour	2	2	2	2	2	2	2
Basal leaf sheath colour	1	1	1	1	1	1	1
Leaf angle	1	1	1	1	1	1	1
Flag leaf angle	3	3	3	3	3	3	3
Leaf senescence	9	9	9	9	9	9	5
Ligule colour	1	1	1	1	1	1	1
Ligule shape	2	2	2	2	2	2	2
Collar colour	1	1	1	1	1	1	1
Auricle colour	1	1	1	1	1	1	1
Culm angle	1	1	1	1	1	1	1
Internode colour	2	2	2	2	2	2	1
Septum colour	2	2	2	2	2	2	1
Panicle type	5	5	5	5	5	5	5
Panicle secondary branching	2	2	2	2	2	2	2
Panicle exsertion	1	1	7	7	1	7	1

Contd.

Table 3. Continued

Character	Njavara genotypes						
	N1	N2	N3	N4	N5	N6	N7
Panicle axis	2	1	2	2	2	1	1
Panicle shattering	1	1	1	1	1	1	1
Panicle threshability	1	1	1	1	1	1	1
Spikelet awning	0	0	7	1	7	0	0
Awn colour	0	0	1	1	1	0	0
Apiculus colour	2	2	3	2	3	2	3
Stigma colour	1	1	1	1	1	1	1
Lemma and palea colour	0	1	3	1	3	1	3
Lemma and palea pubesence	3	1	3	3	3	1	1
Sterile lemma colour	1	1	1	1	1	1	1
Spikelet sterility	1	1	1	1	1	1	1
Seed coat colour	5	5	2	4	4	4	2
Endosperm type	1	1	1	1	1	1	1
Grain scent	0	0	0	0	0	0	0
Length of sterile glumes	3	5	3	3	3	3	3
Pest and disease incidence	1	1	1	1	1	1	1

Contd.

Table 3. Continued

Character	Njavara genotypes						
	N8	N9	N10	N11	N12	N13	Ptb-10
Panicle axis	2	2	2	2	2	2	2
Panicle shattering	1	1	1	1	1	1	1
Panicle threshability	1	1	1	1	1	1	1
Spikelet awning	0	0	7	5	0	1	0
Awn colour	0	0	1	1	0	1	0
Apiculus colour	2	2	3	3	2	3	2
Stigma colour	1	1	1	1	1	1	1
Lemma and palea colour	1	1	3	3	1	3	1
Lemma and palea pubesence	1	1	3	3	1	3	3
Sterile lemma colour	1	1	1	1	1	1	1
Spikelet sterility	1	1	1	1	1	1	1
Seed coat colour	5	5	2	2	5	2	4
Endosperm type	1	1	1	1	1	1	1
Grain scent	0	0	0	0	0	0	0
Length of sterile glumes	5	3	3	3	3	3	3
Pest and disease incidence	1	1	1	1	1	1	1

N2, N6 and N7 had straight panicles and the rest had droopy panicles. High variability was observed with respect to spikelet awning. The genotypes N4 and N13 had short and partly awned spikelets, while the genotypes N3, N5 and N10 had long and partly awned spikelets. The genotype N11 exhibited short and fully awned spikelets and rest of the genotypes did not possess awns. Regarding apiculus colour, the genotypes N3, N5, N7, N10, N11 and N13 were observed to have brown colour and the rest had straw colour. High variability was noticed among Njavara genotypes with respect to lemma and palea colour (Plate 1). Straw colour was observed for the grain of the genotype N1, gold furrows on straw background for the genotypes N2, N4, N6, N8, N9, N12 and Ptb-10 and brown furrows on straw for rest of the genotypes. Regarding lemma and palea pubescence, glabrous glumes were observed for the genotypes N2, N6, N7, N8, N9 and N12 and hairs on upper portion of glumes for rest of the genotypes. Variability was also observed in the case of seed coat colour. The genotypes N3, N7, N10, N11 and N13 exhibited light brown seed coat whereas the genotypes N4, N5, N6 and Ptb-10 showed brown colour and the rest had red colour. Regarding length of sterile glumes, long glumes were observed for the genotypes N2 and N8 and medium glumes for the rest of the genotypes.

4.1.2 Quantitative characters

The quantitative characters of thirteen genotypes of the rice cultivar, Njavara are presented in Table 4. The mean performances of Njavara genotypes with respect to grain yield and associated quantitative characters are presented in Table 5.

4.1.2.1 Seedling height

The seedling height of Njavara genotypes at 5th leaf stage was found to vary from 29.57 cm to 41.80 cm. The check variety, Ptb-10 recorded the lowest (29.57 cm) followed by the genotypes N13 (30.82 cm) and N2 (32.20 cm). The

Plate 1. Variation among Njavara genotypes with respect to lemma and palea colour

Legend: 1 - N1, 2 - N2, 3 - N3, 4 - N4, 5 - N5, 6 - N6, 7 - N7, 8 - N8, 9 - N9, 10 - N10, 11 - N11, 12 - N12, 13 - N13, 14 - Ptb-10


1
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14

Table 4. Quantitative characters of Njavara genotypes

Character	Njavara genotypes						
	N1	N2	N3	N4	N5	N6	N7
Seedling height (cm)	40.45	32.20	41.80	33.20	38.23	34.05	35.26
Leaf length (cm)	50.00	42.43	44.70	34.97	46.13	49.23	49.73
Lead width (cm)	0.99	1.11	0.79	1.16	0.88	1.25	1.12
Panicle length (cm)	24.38	20.74	19.87	21.35	20.21	23.06	23.57
Ligule length (cm)	1.55	1.84	1.880	1.66	1.830	2.40	2.00
Days to 50% heading	44.67	50.00	44.33	56.67	45.33	56.67	51.67
Culm length (cm)	87.63	77.50	66.77	54.27	68.73	94.33	87.47
Culm number	4.37	7.03	6.23	4.77	5.83	4.73	6.00
Culm diameter (mm)	5.40	5.98	5.52	6.00	5.17	6.40	6.05
1000 grain weight (g)	28.93	28.50	23.53	26.97	25.23	30.07	29.20
Grain length (mm)	8.98	9.10	8.30	9.00	8.77	8.77	9.27
Grain width (mm)	3.30	3.22	2.92	3.00	3.00	3.33	3.10
Grain yield (kg/ha)	2554	2621	2330	1009	2477	1370	2148
Straw yield (kg/ha)	6081	4095	3464	2097	3078	1752	3224
Days from seeding to maturity	64.67	68.33	66.33	78.67	66.33	71.67	67.67

Contd.

Table 4. Continued

Character	Njavara genotypes						
	N8	N9	N10	N11	N12	N13	Ptb-10
Seedling height (cm)	38.62	34.34	37.87	37.83	33.28	30.82	29.57
Leaf length (cm)	40.73	37.77	35.50	43.90	36.90	46.03	41.00
Leaf width (cm)	0.90	0.70	0.85	0.66	0.69	0.82	1.28
Panicle length (cm)	20.64	18.30	20.21	18.94	19.87	20.78	25.19
Ligule length (cm)	2.08	1.26	1.38	1.65	1.24	1.97	2.03
Days to 50% heading	46.00	44.00	44.00	45.67	44.67	46.00	63.00
Culm length (cm)	69.63	72.35	74.40	64.50	75.03	66.27	64.50
Culm number	8.13	7.87	7.33	9.13	6.97	7.97	6.60
Culm diameter (mm)	5.08	4.53	4.57	4.37	4.20	5.22	6.35
1000 grain weight (g)	18.50	19.00	24.70	22.83	18.63	21.63	27.20
Grain length (mm)	8.13	7.60	8.68	8.33	7.63	8.23	8.90
Grain width (mm)	2.65	2.80	3.02	2.92	2.80	2.90	3.15
Grain yield (kg/ha)	919	2167	1172	2684	1200	2386	2372
Straw yield (kg/ha)	2637	3307	2254	6084	2467	4161	6818
Days from seeding to maturity	62.33	60.00	70.00	63.67	61.67	64.33	88.00

Table 5. Mean performance of Njavara genotypes evaluated for quantitative characters

S.No.	Njavara genotypes	Seedling height (cm)	Leaf length (cm)	Leaf width (cm)	Panicle length (cm)	Ligule length (cm)	Days to 50% heading	Culm length (cm)	Culm number	Culm diameter (mm)
1	N1	40.45^{AB}	50.00^{A}	0.99^{C}	24.38^{AB}	1.55^{DEF}	44.67^{D}	87.63^{B}	4.37^{F}	5.4^{BCD}
2	N2	32.20^{DEF}	42.43^{CD}	1.11^{C}	20.74^{C}	1.84^{BCD}	50.00^{C}	77.50^{C}	7.03^{BCD}	5.98^{AB}
3	N3	41.80^{A}	44.70^{ABC}	0.79^{E}	19.87^{CDE}	1.88^{BC}	44.33^{D}	66.77^{EF}	6.23^{CDE}	5.52^{ABC}
4	N4	33.20^{DE}	34.97^{F}	1.16^{B}	21.35^{C}	1.66^{CDE}	56.67^{B}	54.27^{G}	4.77^{EF}	6.00^{AB}
5	N5	38.23^{BC}	46.13^{ABC}	0.88^{D}	20.21^{CD}	1.83^{BCD}	45.33^{D}	68.73^{DEF}	5.83^{DEF}	5.17^{BCDE}
6	N6	34.05^{D}	49.23^{AB}	1.25^{A}	23.06^{B}	2.40^{A}	55.67^{B}	94.33^{A}	4.73^{EF}	6.40^{A}
7	N7	35.26^{CD}	49.73^{A}	1.12^{B}	23.57^{B}	2.00^{B}	51.67^{C}	87.47^{B}	6.00^{DEF}	6.05^{AB}
8	N8	38.62^{B}	40.73^{CDE}	0.90^{D}	20.64^{C}	2.08^{B}	46.00^{D}	69.63^{DEF}	8.13^{AB}	5.08^{CDE}
9	N9	34.34^{D}	37.77^{DEF}	0.70^{F}	18.30^{E}	1.26^{FG}	44.00^{D}	72.35^{CDE}	7.87^{ABC}	4.53^{DEF}
10	N10	37.87^{BC}	35.50^{EF}	0.85^{DE}	20.21^{CD}	1.38^{EFG}	44.00^{D}	74.4^{CD}	7.33^{BCD}	4.57^{DEF}
11	N11	37.83^{BC}	43.90^{BC}	0.66^{F}	18.94^{DE}	1.65^{CDE}	45.67^{D}	64.50^{F}	9.13^{A}	4.37^{EF}
12	N12	33.28^{DE}	36.90^{EF}	0.69^{F}	19.87^{CDE}	1.24^{G}	44.67^{D}	75.03^{CD}	6.97^{ABC}	4.20^{F}
13	N13	30.82^{EF}	46.03^{ABC}	0.82^{DE}	20.78^{C}	1.97^{B}	46.00^{D}	66.27^{EF}	7.97^{ABC}	5.22^{BCDE}
14	Ptb-10 (check variety)	29.57^{F}	46.10^{ABC}	1.28^{A}	25.19^{A}	2.03^{B}	63.00^{A}	64.50^{F}	6.60^{BCD}	6.35^{A}

The figures with same alphabets in superscript do not differ significantly at 5% level.

Contd.

Table 5. Continued

Sl.No.	Njavara genotypes	1000 grain weight (g)	Grain length (mm)	Grain width (mm)	Grain yield hectare^{-1} (kg)	Straw yield hectare^{-1} (kg)	Days from seeding to maturity
1	N1	28.93 ^{ABC}	8.98 ^{BC}	3.30 ^{A}	2554 ^{ABC}	6081 ^{A}	64.67 ^{FG}
2	N2	28.50 ^{ABC}	9.10 ^{AB}	3.22 ^{B}	2621 ^{AB}	4095 ^{B}	68.33 ^{DE}
3	N3	23.53 ^{EFG}	8.30 ^{E}	2.92 ^{E}	2330 ^{ABCD}	3464 ^{BC}	66.33 ^{EF}
4	N4	26.97 ^{CD}	9.00 ^{AB}	3.00 ^{D}	1009 ^{FG}	2097 ^{EF}	78.67 ^{B}
5	N5	25.23 ^{DE}	8.77 ^{CD}	3.00 ^{D}	2477 ^{ABC}	3078 ^{BCDE}	66.33 ^{EF}
6	N6	30.07 ^{A}	8.77 ^{CD}	3.33 ^{A}	1370 ^{EFG}	1752 ^{F}	71.67 ^{C}
7	N7	29.20 ^{AB}	9.27 ^{A}	3.10 ^{C}	2148 ^{ABCDE}	3224 ^{BCDE}	67.67 ^{DE}
8	N8	18.50 ^{H}	8.13 ^{E}	2.65 ^{G}	919 ^{G}	2637 ^{CDEF}	62.33 ^{GHI}
9	N9	19.00 ^{H}	7.60 ^{F}	2.80 ^{F}	2167 ^{ABCDE}	3307 ^{BCD}	60.00 ^{I}
10	N10	24.70 ^{EF}	8.68 ^{D}	3.02 ^{D}	1172 ^{FG}	2254 ^{DEF}	70.00 ^{CD}
11	N11	22.83 ^{FG}	8.33 ^{E}	2.92 ^{E}	2684 ^{A}	6084 ^{A}	63.67 ^{GH}
12	N12	18.63 ^{H}	7.63 ^{F}	2.80 ^{F}	1200 ^{FG}	2467 ^{CDEF}	61.67 ^{HI}
13	N13	21.63 ^{G}	8.23 ^{E}	2.90 ^{E}	2386 ^{ABC}	4161 ^{B}	64.33 ^{FG}
14	Ptb-10 (Check variety)	27.20 ^{BCD}	8.90 ^{BCD}	3.15 ^{BC}	2372 ^{ABC}	6818 ^{A}	88.00 ^{A}

genotype, N3 was the tallest (41.80 cm) followed by N1 (40.45 cm) and N8 (38.62 cm).

4.1.2.2 Leaf length

The Njavara genotype, N4 recorded the shortest leaf length (34.97 cm) followed by N10 (35.50 cm) and N9 (37.77 cm). The longest leaves were observed for N1 (50 cm) followed by N7 (49.73 cm).

4.1.2.3 Leaf width

The narrowest leaves were observed for the genotype N11 (0.66 cm) followed by N12 (0.69 cm). Ptb-10 was observed to have the widest leaves (1.28 cm) followed by the genotypes N6 (1.25 cm) and N7 (1.12 cm).

4.1.2.4 Panicle length

The Njavara genotype N9 had the shortest panicles (18.30 cm) followed by N11 (18.94 cm). Ptb-10 had the longest panicles (25.19 cm) followed by the genotypes N1 (24.38 cm) and N7 (23.57 cm).

4.1.2.5 Ligule length

The ligule length ranged between 1.24 cm (N12) and 2.40 cm (N6).

4.1.2.6 Days to 50 per cent heading

The Njavara genotypes N9 and N10 were the earliest for 50 per cent heading (44 days) followed by N4 (44.33 days). Ptb-10 took the maximum number of days (63 days) for 50 per cent heading followed by the genotypes N4 and N6 (56.67 days).

4.1.2.7 Culm length

The Njavara genotype N4 recorded the shortest culm length (54.27 cm) followed by N11 (64.50 cm). The genotype N6 was the tallest (94.33 cm).

4.1.2.8 Culm number

The Njavara genotype N1 produced the minimum number of culms (4.37) followed by N6 (4.73) and N4 (4.77). Higher tillering than Ptb-10 was observed for the genotype N11 which produced 9.13 tillers per hill.

4.1.2.9 Culm diameter

The thinnest culms were expressed by N12 (4.20 mm) and the thickest culms by N6 (6.40 mm).

4.1.2.10 1000 grain weight

The average 1000 grain weight ranged from 18.50 g for N8 to 30.07 g for N6. It was observed that the 1000 grain weight of few genotypes such as N1, N6 and N7 exceeded that of Ptb-10 which had a test weight of 27.20 g.

4.1.2.11 Grain length

The Njavara genotype N9 had the shortest grains (7.60 mm) followed by N12 (7.63 mm). The genotype N7 possessed the longest grains (9.27 mm).

4.1.2.12 Grain width

Grain width ranged between 2.65 mm (N8) and 3.33 mm (N6).

4.1.2.13 Grain yield

In general Njavara genotypes showed low yield potential. The Njavara genotype N8 recorded the lowest yield of 919 kg ha^{-1} followed by N4 (1009 kg ha^{-1})

and N10 (1172 kg^{-1}). The genotype N11 recorded the highest yield of 2684 kg ha^{-1} followed by N2 (2621 kg ha^{-1}) and N1 (2554 kg ha^{-1}).

4.1.2.14 Straw yield

The genotype N6 was observed to have the lowest straw yield of 1752 kg ha^{-1} followed by N4 (2097 kg ha^{-1}). Ptb-10 had the highest straw yield of 6818 kg ha^{-1} followed by the genotype N11 (6084 kg ha^{-1}).

4.1.2.15 Days from seeding to maturity

Generally Njavara genotypes exhibited short growth duration. Njavara genotype N9 took the minimum number of days to mature (60.00 days) followed by N12 (61.67 days) and N8 (62.33 days). Ptb-10 took the maximum number of days to mature (88.00 days) followed by the genotypes N4 (78.67 days) and N6 (71.67 days).

Even though the genotypes N9 and N12 exhibited maximum similarity between them, on the basis of above mentioned qualitative and quantitative characters, they were differing in characters like leaf length, panicle length, ligule length, culm number, culm diameter, grain yield, straw yield and days from seeding to maturity.

4.2 Biochemical characterization

Thirteen genotypes of Njavara which were characterized morphologically, were analysed for variation in protein, peroxidase and esterase banding patterns.

Quiscent seed (viable seed), germinated seed (5 days after soaking) and leaf samples at 30 days after sowing were taken for the study.

4.2.1 Protein polymorphism

4.2.1.1 Quiscent seed

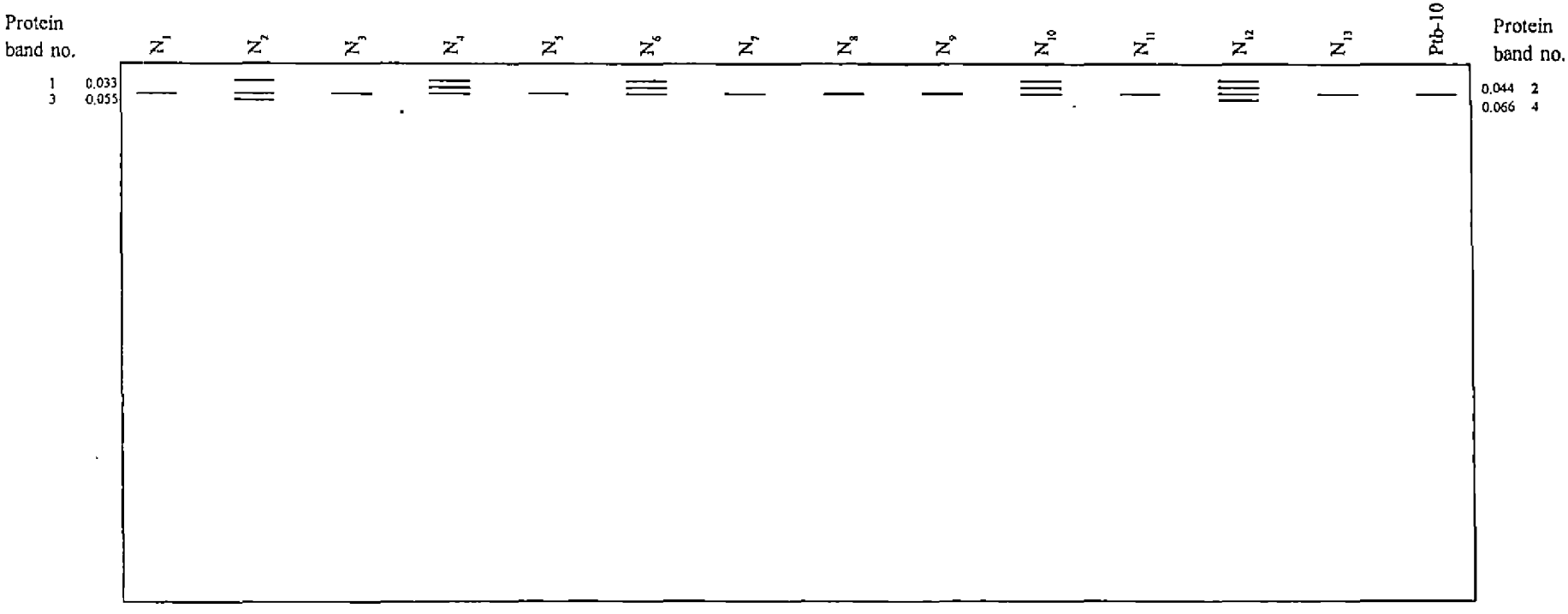
Studies with respect to protein variations in quiscent seed revealed four protein bands (Fig.1 and Plate 2). The protein band number 3 (Rm = 0.055) was common for all the genotypes. The protein band number1 (Rm = 0.033) was present only in N2, N4, N6, N10 and N12. The protein band number 2 (Rm = 0.044) was observed in the genotypes N4, N6, N10 and N12. The protein band number 4 (Rm = 0.066) was present only in genotypes N2 and N12.

Based on similarity indices (SI) with Ptb-10, Njavara genotypes can be placed in three groups. Group-1 consisted of the genotypes N1, N3, N5, N7, N8, N9, N11 and N13 with a SI of 100 per cent. The genotypes N2, N4, N6 and N10 with a SI of 50 per cent were placed in group-2. The genotype N12 with the lowest SI of 40 per cent was assigned to group-3 (Table 6). The similarity indices among Njavara genotypes (Table 7) showed that they can be placed in four groups since the genotype N2 can be distinguished from other genotypes.

4.2.1.2 Germinated seed

In germinated seed samples, a total of 14 protein bands were obtained (Fig.2 and Plate 3). The protein bands number 1, 2, 3, 5, 6, 7 and 14 with Rm values 0.022, 0.039, 0.045, 0.096, 0.147, 0.215 and 0.517 were common for all the genotypes including the check. The protein band number 4 (Rm = 0.073) was present in the genotypes N1, N2, N3, N4, N5 and N6. The protein band number 8 (Rm = 0.255) was found only in the genotype N3. The protein band number 9 (Rm = 0.272) was present in majority of the genotypes except N3, N6, N7 and N8. The protein band number 10 (Rm = 0.307) too was observed in most of the genotypes except N7, N8, N10, N11 and N13. The protein band number 11 (Rm = 0.426) was present only in the genotypes N1 and N4. The protein band number 12 (Rm = 0.443) was observed in majority of the genotypes except N1, N4

Fig.1. Protein banding pattern in quiscent seed of Njavara genotypes



N1 - N13 = Njavara genotypes; Ptb-10 = Check

Plate 2. Protein banding pattern in quiscent seeds of Njavara genotypes

Legend: 1 - N1, 2 - N2, 3 - N3, 4 - N4, 5 - N5, 6 - N6, 7 - N7,
8 - N8, 9 - N9, 10 - N10, 11 - N11, 12 - N12, 13 - N13,
14 - Ptb-10

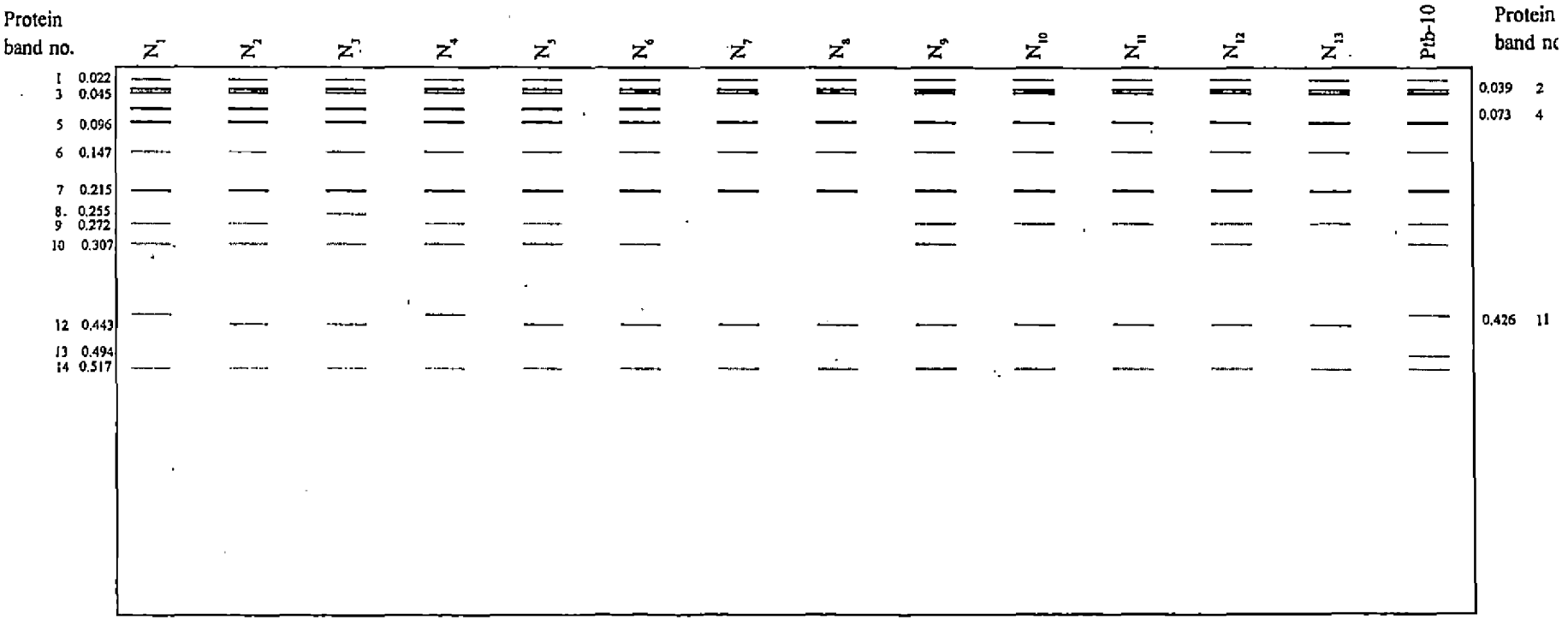
Table 6. Groups of Njavara genotypes based on protein banding pattern in quiscent seed, germinated seed and leaf.

Sl. No.	Njavara genotypes	Quiscent seed		Germinated seed		Leaf	
		Group	SI with Ptb-10 (%)	Group	SI with Ptb-10 (%)	Group	SI with Ptb-10 (%)
1	N1	G-1	100	G-1	90.9	G-4	95.6
2	N2	G-2	50	G-3	81.8	G-3	97.7
3	N3	G-1	100	G-7	72.7	G-5	95.4
4	N4	G-2	50	G-1	90.9	G-1	100
5	N5	G-1	100	G-3	81.8	G-4	95.6
6	N6	G-2	50	G-5	76.2	G-4	95.6
7	N7	G-1	100	G-6	73.7	G-2	97.8
8	N8	G-1	100	G-6	73.7	G-4	95.6
9	N9	G-1	100	G-2	85.7	G-2	97.8
10	N10	G-2	50	G-4	80.0	G-4	95.6
11	N11	G-1	100	G-4	80.0	G-4	95.6
12	N12	G-3	40	G-2	85.7	G-2	97.8
13	N13	G-1	100	G-4	80.0	G-2	97.8

Table 7. Similarity indices among Njavara genotypes based on protein banding pattern in quiscent seed.

Njavara genotypes	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13
N1	1												
N2	0.500	1											
N3	1	0.500	1										
N4	0.500	0.667	0.500	1									
N5	1	0.500	1	0.500	1								
N6	0.500	0.667	0.500	1	0.500	1							
N7	1	0.500	1	0.500	1	0.500	1						
N8	1	0.500	1	0.500	1	0.500	1	1					
N9	1	0.500	1	0.500	1	0.500	1	1	1				
N10	0.500	0.667	0.500	1	0.500	1	0.500	0.500	0.500	1			
N11	1	0.500	1	0.500	1	0.500	1	1	1	0.500	1		
N12	0.400	0.857	0.400	0.857	0.400	0.857	0.400	0.400	0.400	0.857	0.400	1	
N13	1	0.500	1	0.500	1	0.500	1	1	1	0.500	1	0.400	1

Fig.2. Protein banding pattern in germinated seeds (5 days after soaking) of Njavara genotypes

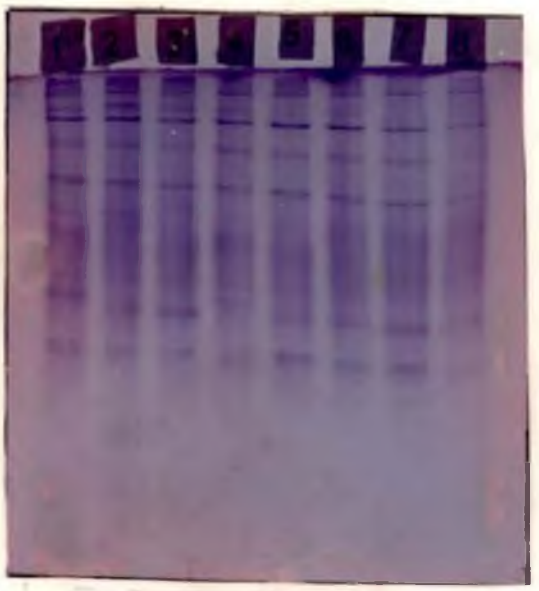


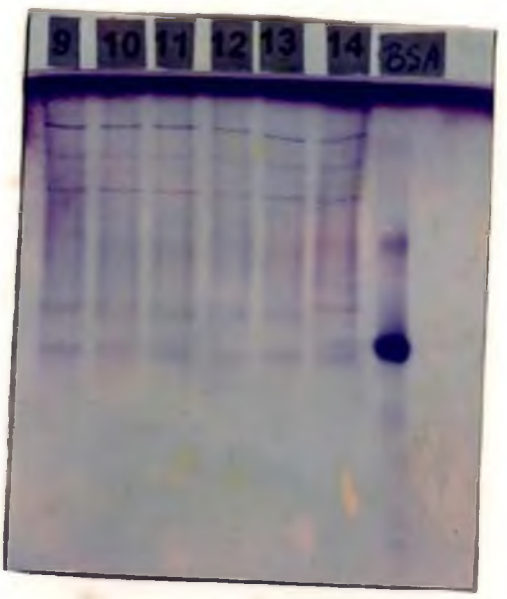
N1 - N13 = Njavara genotypes; Ptb-10 = Check

--- light —— dark

Plate 3. Protein banding pattern in germinated seeds (5 days after soaking) of Njavara genotypes

Legend: 1 - N1, 2 - N2, 3 - N3, 4 - N4, 5 - N5, 6 - N6, 7 - N7, 8 - N8, 9 - N9, 10 - N10, 11 - N11, 12 - N12, 13 - N13, 14 - Ptb-10

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and Ptb-10. The protein band number 13 (Rm = 0.494) was present only in Ptb-10 and was absent in all the Njavara genotypes.

Based on similarity indices of Njavara genotypes with Ptb-10, the genotypes N1 and N4 with a SI of 90.9 per cent were placed in group-1. The genotypes N9 and N12 had a SI of 85.7 per cent and were assigned to group-2. Group-3 consisted of the genotypes N2 and N5 with a SI of 81.8 per cent. The genotypes N10, N11 and N13 having a SI of 80 per cent were placed in group-4. The genotype N6 (SI = 76.2%) was assigned to group-5. Group-6 consisted of the genotypes N7 and N8 with a SI of 73.7 per cent. The genotype N3 had the lowest SI of 72.7 per cent and was placed in group-7 (Table 6). Similarly Njavara genotypes were divided into seven groups based on similarity indices among themselves (Table 8) and this was in confirmity with the grouping based on similarity indices of Njavara genotypes with Ptb-10.

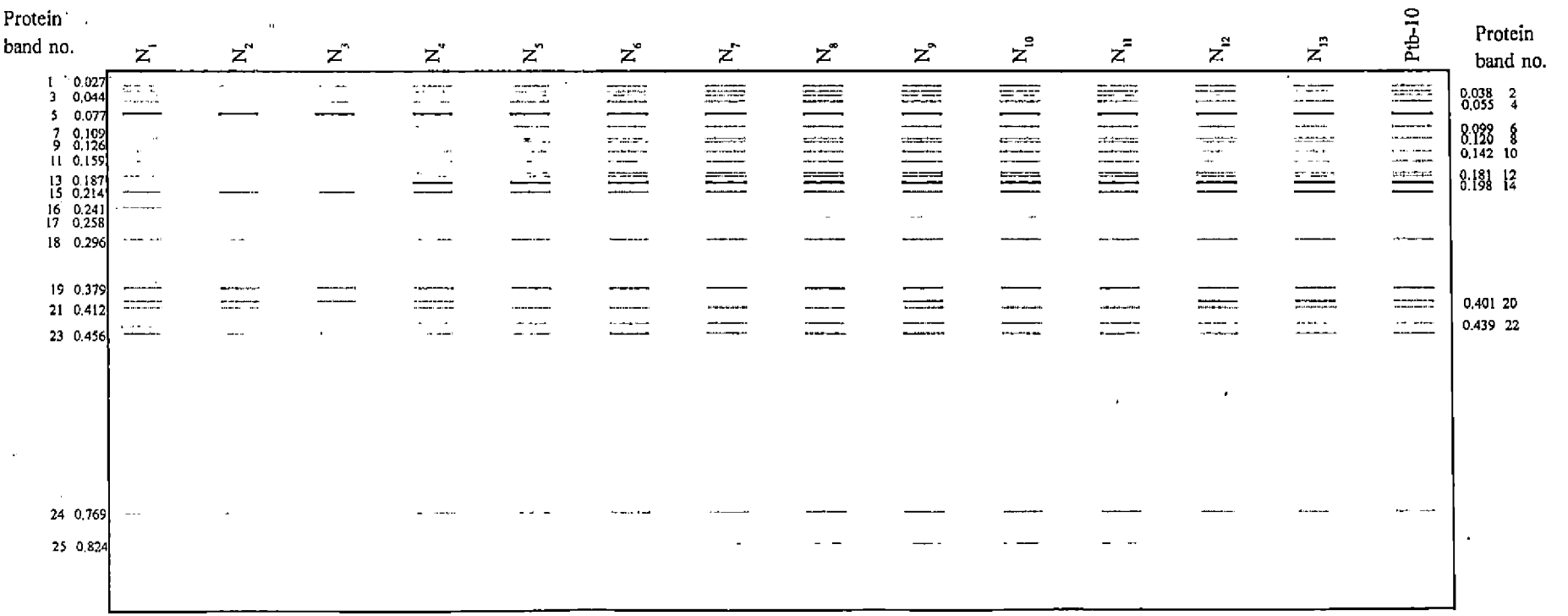
4.2.1.3 Leaf

Maximum number of protein bands were resolved for leaf samples (Fig.3 and Plate 4). A total of 25 protein bands were obtained. The protein bands number 1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 13, 15, 17, 18, 19, 22, 23, 24 and 25 with Rm values 0.027, 0.038, 0.044, 0.055, 0.077, 0.099, 0.120, 0.142, 0.159, 0.181, 0.187, 0.214, 0.258, 0.296, 0.379, 0.439, 0.456, 0.769 and 0.824 respectively were found in all the genotypes. The protein band number 7 (Rm = 0.109) was present only in the genotype N1. The protein band number 9 (Rm = 0.126) was observed in majority of the genotypes except N1, N2, N3 and N4. The protein band number 14 (Rm = 0.198) was also observed in most of the genotypes except N1, N2 and N3. The protein band number 16 (Rm = 0.241) was observed only in N1. The protein band number 20 (Rm = 0.401) was present in most of the genotypes except N5, N6, N7, N8, N10 and N11. The protein band number 21 (Rm = 0.412) was present in all the genotypes except N3.

Table 8. Similarity indices among Njavara genotypes based on protein banding pattern in germinated seed.

Njavara genotypes	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13
N1	1												
N2	0.909	1											
N3	0.818	0.909	1										
N4	1	0.909	0.818	1									
N5	0.909	1	0.909	0.909	1								
N6	0.857	0.952	0.900	0.909	0.952	1							
N7	0.737	0.842	0.842	0.737	0.842	0.889	1						
N8	0.737	0.842	0.842	0.737	0.842	0.890	1	1					
N9	0.857	0.952	0.857	0.857	0.952	0.900	0.889	0.889	1				
N10	0.800	0.900	0.800	0.800	0.900	0.842	0.941	0.941	0.947	1			
N11	0.800	0.900	0.800	0.800	0.900	0.842	0.941	0.941	0.947	1	1		
N12	0.857	0.952	0.857	0.857	0.952	0.900	0.889	0.889	1	0.947	0.947	1	
N13	0.810	0.900	0.800	0.800	0.900	0.842	0.941	0.941	0.947	1	1	0.947	1

Fig.3. Protein banding pattern in leaves (30 DAS) of Njavara genotypes

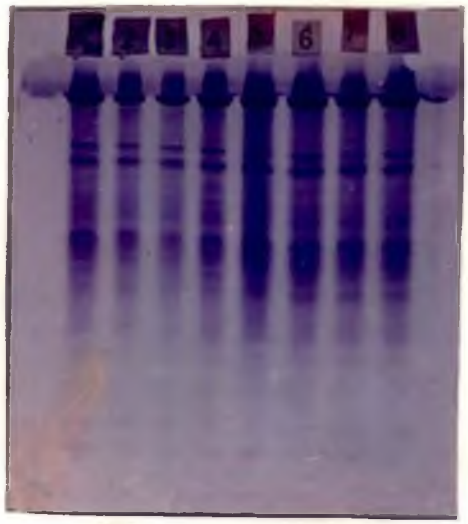


N1 - N13 = Njavara genotypes; Ptb-10 = Check

- – light —— medium —— dark

Plate 4. Protein banding pattern in leaves (30 DAS) of Njavara genotypes

Legend: 1 - N1, 2 - N2, 3 - N3, 4 - N4, 5 - N5, 6 - N6, 7 - N7, 8 - N8, 9 - N9, 10 - N10, 11 - N11, 12 - N12, 13 - N13, 14 - Ptb-10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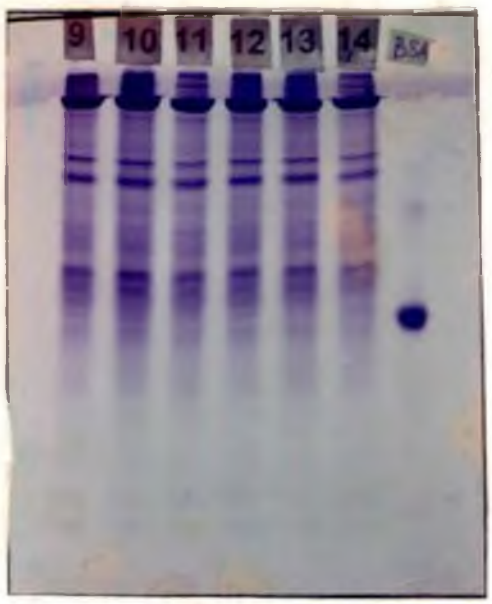
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Based on similarity indices with Ptb-10, Njavara genotypes were classified into five groups. The genotype N4 with a SI of 100 per cent was placed in group-1. Group-2 consisted of the genotypes N7, N9, N12 and N13 with a SI of 97.8 per cent. The genotype N2 (SI = 97.7%) was assigned to group-3. Group-4 consisted of the genotypes N1, N5, N6, N8, N10 and N11 with a SI of 95.6 per cent. The genotype N3 had the lowest SI of 95.4 per cent and was placed in group-5 (Table 6). Based on similarity indices of Njavara genotypes among themselves (Table 9) they can be divided into six groups as the genotype N1 was found to be distinct from other genotypes.

4.2.2 Isozyme analysis

4.2.2.1 Peroxidase

4.2.2.1.1 Quiscent seed

No peroxidase band was observed in quiscent seed sample.

4.2.2.1.2 Germinated seed

Germinated seed sample expressed more peroxidase bands than quiscent seed. Five bands were resolved for germinated seed samples (Fig.4 and Plate 5). The isozyme band PRX-1 (Rm = 0.472) was present only in the genotypes N7 and N10. The band PRX-2 (Rm = 0.483) was observed in majority of the Njavara genotypes except N7, N8 and N10. PRX-3 (Rm = 0.516) was present only in the genotypes N1, N4, N9, N12 and Ptb-10. The bands PRX-4 and PRX-5 with Rm values 0.560 and 0.593 respectively were present in all the genotypes, including Ptb-10.

Based on similarity index (SI) values, the genotypes N1, N4, N9 and N12 showing 100 per cent similarity with the check variety, Ptb-10 were placed in group-1. Group-2 consisted of N2, N3, N5, N6, N7, N10, N11 and N13 which had a SI of 85.7 per cent, while the genotype N8 having a SI of 66.6 per cent was assigned to group-3 (Table 10). Same number of groups and the same grouping pattern of

Table 9. Similarity indices among Njavara genotypes based on protein banding pattern in leaf

Njavara genotypes	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13
N1	1												
N2	0.978	1											
N3	0.955	0.977	1										
N4	0.957	0.955	0.955	1									
N5	0.913	0.933	0.909	0.957	1								
N6	0.913	0.933	0.909	0.957	0.952	1							
N7	0.936	0.957	0.933	0.979	0.979	0.979	1						
N8	0.913	0.933	0.909	0.957	1	1	0.979	1					
N9	0.936	0.957	0.933	0.979	0.979	0.979	1	0.979	1				
N10	0.800	0.900	0.800	0.800	0.900	0.842	0.941	0.941	0.947	1			
N11	0.913	0.933	0.909	0.957	1	1	0.979	1	0.979	1	1		
N12	0.936	0.957	0.933	0.979	0.979	0.979	1	0.979	1	0.979	0.979	1	
N13	0.936	0.957	0.933	0.979	0.979	0.979	1	0.979	1	0.979	0.979	1	1

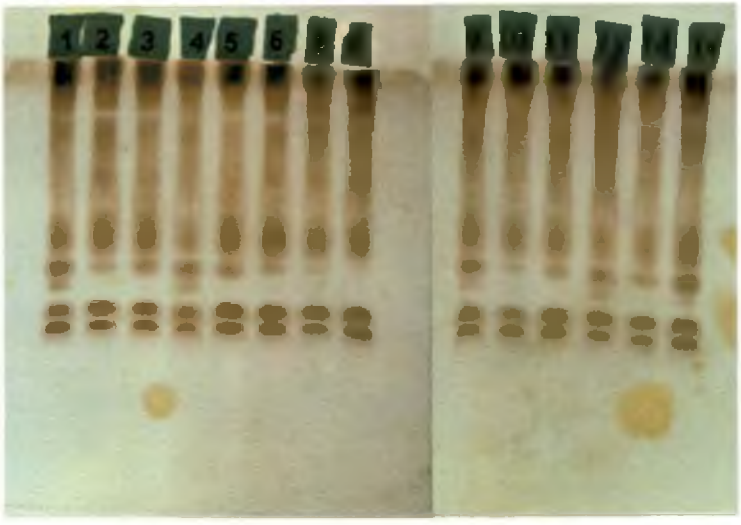


Plate 6: Peroxidase banding pattern in germinated seeds (5 days after soaking) of Njavara genotypes

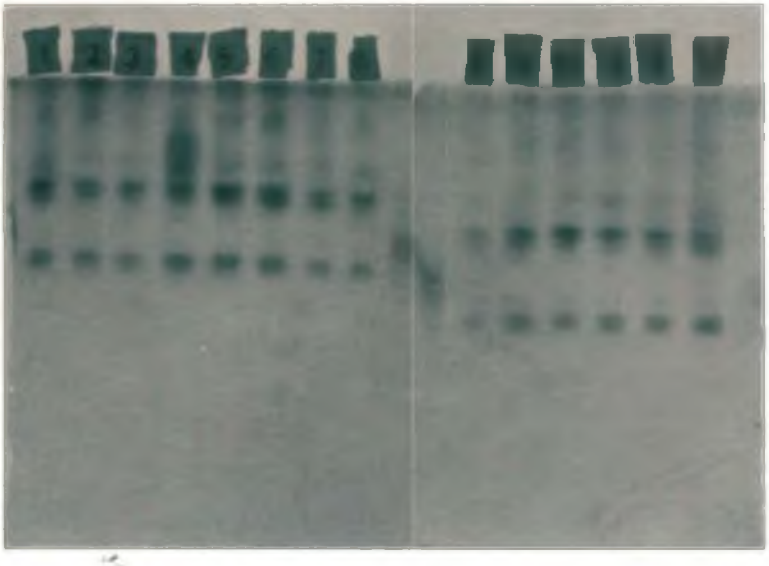
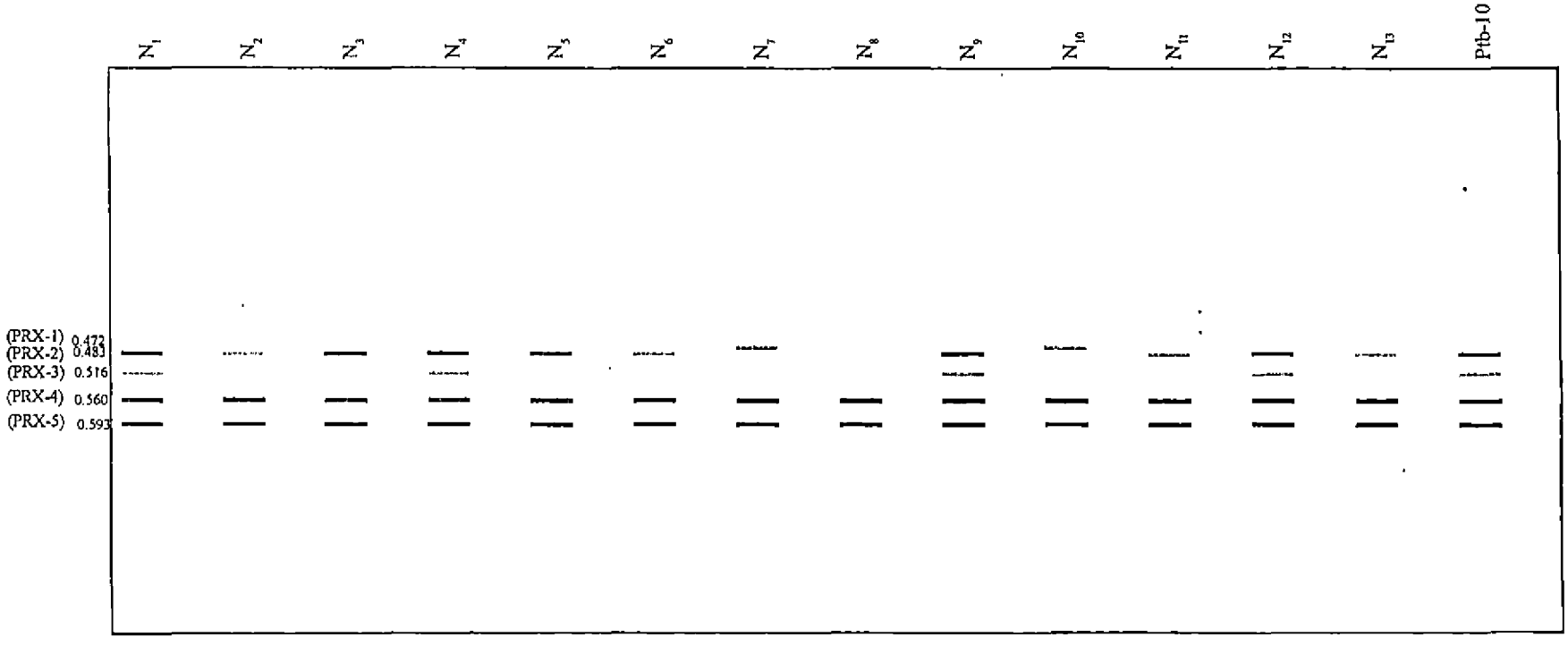


Plate 7: Esterase banding pattern in germinated seeds (5 days after soaking) of Njavara genotypes

Legend:1-N1, 2-N2, 3-N3, 4-N4, 5-N5, 6-N6, 7-N7, 8-N8, 9-N9, 10-N10, 11-N11, 12-N12, 13-N13, 14-Ptb-10

Fig.4. Zymogram of peroxidase in germinated seeds (5 Days after soaking) of Njavara genotypes



N1 - N13 = Njavara genotypes; Ptb-10 = Check

----light —— dark

Table 10. Groups of Njavara genotypes based on peroxidase banding pattern in germinated seed and leaf

Sl. No.	Njavara genotypes	Germinated seed		Leaf	
		Group	SI with Ptb-10 (%)	Group	SI with Ptb-10 (%)
1	N1	G-1	100	G-3	80.0
2	N2	G-2	85.7	G-3	80.0
3	N3	G-2	85.7	G-7	66.6
4	N4	G-1	100	G-5	71.4
5	N5	G-2	85.7	G-7	66.6
6	N6	G-2	85.7	G-7	66.6
7	N7	G-2	85.7	G-6	70.5
8	N8	G-3	66.6	G-8	54.5
9	N9	G-1	100	G-2	82.3
10	N10	G-2	85.7	G-3	80.0
11	N11	G-2	85.7	G-4	75.0
12	N12	G-1	100	G-1	88.8
13	N13	G-2	85.7	G-7	66.6

Njavara genotypes as above was noticed based on similarity indices among themselves (Table 11).

4.2.2.1.3 Leaf

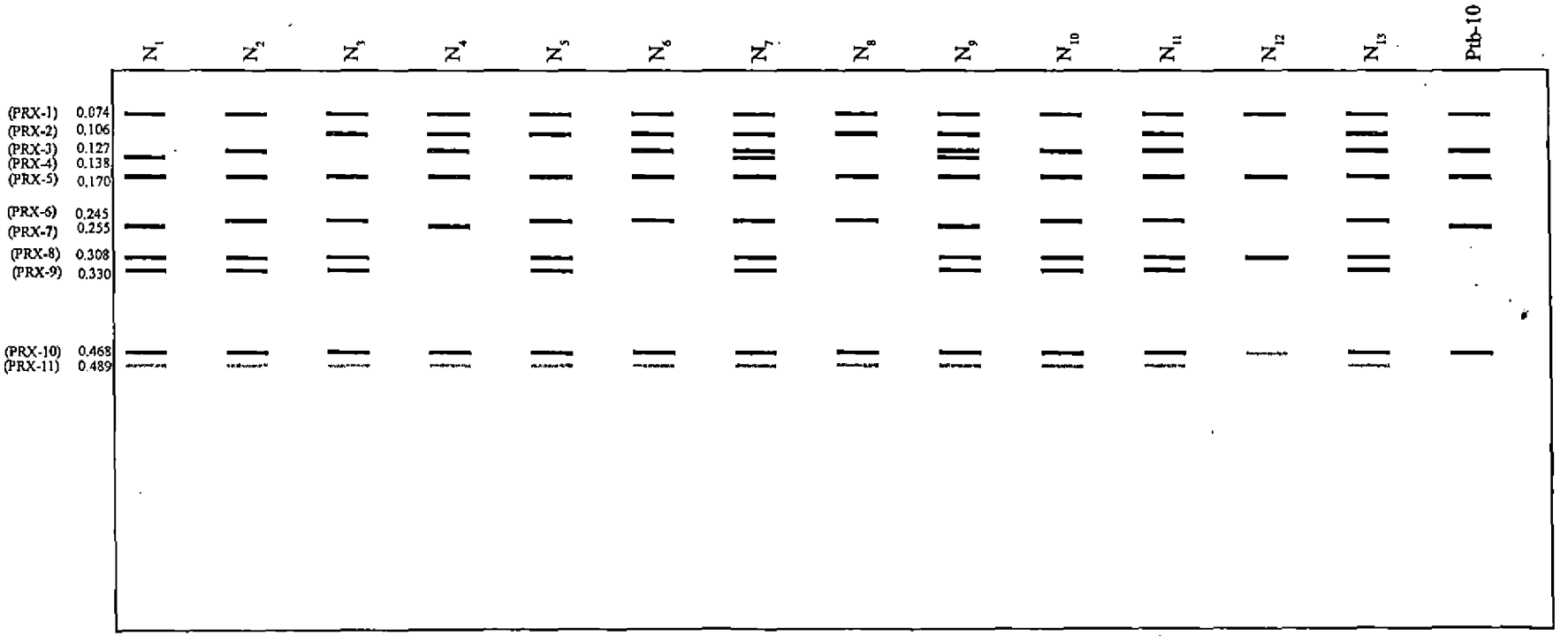
More number of peroxidase isozyme bands were observed in young leaves (Fig.5 and Plate 6). Eleven bands were resolved for leaf samples. The isozyme bands PRX-1, PRX-5 and PRX-10 with Rm values 0.074, 0.170 and 0.468 respectively were common for all the genotypes analysed. PRX-2 (Rm = 0.106) was present in most of Njavara genotypes except N1, N2, N10, N12 and Ptb-10. Similarly, the band PRX-3 (Rm = 0.127) was observed in N2, N4, N6, N7, N9, N10, N11, N13 and Ptb-10. PRX-4 (Rm = 0.138) was present only in N1, N7 and N9. PRX-6 (Rm = 0.245) was present in most of the genotypes except N1, N4, N9, N12 and Ptb-10. The isozyme band PRX-7 (Rm = 0.255) was present only in N1, N4, N9 and Ptb-10. PRX-8 (Rm = 0.308) was observed in majority of the genotypes except N4, N6, N8 and Ptb-10. The band PRX-9 (Rm = 0.330) was also present in most of the genotypes except N4, N6, N8, N12 and Ptb-10. The isozyme band PRX-11 (Rm = 0.489) was present in all the Njavara genotypes except N12. This band was absent in Ptb-10.

Based on similarity index (SI) values, N12 having the highest SI of 88.8 per cent with Ptb-10 was placed in group-1. The genotype N9 having a SI of 82.3 per cent was assigned to group-2. Group-3 consisted of N1, N2 and N10 with a SI of 80 per cent. The genotypes N11 (SI = 75%) and N4 (SI = 71.4%) were placed in group-4 and group-5 respectively. The genotypic N7 with a SI of 70.5 per cent was placed in group-6. Group-7 consisted of the genotypes N3, N5, N6 and N13 with a SI of 66.6 per cent. The genotype N8 with the least SI of 54.5 per cent was placed in group-8 (Table 10). Similarity indices among Njavara genotypes (Table 12) showed that Njavara genotypes can be classified into 10 groups as the genotypes N1 and N6 form two separate groups being distinct from other genotypes.

Table 11. Similarity indices among Njavara genotypes based on peroxidase isozyme pattern in germinated seed

Njavara genotypes	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13
N1	1												
N2	0.857	1											
N3	0.857	1	1										
N4	1	0857	0.857	1									
N5	0.857	1	1	0.857	1								
N6	0.857	1	1	0.857	1	1							
N7	0.857	1	1	0.857	1	1	1						
N8	0667	0.800	0.800	0.667	0.800	0.800	0.800	1					
N9	1	0.857	0.857	1	0.857	0.857	0.857	0.667	1				
N10	0.857	1	1	0857	1	1	1	0.800	0.857	1			
N11	0.857	1	1	0.857	1	1	1	0.800	0.857	1	1		
N12	1	0.857	0.857	1	0.857	0.857	0.857	0.667	1	0.857	0.857	1	
N13	0.857	1	1	0.857	1	1	1	0.800	0.857	1	1	0.857	1

Fig.5. Zymogram of peroxidase in leaves (30 DAS) of Njavara genotypes

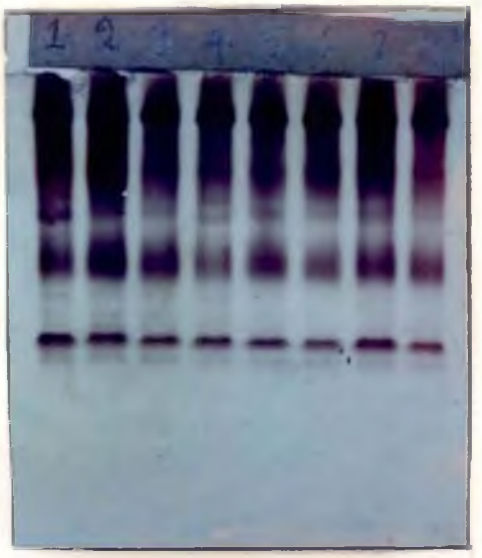


N1 - N13 = Njavara genotypes; Ptb-10 = Check

------light ——— dark

Plate 6. Peroxidase banding pattern in leaves (30 DAS) of Njavara genotypes

Legend: 1 - N1, 2 - N2, 3 - N3, 4 - N4, 5 - N5, 6 - N6, 7 - N7, 8 - N8, 9 - N9, 10 - N10, 11 - N11, 12 - N12, 13 - N13, 14 - Ptb-10


1 2

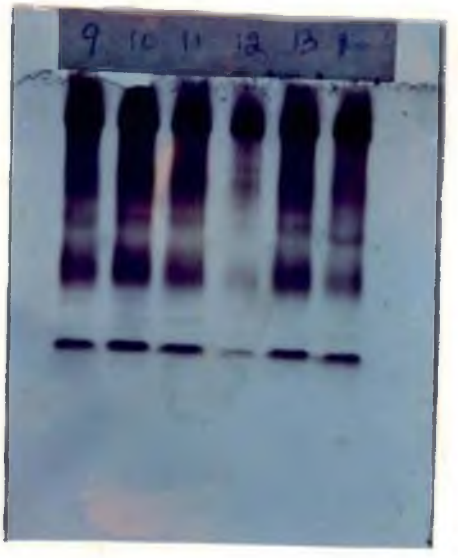

9 10 11 12 13

Table 12. Similarity indices among Njavara genotypes based on peroxidase isozyme pattern in leaf

Njavara genotypes	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13
N1	1												
N2	0.750	1											
N3	0.750	0.857	1										
N4	0.667	0.667	0.667	1									
N5	0.750	0.875	1	0.667	1								
N6	0.571	0.800	0.800	0.857	0.800	1							
N7	0.778	0.889	0.889	0.706	0.889	0.824	1						
N8	0615	0.714	0.857	0.770	0.857	0.923	0.750	1					
N9	0.889	0.778	0.778	0.824	0.778	0.706	0.900	0.625	1				
N10	0.750	1	0.875	0.667	0.875	0.800	0.889	0.714	0.778	1			
N11	0.706	0.941	0.941	0.750	0.941	0.875	0.947	0.800	0.842	0.941	1		
N12	0.500	0.667	0.545	0.730	0.500	0.727	0.571	0.600	0.571	0.667	0.615	1	
N13	0.706	0.941	0.941	0.750	0.941	0.875	0.947	0.800	0.842	0.941	1	0.615	1

4.2.2.2 Esterase

4.2.2.2.1 Quiscent seed

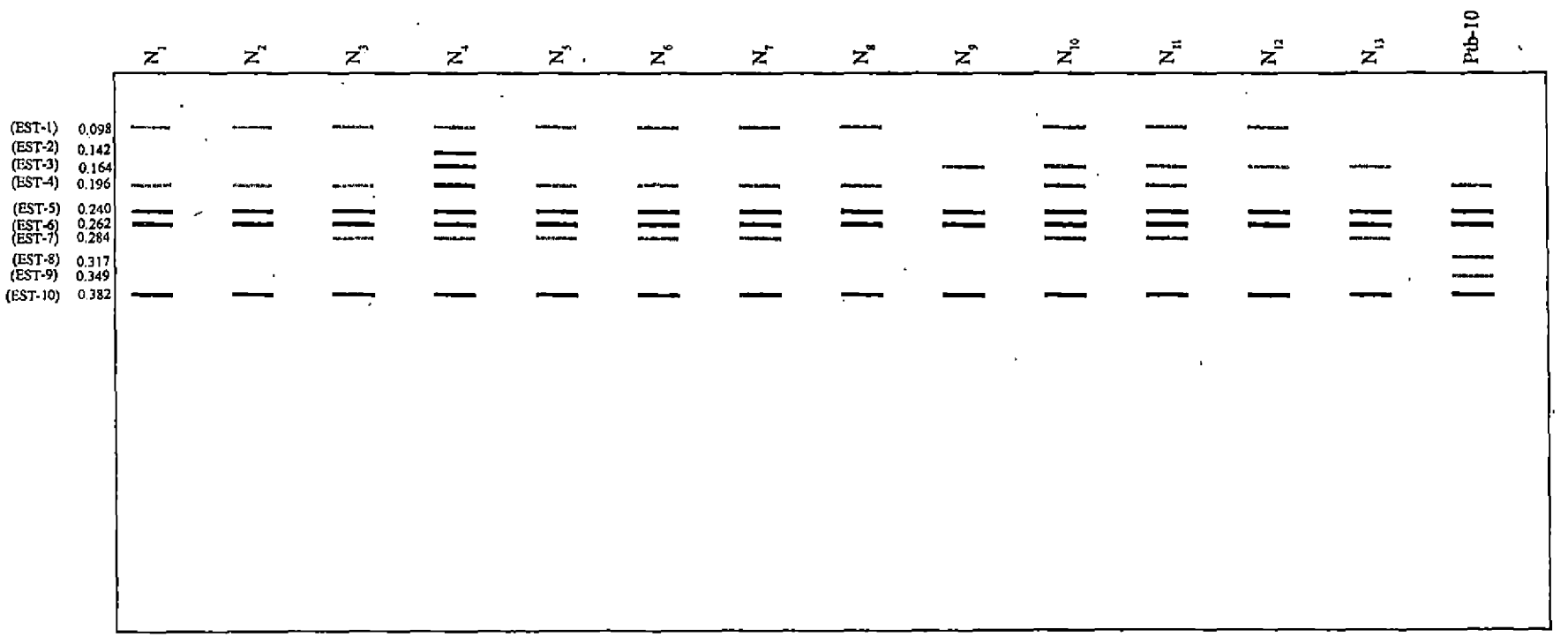
No esterase band was observed in quiscent seed sample.

4.2.2.2.2 Germinated seed

More number of esterase bands were observed in germinated seed (Fig. 6 and Plate 7). A total of ten esterase bands were obtained in germinated seed sample. The bands EST-5, EST-6 and EST-10 with Rm values 0.240, 0.262 and 0.382 respectively were common for all the genotypes. The isozyme band EST-1 (Rm = 0.098) was found in majority of the genotypes except N9, N13 and Ptb-10. The band EST-2 (Rm = 0.142) was present only in N4. The band EST-3 (Rm = 0.164) was observed in N4, N9, N10, N11, N12 and N13. The isozyme band EST-4 (Rm = 0.196) was found in majority of the genotypes except N9, N12 and N13. Most of the genotypes except N1, N2, N8, N9, N12 and Ptb-10 expressed the presence of the band EST-7 (Rm = 0.284). It is to be specifically mentioned that two bands viz., EST-8 and EST-9 with Rm values 0.317 and 0.349 respectively were present only in the check variety, Ptb-10 and were absent in all Njavara genotypes.

Based on similarity indices of Njavara genotypes with Ptb-10, the genotypes N1, N2 and N8 with the highest SI of 72.7 per cent were placed in group-1. This was followed by the genotypes N3, N5, N6 and N7 with a SI of 66.6 per cent and were assigned to group-2. Group-3 consisted of the genotypes N10 and N11 with a SI of 61.5 per cent. The genotypes N9 (SI = 60%) and N4 (SI = 57.1%) were placed in group-4 and group-5 respectively. Group-6 consisted of N12 and N13 with the lowest SI of 54.50 per cent (Table 13). Based on similarity indices among themselves (Table 14), Njavara genotypes can be classified into 7 groups because the genotypes N12 and N13 were distinct from each other and form two separate groups.

Fig.6. Zymogram of esterase in germinated seeds (5 days after soaking) of Njavara genotypes



N1 - N13 = Njavara genotypes; Ptb-10 = Check

—— light —— dark

Table 13. Groups of Njavara genotypes based on esterase banding pattern in germinated seed.and leaf

Sl. No.	Njavara genotypes	Germinated seed		Leaf	
		Group	SI with Ptb-10 (%)	Group	SI with Ptb-10 (%)
1	N1	G-1	72.7	G-1	100
2	N2	G-1	72.7	G-4	76.9
3	N3	G-2	66.6	G-2	92.3
4	N4	G-5	57.1	G-1	100
5	N5	G-2	66.6	G-2	92.3
6	N6	G-2	66.6	G-2	92.3
7	N7	G-2	66.6	G-2	92.3
8	N8	G-1	72.7	G-3	83.3
9	N9	G-4	60.0	G-5	72.7
10	N10	G-3	61.5	G-3	83.3
11	N11	G-3	61.5	G-5	72.7
12	N12	G-6	54.5	G-3	83.3
13	N13	G-6	54.5	G-3	83.3

Table 14. Similarity indices among Njavara genotypes based on esterase isozyme pattern in germinated seed

Njavara genotypes	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13
N1	1												
N2	1	1											
N3	0.909	0.909	1										
N4	0.909	0.909	1	1									
N5	0.769	0.769	0857	0.857	1								
N6	0.909	0.909	1	0.857	1	1							
N7	0.909	0.909	1	0.857	1	1	1						
N8	1	1	0.909	0.769	0.909	0.909	0.909	1					
N9	0.667	0.667	0.600	0.667	0.600	0.600	0.600	0.667	1				
N10	0.833	0.833	0.923	0.933	0.923	0.923	0.923	0.833	0.727	1			
N11	0.833	0.833	0.923	0.933	0.923	0.923	0.923	0.833	0.727	1	1		
N12	0.800	0.800	0.727	0.769	0.727	0.727	0.727	0.800	0.889	0.833	0.833	1	
N13	0.600	0.600	0.727	0.769	0.727	0.727	0.727	0.600	0.889	0.833	0.833	0.800	1

4.2.2.2.3 Leaf

Eight bands were resolved from leaf samples (Fig. 7 and Plate 8). The isozyme bands EST-1, EST-3, EST-7 and EST-8 with Rm values 0.076, 0.391, 0.532 and 0.554 respectively were common for all the Njavara genotypes and also for Ptb-10 whereas EST-2 (Rm = 0.369) was present only in N1, N4 and Ptb-10. The isozyme band EST-4 (Rm = 0.413) was observed in the genotypes N1, N3, N4, N5, N6, N7 and Ptb-10. EST-5 (Rm = 0.434) was present only in N2. The band EST-6 (Rm = 0.510) was found in majority of the Njavara genotypes except N9 and N11 and Ptb-10.

Based on similarity indices of Njavara genotypes with Ptb-10, the genotypes N1 and N4 showing 100 per cent similarity were placed in group-1. The genotypes N3, N5, N6 and N7 with a SI of 92.3 per cent were placed in group-2. Group-3 consisted of the genotypes N8, N10, N12 and N13 with a SI of 83.3 per cent. The genotype N2 (SI = 76.9%) was assigned to group-4. Group-5 consisted of the genotypes N9 and N11 with the lowest SI of 72.7 per cent (Table 13). Similarly same number of groups and the same grouping pattern of Njavara genotypes as above was noticed based on similarity indices among Njavara genotypes (Table 15).

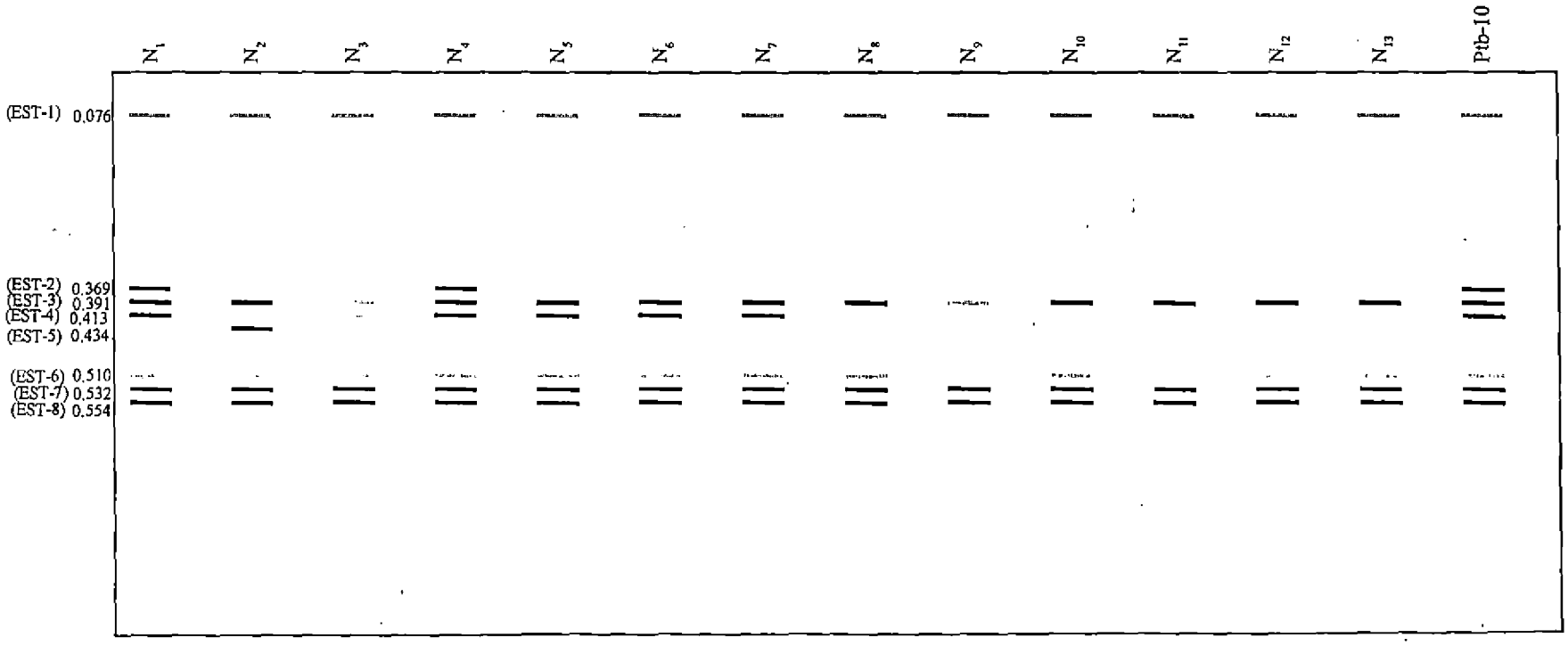
4.3 Nutritional qualities of Njavara grain

4.3.1 Soluble carbohydrate

The Njavara genotype N1 and N11 had the lowest soluble carbohydrate content of 1.38 per cent and 1.43 per cent respectively and were on par with that of Ptb-10. The genotypes N5, N7 and N13 expressed the highest soluble carbohydrate content of 2.93, 2.78 and 2.82 per cent respectively (Table 16).

Based on the analysis of soluble carbohyhdrate content in grain, Njavara genotypes were classified into low (upto 1.5%), medium (1.5%-2.5%) and high (>2.5%) soluble carbohydrate groups (Table 17). As a result, the genotypes N1 and N11 had low soluble carbohydrate content while the genotypes N2, N3, N4, N6, N8,

Fig.7. Zymogram of esterase in leaves (30 DAS) of Njavara genotypes



N1 - N13 = Njavara genotypes; Ptb-10 = Check

light ..... medium ——— dark

Plate 8. Esterase banding pattern in leaves (30DAS) of Njavara genotypes

Legend: 1 - N1, 2 - N2, 3 - N3, 4 - N4, 5 - N5, 6 - N6, 7 - N7, 8 - N8, 9 - N9, 10 - N10, 11 - N11, 12 - N12, 13 - N13, 14 - Ptb-10


1 2 3 4 5 6

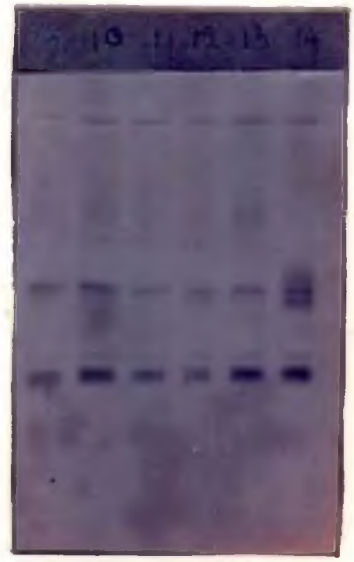

10 11 12 13 14

Table 15. Similarity indices among Njavara genotypes based on esterase isozyme pattern in leaf.

Njavara genotypes	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13
N1	1												
N2	0.769	1											
N3	0.923	0.833	1										
N4	1	0.769	0.923	1									
N5	0.923	0.833	1	0.923	1								
N6	0.923	0.833	1	0.923	1	1							
N7	0.923	0.833	1	0.923	1	1	1						
N8	0.833	0.909	0.909	0.833	0.909	0.909	0.909	1					
N9	0.727	0.800	0.800	0.727	0.800	0.800	0.800	0.889	1				
N10	0.833	0.909	0.909	0.833	0.909	0.909	0.909	1	0.889	1			
N11	0.727	0.800	0.800	0.727	0.800	0.800	0.800	0.889	1	0.889	1		
N12	0.833	0.909	0.909	0.833	0.909	0.909	0.909	1	0.889	1	0.889	1	
N13	0.833	0.909	0.909	0.833	0.909	0.909	0.909	1	0.889	1	0.889	1	1

Table 16. Mean performance of Njavara genotypes evaluated for nutritional quality parameters.

Sl. No.	Njavara genotypes	Soluble carbohydrate (%)	Protein content (%)	Free aminoacid content (mg g^{-1})	Fat content (%)	Amylose content (%)	Amylase activity (µg)
1	N1	1.38^{F}	10.14^{E}	0.147^{CDE}	0.58^{EF}	23.00^{AB}	28.00^{D}
2	N2	1.61^{EF}	13.39^{A}	0.190^{A}	0.55^{EFG}	20.16^{BCD}	16.67^{H}
3	N3	2.15^{BC}	11.85^{BC}	0.156^{ABC}	0.83^{BC}	21.90^{ABC}	22.00^{EF}
4	N4	2.15^{BC}	11.65^{BC}	0.160^{ABC}	1.18^{BC}	22.11^{ABC}	17.00^{H}
5	N5	2.93^{A}	12.04^{BC}	0.118^{DEF}	0.43^{FGH}	21.80^{ABC}	19.67^{FG}
6	N6	1.86^{DE}	10.53^{DE}	0.139^{CDE}	1.03^{CD}	18.88^{CD}	46.67^{A}
7	N7	2.78^{A}	9.47^{E}	0.140^{CDE}	0.90^{CD}	16.99^{D}	40.67^{B}
8	N8	2.10^{BCD}	12.60^{AB}	0.153^{BCD}	1.34^{B}	20.78^{BC}	19.67^{FG}
9	N9	2.26^{B}	11.46^{BCD}	0.114^{EF}	0.520^{FG}	23.27^{AB}	29.67^{D}
10	N10	2.23^{BC}	11.29^{CD}	0.099^{F}	0.56^{EF}	19.75^{BCD}	35.67^{C}
11	N11	1.43^{F}	11.88^{BC}	0.090^{F}	0.33^{FGH}	20.53^{BC}	17.33^{GH}
12	N12	1.96^{CD}	12.30^{BC}	0.160^{ABC}	0.25^{GH}	21.21^{ABC}	16.33^{H}
13	N13	2.82^{A}	11.65^{BC}	0.147^{CDE}	0.19^{H}	21.63^{ABC}	23.00^{E}
14	Ptb-10 (check variety)	1.52^{F}	9.53^{E}	0.188^{AB}	2.16^{A}	24.43^{A}	23.33^{E}

The figures with same alphabets in superscript do not differ significantly at 5% level

Table 17. Comparitive evaluation of quiscent seed quality among Njavara genotypes

Sl. No.	Nutritional quality parameter	Grouping of Njavara genotypes		
		Low/Small	Medium	High/Large
1	Soluble carbohydrate content	N1 and N11	N2, N3, N4, N6, N8, N9, N10, N12 and Ptb-10	N5, N7 and N13
2	Protein content	N7 and Ptb-10	N1, N3, N4, N6, N9, N10, N11 and N13	N2, N5, N8 and N12
3	Present of free aminoacids	N1, N2, N3, N4, N5, N6, N7, N9, N10 and N12	N1, N2, N3, N4, N5, N6, N7, N8, N9, N10, N11, N12, N13 and Ptb-10	N8, N9, N10, N11, N13 and Ptb-10
4	Fat content	N1, N2, N3, N5, N7, N9, N10, N11, N12 and N13	N4, N6 and N8	Ptb-10
5	Amylose content	N6, N7 and N10	N1, N2, N3, N4, N5, N8, N9, N11, N12, N13 and Ptb-10	
6	Starch grain size	N2, N9 and N12	N1, N4, N5, N6, N7, N8, N10, N11 and N13	N3 and Ptb-10
7	Amylase activity	N2, N4, N5, N8, N11 and N12	N1, N3, N9, N10, N13 and Ptb-10	N6 and N7

N9, N10, N12 and Ptb-10 had medium soluble carbohydrate content. The genotypes N5, N7 and N13, which exhibited high soluble carbohydrate were having a lemma and palea colour of brown furrows on straw.

4.3.2 Protein content

The protein content in Njavara grains ranged between 9.47 and 13.39 per cent. The grains of N7 and N1 were poor in protein with a protein content of 9.47 and 10.14 per cent respectively and were on par with that of Ptb-10 (9.53%) (Table 16). The genotype N2 was found to have the highest protein content of 13.39 per cent followed by N8 (12.60%).

As earlier, based on protein content in grain, Njavara genotypes were divided into three groups viz., low (upto 10%), medium (10-12%) and high (>12%) protein groups (Table 17). The genotypes N7 and Ptb-10 were found to have low protein content while the genotypes N1, N3, N4, N6, N9, N10, N11 and N13 had medium protein content. High protein content was observed for the genotypes N2, N5, N8 and N12 out of which the genotype N5 had a lemma and palea colour of brown furrows on straw and the rest exhibited gold furrows on straw.

4.3.3 Presence of free amino acids

The free amino acid content in Njavara grain ranged between 0.090 mg/g and 0.190 mg/g. The genotypes N2, N3, N4, N8, N12 and Ptb-10 expressed higher free amino acid content (>0.150 mg/g) than the rest of the genotypes (Table 16).

The results of paper chromatography performed with respect to free amino acids were presented in Table 18 and Plate 9. A total of nine different spots were resolved with 3 to 5 spots per genotype. The Rf values ranged from 0.140 to 0.437. The genotype N10 showed a maximum of 5 spots while the genotypes N1, N4, N7 and N8 had a minimum of 3 spots. Four spots were observed for the check variety, Ptb-10.

Table 18. Paper chromatography of free amino acids in Njavara grain

Sl. No.	Njavara genotype	No. of spots	Rf value
1	N1	3	0.190 0.265 0.383
2	N2	4	0.190 0.240 0.278 0.376
3	N3	4	0.179 0.220 0.260 0.360
4	N4	3	0.190 0.260 0.370
5	N5	4	0.185 0.230 0.275 0.370
6	N6	4	0.185 0.220 0.260 0.370
7	N7	3	0.190 0.240 0.367
8	N8	3	0.264 0.354 0.437
9	N9	4	0.146 0.228 0.350 0.430
10	N10	5	0.139 0.225 0.268 0.322 0.425
11	N11	4	0.206 0.250 0.290 0.420

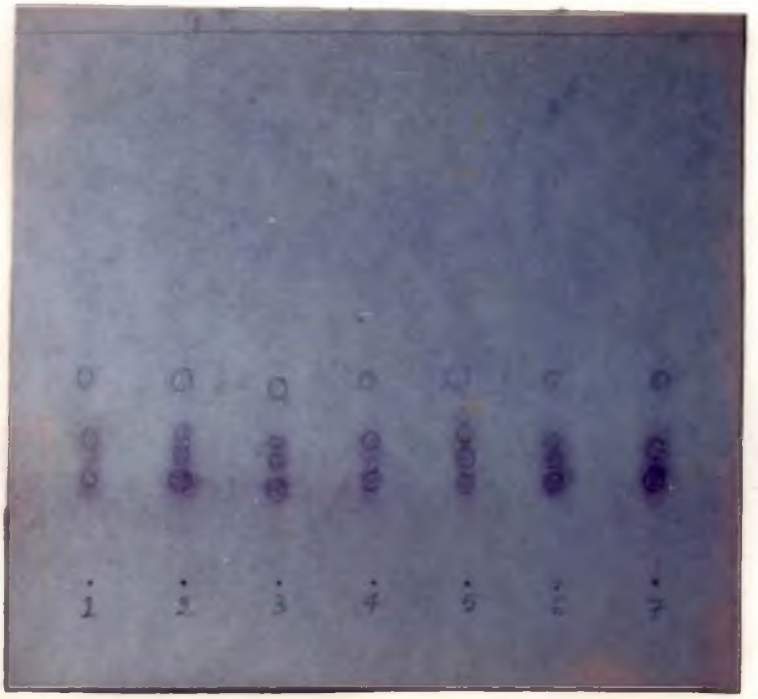
Contd.

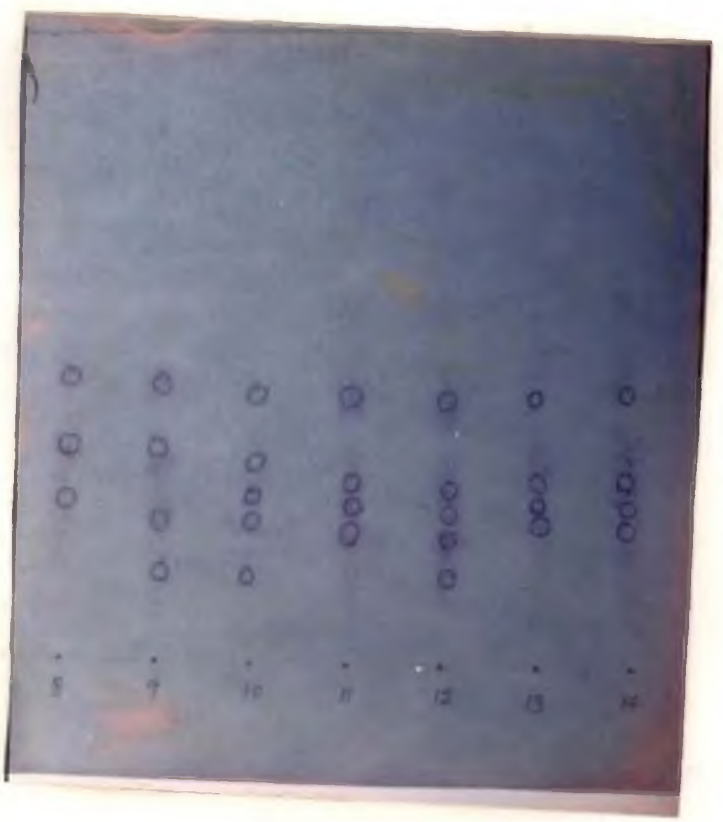
Table 18. Continued

Sl. No.	Njavara genotype	No. of spots	Rf value
12	N12	4	0.140 0.198 0.240 0.276
13	N13	4	0.224 0.255 0.289 0.416
14	Ptb-10 (check variety)	4	0.213 0.253 0.294 0.427

Plate 9. Paper chromatography of free amino acids in the grains of Njavara genotype

Legend: 1 - N1, 2 - N2, 3 - N3, 4 - N4, 5 - N5, 6 - N6, 7 - N7, 8 - N8, 9 - N9, 10 - N10, 11 - N11, 12 - N12, 13 - N13, 14 - Ptb-10





Total free amino acid content was found to be varying among Njavara genotypes. But paper chromatographic study of free amino acids showed three groups as observed in soluble carbohydrate and protein contents. The Rf values of the observed free amino acids were organized into three classes and accordingly spots with Rf values up to 0.2 were placed in low group while the spots with Rf values ranging from 0.2 to 0.4 were assigned to medium group. The high group included spots with Rf values of 0.4 and above. Based on this classification, the genotypes N1, N2, N3, N4, N5, N6,N7,N9,N10 and N12 had spots with Rf values below 0.2 while all the genotypes including Ptb-10 had spots with Rf values ranging between 0.2 to 0.4. The genotypes N8, N9, N10, N11, N13 and Ptb-10 were found to have spots with Rf values above 0.4 (Table 17).

4.3.4 Fat content

Compared to the check variety, the Njavara genotypes in general, expressed a low fat content. While Ptb-10 recorded the highest fat content of 2.16 per cent, the genotypes N5, N11, N12 and N13 recorded the lowest fat content of 0.43, 0.33, 0.25 and 0.19 per cent respectively (Table 16).

The Njavara genotypes, based on fat content in grain, were divided into low (up to 1%), medium (1-2%) and high (>2%) fat groups. As a result, the genotypes N1, N2, N3, N5, N7, N9, N10, N11, N12 and N13 had low fat content while the genotypes N4, N6 and N8 possessed medium fat content. High fat content was observed only for Ptb-10 (Table 17).

4.3.5 Amylose content

Like other Indian rice varieties Njavara comes to the group of nonwaxy rices with a comparitively higher amylose content ranging from 16.99 per cent for N7 to 23.27 per cent for N9. Ptb-10 recorded an amylose content of 24.27 per cent (Table 16).

Based on amylose content in grain Njavara genotypes were divided into low (10 to 20%), medium (20-25%) and high (25-30%) amylose groups. Accordingly, low amylose content was noticed for the genotypes N6, N7 and N10 while rest of the genotypes had medium amylose content (Table 17).

4.3.6 Starch grain size

The Njavara genotypes as well as Ptb-10 had multifaceted starch grains. In the present study, the largest starch grains were identified in Ptb-10 (5.73 μ) and N3 (5.62 μ) (Table 19). Based on starch grain size, the genotypes were divided into eight groups. The check variety, Ptb-10 and the Njavara genotype N3 were placed in group-1. The genotype N5 was observed to have a starch grain size of 5.41 μ and was assigned to group-2. The genotypes N6, N10 and N13 having a starch grain size of 5.31 μ could be placed in group-3. Group-4 consisted of the genotype N7 with a starch grain size of 5.20 μ. The genotypes N1, N4 and N11 with a starch grain size of 5.10 μ were assigned to group-5, while the genotype N8 with a starch grain size of 5.00 μ was placed in group-6. Group-7 consisted of the genotype N2 having a starch grain size of 4.89 μ. The smallest starch grains (4.79 μ) were observed for the genotypes N9 and N12 which were placed in group-8.

4.3.7 Amylase activity

Amylase activity ranged from 16.33 μg for N12 to 46.67 μg for N6. Amylase activity of 23.33 μg was recorded for the check variety, Ptb-10 (Table 16).

Analysis of amylase activity in grain revealed that Njavara genotypes can be divided in to low (upto 20μg), medium (20-40μg) and high (>40μg) amylase activity groups. Accordingly, the genotypes N2, N4, N5, N8, N11 and N12 showed low amylase activity while the genotypes N1, N3, N9, N10, N13 and Ptb-10 exhibited medium amylase activity. The genotypes N6 and N7 had high amylase activity (Table 17).

Table 19. Starch grain size in the grain of Njavara genotypes

Sl. No.	Njavara genotypes	Starch grain size (μ)	Group
1	N1	5.10 ^{DE}	G-5
2	N2	4.89 ^{FG}	G-7
3	N3	5.62 ^{A}	G-1
4	N4	5.10 ^{DE}	G-5
5	N5	5.41 ^{B}	G-2
6	N6	5.31 ^{BC}	G-3
7	N7	5.20 ^{CD}	G-4
8	N8	5.00 ^{EF}	G-6
9	N9	4.79 ^{G}	G-8
10	N10	5.31 ^{BC}	G-3
11	N11	5.10 ^{DE}	G-5
12	N12	4.79 ^{G}	G-8
13	N13	5.31 ^{BC}	G-3
14	Ptb-10 (check variety)	5.73 ^{A}	G-1

The figures with same alphabets in superscript do not differ significantly

4.3.8 Flavonoids

Flavonoids were not detected in the solvent extracts of grain in all the Njavara genotypes and also in Ptb-10.

4.4 Genetic variability

The extent of genetic variability with respect to 15 quantitative and seven nutritional quality characters, in a set of 13 Njavara genotypes, were estimated in the present study.

The abstract of analysis of variance of these characters is given in Table 20. The data on range, mean and estimates of genetic parameters for the above twenty two characters are presented in Table 21.

Results from the analysis of variance, revealed high significant differences among the 13 genotypes for all quantitative and nutritional quality characters studied.

Among the quantitative characters, seedling height varied from 29.57 to 41.80 cm with an average of 35.53 cm. Length and width of leaf varied from 34.97 to 50 cm and 0.66 to 1.28 cm with means 43.15 cm and 0.94 cm respectively. Panicle length and ligule length varied from 18.30 to 25.19 cm and 1.24 to 2.40 cm with an average of 21.22 cm and 1.77 cm respectively. In the case of number of days to 50 per cent heading, the range of variation was from 44 to 63 days with an average of 41.70 days. Culm length varied from 54.27 to 94.33 cm, average being 73 cm. With respect to mean culm number and mean culm diameter, the variability ranged from 4.37 to 9.13 and 4.20 to 6.40 mm with an average of 6.60 and 5.34 mm respectively. 1000 grain weight ranged from 18.50 to 30.07 g with a mean of 24.64 g. Grain yield and straw yield varied from 919 to 2684 kg ha^{-1} and 1752 to 6818 kg ha^{-1} with an average of 1722 kg ha^{-1} and 3679 kg ha^{-1} respectively. Days from seeding to maturity ranged from 60 to 88 days and had a mean of 68 days.

Table 20. Analysis of variance for grain yield and associated quantitative and qualitative characters in Njavara genotypes.

Source of variation	Mean sum of squares									
	Seedling height	Leaf length	Lead width	Panicle length	Ligule length	Days to 50% heading	Culm length	Culm number	Culm diameter	1000 grain weight
Replications	3.557	0.543	0.001	2.750	0.060	0.881	10.556	1.430	0.029	1.201
Treatments	26.783**	82.162**	0.133**	12.656**	0.333**	105.665**	349.461**	5.999**	1.647**	49.619**
Error	1.705	8.835	0.002	0.776	0.028	1.522	11.996	0.913	0.225	1.397

Grain length	Grain width	Grain yield	Straw yield	Maturity duration	Soluble carbohy-drate	Fat content	Amylose content	Free amino-acid content	Protein content	Amylase activity	Starch grain size
0.086	0.009	1280437.6	263686.3	3.167	0.063	0.001	0.718	0.000	0.735	193.700	0.720
0.846**	0.117**	1104108.1**	7701033.2**	165.313**	0.765**	0.836**	10.778**	0.003**	3.864**	276.260**	0.239**
0.021	0.002	201865.6	357995.3	2.038	0.024	0.027	3.392	0.000	0.351	2.046	0.006

**Significant at 1% level

Table 21. Range, mean and estimates of genetic parameters for grain yield and associated quantitative and qualitative characters in Njavara genotypes

Sl. No.	Character	Range	Mean±SEM*	Genotypic variance (Vg)	Phenotypic variance (Vp)	Heritability in broad sense (H^2)	Phenotypic coefficient of variation (PCV)	Genotypic coefficient of variation (GCV)	Genetic advance (GA)	Genetic gain
1	Seedling height (cm)	29.57 (Ptb-10) 41.80 (N3)	35.53±1.69	12.54	14.24	88.0	10.62	9.96	6.84	19.26
2	Leaf length (cm)	34.97 (N4) 50.00 (N1)	43.15±1.82	24.44	33.28	73.5	13.36	11.45	8.72	20.22
3	Leaf width (cm)	0.66 (N11) 1.28 (Ptb-10)	0.94±0.07	0.04	0.05	95.6	22.68	22.18	0.42	44.60
4	Panicle length (cm)	18.30 (N9) 25.19 (Ptb-10)	21.22±0.37	0.63	1.40	44.7	5.58	3.73	1.09	5.13
5	Ligule length (cm)	1.24 (N12) 2.40 (N6)	1.77±0.11	0.10	0.13	78.4	20.35	18.02	0.58	32.87
6	Days to 50% heading	44.00 (N9, N10) 63.00 (Ptb-10)	41.70±1.90	34.71	36.23	95.8	14.43	14.13	11.88	28.49
7	Culm length (cm)	54.27 (N4) 94.33 (N6)	73.00±3.50	112.49	124.48	90.4	15.26	14.50	20.76	28.41
8	Culum number	4.37 (N1) 9.13 (N11)	6.60±0.51	1.70	2.60	65.0	24.32	19.60	2.16	32.56
9	Culm diameter (mm)	4.20 (N12) 6.40(N6)	5.34+0.26	0.47	0.70	67.8	15.64	12.88	1.17	21.85
10	1000 grain weight (g)	18.50 (N8) 30.07 (N6)	24.64+1.32	16.07	17.47	92.0	16.96	16.27	7.92	32.15
11	Grain length (mm)	7.60 (N9) 9.27 (N7)	8.56±0.17	0.28	0.29	92.9	6.35	6.12	1.04	12.16

Contd.

*SEM - Standard Error of Mean

Table 21. Continued

Sl. No.	Character	Range	Mean±SEM	Genotypic variance (Vg)	Phenotypic variance (Vp)	Heritability in broad sense (H^2)	Phenotypic coefficient of variation (PCV)	Genotypic coefficient of variation (GCV)	Genetic advance (GA)	Genetic gain
12	Grain width (mm)	2.65 (N8) 3.33 (N6)	3.00±0.06	0.04	0.04	95.0	6.67	6.51	0.39	13.07
13	Grain yield (kg/ha)	919 (N8) 2684 (N11)	1722±224	300747.50	502613.09	59.8	41.14	31.83	873.88	50.72
14	Straw yield (kg/ha)	1752 (N6) 6818 (Ptb-10)	3679±529	2447679.25	2805674.50	87.2	45.51	42.51	3010.25	81.80
15	Days from seeding to maturity	60.00 (N9) 88.00(Ptb-10)	68.00±2.38	54.46	56.46	96.4	11.03	10.83	14.92	21.90
16	Soluble carbohydrate (%)	1.38 (N1) 2.93 (N5)	2.00±0.16	0.25	0.27	91.1	24.98	23.84	0.97	46.90
17	Fat content (%)	0.19 (N13) 2.16 (Ptb-10)	0.77±0.17	0.27	0.29	90.9	70.19	66.91	1.02	131.43
18	Amylose content (%)	16.99 (N7) 24.43(Ptb-10)	21.20±0.76	2.46	5.85	42.1	11.42	7.41	2.09	9.90
19	Free amino acid content (mg/g)	0.09 (N11) 0.19 (N2)	0.14±0.01	0.001	0.001	100.0	22.11	22.11	0.06	45.55
20	Protein content (%)	9.47 (N7) 13.39 (N2)	11.41±0.39	1.17	1.52	76.9	10.80	9.48	1.95	17.13
21	Amylase (µg) activity	16.33 (N12) 46.67 (N6)	25.40±3.00	91.52	93.56	97.8	38.07	37.65	19.49	76.71
22	Starch grain size (µ)	4.79 (N12) 5.73 (Ptb-10)	5.19±0.08	0.08	0.08	92.8	5.57	5.37	0.55	10.66

Grain length and grain width varied from 7.60 to 9.27 mm and 2.65 to 3.33 mm with an average of 8.56 mm and 3 mm respectively. Soluble carbohydrate and fat contents varied from 1.38 to 2.93 per cent and 0.19 to 2.16 per cent with an average of 2 per cent and 0.77 per cent respectively. Among cooking quality characters, amylose content ranged from 16.99 to 24.43 per cent and had an average of 21.20 per cent. Free amino acid content and protein contents varied from 0.09 to 0.19 mg/g and 9.47 to 13.39 per cent with an average of 0.14 mg/g and 11.41 per cent respectively. Amylase activity ranged from 16.33 to 46.67 µg, with a mean of 25.40 µg. Starch grain size varied from 4.79 to 5.73 µ with an average of 5.19 µ.

4.5 Genotypic and phenotypic coefficients of variation

Among nutritional quality characters high pcv and gcv was observed for fat content (70.19%) and (66.91%). Moderate estimates of pcv and gcv were observed with respect to the quantitative characters straw yield (45.51% and 42.51%) and grain yield (41.14% and 31.83%). Amylase activity (38.07% and 37.65%) among nutritional quality characters had moderate estimates of pcv and gcv. Low variability was observed with respect to the characters, seedling height, leaf length, leaf width, panicle length, ligule length, days to 50 per cent heading, culm length, culm number, culm diameter, 1000 grain weight, grain length, grain width and days from seeding to maturity among quantitative characters and soluble carbohydrate content, amylose content, free amino acid content, protein content and starch grain size among nutritive quanlity characters.

4.6 Heritability

Among quantitative characters, heritability (in broadsense) estimates ranged from 44.7 per cent (panicle length) to 96.4 per cent (days from seeding to maturity). Heritability estimates of qualitative characters ranged from 42.1 per cent (amylose content) to 100 per cent (free amino acid content). Heritability estimates with respect to quantitative characters namely, days from seeding to maturity

(96.4%), days to 50 per cent heading (95.8%), leaf width (95.6%), grain length (92.9%), 1000 grain weight (92%), culm length (90.4%), seedling height (88%), straw yield (87.2%), ligule length (78.4%), leaf length (73.5%), culm diameter (67.8%) and culm number (65%) and grain yield (59.5%) were found to be high. Panicle length (44.7%) exhibited moderate heritability. All nutritional quality characters except amylose content (42.1%) exhibited high heritability.

4.7 Genetic gain

Genetic gain among quantitative characters varied from 5.13 per cent for panicle length to 81.80 per cent for straw yield. Among nutritional quality characters, genetic gain ranged from 9.90 per cent for amylose content to 131.43 per cent for fat content. Among quantitative characters genetic gain was high for straw yield (81.80%), grain yield (50.72%), leaf width (44.60%), ligule length (32.87%), culm number (32.56%), 1000 grain weight (32.15%), days to 50 per cent heading (28.49%), culm length (28.41%), days from seeding to maturity (21.90%) and culm diameter (21.85%). Among nutritional quality characters genetic gain was high for fat content (131.43%), amylase activity (76.71%), soluble carbohydrate content (46.90%) and free amino acid content (45.55%). Estimates of genetic gain were moderate for the quantitative characters namely, leaf length (20.22%), seedling height (19.26%), grain width (13.07%) and grain length (12.16%) and for the nutritional quality characters namely, protein content (17.13%) and starch grain size (10.66%). Genetic gain was low for panicle length and amylose content.

4.8 Correlation studies

The genotypic and phenotypic correlation coefficients between grain yield hectare^{-1} and twenty one yield component characters *inter se* are presented in Table 22.

Grain in kg ha^{-1} was found to be positively and significantly correlated at genotypic level with straw yield (0.627**) and leaf length (0.506*).

Table 22. Phenotypic (upper diagonal) and genotypic (lower diagonal) correlation coefficients between yield and yield component characters in Njavara genotypes

	1	2	3	4	5	6	7	8	9	10	11
1		-0.261	-0.186	-0.201	0.037	-0.157	-0.010	-0.013	-0.146	0.090	-0.227
2	-0.264		0.188	0.415	-0.398	0.107	-0.228	0.078	0.667*	0.427	0.753*
3	-0.355	0.266		0.194	0.060	-0.386	-0.042	-0.132	-0.120	-0.286	0.030
4	-0.294	0.532*	0.278		-0.007	-0.258	-0.364	0.090	0.487	0.263	0.468
5	0.023	-0.480*	-0.028	0.064		-0.602*	0.107	-0.407	-0.437	-0.169	-0.453
6	0.169	0.121	-0.612**	-0.319	-0.682**		0.038	0.351	0.358	0.289	0.165
7	-0.017	-0.239	-0.148	-0.481*	0.095	0.032		0.097	-0.391	-0.132	-0.606*
8	-0.028	0.130	-0.216	0.170	-0.587**	0.401	0.121		0.364	0.660*	0.183
9	-0.156	0.706**	-0.138	0.611**	-0.509*	0.370	-0.409	0.371		0.594*	0.839*
10	0.087	0.527*	-0.329	0.396	-0.266	0.318	-0.139	0.639**	0.640**		0.497
11	-0.239	0.820**	0.108	0.564**	-0.495*	0.164	-0.625**	0.194	0.869**	0.544**	
12	-0.108	-0.143	-0.636**	-0.031	-0.386	0.751**	0.131	0.563**	0.266	0.236	-0.064
13	0.040	-0.268	-0.0008	-0.424	-0.513	-0.380	-0.109	-0.334	-0.695**	-0.231	-0.420
14	-0.096	0.720**	-0.102	-0.701**	-0.520**	0.354	-0.308	0.580**	1.006**	0.820**	0.850**
15	-0.252	0.666**	0.041	0.560*	-0.816**	0.409**	-0.240	0.620**	0.836**	0.508*	0.692**
16	-0.205	0.304	-0.343	0.268	-0.561**	0.454*	-0.092	0.569**	0.817**	0.474*	0.578**
17	-0.075	0.356	-0.357	0.274	-0.425	0.251	-0.057	0.443	0.797**	0.466*	0.559**
18	-0.346	0.196	-0.179	0.264	-0.508*	0.474*	-0.129	0.602**	0.731**	0.384	0.489*
19	-0.104	-0.290	-0.274	0.090	0.070	-0.171	-0.097	0.506*	-0.081	0.225	-0.038
20	-0.540**	0.173	0.581*	0.153	-0.313	-0.278	-0.060	0.459	0.017	-0.009	0.167
21	-0.231	0.803**	0.305	0.478*	-0.471*	0.071	-0.467*	0.062	0.775**	0.372	0.904**
22	0.119	0.408	0.197	0.075	-0.504	0.191	0.077	0.460	0.360	0.538*	0.400

Contd.

Table 22. Continued

	12	13	14	15	16	17	18	19	20	21	22
1	-0.090	0.006	-0.098	-0.226	-0.174	-0.062	-0.340	-0.045	-0.485	-0.233	0.115
2	-0.130	-0.230	0.505*	0.532*	0.286	0.359	0.179	-0.196	0.171	0.741*	0.348
3	-0.398	-0.009	-0.078	-0.039	-0.190	-0.203	-0.129	-0.026	-0.354	0.145	0.119
4	-0.027	-0.201	0.503*	0.390	0.224	0.260	0.226	0.068	0.152	0.375	0.134
5	-0.334	0.320	-0.340	-0.656*	-0.418	-0.344	-0.458	0.052	-0.303	-0.443	-0.388
6	0.706*	-0.284	0.271	0.364	0.427	0.251	0.460	-0.145	-0.258	0.070	0.173
7	0.127	-0.060	-0.264	-0.206	-0.067	-0.047	-0.132	-0.027	-0.064	-0.460	0.061
8	0.479	-0.313	0.452	0.581*	0.503	0.384	0.510	0.428	0.399	0.045	0.403
9	0.874	-0.546*	0.836*	0.781*	0.778*	0.772*	0.696*	-0.090	0.040	0.749*	0.343
10	0.184	-0.216	0.602*	0.474	0.403	0.393	0.308	0.238	0.011	0.326	0.458
11	-0.063	-0.322	0.671*	0.651*	0.547*	0.524	0.470	-0.039	0.145	0.892*	0.375
12		-0.284	0.239	0.390	0.423	0.205	0.541	-0.036	-0.107	-0.235	-0.161
13	-0.430		-0.447	-0.489	-0.606*	-0.478	-0.561*	0.170	0.177	-0.314	-0.212
14	0.244	-0.802**		0.717*	0.71*	0.642*	0.639*	-0.001	0.095	0.598*	0.410
15	0.047	-0.702**	0.804**		0.66*	0.595*	0.653*	-0.027	0.301	0.610*	0.749*
16	0.438	-0.765**	0.864**	0.741**		0.887*	0.902*	0.169	0.137	0.538	0.344
17	0.206	-0.655**	0.839**	0.714**	0.935**		0.746**	0.127	0.155	0.567*	0.339
18	0.573**	-0.742**	0.751**	0.731**	0.960**	0.774**		0.159	0.236	0.467	0.284
19	-0.018	0.411	0.223	-0.054	0.252	0.230	0.243		0.429	-0.075	0.021
20	-0.134	0.183	0.085	0.379	0.178	0.155	0.253	0.627**		0.201	0.209
21	-0.245	-0.430	0.727**	0.639**	0.576**	0.605**	0.476**	-0.058	0.230		0.567**
22	-0.155	-0.253	0.508*	1.112**	0.388	0.390	0.306	0.238	0.290	0.690**	

** Significant at 1% level, * Significant at 5% level

1. Soluble carbohydrate content (%)
2. Fat content (%)
3. Amylose content (%)
4. Free amino acid content (mg/g)
5. Protein content (%)
6. Amylase activity (µg)
7. Seelding height (cm)
8. Leaf length (cm)
9. Leaf width (cm)
10. Ligule length (cm)
11. Days to 50% heading
12. Culm length (cm)
13. Culm number
14. Culm diameter (mm)
15. Panicle length (cm)
16. 1000 grain weight (g)
17. Grain length (mm)
18. Grain width (mm)
19. Grain yield (kg/ha)
20. Straw yield (kg/ha)
21. Days from seeding to maturity
22. Starch grain size (µ)

Grain yield was not significantly correlated with any of the physico-chemical characters and cooking qualities. Only genotypic correlation coefficients among yield and yield components are dealt in detail.

Correlation coefficients among yield components showed that seedling height had significant positive association with days to 50% heading, days from seeding to maturity and free amino acid content. The character leaf length was positively and significantly correlated with ligule length, grain width, panicle length, culm diameter, 1000 grain weight, culm length and grain yield while its association with protein content was found to be significantly negative. Leaf width showed significant positive correlation with ligule length, days to 50% heading, culm diameter, panicle length, 1000 grain weight, grain length, grain width, days from seeding to maturity, fat content and free amino acid content while it had significant negative association with culm number and protein content. The character ligule length was found to be positively and significantly correlated with leaf length, leaf width, days to 50% heading, culm diameter, panicle length, 1000 grain weight, grain length, starch grain size and fat content. Days to 50% heading exhibited significant positive correlation with leaf width, ligule length, culm diameter, panicle length, 1000 grain weight, grain length, grain width, days from seeding to maturity, fat content and free amino acid content but had significant negative association with seedling height and protein content.

Culm length was found to be positively and significantly correlated with leaf length, grain width and amylase activity and negatively associated with amylose content. Culm number exhibited significant positive association with protein content and showed significant negative correlation with leaf width, culm diameter, panicle length, 1000 grain weight, grain length and grain width. The character culm diameter was positively and significantly correlated with panicle length, 1000 grain weight, grain length, grain width, days from seeding to maturity, starch grain size, fat content and free amino acid content while it had significant negative association with protein content. Panicle length had significant positive correlation with 1000

grain weight, grain length, grain width, days from seeding to maturity, starch grain size, fat content and free amino acid content and showed significant negative correlation with protein content. 1000 grain weight exhibited significant positive association with grain length, grain width, days from seeding to maturity and amylase activity and significant negative association with amylose content and protein content. Correlation studies among physico-chemical properties of rice namely, grain length, grain width showed that the character grain length was positively and significantly correlated with grain width and days from seeding to maturity. Grain width was also significantly correlated with days from seeding to maturity in the positive direction. Straw yield showed significant positive correlation with amylose content. days from seeding to maturity exhibited significant positive association with starch grain size and free amino acid content but showed significant negative association with protein content. The character starch grain size also showed significant negative correlation with protein content.

Correlation studies among quality characters revealed that fat content showed significant positive correlation with leaf width, ligule length, days to 50 per cent heading, culm diameter, panicle length and free amino acid content but had significant negative association with protein content. Amylose content was significantly and positively correlated with straw yield while it exhibited significant negative association with culm length, 1000 grain weight and amylase activity. Free amino acid content had significant positive association with leaf width, days to 50% heading, culm diameter, panicle lenth and days from seeding to maturity and showed significant negative correlation with seedling height. Protein content was significantly and positively correlated with culm number and negatively associated with leaf length, leaf width, days to 50 per cent heading, culm diameter, panicle length, 1000 grain weight, grain width, days from seeding to maturity, starch grain size and amylase activity. Amylase activity exhibited significant positive association with culm length, 1000 grain weight and grain width.

4.9 Path analysis for yield

Two yield components namely, straw yield and leaf length, which had significant genotypic correlation with yield, were taken for path analysis. Besides, other yield contributing characters such as grain length and grain width were also included in path analysis. The estimates of direct and indirect effects of the four yield component characters on yield are presented in Table 23.

The residual effect of path analysis was found to be 0.5229. It was observed that the characters namely, leaf length, grain length and straw yield exerted positive direct influence on yield. The character grain width had negative direct effect on grain yield. Straw yield had the highest positive direct effect (0.500) on yield followed by leaf length (0.333) and grain length (0.181). Negative direct effect was exhibited by grain width (-0.223). The highest positive indirect effect with yield was exhibited by leaf length via. straw yield (0.231). This was followed by grain width via. leaf length (0.201), straw yield via. leaf length (0.153), grain length via. leaf length (0.148), grain width through grain length (0.140) and grain width through straw yield (0.127). The highest negative indirect effect with yield was exhibited by grain length via. grain width (-0.172). This was followed by leaf length via. grain width (-0.135).

4.10 Path analysis for free amino acid content

Seven characters namely, seedling height, leaf width, days to 50 per cent heading, culm number, culm diameter, panicle length and days from seeding to maturity, showing significant genotypic correlations with free amino acid content were considered for path analysis. The estimates of direct and indirect effects of the seven selected characters on free amino acid content are presented in Table 24.

The residual effect of path analysis was found to be 0.6550. It was observed that the characters namely, leaf width and days to 50 per cent heading exerted positive direct influence on free amino acid content. Seedling height, culm

Table 23. Direct and indirect effects of four yield components on grain yield of Njavara

Characters	Leaf length (cm)	Grain length (cm)	Grain width (cm)	Yield of straw (kg/ha)	Total correlation with yield
Leaf length (cm)	**0.333**	0.081	-0.135	0.231	0.506*
Grain length (mm)	0.148	**0.181**	-0.172	0.077	0.231
Grain width (mm)	0.201	0.140	**-0.223**	0.127	0.243
Yield of straw (kg/ha)	0.153	0.028	-0.056	**0.500**	0.627**

Bold figures represent direct effects; Residual effect = 0.5299

Table 24. Direct and indirect effects of seven free amino acid components on free amino acid content of Njavara grain

Characters	Seedling height (cm)	Lead width (cm)	Days to 50 % heading	Culm number	Culm diameter (mm)	Panicle length (cm)	Days from seedling to maturity	Total correction with free amino acid content
Seeding height (cm)	**-0.315**	-0.552	-0.346	0.076	0.423	0.031	0.197	-0.485*
Leaf width (cm)	0.129	**1.348**	0.481	0.476	-1.382	-0.108	-0.328	0.611**
Days to 50% heading	0.197	1.172	**0.553**	0.288	-1.170	-0.090	-0.382	0.564**
Culm number	0.035	-0.945	-0.234	**-0.679**	1.115	0.092	0.183	-0.424
Culm diameter (mm)	0.097	1.364	0.474	0.554	**-1.366**	-0.104	-0.309	0.701**
Panicle length (cm)	0.075	1.130	0.383	0.482	-1.104	**-0.129**	-0.270	0.560*
Days from seeding to maturity.	0.147	1.046	0.501	0.295	-1.001	-0.083	**-0.422**	0.479*

Bold figures represent direct effects; Residual effect = 0.6550

number, culm diameter, panicle length and days from seeding to maturity showed negative direct influence on free amino acid content. Leaf width had the highest positive direct effect (1.348) on free amino acid content followed by days to 50 per cent heading (0.553). The highest negative direct effect was observed for culm diameter (-1.366). This was followed by culm number (-0.679), days from seeding to maturity (-0.422), seedling height (-0.315) and panicle length (-0.129). The highest positive indirect effect on free amino acid content was exhibited by culm diameter via. leaf width (1.364). This was followed by days to 50 per cent heading via. leaf width (1.172), panicle length via. leaf width (1.130), culm number via. culm diameter (1.115), days from seeding to maturity through leaf width (1.046), culm diameter via. culm number (0.554) and days from seeding to maturity through days to 50 per cent heading (0.501). The highest negative indirect effect with free amino acid content was exhibited by leaf width via. culm diameter (-1.382). This was followed by days to 50 per cent heading via. culm diameter (-1.170), panicle length via. culm diameter (-1.104), days from seeding to maturity through culm diameter (-1.001) and culm number via. leaf width (-0.945).

4.11 Path analysis for protein content

Five characters namely, leaf length, lead width, days to 50 per cent heading, panicle length and 1000 grain weight, showing significant genotypic correlations with protein content were considered for path analysis. The estimates of direct and indirect effects of the five selected characters on protein content are presented in Table 25.

The residual effect of path analysis was found to be 0.1174. It was observed that the characters namely, leaf length and leaf width exerted positive direct influence on protein content. Days to 50 per cent heading, panicle length and 1000 grain weight showed negative direct influence on protein content. Leaf width had the highest positive direct effect (1.648) on protein content followed by leaf length (0.107). The highest negative direct effect was observed for panicle length

Table 25. Direct and indirect effects of five protein components on protein content of Njavara grain

Characters	Leaf length (cm)	Lead width (cm)	Days to 50% heading	Panicle length (cm)	1000 grain weight (g)	Total correlation with protein content
Leaf length (cm)	**0.107**	0.612	-0.128	-0.868	-0.315	-0.587**
Leaf width (cm)	0.040	**1.648**	-0.574	-1.174	-0.451	-0.510*
Days to 50% heading	0.021	1.433	**-0.660**	-0.971	-0.319	-0.495*
Panicle length (cm)	0.066	1.381	-0.458	**-1.400**	-1.410	-0.815**
1000 grain weight (g)	0.061	1.348	-0.382	-1.041	**-0.552**	-0.561**

Bold figures represent direct effects; Residual effect = 0.1174

(-1.400) followed by days to 50 per cent heading (-0.660) and 1000 grain weight (-0.552). The highest positive indirect effect with protein content was exhibited by days to 50 per cent heading via. leaf width (1.433). This was followed by panicle length via. leaf width (1.381), 1000 grain weight via. leaf width (1.348) and leaf length through leaf width (0.612). The highest negative indirect effect with protein content was exhibited by leaf width via. panicle length (-1.174). This was followed by 1000 grain weight via. panicle length (-1.041), days to 50 per cent heading through panicle length (-0.971), leaf length via. panicle length (-0.868) and leaf width via. days to 50 per cent heading (-0.574).

DISCUSSION

DISCUSSION

Characterization of plant varieties is an essential prerequisite for the protection of our `sovereign rights on biodiversity' with special reference to the indigenous genetic resources. Its importance has to be seen in the context of extension of Intellectual Properts Rights (IPR) to agricultural sector and India being a signatory to GATT accord.

Morphological traits are older and widely used markers for varietal characterization because of their simplicity, rapidity and cost effectiveness and hence widely used in developing countries. But the morphological characters cannot be solely relied upon since they are highly variable with environment. To rectify this lacuna, biochemical and molecular markers are to be utilized as supplements to morphological characterization of plant varieties and species.

Njavara is the unique traditional rice cultivar from Kerala widely utilized in Ayurveda system of medicine. The oldest Ayurveda texts like Ashtanga Hridaya and Sushrutha Samhitha had described two broad types of Njavara and recent studies by Menon and Potty (1996) had also confirmed occurrence of ecotypes in this cultivar. Hence it is essential that indepth studies are to be undertaken to reveal the extent of variability existing in Njavara so that the best types can be selected not only for general cultivation but also for utilizing in special diets and Ayurveda treatments. Moreover, types with better nutritional qualities can be utilized in rice improvement programmes. In this perspective the present study assumes significance.

5.1 Morphological characterization

The data on morphological characters indicated that the thirteen Njavara genotypes were morphologically distinct among themselves. The diversity among Njavara genotypes may be due to their adaptation to the diverse locations under

which they are cultivated and evolved, representing different agroclimatic regions of Kerala. Though the genotypes N9 and N12 exhibited maximum similarity between themselves on the basis of qualitative and quantitative characters studied, they were differing in characters like leaf length, panicle length, ligule length, culm number, culm diameter, grain yield, straw yield and days from seeding to maturity. Hence based on morphological character, Njavara genotypes could be classified into 13 distinct groups.

5.1.1 Qualitative characters

Among the morphological characters studied, qualitative characters like leaf blade pubescence, leaf blade colour, basal leaf sheath colour, leaf angle, ligule colour, ligule shape, collar colour, auricle colour, culm angle, internode and septum colour, secondary branching of panicle, panicle shattering, panicle threshability, stigma colour, sterile lemma colour, spikelet sterility, endosperm type, grain scent and pest and disease incidence showed no variation while the observed variation was low for flag leaf angle, leaf senescence, panicle type, panicle exsertion, panicle axis, apiculus colour, lemma and palea pubescence and length of sterile glumes and hence were of limited use in distinguishing Njavara genotypes from the check variety and also in the differentiation of Njavara genotypes among themselves. Among the qualitative characters panicle type, spikelet awning, lemma and palea colour, lemma and palea pubescence and seed coat colour showed great variability and hence can be considered as markers for the identification of Njavara types. Njavara genotypes exhibited a wide array of lemma and palea colour like straw colour, gold furrows on straw background and brown furrows on straw. This is contradictory to the classification of Njavara as black and golden yellow glumed types by Menon and Potty (1996). According to Gupta and Agrawal (1987) lemma and palea colour and spikelet awning are altered by external factors and should be used as secondary diagnostic characters in varietal identification. The amylose content estimates from biochemical analysis confirmed the nonwaxy nature of

endosperm of this cultivar. The nonscented nature of grains was corroborated by the absence of flavonoids in the grain of all Njavara genotypes and Ptb-10.

In general, Njavara genotypes had glabrous and green leaves, green basal leaf sheath, erect leaves, white and 2-cleft ligules, pale green collar and auricle, erect culms, light gold internode and septum, heavy secondary branching of panicle, very low shattering of panicle, white stigmas, straw coloured sterile lemmas, highly fertile spikelets, nonwaxy endosperm, non scented grains and low damage by pests and diseases with respect to qualitative traits. Njavara genotypes showed panicle threshability comparable to that of Ptb-10 and hence will not be a problem to farmers at the time of threshing. Moreover, a high panicle threshability may often lead to grain loss in the field especially when there is a delay in harvesting.

5.1.2 Quantitative characters

Quantitative characters like seedling height, leaf length, leaf width, panicle length, ligule length, days to 50 per cent heading, culm length, culm number, culm diameter, 1000 grain weight, grain length, grain width, grain yield, straw yield and days from seeding to maturity showed variation and were found to be more useful for the characterization of Njavara genotypes. Gupta and Agrawal (1987) reported that grain length and grain length/breadth ratio were quite stable characters and can be used as primary diagnostic characters in varietal identification which is in accordance with our observation.

With respect to variation in quantitative traits, Njavara genotypes exhibited a seedling height ranging from 30.82 cm to 41.80 cm, leaf length varying from 34.97 cm to 50.00 cm, leaf length varying from 34.97 cm to 50.00 cm, 0.66 to 1.25 cm wide leaves, 18.30 cm to 24.38 cm long panicles, ligules of 1.24 cm to 2.40 cm long, days to 50 per cent heading ranging from 44 days to 57 days, culm length varying from 54.20 cm to 94.30 cm, mean culm number ranging from 4.4 to 9.1, 4.2 mm to 6.4 mm thick culms, 1000 grain weight ranging from 18.5 g to

30.0 g, 7.6 mm to 9.3 mm long grains, 2.65 mm to 3.33 wide grains, grain yield hectare^{-1} ranging from 919 kg ha^{-1} to 2684 kg ha^{-1}, straw yield hectare^{-1} varying from 1752 kg ha^{-1} to 6084 kg ha^{-1} and days from seeding to maturity ranging from 60 days to 79 days. Extra short duration of 60 days to 79 days for Njavara genotypes is in agreement with the earlier reports on Njavara (Elsy *et al.*, 1992; Menon and Potty, 1996). The early maturing varieties are of special importance for tracts with low and uncertain rainfall or tracts where the growing season is short due to the early setting in of low temperature or tracts which are subject to drought. The early maturing varieties are adapted to a wider range of sowing (Ghose *et al.*, 1960). Hence extra short duration is a desirable character and Njavara can be considered as a potential donor for the evolution of rice varieties of extra short duration. The Njavara genotypes N11, N2, N1, N5 and N13 had grain yields of 2684, 2621, 2554, 2477 and 2386 kg ha^{-1} respectively and can be recommended for cultivation as their yields were on par with that of Ptb-10. It can be seen that the per day productivity of these Njavara genotypes is high because of their extra short duration. Because of their medicinal properties, farmers can realise high market price for Njavara grain. After indepth studies of the Njavara genotypes regarding their medicinal properties, suitable Njavara genotypes can be recommended for cultivation so that rice cultivation would be more economically viable to the farmers in Kerala, especially in areas where water scarcity is a problem in rice cultivation.

5.2 Biochemical characterization

5.2.1 Protein polymorphism

5.2.1.1 Quiscent seed

Based on protein banding pattern in quiscent seed, Njavara genotypes could be divided into 3 groups based on their similarity with the check variety, Ptb-10. But based on similarity indices among themselves, Njavara genotypes were divided into 4 groups since the genotype N2 could be distinguished from other genotypes. Protein polymorphism in quiscent seed was very low and found to be not efficient in characterizing Njavara genotypes.

5.2.1.2 Germinated seed

The protein banding pattern in germinated seed revealed that Njavara genotypes could be divided into 7 groups based on their similarity with the check variety, Ptb-10 which is in agreement with the grouping based on similarity indices among Njavara genotypes. The protein band number12 (Rm = 0.443) was present in most of the Njavara genotypes (except N1 and N4) and could be used as a marker to separate Njavara genotypes from other rice varieties. The protein band number13 (Rm = 0.494) was specific for Ptb-10, hence Njavara genotypes could be identified by the absence of this band. The Njavara genotype N3 could be distinguished from other genotypes because of a specific protein band number 8 (Rm = 0.255). Protein banding pattern in germinated seed showed some degree of correlation with morphological characterization, but it cannot be fully relied upon as it showed similarity between Njavara genotypes which exhibited significant morphological differences. Guidolin *et al.* (1995) were able to differentiate morphologically similar cultivars based on electrophoretic analysis of proteins of caryopsis in rice cultivars.

5.2.1.3 Leaf

With regard to protein banding pattern in leaf, Njavara genotypes could be divided into five groups based on their similarity with Ptb-10 which is not in agreement with the grouping based on similarity indices among Njavara genotypes. Similarity indices among Njavara genotypes indicated that they could be classified into six groups since N1 was found to be distinct from other genotypes. The genotype N1 could be easily identified from other genotypes because two protein bands viz., no.7 and 16 with Rm values 0.109 and 0.241 respectively, were unique to it. Observed polymorphism with respect to protein banding pattern in leaf was low and the Njavara genotypes could not be distinguished efficiently. Vodenicharova (1989) used protein as a marker in studying population structure, phylogenetic relationship between species , the functioning of plant genome, the

selection of promising genotypes as parents for hybridization, gene mapping and the construction of genotypes with the required combination of characters.

5.2.2 Isozyme analysis

The results of isozyme analysis with respect to peroxidase and esterase were discussed as follows.

5.2.2.1 Peroxidase

5.2.2.1.1 Quiscent seed

No peroxidase band was observed in quiescent seed. This might be due to the very low activity of peroxidase in quiscent seed (Tomar and Gupta, 1981).

5.2.2.1.2 Germinated seed

Based on peroxidase isozyme patterns in germinated seed and the corresponding similarity indices of Njavara genotypes with the check variety, Ptb-10, Njavara genotypes were divided into three broad groups. Which was in agreement with the grouping based on similarity indices among Njavara genotypes. Peroxidase polymorphism in germinated seed sample was observed to be low and it is not easy to distinguish Njavara genotypes from one another and from the check variety, Ptb-10 based on this peroxidase polymorphism in germinated seed. But it is to be noted that the band PRX-1 (Rm = 0.472) was present in N7 and N10 and hence is a probable marker for identifying these genotypes. Pawar and Gupta (1975) found variation in the peroxidase isozyme patterns in tall and dwarf varieties of rice. A number of rice geneticists had used isozymes to determine the genetic divergence among cultivars and their wild relatives (Nakagahra and Hayashi, 1977; Glaszmann, 1986; Second, 1986).

5.2.2.1.3 Leaf

High activity of peroxidase isozyme was observed in young leaves resulting in 11 bands. The band PRX-11 (Rm = 0.489) was present in all the Njavara genotypes except N12 and absent in Ptb-10. Hence it can be considered as a marker to identify Njavara genotypes, even though further studies are required to prove its utility as a biochemical marker for the identification of this unique rice cultivar. Weeden (1989) had reported about the application of isozyme polymorphism to reveal genetic diversity in germplasm collections and cultivar identification.

Based on peroxidase isozyme pattern in leaf and the corresponding similarity indices of Njavara genotypes with Ptb-10, they can be divided into eight groups. But based on similarity indices among Njavara genotypes, they can be classified into 10 groups since N1 and N6 form two separate groups being distinct from other genotypes. This deviation arises mainly due to the formula for calculating similarity index wherein in the number of homologous and non-homologous bands are equal for two distinct genotypes with reference to the check variety. Hence the two genotypes show 100 per cent similarity with each other though they are different based on similarity index between them.

5.2.2.2 Esterase

5.2.2.2.1 Quiscent seed

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

5.2.2.2.2 Germinated seed

Based on esterase banding pattern in germinated seed and the corresponding similarity indices of Njavara genotypes with the check variety, Ptb-10, they can be divided into six broad groups. But based on similarity indices among Njavara genotypes, they can be classified into seven groups because the

genotypes N12 and N13 are distinct from each other. The isozyme band, EST-1 (Rm = 0.098) could be used as a marker to distinguish Njavara genotypes from other rice varieties as it was observed in majority of them except N9 and N13. The isozyme band EST-8 (Rm = 0.317) and EST-9 (Rm = 0.349) were specific for Ptb-10 and the Njavara genotypes could be identified by the absence of these bands. The genotype N4 could be distinguished from other Njavara genotypes because the band EST-2 (Rm = 0.142) was specific for it. Hence it is suggested that esterase banding pattern could contribute for cultivar identification and also for revealing genetic diversity among Njavara genotypes. Application of isozyme banding patterns for cultivar identification had been reported by Weeden (1989). Detailed studies are needed to prove the usefulness of these unique bands for cultivar identification.

5.2.2.2.3 Leaf

Based on esterase isozyme pattern in leaf and their corresponding similarity indices with the check variety, Ptb-10, Njavara genotypes were classified into 5 groups which was in agreement with the grouping based on similarity indices among Njavara genotypes. The isozyme band EST-5 (Rm = 0.434) was specific for the genotype N2 and it could be easily identified. This also needs confirmation.

Out of the biochemical markers tested, peroxidase banding pattern in leaf showed maximum polymorphism and was able to classify Njavara genotypes into 10 groups. This was followed by esterase banding pattern in germinated seed and protein banding pattern in germinated seed with respect to polymorphism. Detailed studies are required to prove the efficiency of these isozyme markers to distinguish Njavara genotypes. The isozyme markers such as peroxidase banding pattern in germinated seed, esterase pattern in leaf and protein banding pattern in quiscent seed and leaf revealed low polymorphism and were considered as less efficient isozyme markers to distinguish Njvara genotypes. Results from the analysis of quiscent seed samples with respect to isozyme and protein banding indicated that it is not feasible

to assay quiscent seed for characterization of Njavara genotypes. The genotypes N9 and N12 were found to be similar with respect to three markers viz., peroxidase banding pattern in germinated seed, protein banding patterns in germinated seed and leaf confirming their morphological similarity to a certain extent. The protein bands number 7 (Rm=0.109) and 16 (Rm=0.241) in leaf sample were specific for the genotype N1 while the isozyme band EST-5 (Rm=0.434) in leaf was unique for the genotype N2. With respect to germinated seed samples, protein band number 8 (Rm=0.255) was present only in the genotype N3 whereas the isozyme band EST-2 (Rm=0.142) was specific for the genotype N4. Hence the Njavara genotypes N1, N2, N3 and N4 could be easily identified because of their unique marker bands. Once intensive studies are conducted to reveal the medicinal properties of diverse Njavara genotypes these marker bands will make the task of identification of outstanding Njavara genotypes easy.

The banding pattern of soluble proteins and isozymes revealed that the function of proteins and isozymes of germinating seed and leaf are the same. All the above bands were recorded in active cells of germinating seed and leaf. Maximum number of protein bands observed in leaf correlated with high protein content of Njavara grain which may be contributing to the medicinal properties. It can also be inferred that the isozyme and protein patterns are contributing to growth and development in both germinated seed and growing plant whereas additional bands in leaf contribute to metabolism and accumulation of primary product.

In the present study germinated seed was taken five days after soaking and leaf at 30 days after sowing. Expression of protein and isozyme patterns may be different for other stages. A more detailed study of isozyme and protein patterns at different stages of growth and development and enzyme activity studies are required to establish specific biochemical markers for genotypic characterization. None of the biochemical markers studied showed 100 per cent correlation with morphological characterization and did not exhibit consistency among themselves in grouping

Njavara genotypes. In the light of this information, the strategies suggested for characterization of Njavara genotypes are

a) selecting some more isozymes to reveal the polymorphism coupled with enzyme activity studies.
b) opting for molecular markers in case of failure of the first strategy.

The peroxidase band PRX-1 (Rm=0.472) from germinated seed was unique for the cultivars N7 and N10 and hence when coupled with morphological markers, these isozyme markers can distinguish these genotypes.

Unique bands like EST-8 (Rm=0.317) and EST-9 (Rm=0.349) were present only in the germinated seeds of Ptb-10 and absent in all the Njavara genotypes. Similarly, EST-1 (Rm=0.098) was present in germinating seeds of most of the Njavara genotypes and absent in Ptb-10. And hence these bands could be considered as isozyme markers to distinguish Njavara cultivar from other rice genotypes, eventhough detailed studies can only prove their consistency.

5.3 Nutritional qualities of Njavara grain

Njavara being a medicinal rice cultivar, its evaluation for the nutritional qualities is important to reveal its medicinal properties. The results of biochemical analysis with respect to various nutritive quality parameters are discussed below.

5.3.1 Soluble carbohydrate content

High soluble carbohydrate content reduces gelatinization time and aids in faster cooking of rice (Swaminathan, 1987). High content of soluble carbohydrate is a desirable quality especially in rice which is consumed by absorption method of cooking as in the case of Njavara. Soluble carbohydrate in Njavara genotypes was found to be high compared to the check variety, Ptb-10 (1.52 per cent). The genotypes N5 (2.93 per cent), N13 (2.82 per cent) and N7 (2.78 per cent) have the highest soluble carbohydrate

content and hence desirable as component of weaning and invalid food due to reduced gelatinization period and easy digestibility.

The genotypes N5, N7 and N13 which exhibited high soluble carbohydrate were having a lemma and palea colour of brown furrows on straw which would have been referred as black glumed type in Sushrutha Samhitha (Sushruthacharya, 2500 B.C.). Sushrutha Samhitha had described the black glumed type as the superior type.

Even though the high yielding Njavara genotypes viz., N1, N2, N5, N11 and N13 were found to have medium amylose content, most of the Njavara genotypes contained medium to high soluble carbohydrate content. It indicated that Njavara genotypes may contain soluble carbohydrates other than amylose. Fu *et al.* (1991) reported anthocyanidin, a glucoside acting as heart stimulant, as one of the medicinally active constituents of black glutinous rice.

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. In the present study, most of the Njavara genotypes have high protein content than Ptb-10 (9.53%). The genotypes N2 (13.39%) and N8 (12.60%) having higher protein content can be recommended to be included in weaning and invalid foods, diets during pregnancy and lactation during which the protein requirement is high. Since protein content is highly influenced by environment (Juliano, 1978), further studies are needed to confirm the high protein content in the grains of these Njavara genotypes. In the isozyme polymorphism studies specific bands were identified to distinguish N2 from other Njavara genotypes.

. Of the four genotypes which exhibited high protein content (>12%), three genotypes viz., N2, N8 and N12 showed lemma and palea colour of straw or shades of straw which would have been described as white glumed types in traditional Ayurveda texts like Ashtanga Hridaya (Vagbhata, 500 B.C.) and Sushrutha Samhitha (Sushruthacharya, 2500 B.C.). The results from protein content analysis is in agreement with Ashtanga Hridaya which suggested white glumed Njavara type as the best one. In the present study, only one genotype with high protein content exhibited a lemma and palea colour of brown furrows on straw whereas the other three exhibited shades of straw.

The high yielding Njavara genotypes viz., N1, N2, N5, N11 and N13 were observed to have medium to high protein content and this indicated the possibility of their utilization in rice improvement programmes aimed to combine quality with yield potential.

5.3.3 Presence of free amino acids

Free amino acids content varies widely with environmental factors. Menon and Potty (1996) analysed the free amino acid content and the probable free amino acids in Njavara grain under varying degrees of shade and in wetland and upland situations. In their study, free amino acid content ranged from 0.089 mg/g to 0.814 mg/g and out of the free amino acids identified isoleucine, L-histidine monochloride, leucine, methionine and threonine turned out be essential and hence have nutritive value.

In the present study, free amino acid content was found to be lower than Ptb-10 (0.188 mg/g), in majority of the Njavara genotypes. The genotypes N2 (0.190 mg/g), N4 (0.160 mg/g), N3 (0.156 mg/g) and N12 (0.150 mg/g) have high free amino acid content. The number of free amino acids observed ranged from three in the genotypes N1, N4, N7 and N8 to five in N10. Taking into consideration of free amino acid content and number of free amino acids observed the genotypes

N2, N3 and N12 may have better nutritive value. Four free amino acids were observed for Ptb-10.

The results also showed that total free amino acids content was varying among Njavara genotypes because the type of free amino acids available in the grain were different. Paper chromatographic study of free amino acids revealed that the high yielding Njavara genotypes viz., N1, N2, N5, N11 and N13 had free amino acids falling within the Rf value range of 0.2-0.4. It was also supported by the total number of spots observed i.e. three spots were observed for the genotype N1 while four spots were observed for genotypes N2, N5, N11 and N13. The exceptional qualities of these genotypes can be related to the free amino acids and banding patterns of soluble protein and isozymes. Even though the high yielding Njavara genotypes expressed free amino acids falling in the Rf value range of 0.2 to 0.4, there were certain free amino acids coming within the Rf value of 0.2 and above 0.4. These variations were directly related to the phenotypic expression as well as banding pattern of soluble proteins and isozymes (Tables 3 & 4, Fig.1-7). Since free amino acids are precursors of secondary metabolites and proteins, they are the primary source of medicinal properties of Njavara. Free amino acids coupled with soluble carbohydrates may be contributing to the production of active proteins and secondary metabolites.

5.3.4 Fat content

Fats are mainly deposited in the aleurone layer and embryo of the seed. They are not only an essential energy source for germination and growth, but also an important nutritional source for human beings (Okuno, 1997). The source of fat in rice is invisible (Achaya, 1986) and the total fat content of rice do not have much nutritional significance. In the present study, all the Njavara genotypes had very low fat content compared to Ptb-10.

Here again, the accumulation of primary metabolites as protein and soluble carbohydarates including glucosides might have taken place in Njavara

genotypes leading to low fat content and such a result was not to be observed in Ptb-10 resulting in high fat content.

5.3.5 Amylose content

Starch, the nutritional reservoir in rice exists in two different forms; amylose, the unbranched type of starch with glucose residues with 1-4 linkage and amylopectin, the branched form with 1-4 and 1-6 cross linkage (Aberg, 1994). According to Juliano (1970) amylose is the linear molecular component of rice starch and is the texture determinant during cooking. The nonwaxy rices cook dry, are less tender and become hard upon cooling. Rice starch is reported to be composed of 15 to 25 per cent amylose and 75 to 85 per cent amylopectin.

In the present study, majority of the Njavara genotypes had intermediate amylose content ranging between 16.99% to 23.27%. Since intermediate amylose rices are preferred in most of the rice growing regions of the world (Anon., 1979b), the Njavara genotypes may not have any problem with respect to consumer preference. The genotype N7 had the lowest amylose content of 16.99 per cent and hence easy to digest because of high amylopectin.

The genotype N2, having intermediate amylose content coupled with high protein content is more desirable from nutritional point of view.

The high yielding Njavara genotypes, as mentioned earlier, were found to have medium amylose content while Ptb-10 too had medium amylose content. Even though amylose content was low for the genotype N7, its free amino acid spots were found to be coming in the Rf value range of 0.2 to 0.4 implying that the precursors of the medicinal principles are same for this genotype as any other Njavara genotype.

5.3.6 Starch grain characterization

Starch grains vary in structure as well as in size and are so characteristic of the species that an expert can determine the source of isolated grains by microscopic examination (Greulach, 1973). In the present study, the Njavara genotypes as well as Ptb-10 had multifaced and compound starch grains. Variation among the genotypes was observed for the size of starch grain. The Njavara genotypes N9 and N12 had the smallest starch grains (4.79 μ) while Ptb-10 had the largest starch grains (5.73 μ). Within the Njavara genotypes, there were significant differences with respect to starch grain size and they were divided into 8 groups. This revealed the possibility of using starch grain size for varietal characterization in conjunction with morphological and biochemical characterization.

The high yielding Njavara genotypes were observed to have small to medium sized starch grains while Ptb-10 had the largest starch grains. This indicated the presence of other biomolecules which are imparting medicinal properties to Njavara genotypes.

5.3.7 Amylase activity

Alpha-amylase is the major enzyme of starch degradation in the endosperm of rice and reported to be correlated with seedling vigour (Anon., 1979b). In the present study, the genotypes N6 and N7 had high amylase activities of 46.67 μg and 40.67 μg respectively. The genotype N7 had the lowest amylose content (16.99%) and high amylase activity (40.67 μg) and hence desirable for its digestibility.

The high yielding Njavara genotypes were found to have low to medium amylase activities while Ptb-10 had medium amylase activity.

5.3.8 Flavonoids

Absence of flavonoids indicated the nonaromatic nature of Njavara grain. Based on the results of the biochemical analyses with respect to nutritional quality parameters, the high yielding Njavara genotypes viz., N1, N2, N5, N11 and N13 originating from different locations of Kerala were found to have medium to high protein content, low fat content, small to medium sized starch grains. In contrast, the check variety, Ptb-10 had low protein content, high fat content and largest starch grains. From digestibility point of view the genotype N7 was found to be superior as it had high soluble carbohydrate content, low amylose content and high amylase activity.

5.4 Genetic variability

Success in crop improvement programme depends on the magnitude of genetic variability and the extent to which the desirable characters are heritable. The estimates of variability in respect of yield, quality and their heritable components in the material with which the breeder is working, are therefore, pre-requisites for any breeding programme. Hence it becomes necessary to split the phenotypic variability into heritable and non-heritable components with the help of certain genetic parameters such as genotypic and phenotypic coefficients of variation, heritability, genetic advance and genetic gain. The results obtained in the present study are discussed as below.

The analysis of variance showed high significant differences for all the characters studied, suggesting the presence of substantial genetic variability among the genotypes. Variability for different characters was previously observed by several workers like Govindaswami *et al.* (1973), Lal *et al.* (1983), Singh *et al.* (1984), Sardana and Sasikumar (1987), Chaubey and Richharia (1990) and Gomathinayagam *et al.* (1990) for number of days to 50 per cent heading, culm length, panicle length, 1000 grain weight.

Rice with low amylose is moist and chewy, while high amylose rice cooks dry, fluffy and readily hardens upon cooling. Preferences for varieties with different amylose contents varies widely from region to region. By and large, the varieties with intermediate amylose content are preferred in South and South East Asia, the world's largest import market (Chauhan and Nanda, 1983). Among the 13 Njavara genotypes included in the study, ten genotypes (except N6, N7 and N10) had intermediate level of amylose content, which are more preferred in cooking qualities than the rest.

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). In the present study, high protein was observed in most of the Njavara genotypes, the range being 9.47 per cent to 13.39 per cent in brown rice. Hence the grain of Njavara genotype is highly nutritious in terms of protein.

High soluble carbohydrate content is a desirable quality as it reduces gelatinzation time and aids in faster cooking of rice. In the present study, majority of the Njavara genotypes had high soluble carbohydrate content, the range being 1.38 per cent to 2.93 per cent (compared to the reported range of 0.8% - 1.36%) in brown rice.

Fat in rice grain is mainly composed of polyunsaturated fatty acids (invisible fat), hence desirable (Achaya, 1986). In the present study, majority of the Njavara genotypes had low fat content, the range being 0.195 per cent to 2.16 per cent with a mean of 0.77 per cent.

Menon and Potty (1996) reported that free amino acid content in Njavara grain ranged from 0.089 mg/g to 0.814 mg/g and the free amino acids identified turned out to be essential and hence have nutritive value. In the present study, free amino acid content ranged from 0.090 mg/g to 0.190 mg/g in brown rice.

Amylase activity was reported to be correlated with seedling vigour as it is the major enzyme of starch degradation in the endosperm of rice. In the present study the amylase activity ranged from 16.33 µg to 46.70 µg.

Starch grains vary in structure as well as in size and are so characteristic of the species that an expert can determine the source of isolated grains by microscopic examination (Greulach, 1973). In the present study multifaceted starch grains were observed in all the Njavara genotypes and the size of starch grains ranged from 4.79 µ to 5.73 µ.

5.5 Phenotypic and genotypic coefficients of variantion

High magnitude of phenotypic coefficient of variation (pcv) and genotypic coefficient of variation (gcv) observed for fat content indicated the existence of large variability and scope of genetic improvement of this trait through selection. Moderate level of variability observed for the quantitative characters namely, straw yield and grain yield indicated the usefulness of these characters in rice improvement programme. Similar result of moderate variability for grain yield plant^{-1} was reported by Murthy *et al.* (1999). Low variability for quantitative characters namely, seedling height, leaf length, leaf width, ligule length, days to 50 per cent heading, panicle length, grain length, grain width, culm length, culm number, culm diameter, 1000 grain weight and days from seeding to maturity and nutritional quality characters viz., soluble carbohydrate content, amylose content, free amino acid content, protein content and starch grain size, reflects little possibility of improving these characters through selection. Similar findings of low variability were reported by Pathak and Patel (1989) for number of days to 50 per cent flowering, number of days to maturity and panicle length, Vanaja (1998) for grain length and grain breadth.

Considerable influence of environmental factors was observed in the case of panicle length, grain yield hectare^{-1} and amylose content as these characters showed high pcv than gcv. Closeness between genotypic and phenotypic

coefficients of variation observed in quantitative characters such as seedling height, leaf width, ligule length, days to 50 per cent heading, culm length, 1000 grain weight, grain length, grain width, straw yield hectare^{-1} and days from seeding to maturity and nutritional quality characters like soluble carbohydrate content, fat content, free amino acid content, amylase activity and starch grain size suggested that these characters might be less influenced by environmental factors.

5.6 Heritability

Knowledge of the heritable fraction of the variability enables the plant breeder to base selection on the phenotypic performances. In the present study quantitative characters namely, days from seeding to maturity, days to 50 per cent heading, leaf width, grain length, 1000 grain weight, culm length, seedling height, straw yield, ligule length, leaf length, culm diameter, culm number and grain yield and all the nutritional quality characters except amylose content exhibited high degree of broad sense heritability. This result revealed that these characters are less influenced by environment and there could be greater correspondence between phenotypic and breeding values. Similar reports were also made by Singh *et al.* (1984), Kihupi and Doto (1989) and Roy and Kar (1992) for number of days to 50 per cent heading and maturity, culm length and 1000 grain weight, Anandakumar (1992) for culm length and Vanaja (1998) for number of total tillers plant^{-1} and yield hectare^{-1}. Moderate heritability estimate for panicle length was in agreement with the result of Roy and Kar (1992). Moderate heritability was also observed for the nutritional quality character viz., amylose content.

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 inorder to ascertain its

relative utility. High expected genetic gain was observed for quantitative characters namely, straw yield hectare^{-1}, grain yield hectare^{-1}, leaf width, ligule length, culm number, 1000 grain weight, days to 50 per cent heading, culm length, days from seeding to maturity and culm diameter and for nutritional quality characters namely, fat content, amylase activity, soluble carbohydrate content and free amino acid content. This indicated that considerable level of improvement could be achieved in these traits by selection from segregating generations. Similarly moderate estimate of genetic gain was observed for quantitative characters like leaf length, seedling height, grain width and grain length and for nutritional quality characters such as protein content and starch grain size. Expected gnetic gain was low for panicle length and amylose content. High expected genetic gain was reported by Singh *et al.* (1984), Reddy *et al.* (1988) and Rao and Shrivastav (1994) for culm length and culm number, Singh *et al.* (1984) for 1000 grain weight, Vanaja (1998) for yield hectare^{-1}, culm number, culm length and 1000 grain weight. Moderate expected genetic gain was reported by Vanaja (1998) for grain length and grain breadth.

Acording 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 advance would be expected. In the present investigation, in the case of nutritional quality character fat content, heritability (broad sense) was high coupled with a high expected genetic gain and high genotypic coefficient of variation. High heritability and high expected genetic gain coupled with moderate gcv were exhibited by grain yield and straw yield among quantitative characters and amylase activity among nutritional quality characters. Similar result for grain yield plant^{-1} was reported by Murthy *et al.* (1999). High heritability and high expected genetic gain coupled with low gcv were exhibited by leaf width, ligule length, days to 50 per cent heading, culm length, culm number, culm diameter, 1000 grain weight and days from seeding to maturity among quantitative characters and soluble carbohydrate content and free amino acid

content among nutritional quality characters. These results suggested that the quantitative characters namely, grain yield, straw yield, leaf width, ligule length, days to 50 per cent heading, culm length, culm number, culm diameter, 1000 grain weight and days from seeding to maturity and nutritional quality characters like fat content, amylase activity, soluble carbohydrate content and free amino acid contents were under additive genetic control and hence can be relied upon for further improvement in yield through selection based on phenotypic performance. Similar results of high estimate of heritability coupled with high expected genetic gain were observed by Chaubey and Richharia (1990) for culm length, Pathak and Patel (1989) and Roy and Kar (1992) for culm length and 1000 grain weight, Singh *et al.* (1984) for culm length and grain yield hectare^{-1}. High heritability estimate with low gcv estimate and moderate estimate of expected genetic gain were observed for the characters like seedling height, leaf length, grain length and grain width among quantitative characters and protein content and starch grain size among nutritional quality characters which might be due to the variation in environmental components involved in these traits. The present result of high heritability coupled with moderate expected genetic gain and low gcv for grain length and grain width is in agreement with the results of Hussain *et al.* (1987). Moderate estimate for heritability and low value for gcv and genetic gain observed in case of panicle length and amylose content indicated that lesser scope for improving these characters through selection.

In general the quantitative characters namely, grain yield, straw yield, leaf width, culm number, culm diameter, 1000 grain weight, days from seeding to maturity, ligule length, days to 50 per cent heading and culm length and nutritional quality characters like fat content, amylase activity, soluble carbohydrate content and free amino acid contents provided a good base for selection as they are controlled by genes with additive effects.

5.8 Correlation studies

Studies on association of characters gain improtance 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 or 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. The results of correlation studies between grain yield hectare^{-1} and twenty one yield components are discussed below.

Among the correlation coefficients of 21 characters with grain yield, for the characters ligule length and culm length, the phenotypic correlation coefficients were higher than genotypic correlation coefficients, which indicated the influence of environment on these characters. Out of 21 characters, only two yield components namely, leaf length and straw yield hectare^{-1} were significantly correlated with yield at genotypic level.

The highest significant positive genotypic correlation of yield was with straw yield hectare^{-1} followed by leaf length. This revealed that improvement of grain yield hectare^{-1} could be achieved by exercising selection simultaneously for increased straw yield hectare^{-1} and leaf length.

The high degree of significant positive association at genotypic level between straw yield and grain yield suggested that straw yield was a highly reliable component of yield and could very well be utilized as an yield indicator in yield trials. The significant positive association of leaf length with grain yield hectare^{-1} indicated the favourable influence of increased leaf length on grain yield. The above

result was in agreement with the report of Selvarani and Rangasamy (1998) for drymatter production.

In the present study, absence of significant correlation of the nutritional quality characters with yield suggested that these characters could be recombined as desired.

Inter correlations among yield components revealed that selection for dwarf seedlings would bringforth correlated response for desirable characters such as high free amino acid content, reduced number of days to 50 per cent heading and reduced number of days from seeding to maturity. Similarly positive genotypic correlations of ligule length, culm length, culm diameter and panicle length, 1000 grain weight and grain width with leaf length revealed that selection for increased ligule length, taller and thick culms, long panicles, high 1000 grain weight and bold grains would increase leaf length which has significant positive correlation with yield. But selection for longer leaves would reduce protein content in grain as leaf length and protein content were negatively correlated. The positive association of culm length and diameter with panicle length was in confirmity with the report of Enyi (1956) while the positive correlation of 1000 grain weight with culm length observed in the present study was in agreement with the result of Awan and Cheema (1986). The significant correlations of leaf width, ligule length and days to 50 per cent heading with other yield components at genotypic level revealed that selection for thick culms, long panicles, high 1000 grain weight, long and bold grains and increased number of days from seeding to maturity would result in high fat content and free amino acid content but would result in reduced protein content since leaf width, ligule length and number of days to 50 per cent heading exhibited significant negative correlation with protein content. The positive correlations of panicle length and 1000 grain weight with days to 50 per cent heading observed in the present study was in agreement with the report of Reddy and Reddy (1981). The significant positive correlation of culm length with grain width indicated that selection for

shorter culms would reduce grain width, which favours high protein and amylose contents and low amylase activity.

The significant negative correlations of culm number with other yield components indicated that selection for thinner culms, shorter panicles, reduced 1000 grain weight, reduced grain length and grain width would increase protein content but decrease free amino acid content. The significant positive inter correlations of culm diameter and panicle length with other yield components revealed that selection for increased 1000 grain weight, long and bold grains, increased number of days from seeding to maturity and larger starch grains favoured high fat and free amino acid contents, high yield but will reduce protein content. The significant positive correlations of 1000 grain weight and grain width with other yield components indicated that selection for short and narrow leaves, shorter ligules, decreased number of days to 50 per cent heading, thin culms, shorter panicles, reduced number of days from seeding to maturity and high culm number would favour high protein content. The significant positive correlation of grain length with grain width indicated that selection for shorter grains favoured high protein content and low amylase activity. The correlation of days from seeding to maturity with other yield components revealed that selection for increased number of days from seeding to maturity favoured high fat and free amino acid contents but decreased protein content.

In breeding programmes to improve a number of traits simultaneously, a significant correlation of traits with yield would be considered desirable. In the present study, significant positive correlation of leaf length and straw yield hectare^{-1} with grain yield hectare^{-1} suggested that yield could be improved by exploiting these traits. Among the nutritional quality characters, significant positive correlations of fat and free amino acid contents with other yield components indicated that selection for wider leaves, longer ligules, increased number of days to 50 per cent heading, thick culms, longer panicles and increased number of days from seeding to maturity favoured high fat and free amino acid contents. Soluble carbohydrate content did not

show correlation with none of the characters studied. The significant association of amylose content with other yield components revealed that selection for low amylase activity, shorter culms and high straw yield favour high amylose content. The significant negative correlation of protein content with other yield components indicated that selection for low amylase activity, short and narrow leaves, shorter panicles, low 1000 grain weight, slender grains, reduced number of days from seeding to maturity and smaller starch grains favoured high protein content. The negative association of 1000 grain weight with protein content observed in the present study was in agreement with the report of Kambayashi *et al.* (1984). The significant positive correlations of amylase activity with other yield components indicated that selection for tall culms, high 1000 grain weight and bold grains favoured high amylase activity.

5.9 Path analysis for yield

Though the correlation studies were helpful in measuring the association between 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 pinpoint the important yield components which can be utilized for formulating selection parameters.

In the present study, path coefficient analysis performed taking four quantitative yield compoents, of which two were significantly correlated with yield at genotypic level was discussed. The cause and effect relationships between yield and its four components are illustrated in Fig.8.

The residual effect of 0.5299 obtained in path analysis indicated that the causative factors included in the analysis were inadequate to explain variability in yield. The 47.11 per cent variation in grain yield was contributed genotypically by four yield components namely, leaf length, grain length, grain width and straw yield hectare^{-1}. The highest positive direct effect was exhibited by straw yield. This was

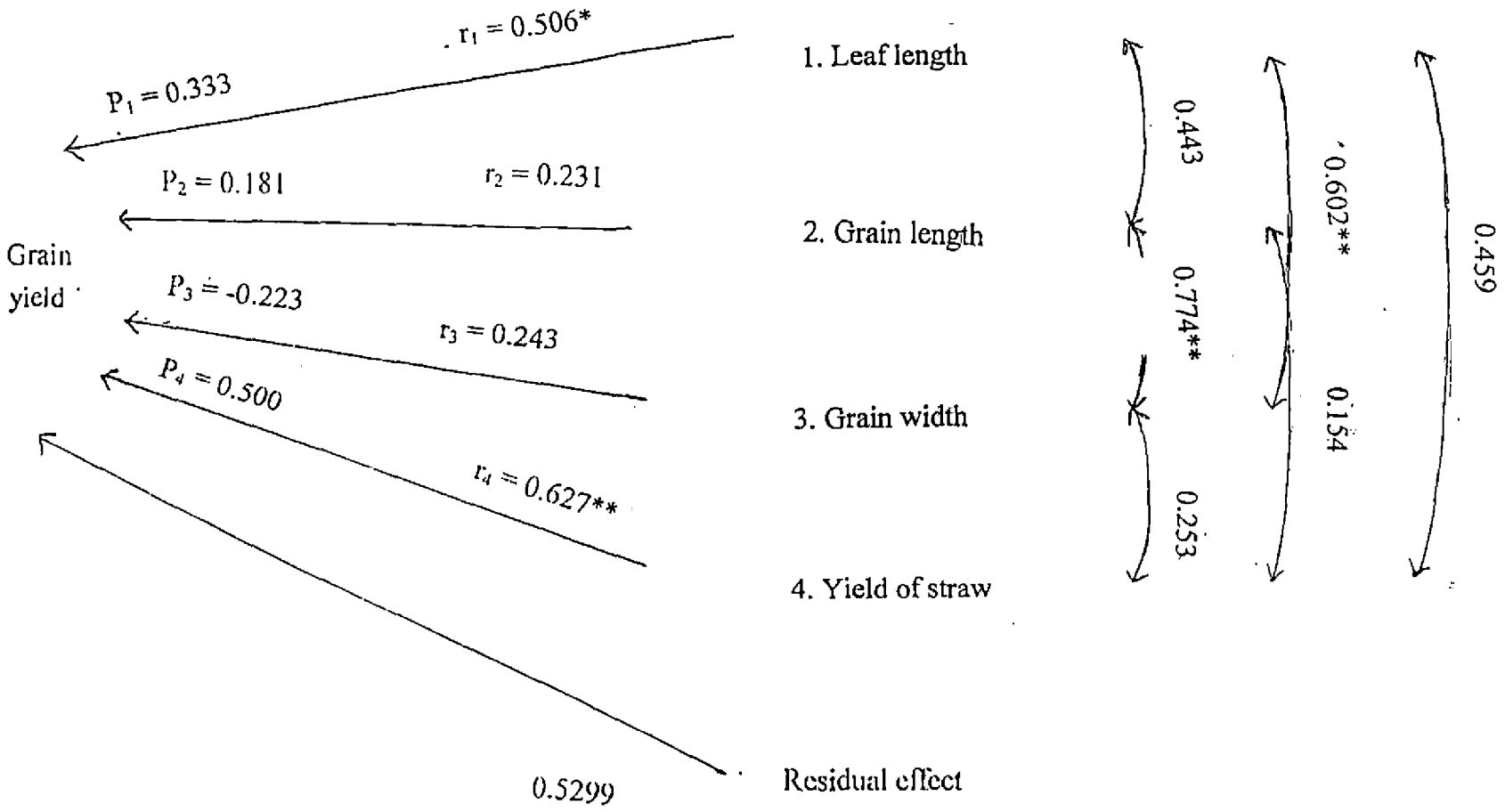


FIG.8. PATH DIAGRAM CONSTRUCTED USING GENOTYPIC CORRELATION COEFFICIENTS AMONG YIELD AND FOUR OF ITS COMPONENT CHARACTERS IN NAVARA

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 positive direct effect and partly by its positive indirect effect through leaf length. Second highest positive direct effect on grain yield was contributed by the character leaf length whose significant positive correlation with yield was explained partly by its positive direct effect and partly by its positive indirect effect through straw yield. Grain length too had positive direct effect on yield. Its positive correlation with yield was due to its direct effect and indirect effect through straw yield, while the indirect effects via. leaf length and grain width cancelling out each other. Grain width though exhibited positive correlation with yield, exerted negative direct effect. Its positive correlation with yield was mainly due to its positive indirect effects via. leaf length, grain length and yield of straw.

5.10 Path analysis for free amino acid content

Path analysis with respect to free amino acid content was done considering seven characters having significant genotypic correlation with free amino acid content. The cause and effect relationship between free amino acid content and its seven components are illustrated in Fig.9.

The residual effect of 0.6550 obtained in path analysis indicated that the causative factors included in the analysis were inadequate to explain variability in free amino acid content. The 34.50 per cent variation in free amino acid content was contributed genotypically by seven free amino acid components namely, seedling height, leaf width, days to 50 per cent heading, culm number, culm diameter, panicle length and days from seeding to maturity. Leaf width had the highest positive direct effect on free amino acid content followed by days to 50 per cent heading. The positive correlation of leaf width with free amino acid content was mainly due to its positive direct effect. In the case of days to 50 per cent heading too, its positive correlation with free amino acid content was mainly due to its positive direct effects while the indirect effects cancelled out each other. The direct effects of three other

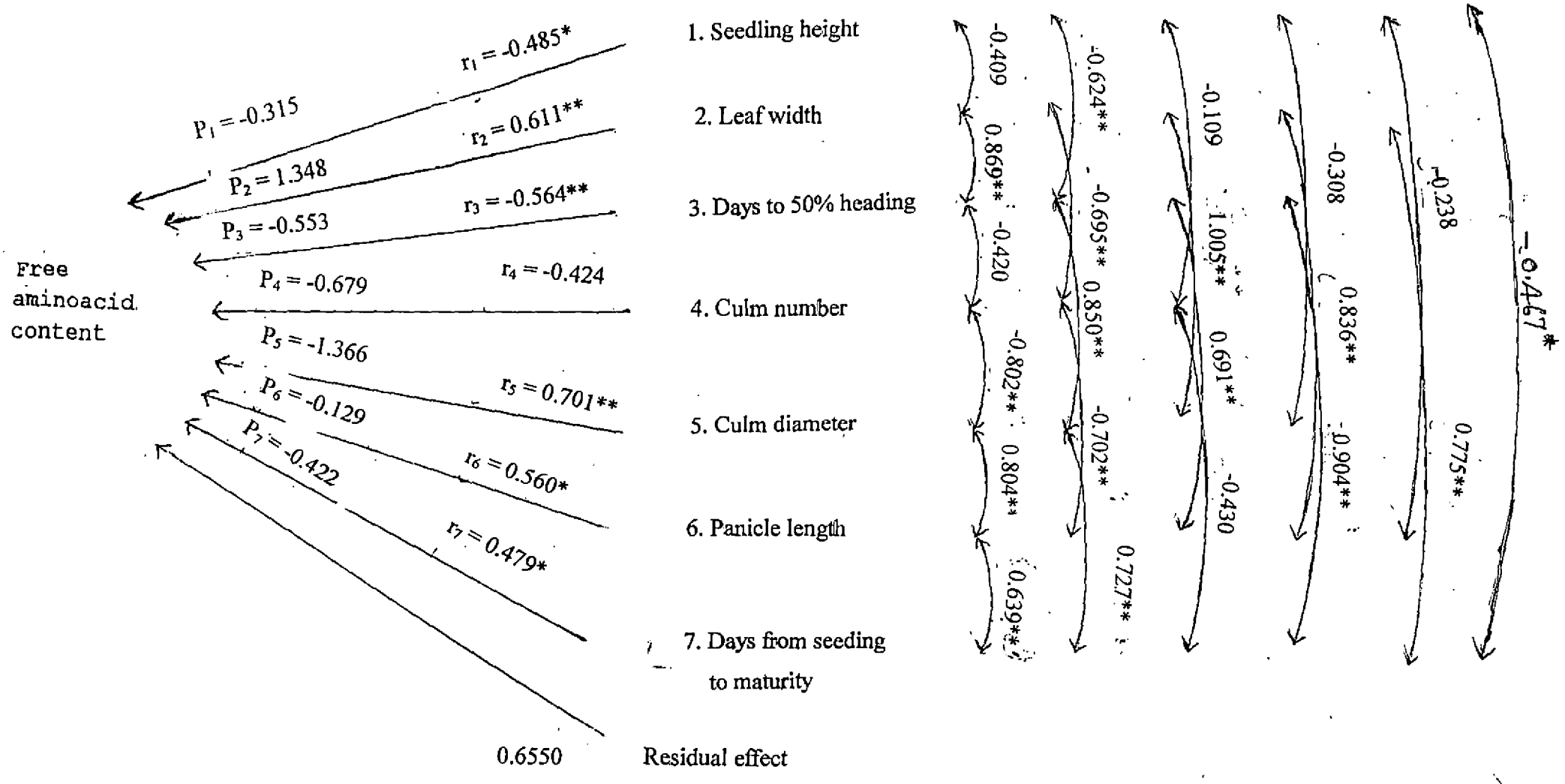


FIG.9. PATH DIAGRAM CONSTRUCTED USING THE GENOTYPIC CORRELATION COEFFICIENTS AMONG FREE AMINOACID CONTENT AND SEVEN OF ITS COMPONENT CHARACTERS IN NJAVARA.

components such as culm diameter, panicle length and days from seeding to maturity were found to be negative though these components registered significant positive correlations which is mainly due to their positive indirect effects via. seedling height, leaf width, days to 50 per cent heading and culm number. Seedling height and culm number too had negative direct effects because of their significant negative genotypic correlations with free amino acid content.

5.11 Path analysis for protein content

Five characters showing significant genotypic correlations with protein content were considered for path analysis. The cause and effect relationships between protein content and its five components are illustrated in Fig.10.

The residual effect of 0.1174 obtained in path analysis indicates that the causative factors included in the analysis were adequate to explain variability in protein content. The 88.26 per cent variation in protein content was contributed genotypically by five protein components namely, leaf length, leaf width, days to 50 per cent heading, panicle length and 1000 grain weight. Leaf width had the highest positive direct effect followed by leaf length though they showed significant negative genotypic correlations with protein content. This was mainly because of their negative indirect effects through days to 50 per cent heading, panicle length and 1000 grain weight. The highest negative direct effect observed for panicle length coupled with its negative indirect effects via. days to 50 per cent heading and 1000 grain weight were mainly responsible for its high significant correlation with protein content at genotypic level. Days to 50 per cent heading and 1000 grain weight too had negative direct effects resulting in their negative correlation with protein content while the indirect effects via. other component characters cancelled out each other.

In conclusion, fat content exhibited large variability indicating scope of improvement of this trait through selection. Straw yield and grain yield showed

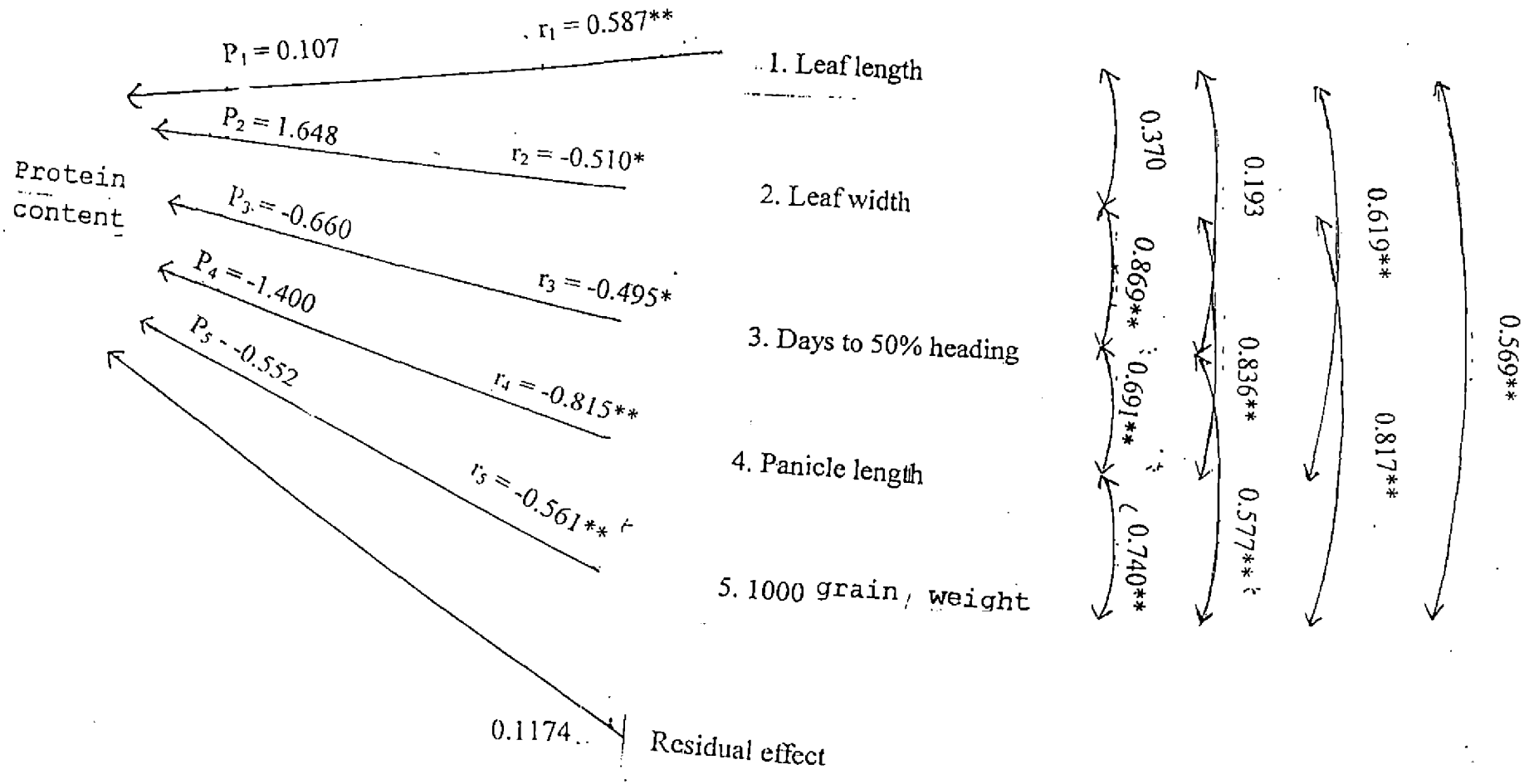


FIG.10. PATH DIAGRAM CONSTRUCTED USING THE GENOTYPIC CORRELATION COEFFICIENTS AMONG PROTEIN CONTENT AND FIVE OF ITS COMPONENT CHARACTERS IN NJAVARA

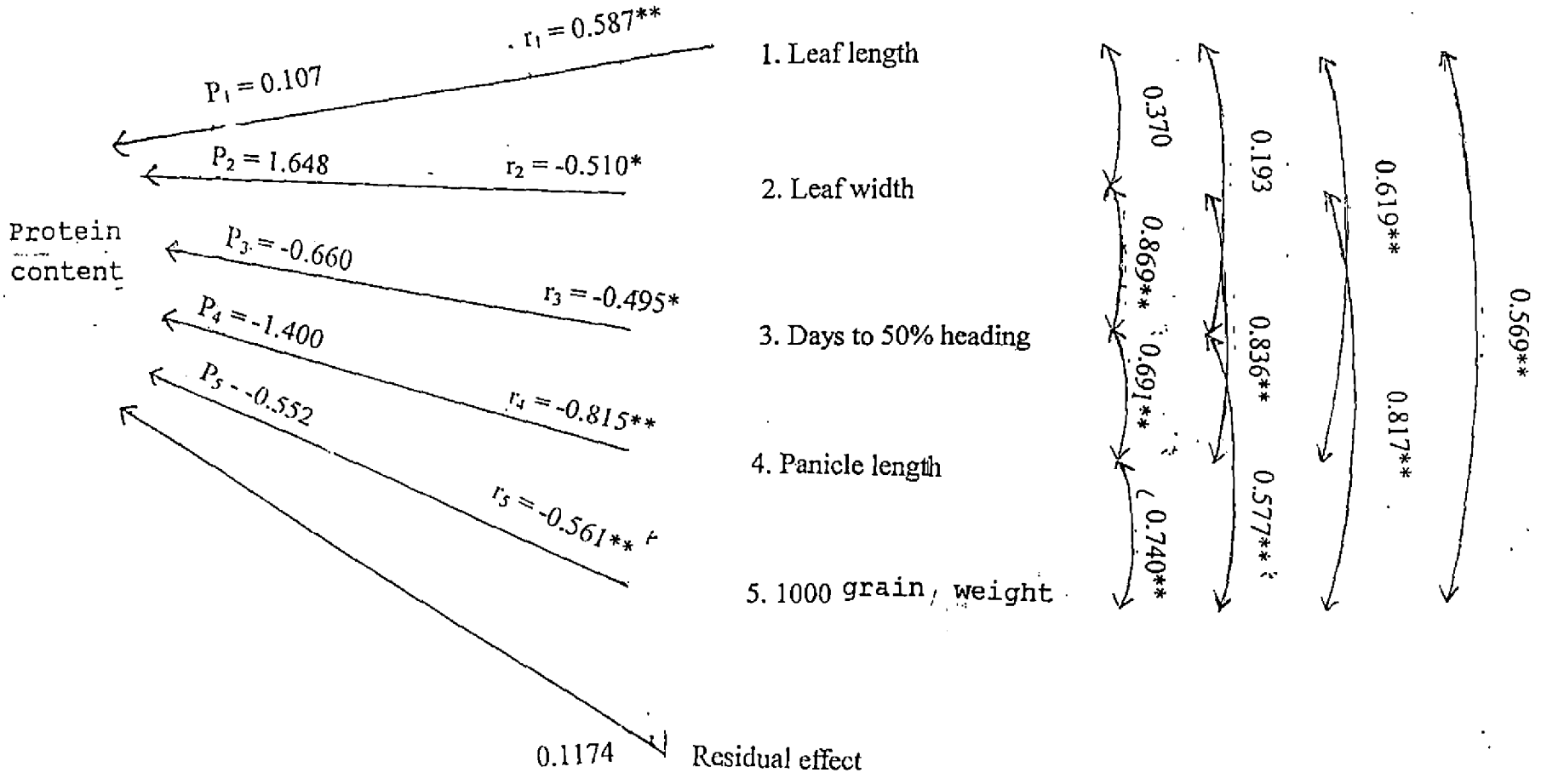


FIG.10. PATH DIAGRAM CONSTRUCTED USING THE GENOTYPIC CORRELATION COEFFICIENTS AMONG PROTEIN CONTENT AND FIVE OF ITS COMPONENT CHARACTERS IN NJAVARA

moderate variability which suggested that these traits were useful in rice improvement programme. In addition, the above mentioned traits were found to be under additive genetic control indicating that they provided a good genetic base for selection. Correlation and path studies revealed that grain yield could be improved by simultaneous selection for high straw yield and longer leaves. Free amino acid and fat content could be improved by exercising selection for wider leaves, longer ligules, increased number of days to 50 per cent heading, thick culms, longer panicles and long growth duration. Similarly selection for low amylase activity, short and narrow leaves, shorter panicles, low 1000 grain weight, slender grains, short duration genotypes and smaller starch grains, favoured high protein content while selection for tall culms, high 1000 grain weight and bold grains favoured high amylase activity. Thus high yielding Njavara genotypes and genotypes with high protein content, high free amino acid content and high amylase activity could be evolved by exercising selection in respect of the characters mentioned above.

SUMMARY

SUMMARY

Investigations were undertaken in the Department of Genetics and Plant Breeding and Biochemistry Laboratory, College of Horticulture, Vellanikkara during 1997-1999 to characterize the rice (*Oryza sativa* L.) cultivar, Njavara based on morphological and biochemical characteristics and to evaluate its nutritional qualities.

Thirteen Njavara genotypes collected from different locations of Kerala formed the material for the present study. The genotypes were laid out in a Randomised Complete Block Design (RCBD) with three replications in plots of 2.0 m x 1.0 m with 10 cm x 15 cm spacing during *kharif* season. The morphological observations were recorded at different stages of plant growth following the 'rice descriptor' published by the IRRI, Philippines.

The salient findings could be summarised as follows:

1. The thirteen Njavara genotypes included in the present study were found to be morphologically distinct among themselves. In general, Njavara genotypes had glabrous and green leaves, green basal leafsheath, erect leaves, white and 2-cleft ligules, palegreen collar and auricle, erect culms, light gold internode and septum, heavy secondary branching of panicle, very low shattering of panicle, white stigmas, straw coloured sterile lemmas, highly fertile spikelets, nonwaxy endosperm, non scented grains and low pest and disease incidence. With respect to other qualitative and quantitative characters Njavara genotypes showed variation the maximum range being in the case of spikelet awning, lemma and palea colour and brown rice colour.
2. The extra short duration nature of the Njavara cultivar makes it ideal for cultivation in drought prone areas and as a donor parent for evolving extra short duration high yielding rice varieties.

3. The Njavara genotypes N1, N2, N5, N11 and N13 were found to be yielding on par with that of the check variety Ptb-10. After indepth studies to reveal their unique medicinal properties, suitable Njavara genotypes can be recommended for cultivation to be utilized as a general food grain, specific diet food grain and also in Ayurveda treatment.
4. Biochemical characterization indicated the possibility of utilizing peroxidase, esterase and protein polymorphism for identifying the Njavara genotypes especially N1, N2, N3 and N4 and also for the identification of Njavara as a cultivar.
5. Evaluation of Njavara genotypes with respect to nutritional quality of grain revealed that the genotypes N5, N7 and N13 had high soluble carbohydrate content and can be utilized as components of invalid and weaning foods because of reduced gelatinization period and easy digestibility.
6. With respect to protein content, majority of the Njavara genotypes expressed high protein content than Ptb-10. The genotypes N2 and N8 were found to be superior and they too can be included in weaning and invalid foods and diets during pregnancy and lactation.
7. The genotypes N2, N3 and N12 appeared to have better nutritive value based on number and content of free amino acids which are the precursors and primary source of medicinal properties of Njavara.
8. All the Njavara genotypes had low fat content while intermediate amylose content was observed in most of the Njavara genotypes suggesting that they might not have any problem with respect to consumer preference and cooking quality.
9. With respect to starch grain size, smaller grains were observed for all the Njavara genotypes compared to Ptb-10. Starch grain characterization revealed the possibility of using starch grain size for varietal characterization in conjunction with morphological and biochemical characterization.

10. Amylase activity was observed to be high for the genotypes N6 and N7. Besides high amylase activity, the genotype N7 had low amylose content and high soluble carbohydrate content and hence desirable due to its digestibility.
11. Absence of flavonoids in Njavara grain indicated its nonaromatic nature.
12. Variability studies indicated that fat content, straw yield and grain yield provided a good genetic base for selection. Correlation and path studies revealed that grain yield could be improved by simultaneous selection for high straw yield and longer leaves while selection for wider leaves, longer ligules, increased number of days to 50 per cent heading, longer panicles and long duration genotypes favoured high free amino acid content. Low amylase activity, short and narrow leaves, shorter panicles, low 1000 grain weight, slender grains, short growth duration and smaller starch grains were observed to favour high protein content and selection for tall culms, high 1000 grain weight and bold grains was found to favour high amylase activity.

Suggested future line of work

1. Collection and maintenance of all possible genetic variability existing in this unique rice cultivar.
2. Indepth studies to identify isozyme markers coupled with enzyme activity studies for characterization of Njavara genotypes and also at different growth stages.
3. Opting for molecular markers in case of failure of biochemical markers.
4. Detailed studies to identify the medicinal and nutritive principles in Njavara grain.

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CHARACTERISATION AND EVALUATION OF THE RICE (*Oryza sativa* L.) CULTIVAR NJAVARA

By

G. SRINIVASA REDDY

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

Department of Plant Breeding and Genetics

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2000

ABSTRACT

Characterization and evaluation of the rice (*Oryza sativa* L.) cultivar Njavara was undertaken in the Department of Plant Breeding and Genetics and Biochemistry Laboratory, College of Horticulture, Vellanikkara during 1997-1999 with an aim to characterize Njavara genotypes based on morphological and biochemical studies and to evaluate its nutritional qualities.

Morphological characterization indicated that the thirteen Njavara genotypes were distinct among themselves and the Njavara genotypes N1 (Njavara type from Mulloorkara, Thrissur), N2 (Njavara type from Chittoor, Palakkad), N5 (Njavara type from Edavanna, Malappuram), N11 (Njavara type from Pattambi, Palakkad) and N13 (Njavara type from Thootha, Palakkad) yielded on par with the check variety Ptb-10, eventhough they were having extra short growth duration compared to Ptb-10.

Biochemical characterization revealed the possibility of utilizing peroxidase, esterase and protein polymorphism for identifying the Njavara genotypes especially N1 (Njavara type from Mulloorkara, Thrissur), N2 (Njavara type from Chittoor, Palakkad), N3 (Njavara type from Chittoor, Palakkad) and N4 (Njavara type from Chittoor, Palakkad) and also for the identification of Njavara as a cultivar. Selecting some other isozymes coupled with enzyme activity studies and opting for molecular markers were the other strategies suggested for characterization of Njavara genotypes.

Majority of the Njavara genotypes expressed high protein content than Ptb-10. The genotypes N2 (Njavara type from Chittoor, Palakkad) and N8 (Njavara type from Alwaye) were recommended to be included in weaning and invalid foods due to high protein content. The genotypes N2 (Njavara type from Chittoor,

Palakkad), N3 (Njavara type from Chittoor, Palakkad) and N12 (Njavara type from Thrissur) appeared to have higher number and content of free amino acids. Most of the Njavara genotypes had intermediate amylose and as such may not have any problem with respect to consumer preference. Starch grain characterization revealed that starch grain size could be used for varietal characterization in conjunction with morphological and biochemical markers. Absence of flavonoids in Njavara grain indicated its nonaromatic nature. The genotype N7 (Njavara type from Tellicherry,Kannur) was found to have better digestibility due to low amylose content, high amylase activity and high soluble carbohydrate content.

Variability studies indicated that fat content, straw yield and grain yield provided a good genetic base for selection. Correlation and path studies revealed that grain yield could be improved by simultaneous selection for high straw yield and longer leaves while selection for wider leaves, longer ligules, increased number of days to 50 per cent heading, longer panicles and long duration genotypes favour high free amino acid content. Low amylase activity, short and narrow leaves, shorter panicles, low 1000 grain weight, slender grains, short duration genotypes and smaller starch grains were observed to favour high protein content and selection for tall culms, high 1000 grain weight and bold grains was found to favour high amylase activity.