Progress Report of the Mc Knight Foundation funded Project

II Year

2003-2004

Title of the Project

Development of high yielding, disease resistant, drought tolerant Finger millet (*Eleusine coracana* Gaertn)

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Objectives of the Project for the second Year

1. **Socio and economic constraint analysis**
2. **Participatory breeding of finger millet**
3. **Characterization of blast isolates for neck and leaf blast**
4. **Development of SSR markers and other markers**
5. **Phenotyping and Map development**
6. **Development of introgression lines**

INTRODUCTION

Finger millet (***Eleusine coracana L.***), is also known as African millet, Koracan, Ragi (in India), Bulo (Uganda), Wimbi (Swahili) and Telebun (Sudan). It is an important cereal crop for subsistence agriculture in the dry areas of Eastern Africa, India and Srilanka. India is one among the major cereal producing countries in the world. World finger millet production is 4.5 million tonns, of which about 2.5 million tonns is produced by Africa. The crop originated in Africa and has been cultivated for thousands of years in the highlands of Uganda and Ethiopia. It was introduced to India at a very early date, probably over 3000 years ago. Though finger millet is reported to have reached Europe at about the commencement of the Christian era, its utilization is restricted mostly to eastern Africa and India.In India, it is cultivated in Tamil Nadu, Andhra Pradesh, Orissa, Bihar, Gujart, Maharastra and in the hilly regions of Uthar pradesh and Himachal Pradesh. Finger millet cultivation occupies a total area of 2.5 million hectares with a production of 2.2 million tonnes. Although it has not entered the international market it is a very important cereal grain in areas of its adaptation. Finger millet is the second most important food and fodder crop of the dry lands in Karnataka. It has a high level of regional or local adaptation. Although grown under dry lands, it provides an assured harvest, thus making it indispensable in specific ecosystems.

Finger millet is considered as a coarse grain because of its fibrous and tough outer layer that irritates the tongue and not readily accepted for people accustomed for the consumption of wheat and rice. Apart from palatability it lacks gluten characteristic of wheat and hence does not lend itself for the preparation of chapathis or baked products. It has remained as the food of the lower socio-economic groups and traditional consumers, because of its coarse texture and intense colour of seedcoat.Finger millet contains a large proportion of carbohydrates and thus provides bulk of energy in diets. It is also rich in proteins, sulphur containing amino acids and because of its low glycemic index with high fibre it is recommended for diabetic patients. Apart from the major nutrients, it also contains iron and calcium, which is deficient in most Indian women. High calcium, high soluble fibre, low fat, high diastatic power of malted grains renders finger millet unique. It has proved to be very effective in controlling blood glucose level of diabetics. Consumption of finger millet prevents constipation and cholesterol.

A variety of products can be prepared out of finger millet. Around 75% of finger millet production is being used in the preparation of traditional products like “mudde”, “dosa”, “idly” and “paddu”. With increasing awareness of good nutrition, healthy living, consumption of finger millet is increasing among the higher income groups as well. Finger millet is considered as wonderful millet because of its good storage quality, high nutritive value and therapeutic value. Hence, there is enormous scope for providing improved cultivars, which are need based and best suited for developing newer products in finger millet so that good value addition could be achieved.

In India, as in other developing countries, pollution growth is keeping pace with agricultural production, making food security an important issue. The Green Revolution has contributed tremendously towards making India self –sufficient in food production. However, the higher-yielding varieties also require higher inputs to reach their potential, a cost that can't be afforded by the more marginal farmers. Approximately 70 percent of India's working force relies on agriculture for their livelihood and a significant portion of them are subsistence farmers. In most years, they produce barely enough food to feed their families, which makes them extremely vulnerable to the effects of adverse conditions on crop yields. Therefore, in addition to addressing the issue of the food security at a global level, efforts need to be directed towards such indigenous crop. This crop, which is being neglected by the researchers, has been grown for decades. Very few studies have been conducted on economic and welfare implications of finger millet. Hence, research towards such indigenous, nutrient rich crop which can be grown in marginal environment will surely benefit the poor communities. Hither to no serious effort has been made to understand the technological needs of the farmer in respect of finger millet varieties. This is essential for the success of varieties developed.

To popularize the best suited drought tolerant and needy varieties of finger millet and to understand what the farmers need the most in varieties of finger millet, an appraisal regarding the desired attributes of the variety and welfare aspects of Finger millet cultivating farmers is needed. The hallmark of the present study is the in-depth evaluation of existing, preferred traits of the varieties and the intrinsic features of the leading variety. This study is part of larger study on breeding entitled "Development of High Yielding, Disease Resistant and Drought Tolerant finger millet (*Eleusine coracana gaertn*)", underway in the Department of Plant Breeding and Genetics. This study was carried out to compliment the above said study with the specific objective of the project. Under standing the requirements of production technology such as disease, drought tolerance, duration, cooking qualities, etc., will greatly enhance the adoption rate of the variety and accordingly the commercial success of the variety. This will result in a higher pay-off for the investment.

Objectives

1. Socio and economic constraint analysis

The primary objective of the study was to understand the technology gap in the production of finger millet in Karnataka in the backdrop of the socio-economic, institutional and other parameters. The specified objectives of the study are

(1) To document the socio-economic conditions of Finger millet cultivating farmers in the study area.
(2) To study the productivity of resources and economics of finger millet cultivation among different groups.

(3) To identify the sources contributing to change in output of finger millet in Karnataka.
(4) To determine the most preferred attributes both with respect to production and consumption of finger millet for different categories of farmers.
(5) To analyse the past trends and future prospects of finger millet cultivation in the state.

Data & Methodology

This section gives a brief overview of the study area, the nature and sources of data, the sampling design and the method of analysis.

Description of the study

Karnataka is the sixth largest state in India with an area of 190.50 lakh hectare. The state is surrounded by the Arabian Sea in the west, the state of Maharastra in the north, Andhra Pradesh and Tamil Nadu in the east and Kerala and Tamil Nadu in the south. It is situated between 11.5 and 18.50 north latitude and 74 east and 78.30 east longitude in the southern plateau. According to 2001 census, Karnataka had a total population of 449.77 lakh persons, of which rural population forms 310.69lakh (69%) and the urban population forms 139.08 lakh persons (31%). The male population accounts for about 229.5 lakh persons (51%) and female population formed about 220.25(49%), with a population density of 235. The average growth rate of population of the state is 22 percent per decade. Out total geographical area 30.62 lakh hectares is under forest cover, 10.17 lakh hectares of permanent pasture, 3.17 lakh hectares under trees and groves. The total farmers in the state are 62,207,98 farmers, out of which 26,095,13(41.9%) are marginal farmers having below 1 hectare,17,068,39 (%) small farmers having 1-2 hectare, 1,204,185(%) semi medium having 2-4 hectares, 594,232(%) medium farmers having 4 - 10 hectares and finally 106,029(%) large farmers having more than 10 hectares. The overall literacy rate in the state is 67.04per cent, comprising male literacy rate 76.29per cent and female literacy rate 57.45 per cent.

The influence of the climate and rainfall distribution pattern plays a significant role on the crop. The state has pleasant and moderate climate with a maximum temperature up to 40^0C over the northeastern parts the state and the minimum temperature of 2.8^0C. The average humidity is 60 percent. The state receives an annual rainfall of 1139 mm from the both south west monsoon and northeast monsoon, which starts from June and extends up to November. Major rain is received from the south west monsoon. The state on an average is categorized as drought prone; the severity of the drought varies from year to year.

As in case of the country, agriculture is the backbone of the economy of the state. The net sown area is 106.09 lakh hectares, and accounts for 69 percent of the total geographical area. The major crops grown are cereals, pulses, oilseeds and cash crops. Important food crops of the state are ragi, paddy, jowar, maize and bajra. Pulse crops like red gram, bengal gram, field been cow pea and horse gram are also grown. The important oil seed crops in the state are ground nut, sunflower, safflower and sesamum. Cotton, sugar cane and mulberry are the commercial crops. The vegetable crops include potato, carrot, cabbage, beetroot, radish, cauliflower, brinjal beans and leafy vegetables. The major fruit crops like mango, guava, sapota, grapes and the flower crops like roses,

chrysanthemum, crossanrda, aster, jasmine, champaka and marigold are grown in the state.

The present study was undertaken to examine the various aspects of finger millet cultivation like production, consumption, cost and returns and the soci -economic aspects of the sample farmers. The main focus of the study is to find out from the farmers what are the features of a variety that the farmer would like to grow. Based on the dominance of area, districts were selected for the study. The five districts selected were Bangalore (urban), Bangalore (rural), Tumkur, Kolar and Mandya. Tumkur ranks first in area under finger millet cultivation, followed by Bangalore (rural), Kolar, Mandaya, Chikkamangalore and Bangalore (urban) as indicated in the below table (Table 1), and same is also represented in Fig. 1.

Table 1: District wise area under finger millet cultivation (2000-2001).

Sl. No.	District	Area (ha)	% Area
1	Bangalore urban	48023	5.10
2	Bangalore rural	145958	15.5
3	Kolar	131856	14.0
4	Tumkur	172270	18.3
5	Mandya	78744	8.4
	Total	576851	61.3
	State total	941375	---

Source: "Fully revised estimates of principal crops in Karnataka" for the year 2000-2001

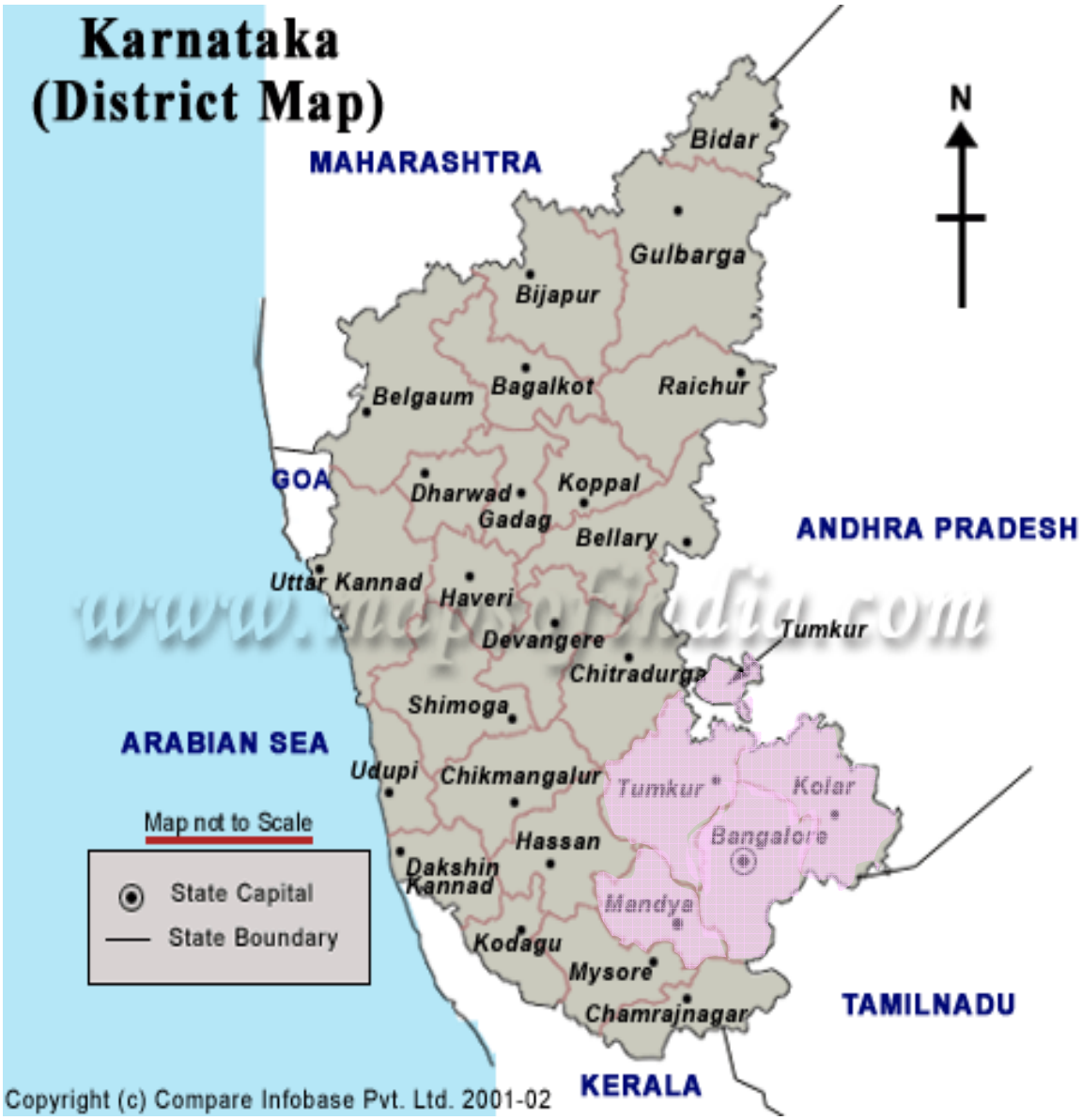

Fig.1: Map Showing the Study Districts

Bangalore urban

The district is situated in the southeastern portion of Karnataka, comes under eastern dry zone. It lies between longitude with a total geographical area of 217,410 hectare and with 3,303 hectare under forest cover. The district at present comprised of tree taluks viz. Anekal, Bangalore north and Bangalore south, with 17 hoblies and 728 villages. Total population of the district according to 2001 census was 652,311,0 persons with sex ratio of 906. The literacy rate was found to be 83.91percent and the percapita income at constant prices was Rs. 19,906. The normal rainfall of the district is about 867mm which is scanty and erratic, mostly received from south – west monsoons. The climate is generally dry with a temperature ranging from 24^{o}C to 36^{o}C. Though the district has witnessed significant industrial development on an impressive scale in past few decades, the economy of the district particularly rural areas depends on agriculture. The important food crops grown are ragi, jowar, paddy, millets, and pulses. Among the

non - food crops, oil seeds and mulberry deserve special mention. Cultivable lands are mainly rain fed and dry farming is the characteristic feature of the district. The irrigation potential of the district is quite low.

Bangalore rural

Bangalore rural district is located in the south eastern corner of Karnataka state. It comes under zone five (eastern dry zone). It is bounded by Kolar on the north - east, by Dharmapuri district of Tamil nadu on the east and south east, by Mysore on the south, Mandaya on the south- west. Spanning a geographical area of 585,431 hectare, with 81,268 ha of forest coverage. It lies between the latitudinal parallels of 12 15 N and 13 35 N on one hand and the longitudinal parallels of 77 05 E and 78 E on the other. According to 2001 census the total population of the district was 1,877,416 persons with a sex ratio of 953. The literacy rate was found to be 65 percent and the percapita income at constant prices was about Rs. 8,630. The normal rainfall of the district was about 817mm which is scanty and irregular. The climate and soil conditions in all parts of the districts are quite conductive for producing a variety of both food and non food crops.The economy of Bangalore rural district has two features. The traditional economy with its roots in the primary sector mainly agriculture and the modern economy characterised by a dominance of the secondary and tertiary sectors. The agricultural economy of the district is almost totally dependent on good seasonal conditions, that is, adequate and timely rainfall. Cultivable lands are mainly rain fed and dry farming is a characteristic feature of the district. The important food crops grown are ragi, jowar, paddy, millets and pulses. Among the non – food crops, oil seeds and mulberry deserve a special mention. In recent years cultivation of sunflower has also gained considerable popularity in the district.

Kolar district

This district is popularly known as the “Golden land” of India, for its famous Kolar Gold Fields. Kolar district is located in the southern region of the state and happens to be the eastern most district of the state. This district also comes under eastern dry zone. It is bounded by Bangalore and Tumkur districts on the west and on all other sides by the adjoining states of Andhra Pradesh and Tamil Nadu. The total geographical area of the district is 779,467 hectare with 703, 24 hectare under forest , has its greatest length of about 135 km from north to south with almost the same distance from east to west. According to 2001 census the total population of the district was 2,523,406 persons with a sex ratio of 970. Under this district, there are 11 taluks, 53 hoblies and 3321 villages. The literacy rate of the district was 63.14 percent and has a per capita income of Rs. 7,466 at constant prices. Agriculture is the backbone of the economy of district. The net area irrigated is 81,887 hectare and nearly 71 percent of which is under irrigation by bore wells, the remaining area under irrigation by tanks. The cultivable land is classified as dry (kushki), wet (tari) and garden (bagayat). The kushki lands are cultivated with crops such as ragi, jowar, pulses and millets. The wetlands usually have the irrigational facilities where crops like paddy, sugarcane, mulberry and virginia tobacco. The garden lands are cultivated with coconuts, areca and betel wine. Fruit crops such as mango, citrus and grapes are grown in almost all the taluks. The district enjoys a pleasant and moderate climate with a maximum temperature of 36^{o} C in may and the minimum temperature of 15^{o} C in December. The average humidity in the district is 65 percent.

Tumkur district

`The district is located in the eastern belt in the southern half of the state. It belongs to the central dry zone with soils suitable for cultivation of most food crops. This district lies between the latitudinal parallels $12^{o}\ 45^{1}$ N and $14^{o}\ 22^{1}$ N and the longitudinal parallels of $76^{o}\ 24^{1}$ E and $77^{o}\ 30^{1}$ E. The shape of the district is some what irregular and the peculiar feature is that the north – eastern portion is totally detached from the remaining areas of the district. it is bounded on the north by Ananthpur district of Andhra Pradesh, on the east by the Kolar district, Bangalore on the south, Mandaya on the west and Hassan and Chitradurga in the north-west. Total population according to 2001 census is 2,579,516 persons, with a sex ratio of 966. The literacy rate of the district is 67.17 percent, with a percapita income of Rs. 8175 at constant prices. Finger millet is the most extensively cultivated food crop of the district. It is grown in all the taluks as a rain fed crop as well as an irrigated crop. The total area under ragi roughly constitutes one – third of the total cropped area in the district. In rural area, finger millet is even now the main staple food. Paddy and jowar are the other two important food crops and paddy is usually raised under irrigated conditions. Horse gram, 'tur' and 'avare' are the pulses grown all over the district. Sugarcane cultivation is of special significance in the taluks of Tiptur, Chiknayakanahalli and Turvekere. This particular belt is also renowned for the production of superior quality coconut. Tiptur is known for its copra all over the country. Taluks of Pavagada, Sira and Madhugiri are leading in ground nut production. Areca, tobacco, Bengal gram and caster seeds are also grown in the district. Even though the district is blessed with important rivers which are flowing through this district, the cultivation is mainly dependent upon channels and numerous minor and major tanks as well as four large reservoirs.

Mandya district

Mandya district is located in the central belt of the southern sector, is included in the southern dry zone. The district is bounded in the north- west by Hassan district, on the east by Bangalore district and on the south and south – west by Mysore district. The boundaries of the district encompass a total geographical area of 498,244 hectare, with 24,765 under forest cover. The district has an irregular shape lying between the east longitudinal parallels of $76^{0}20^{1}$ and the north latitudinal parallels of $12^{o}13^{1}$ and 13o 04^{1}. According to 2001 census the total population of the district was 1,716,718 persons with a sex ratio of 985. The literacy rate of the district is 61.21 percent and has a percapita income of Rs 7,992 at constant prices. The district has totally 7 taluks, 31 hoblies and 1478 villages (*www.mapsofindia.com).*The principal crops grown in the district are paddy, sugarcane, ragi, jowar, horse gram, groundnut, coconut and castor besides chillies and tobacco. Sugarcane is the most important cash crop produced under irrigated conditions. This along with paddy promoted the establishment of numerous agro – based industries. Cereal production in the district account for about 76 percent of the annual gross cropped area while sugarcane accounts for another 18 to 19 percent. Irrigation by canals is a characteristic feature of the district.

Nature and sources of data

The report is based on both primary and secondary data. To study the growth rates and to analyse the trend in finger millet cultivation, time series data were used. The data pertaining to the area, production and yield in selected districts were obtained from "Fully Revised Estimates of Principal Crops" of the Directorate of Economics and Statics, Government of Karnataka, Bangalore.The data on these variables were collected for a period of 20 years i.e., from 1980-81 to1999-2000. A preliminary examination of the time series data indicated two distinct patterns during the selected period. Hence this study will refer to these two distinct pattern periods as I period (1980-1990) and II period (1991-2000) hereafter.

The primary data were obtained from the sample farmers through personal interview by using pre-tested schedule. Data covered the socio-economic back ground of the farmers, their land holding structure, consumption and disposal pattern, crops grown and allied activities practised. The inputs used and the output realised documented both in physical and monetary terms. Finally awareness about ragi products and the varietal information were also documented. Data was collected during November-December months of the year 2002.

Sampling design

According to 2000-2001 estimates, nearly 70 per cent of area under finger millet was distributed in Tumkur, Kolar, Bangalore Urban, Bangalore Rural and Mandya districts. Hence, these five districts were purposively selected for the study. Further, a three stage random sampling procedure was adopted to select the sample respondents. Eight taluks were selected at random from five districts. A total of 32 villages were selected randomly at a rate of 3-5 villages from each taluk. From each one of these 32 sample villages, 6-8 farmers were randomly chosen for the study to make up the pre-determined 180 sample respondents. These 6-8 farmers from each village comprised of all three categories of farmers.

Analytical frame work

For the purpose of analysis, the sample farmers were post-stratified as small, medium and large farm categories by standardizing the size of land holding to dry land equivalent. For this purpose one acre of wetland or garden land was considered as equivalent to two acres of dry land (This classification has been followed by the Small Farmers Development Agency, Government of Karnataka). The classification of farmers into small, medium and large farm categories was done on the following basis.

<5acre - Small farmers
5-10acre- Medium farmers
>10acre- Large farmers.

Analytical techniques used

The analytical techniques used in the report are listed below

- Production function analysis
- Resource productivity and allocative efficiency
- Technical efficiency in finger millet cultivation

- Decomposition Analysis
- Frontier production function and total factor productivity change
- Compound growth rate analysis
- Conjoint analysis
- Cluster analysis
- Discriminant analysis

Emperical Findings and Discussion

This section of the report is presented under the following headings

- General characteristics of the respondents
- Technical details of finger millet cultivation
- Sources of information for farming
- Resource productivity and resource use efficiency
- Output decomposition
- Varietal preferences in different segments
- Growth performance of finger millet

General characteristics of the respondents

The general socio-economic, characteristics of the farmers have been studied. It was found that the family size comprised 4.2 adult males and 4 adult females in the large farmer category with 2.8 children. The average family size of the small farm household was 2.1, 2.0 and 1.7 respectively of adult male, adult female and children. There is not much difference between small and medium category of farmers with regard to adults. The average size of land holding was 27 ac, 8 ac and 4.5 ac of large, medium and small farm categories, respectively. The average income of different category farmers ranged from Rs 30110 to Rs183887.5 per household.

As regard the education level, overall 95 per cent of the respondents belonged to primary, middle and high school level, and many had not gone up to the graduation level. From this, it was concluded that the farmers in the study area had easy access to school.

Technical details of finger millet cultivation

The results on duration of varieties cultivated as shown in Table 3 reveals that majority of the respondents adopt short duration varieties rather than medium and long duration varieties. The reason for wide spread acceptability of short duration varieties may be the late and irregular onset of monsoon. Farmers usually taken up finger millet in the Kharif, to suite the late rains and to cope up with the dry spells farmers do go for short duration varieties.

Table 1: Finger millet varieties used by the farmers in their previous crop season according to duration

(Numbers)

Sl. No.	Duration of variety	Category			
		Small	**Medium**	**Large**	**Total**
1	Short	40 (42.11)	18 (48.65)	27 (56.25)	85 (47.22)
2	Medium	35 (36.84)	11 (29.73)	9 (18.75)	55 (30.55)
3	Long	20 (21.05)	7 (18.92)	5 (10.42)	32 (17.78)
4	Short + long	-	1 (2.70)	3 (6.25)	4 (2.22)
5	Medium + long	-	-	2 (4.17)	2 (1.11)
6	Short + medium	-	-	2 (4.17)	2 (1.11)
Total		95 (100)	37 (100)	48 (100)	180 (100)

Note: Figures in parenthesis indicate percentage to their total

Sources of information for farming

As regards the source of information on finger millet cultivation farmers were largely dependent on fellow farmers and village level workers. The probable reason for the wide popularity of these two sources may be because of their free and easy accessibility. Erection of small section bunds and ploughing across the slope are the two measures regularly undertaken to conserve moisture. As these two practices are followed from a long time, they were all aware of the advantages of these two measures(Table 2).

Table 2: Table showing the sources of technical information for finger millet cultivation

(Numbers)

Category	Source of technical information						
	VLW	**AAO**	**FF**	**UAS**	**KV**	**TV**	**NP**
Large	32 (40.51)	22 (48.89)	28 (19.86)	7 (25.92)	5 (33.33)	16 (38.09)	11 (28.95)
Medium	23 (29.11)	16 (35.55)	40 (28.37)	7 (25.93)	4 (26.67)	11 (26.19)	8 (21.05)
Small	24 (30.38)	7 (15.55)	73 (51.77)	13 (48.15)	6 (40.00)	15 (35.71)	11 (28.95)
Total	79 (100)	45 (100)	141 (100)	27 (100)	15 (100)	42 (100)	38 (100)

Note: VLW = Village level worker

AAO = Assistant agriculture officer

FF = Fellow farmer

UAS = University of Agricultural Sciences

KV = Krishi Vignana Kentdhra

TV = Television reports

NP = News paper

Figures in parenthesis indicate percentage to their total

Resource productivity and resource use efficiency in finger millet cultivation

In this study the productivity of resources use as well as resource use efficiency was examined to know whether any redeployment of resources in finger millet cultivation could improve the profitability of finger millet cultivation. Elasticity of production of each factor in the production of finger millet was analyzed to see whether these factors significantly contributed to the output. For this purpose a Cobb-Douglas production function was fitted to the data and the results are presented in the Table 3.

Table3: Production function estimates of finger millet for different

groups of farmers.

Sl.No.	Item	Category			
		Small	**Medium**	**Large**	**Overall**
1	No. of observations	95	37	48	180
2	Human labour	0.3204** (2.355)	0.2523 (1.183)	0.3798** (2.527)	0.2855*** (3.287)
3	Power (hr)	0.3433*** (3.257)	0.146 (0.7701)	0.1786 (1.126)	0.3083*** (4.118)
4	Inorganic fertilizers (Kg)	0.243** (1.985)	0.4326** (2.100)	0.733 (0.3912	0.255*** (2.809)
5	FYM (cart loads)	0.1053 (1.279)	0.1243 (1.078)	0.376** (2.036)	0.1887*** (3.1026)
6	R2 (R square)	0.57	0.69	0.427	0.629
7	Returns to scale	1.012	0.955	1.008	1.038

Note:

Figures in parenthesis indicate 't' test values.

* Statistically significant at 10 per cent

** Statistically significant at 5 per cent

*** Statistically significant at 1 per cent

Human labour, power, inorganic fertilizer and FYM had significant influence on returns of finger millet. The sum of elasticities was almost equal to unity among all the category farmers. This indicates the existence of constant returns to scale. That is there exists a little scope to increase productivity without changing the technology.

Labour contributed significantly to the yield. Since finger millet cultivation is labour intensive, labour played a major role in increasing the returns. This is observed by significant coefficient for labour in the production function .Power contributed significantly to the output. This is because finger millet required proper land preparation to bring it to the fine tilth. Improper land preparation affected the seed germination and so it should be properly undertaken. Inorganic fertilizers contributed significantly to the output. And finally, FYM also contributed significantly to finger millet cultivation. Science FYM application increases the microbial activity and thus helps for higher output realization, it contributed significantly to the finger millet cultivation. There is vast scope for increasing the use of FYM to realize better output.

Allocative efficiency in cultivation of finger millet

The allocative efficiency of power, inorganic fertilizer and FYM were positive and more than one, indicating the scope for the greater use of these inputs. It can be seen from the Table 4 that additional rupee invested in these inputs is highly economical. Since, the additional revenue obtained will lead to additional profit level.The MVP to MFC ratio of labour was 0.92, which indicated every rupee invested on labour returned only Rs 0.92 in the form of output. This showed over use (above economic optimum) of labour. It is not advisable to further increase the labour force, which would reduce the profit. And every rupee invested on power and inorganic fertilizer gave Rs 1.25 and Rs 1.5, respectively in the form of additional profit. This indicated that resources are under utilized and further, scope for the increased use of these inputs to increase profit.

The MVP to MFC ratio of FYM was 4.06, indicated every rupee of investment on FYM returned Rs 4.06 in the form additional profit. This showed the underutilization (below economic optimum) of FYM. It is advisable to increase the use of FYM, power and inorganic fertilizers to increase the profit.

Table 4: Resource use efficiency/allocative efficiency of various inputs among sample farms

Item	Category			
I. marginal physical products	**Small**	**Medium**	**Large**	**Overall**
Human labour (Mandya)	11	11	18	11
Power (hrs)	34	18	25	35
Inorganic fertilizer (kg/ac)	3	35	6	5
FYM (Cart loads)	63	102	4509	140
II Marginal value product				
Human labour (Rs)	47.41	47.41	77.58	47.41
Power (Rs)	146.54	77.58	107.75	150.85
Inorganic fertilizer (Rs)	12.93	150.85	25.86	21.55
FYM (Rs)	271.53	439.6	1939.5	609
III Ratio of MVP's and factor prices				
Human labour (Mandays)	0.92	0.92	1.50	0.92
Power (hrs)	1.2	0.64	0.89	1.25

Inorganic fertilizer (kg/ac)	0.9	10.5	1.8	1.5
FYM (Cart loads)	1.8	2.9	12.9	4.06

Technical efficiency in finger millet production

The average technical efficiency of finger millet cultivation in the study area was 68.71 per cent(Table 5). Only 30 per cent of the farmers were operating at more than 80 per cent efficiency level. This showed that the technology adoption was lower in this region, which led to lower productivity. Declining area under finger millet in the state and erratic rainfall pattern and limited market opportunities for the crop may be the reasons for low rate of technical efficiency. Crop being grown on marginal lands under dry land conditions with minimum cultural and management practices may also be the reason for lower technology adoption.

Table 5: Distribution of sample farmers according to technical efficiency

Technical efficiency range (%)	**Frequency Distribution**			**Overall**
	Small	**Medium**	**Large**	
21 - 40	6 (50.0)	3 (25.0)	3 (25.0)	12 (6.7)
41 - 60	32 (61.5)	6 (11.54)	14 (26.92)	52 (28.9)
61 - 80	46.77 (29.0)	17 (27.42)	16 (25.81)	62 (34.4)
81 - 100	30 (55.6)	11 (20.37)	13 (24.07)	54 (30.0)
Average technical efficiency	68.18	69.27	69.34	68.71

Note: Figures in parenthesis indicate percentage to their total

Output decomposition

Decomposition of growth in agricultural output has remained of active interest to researchers and policy makers. A breakdown of growth into various components such as technology, input growth and technical change helps for better output projection with target policies

The decomposition analysis as in Fig. 1 reveals that with the same level of per acre inputs, 71.5 per cent more output could be obtained with the adoption of high yielding variety technology. The difference in input use contributed to the extent of 27.7 per cent. Among the different inputs contributing to the productivity difference, power contributed more (11.17%) followed by inorganic fertilizers (10.51%), FYM (68%) and labour (5.32%).

The technological change through biological inputs (neutral technical change) contributed higher than technological change through mechanical inputs. The respective contributions of neutral and non-neutral change were 51.71 per cent and 12.02 per cent. The unexplained variation, which is not contributed for the four explanatory variables under consideration, was 8.57 per cent.

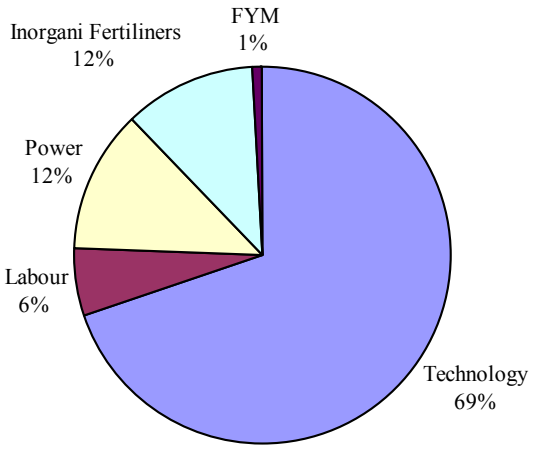

Fig 1: Factors contributing for higher output in finger millet

Total Factor Productivity

The magnitude of the difference in input contribution was found impressive compared to the technology and efficiency in production (Table 6). The technical efficiency was contributed negatively to the output growth. The reason for the negative contribution of technical efficiency could be the bad management practices followed in the production system. The empirical results reveal that TFP growth in finger millet is not accounting for an impressive proportion of output growth. Out of which input growth plays a crucial role in productivity growth.

Table 6: Decomposition of output growth in finger millet production

SL.No	TFP components	Observed	Percentage
1	Technical efficiency	-3.31	-486.7
2	Technical change	1.65	243.2
3	Total factor productivity	1.66	243.5
4	Input growth	2.34	343.5
	Output growth	0.68	100

Varietal preferences in different segments

Conjoint analysis

Finger millet is the second most important food and fodder crop of dry lands in Karnataka. It is grown in many states of India under diverse dry land conditions. It has a high level of regional /local adaptation. Although many improved cultivars have been released, only farmers have accepted a few. Thus it becomes necessary to evaluate the finger millet variety to arrive at the most preferred ideal variety. The results of conjoint analysis are presented in **Fig 2.**

Duration as an attribute was by for the most important attribute to explain the varietal preference and short duration varieties (90 to 90 days) were most preferred, deriving the utility of .2194.As crop is vulnerable to vagaries of rainfall, farmers prefer the short duration varieties, do achieve good yields with in even, minimum showers.
Cooking quality as an attribute hardly bare importance to the total preference cooking quality as a whole had relative importance of only 7.30 per cent. Yield of a variety and ear type had the relative importance 22.16 per cent and 15.68 per cent respectively. High yielding variety and semi-compost attribute of a variety were most preferred (utilities = .4896 and .0639 respectively).

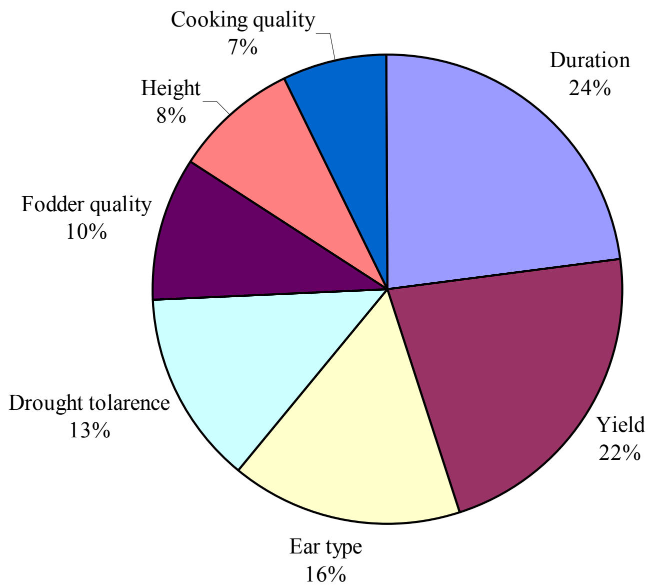

Fig. 2: Group results of conjoint analysis in finger millet

Segmentation of farmers

Five clusters are obtained as shown in Fig 3 and the individual characteristics of each cluster are discussed below

```
C A S E     0         5         10        15        20        25
  Label   Num  +---------+---------+---------+---------+---------+

           29   ⇩⬂
           30   ⇩▫
           24   ⇩▫
           12   ⇩▫
           25   ⇩▫
           26   ⇩⇳⇩⬂
           28   ⇩▫ ⬄
           19   ⇩▫ ⬄
           27   ⇩▫ ⬄
           13   ⇩⬃  ⬄
           22   ⇩⬂ ▫⇩⇩⇩⇩⇩⬂
           23   ⇩▫ ⬄        ⬄
           16   ⇩▫ ⬄        ⬄
           20   ⇩▫ ⬄        ⬄
            1   ⇩▫ ⬄        ⬄
           11   ⇩⇳⇩⬃        ⬄
            8   ⇩▫         ⬄
           10   ⇩▫
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           21   ⇩▫         ⬄                                  ⬄
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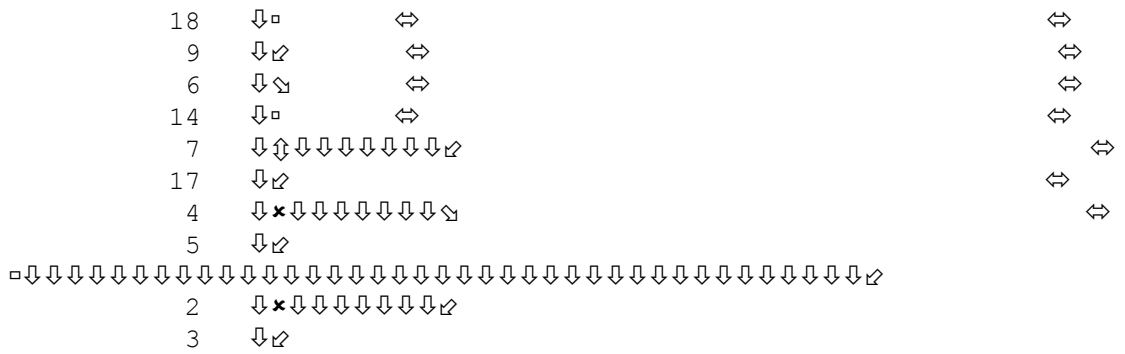

Fig. 3: Dendogram of hierarchial clusters of finger millet farmers

Group 1 including 34 per cent of the sample farmers considered duration, yield and drought tolerance to be the most important attributes for the finger millet variety. This segment was conscious about the uneven rainfall pattern and yields. The segment had an average income of 14100 Rs. Per annum. Average land holding of the group was very less compared to the other groups. Surprisingly this group was more conscious about the duration of variety rather then the yields.

Group 2 including 40 per cent of the respondents considered duration, yield and ear type as the major attributes. This group consisted of rich farmers having an average annual income of 101833.3 Rs /annum varieties tolerant to drought and fodder quality occupied the fourth and fifth place in the relative importance of attributes with 13.46 per cent and 9.98 per cent respectively.

Varieties tolerant to drought and having good fodder quality were most preferred with utilities .3021 and .0187 respectively. This group had an average land holding of 11.25 ac per farm. Thus this group had almost the similar preferences like that of the first group except the type of the ear head.

Group 3 capturing 14 per cent of respondents was similar to the group 2. While this group had an average land holding of 17.5 ac per farm which was also highest among all the target groups.

Group 4 capturing 6 per cent respondents was quite an interesting one and differed heavily from other groups. They considered yield, height of the cultivar and drought tolerance as the major attributes. Thus this group was highly yield conscious.

Group 5 capturing 6 per cent respondents was also highly conscious of yield of a variety. They considered yield, duration and ear type were the major attributes.

The results of the multiple discriminate analysis for farmers (**Table 7 and Fig 4**) reveals that the four functions explains all the variation in the groups. The income of farmer, intercropping, land and use of Indaf –5 varieties were the important variables to distinguish the first cluster from the rest function. Medium duration variety local variety, PR 202, short duration variation and family size were found important in the second function. Drought tolerance, long duration, GPU-28, height, ear type, MR-1 and cooking quality of a variety were found to have maximum influence in the third function. And the rest of the variables were found to have maximum weight age in fourth function.

Table 7: Summary of canonical discriminate functions

I. Eigen values				
Function	**Eigenvalue**	**% of variance**	**Cumulative %**	**Canonical correlation**
1.	217.872[a]	94.6	94.6	0.998
2.	7293[a]	3.2	97.7	0.938
3.	3.004[a]	1.3	99.0	0.866
4.	2.236[a]	1.0	100.0	0.831
a. First 4 canonical discriminate functions were used in the analysis				

II. Wilks lambda				
Test function (S)	**Wilks lambda**	**Chi-square**	**Degrees of freedom**	**Signficnace**
1thorugh	0.000	176.143	72	0.000
2 through	0.009	81.844	51	0.004
3 through 4	0.077	44.825	32	0.660
4	0.309	20.549	15	0.152

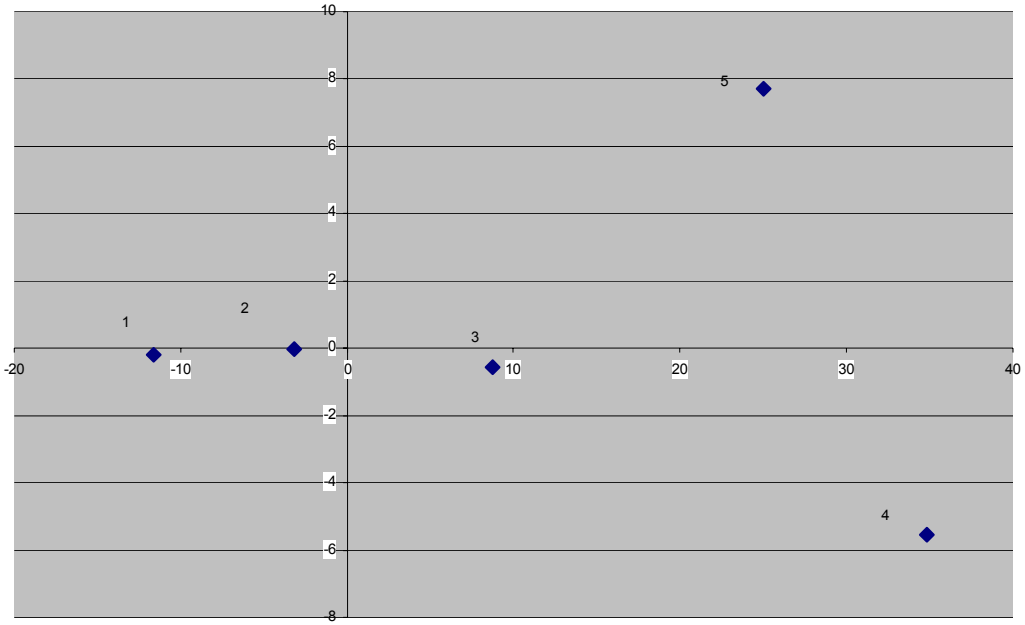

Fig: 4 Placement of groups based on multiple discriminant functions

Growth performance of finger millet

Area analysis

The growth analysis of area under finger millet indicated a negative and significant growth rate in all the districts except Tumkur district(Table 7). The negative trend was also observed for the entire state. The rate of decline was highest in Bangalore Urban. The maximum decelerating growth in area in Bangalore urban could be mainly due to the urbanization. The other reason for the same could be shifting of farmers from agriculture to industrial sector. As the Bangalore Urban district is having huge industries, which can provide employment for rural masses, the area under the crop has been decreased over the years. The same decreasing trend was observed in other districts because the crop has limited scope for further processing and development. Finally, it is a crop attractive for peasants and small holders who are of relatively low-income groups having no irrigation practices.

Table 8: Growth rates of area under finger millet

Sl No.	District	Period I (1980-1990)	PeriodII (1991-2000)	Overall (1980-2000)
1.	**Bangalore (urban)**	-0.201 (-0.148)	-2.413** (-3.092)	-1.944*** (-5.193)
2.	**Bangalore (rural)**	-0.309 (-0.304)	3.582 (1.504)	-0.09 (-0.076)
3.	**Kolar**	-1.209* (-1.920)	1.350 (1.147)	-1.170*** (-3.272)
4.	**Tumkur**	2.028** (3.506)	0.015 (0.014)	0.87** (2.872)
5.	**Mandya**	2.721 (1.7785)	-1.563** (-2.889)	-0.868** (-4.256)
6	**State**	0.421 (0.984)	-1.563** (-2.889)	-0.868*** (-4.256)

Note : **Figures in parenthesis indicate 't' test values**

*** Statistically significant at 10 per cent**

** Statistically significant at 5 per cent

*** Statistically significant at 1 per cent

Positive growth rates in area in Tumkur district was because of finger millet being a constituent of traditional food habits of the poor and marginal farmers. Singh et al. (1993). Dhindsa and Anjusharama (1997) and Choudary and Saini (1990) also documented a picture of negative growth rate in area under different crops.

Production analysis

The production analysis of finger millet revealed a positive and significant growth rates at the state level and few other study districts of Karnataka (Table 8). Except Bangalore Urban and Mandya districts, rest all others showed a impressive acceleration in production. While the area expansion was negative in all most of the districts and in the state. This clearly indicates that the positive growth in production in most of the districts is not due to the area expansion. The strong reason for increased production may emergence of widely accepted varieties of Indaf series. The Indaf varieties were released in 1987 made an impressive impact on finger millet production.Due to their early maturity, good fodder quality and relatively higher yields, the indaf series have revolutionized the finger millet production.

Table 9: Growth rates of production of finger millet

Sl No.	District	Period I (1980-1990)	Period II (1991-2000)	Overall (1980-2000)
1.	**Bangalore (urban)**	-5.880 (-0.867)	21.394 (1.637)	2.857 (0.512)
2.	**Bangalore (rural)**	-7.08 (-1.594)	5.790* (1.990)	4.649*** (3.212)
3.	**Kolar**	10.735*** (3.455)	0.045 (0.018)	5.365*** (4.374)
4.	**Tumkur**	1.511 (1.148)	-0.613 (-0.331)	3.4053*** (5.256)
5.	**Mandya**	-1.968 (-0.563)	4.225 (1.121)	1.112 (0.863)
6.	State	-0.519 (-0.332)	-0.266 (-0.182)	1.5786** (2.628)

Note: Figures in parenthesis indicate 't' test values

*** Statistically significant at 10 per cent**

** Statistically significant at 5 per cent

*** Statistically significant at 1 per cent

Yield analysis

The yield analysis revealed a negative compound growth rates in period I in all the five districts except Kolar(Table 9). Where the compound growth rate in period I was found positive (7.092 per cent). The compound growth rates in period II were positive in most of the districts except Tumkur district. This clearly indicates that yields of finger millet increased only in the period II i.e. after the farmers used high yielding varieties. The average annual compound growth rates in the districts were positive and statistically in most of the districts. Te acceleration in yields in period II may attribute to the several reasons.

Table 10: Growth rates of yields in finger millet

Sl No.	District	Period I (1980-1990)	Period II (1991-2000)	Overall (1980-2000)
1.	**Bangalore (urban)**	-5.690 (-0.808)	25.113* (1.903)	5.070 (0.887)
2.	**Bangalore (rural)**	-6.721 (-1.322)	1.6120 (1.128)	4.959*** (3.848)
3.	**Kolar**	07.045 (-0.887)	0.053 (0.223)	7.045** (2.127)
4.	**Tumkur**	-0.507 (0.474)	-1.153 (-0.821)	2.322** (3.925)
5.	**Mandya**	-4.565* (-2.167)	5.312** (3.037)	1.048 (1.101)
6.	**State**	-0.521 (-0.426)	1.441 (1.361)	2.571*** (4.772)

Note : **Figures in parenthesis indicate 't' test values**

*** Statistically significant at 10 per cent**

** Statistically significant at 5 per cent

*** Statistically significant at 1 per cent

References:

Alias, R. and Md Nasir Shamsudin (2001), Production frontier and technical efficiency: The case of paddy farms in Malaysia. *The Asian Economic Review*, **43**(2): 315-323.

Bisalaiah, S., 1977, Decomposition analysis of output change under new production technology in wheat farming. Some implications to returns on investment. Indian *Journal of Agricultural Economics*, **32** (3): 193-201.

Bisaliah, S., 1978, Decomposition analysis of employment change new production technology in Punjab agriculture. Indian Journal of Agricultural Economics*, **32** (4) : 193-201.*

Farrell, M.J., 1957, The measurement of production efficiency. *Journal of Royal Statistical Society*, **A120**(2): 253-281.

Fully revised estimate of principal crops, directorate of economics and statistics, Karnataka, various issues.

Gaddi, G.M., 1999, Yield gaps and constraints in production of major crops in north Karnataka- An economic analysis Ph.D. (Agri) thesis, University of Agricultural Sciences, Dharwad.

Gaddi,G.M, Mundinamani,S.M., and Basavaraj.H., 2001, Yield gaps and constraints in the production of rabi sorghum in Karnataka – A path co-efficient analysis. *Asian Economic Research Review*, **15**(1): 13-25.

http://www.kar.ni.in
http://www.mapsofindia.com.

Jayaram, H., 1988, Analysis of yield gap in paddy and ragi (irrigated) in Mandya district. Unpublished M.Sc. (Agri) thesis, submitted to the university of agricultural sciences, Bangalore.

Jayson L. Lusk, 2002, A comparison of conjoint analysis response formats: comment, American Journal of Agricultural Economic., **84** (4) : 1165-117.

Job Elsamama and George, M.V., 2003, Technical efficiency in rice production-A frontier production function approach. Agricultural Economics Research Review,***15** (1): 2002.*

Kalirajan, K., 1981, An econometric analysis of yield variability in paddy production. *Canadian Journal of Agriculture Economics*, **29**:283-294.

Kalirajan, K.P. and Shand, R.T., 1985, Type of education and productivity: A quantitative analysis of Tamil Nadu rice farming. *Journal of development studies*. **21** (2): 232-243.

Kalirajan, N.and Shand, R. T., 1986, Estimating location specific and farm specific technical efficiency: An analysis of Malaysian agriculture. *Journal of Economic Development*, **11**: 147-160.

Kevin, J., Boyle, Thomas, P., Holmes, Mario, F., Teisl and Brain Roe, 2001, A comparison of conjoint response formats. *American Journal of Agricultural Economics* , **83** (2):441- 453.

Kranz, D.H. and Tversky, A., 1971, Conjoint Measurement analysis of Composition Rules in Psychology. *Psychological Review*, **78**: 151-169

Russel, N.P., and Yong, T., 1983, Frontier production function and measurement of technical efficiency. *Indian Journal of Agricultural Economics*, **34** (2): 139-149.

Timmer, C.P., 1971, Using a Probabilistic Production Function to measure Technical Efficiency. *Journal of Political Economy*, **79**(4):776-794.

Umesh, K.B. and Bvisaliah, S., 1991, Efficiency of groundnut production in Karnataka, frontier profit function approach. Indian Journal of Agricultural Economics*, **46** (1): 20-23.*

Veeraroys, R.C. and Pareek, S.N., 1999, Resource use efficiency on small farm size vis-à-vis large farm size in Bijapur District. *Indian Journal of Agricultural economics,* **30**: 235.

Objective2.

Participatory breeding of finger millet in Southern Karnataka

"Farmers Participatory Varietal Selection Emulates Centralized Plant Breeding System in Finger Millet (*Eleusine coracona*)"

Farmer's Participatory Varietal selection in Finger Millet (*Eleusine coracona*)

In finger millet several high yielding varieties were developed and released for general cultivation since from last three decades through hybridization between exotic lines introduced from Africa and native Indian germplasm. Farmers in areas where rainfall is uniform accepted some of elite varieties, but there adoption is uneven in the major finger millet growing belts of Karnataka where rainfall is scanty and erratic. Non adoption of high yielding varieties is due to lack of location specific adoption, less number of varieties for farmers choice, farmers vested interests are not taken into consideration, elite varieties are developed by considering broad geographical area neglecting the local environment *etc* (Joshi, *et al*., 1996; Sthapit, *et al*., 1996). In finger millet growing states of India in specific to Karnataka was experienced severe drought consecutively for last three years. Finger millet growing farmers are facing lack of suitable variety/s tolerant to the deficit of rainfall during crop growing period. The local land races, varieties like Indaf 5 (Year of release 1978) and GPU 28 (Year of release 1998) (Seetharam, *et. al*., 2003) are still growing in majority of finger millet cultivating area in Karnataka were could not able to cope with long dry spell. So, the resource poor farmers practicing rainfed agriculture are often bereft of knowledge on the availability of high yielding varieties for their use. The scientists working for marginal environments set the breeding strategy by considering felt needs of the farmers which may differ from the real needs. In order to bridge the gaps in guiding the farmers in varietal selection and scientists in setting breeding goals, a Farmers Participatory Varietal Selection (FPVS) was under taken in finger millet. In Farmers Participatory Varietal Selection client farmers be involved in selection of merely finished or finished products. The four important steps followed in Farmers Participatory Varietal Selection are,

1. Rural appraisal survey
2. Selection of varieties / lines
3. Conduction of farmer's participatory trials
4. Dissemination (Witcombe, *et al.,* 2002)

Farmer's participatory rural appraisal was carried out during *Kharif* 2002 in finger millet growing agro climatic zones like central dry zone (Zone no. 4) and eastern dry zone (Zone no. 5) of Karnataka. The districts falls under the domain of these zones are Bangalore rural, Bangalore urban, Chitradurga, Tumkur and Kolar district. The survey has evidently given an idea of about lack of farmers preferred finger millet varieties. The farmers in these areas are still growing local land races and obsolete varieties, because which are well suited to that area and farmers having confidence of getting minimum yield by growing local land races instead of going for new varieties. In the Rural Appraisal Survey, it clearly indicates the improved technology has taken place in lab is not going to field because of extension activities are not taking place properly. Farmers Participatory Varietal Selection offers new vistas in the field of centralized plant breeding to combat the problems faced by the farmers in marginal environments regarding adoption of varieties developed in different food crops. Success have been accomplished in finger millet by providing farmers preferred varieties / lines through

Participatory Varietal Selection programme in districts like Chitradurga, Bellary, Karnataka state, India (Gowda, *et al*., 2000). The Farmers Participatory Varietal Selection has been success full in crops like rice, sorghum, barely, tomato *etc*.

This report highlights the Farmers Participatory Varietal Selection in finger millet to address the hard-core problems of finger millet growing farmers. This approach is taking a shape with right mixture of scientists, researchers, resources and dedicated hard working farmers well coming this approach whole heartedly to gain access to improved finger millet lines / varieties. In this approach farmers vested interests are taken into consideration in selecting the varieties / lines for Participatory Varietal Selection programme in finger millet.

Materials and Methodologies

Farmers Participatory Varietal Selection (FPVS) in finger millet, based on the farm house hold survey and participatory rural appraisal survey a extensive search was made to find out the advanced lines that are in final stage of release and also already released varieties which are best match with the traits of interest that farmers preferred to have in a new variety. The farmers preferred traits are high yielding, medium plant height, high tillering, medium compact ear head, medium duration (100 – 105 days), and drought resistance, resistance to blast disease *etc*. About 28 superior Recombinant Inbred Lines (RILs) that are best match with the criteria of farmers and also the lines that are scientists feel to have good are enrolled in FPVS (Farmers Participatory Varietal Selection) programme (Table 1). Along with IE 1012 is an exotic line and Indaf 5 (released for commercial cultivation in Karnataka) are the parents of these elite RIL's. One more variety GPU 28 (released for commercial cultivation in Karnataka) utilized as check in the FPVS programme. These elite RIL's were bred through hybridization between IE1012 x Indaf 5 by following a Single Seed Descent (SSD) method. The Table 1 showing material chosen for FPVS programme.

Selection of research sites for Farmers Participatory Varietal Selection in finger millet

In India, Karnataka is one of the leading finger millet growing state. Karnataka alone occupies 40 % of the finger millet growing area and it is divided into 10 different agro climatic zones based on meteorological data (*viz*., rainfall pattern, temperature), soil pattern and cropping pattern. The research sites selected for FPVS come under the purview of Agro climatic Zone 4 (Central dry zone) and zone 5 (Eastern dry zone) (Fig 1). The salient features of these agro climatic zone 4 (central dry zone) is having sandy loam soil characterized with lesser depth, annual rainfall 606.8mm, coefficient of variation in rainfall 25.6%, 80% of the annual rainfall is received from May to October.

Table 1: List of 28 RIL's, two parents and check of finger millet used in farmers managed participatory research trials

Sl. No.	RIL's / Varieties
1	ML 31
2	ML 32
3	ML 171
4	ML 181
5	ML 197
6	ML 203
7	ML 208
8	ML 224
9	ML 255
10	ML 264
11	ML 265
12	ML 280
13	ML 297
14	ML 298
16	ML 300
17	ML 302
18	ML 316
19	ML 322
20	ML 343
21	ML 349
22	ML 371
23	ML 392
24	ML 426
25	ML 430
26	ML 434
27	ML 531
28	ML 553
29	IE 1012
30	Indaf 5
31	GPU 28

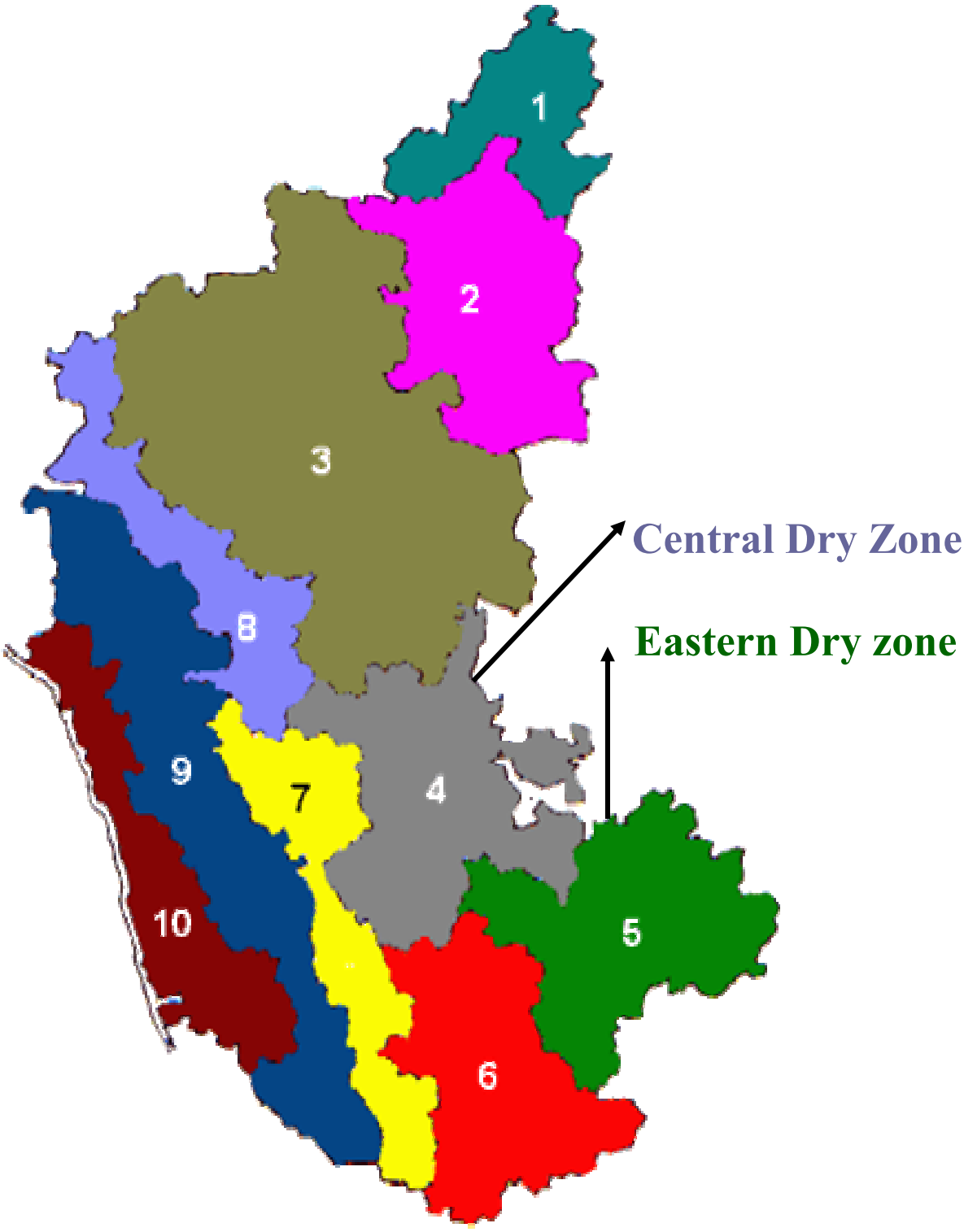

Fig. 1 Map showing Farmers Participatory Varietal Selection Trials in Finger Millet Conducted in Central and Eastern Dry Agro Climatic Zones of Karnataka, *Kharif* 2003

Water holding capacity of 10 to 15 cm, moisture stress on crops is severely stressed and major crops growing are finger millet, sorghum, paddy, groundnut, *etc*. Where as zone no. 5 (Eastern dry zone) is having red loamy soils in majority of the areas and few areas with lateritic soil, these soils are shallow with low available water holding capacity of 14 to 20 cm, annual rain fall 768.2 mm is received in two peaks, one in May and other from September to October, coefficient of variation in rainfall 25.6%, higher degree of moisture stress on crops, major crops growing are finger millet, groundnut, paddy, horse gram *etc*.

The decisive factor in selecting the research sites is due to 16% of the total cultivated area in agro climatic zone 4 (central dry zone) comes under finger millet, where as in zone no. 5 (eastern dry zone) 42% of the total cultivated area comes under finger millet. Even though finger millet is not cost remunerative crop but it is one of the well preferred staple food and also having nutritional (rich in Ca, carbohydrates, minerals, fiber *etc.*) and medicinal value (specifically for diabetes mellitus). Six locations are selected to conduct farmer's participatory varietal selection in finger millet that includes Hatna and Alugona village, Tumkur district comes under agro climatic zone 4. The remaining four locations *viz*., Ardeshahalli village, Shettigere village, Jakkasandra village, GKVK campus, University Agricultural Sciences of Bangalore rural district comes under agro climatic zone 5. In five locations participatory varietal selection conducted were farmers managed participatory research trials excluding location in GKVK campus, University of Agricultural sciences, Bangalore it was on station scientists managed FPVS trial. Only 8 RIL's including Indaf 5 and GPU 28 were selected for farmer's participatory varietal selection in Hatna and Alugona village, Tumkur District, where as 24 elite RIL's including parents and check in Shettigere village and 27 elite RIL's including parents and check in remaining villages.

The sowing was undertaken in the month of July last week in all the locations in an unreplicated trial of plot size 3 x 2.25 m^2. Farmers were taken to the finger millet trials at three important crop growing stages *viz*., vegetative stage (45 days), flowering stage and maturity stage. The reason is to get feed back about the crop and imbue confidence in farmers regarding elite RIL's / varities of finger millet. The final evaluation of superior finger millet elite RIL's including varieties was under taken during maturity stage (105 days). For this a questionnaire was prepared in local language Kannada to implore the information regarding finger millet and also ask them to rank elite RIL's / varieties that were included in FPVS programme. The information solicited from farmers regarding finger millet were size of land holdings, area of finger millet cultivation, varieties they are growing and traits they are preferred while ranking the elite RIL's or varieties and most preferred traits.

Results and Discussions

It was observed that total 150 farmers involved in farmers participatory varietal selection in finger millet conducted in different locations of Karnataka *viz*., GKVK on station scientists managed farmers participatory varietal selection (25 farmers),

Aradeshahalli (30 farmers), Jakkasandra (28 farmers), Shettigere (32 farmers), Hatna (22 farmers), and Alugona (13 farmers) were farmers managed participatory varietal selection. Total 150 farmers from all locations fallen into different land holdings *viz*., small farmers (64 farmers), marginal farmers (53 farmers) and large farmers (33 farmers) Table 2. The pie chart showing percent of finger millet growing farmers participating in FPVS having small, marginal and large land holdings (Fig 2). It clearly indicates fragmentation of land holdings of farmers (42 % of the farmers are small, 35 % are marginal and only 22 % of the farmers are large). The fragmentation in land holdings of farmers may be a reason for non-adoption of improved varieties of finger millet.

Table 2 Land holdings of farmers participated in Farmers Participatory Varietal Selection in Finger millet from different locations Kharif *2003*

Sl. No.	Type of farmers	GKVK	Hatna	Alugona	Jakkasandra	Aradeshalli	Shettigere	Total	%
1	Small	16	8	3	7	15	15	64	43.00
2	Marginal	7	8	8	11	11	8	53	35.00
3	Large	2	6	2	10	4	9	33	22.00

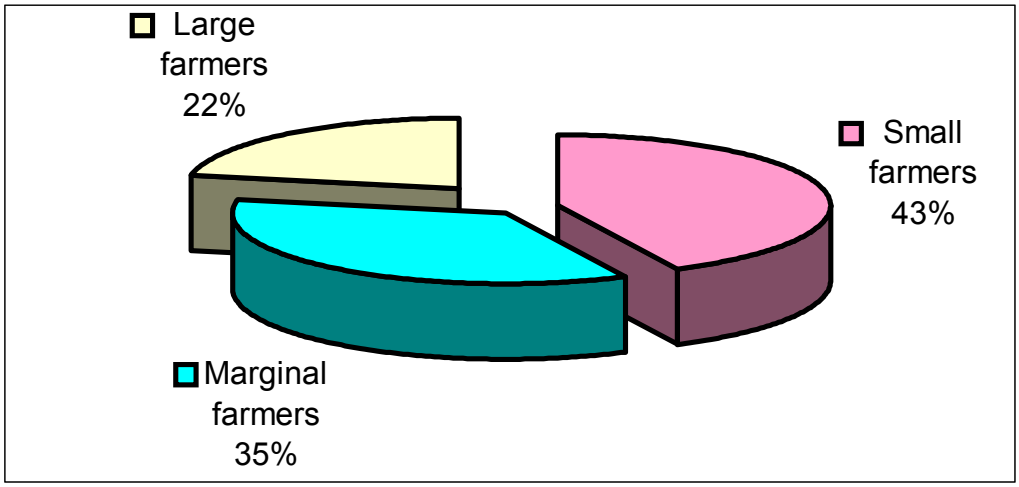

Fig. 2 Pie chart showing % of land holdings of farmers participated in FPVS

Preference of farmers for existing varieties of finger millet in different locations

The Table 3 showing preference of farmers for existing varieties (includes local land races and varieties released by university of Agricultural sciences for commercial cultivation). Out of 150 farmers some of them preferred more than one variety among existing local land races / varieties. Majority of the farmers preferred Indaf 5 (year of release 1978), HR 911 (year of release 1986), GPU 28 (year of release 1998) released by University of Agricultural Sciences, Bangalore (Seetharam, *et al*., 2003). It gives clear-cut evidence that farmers are still cultivating age-old varieties of finger millet. Few farmers are still growing local varieties *viz*., Kaddimuruku ragi, Gutte ragi, Hynu ragi, Doddabalpur ragi, Karikaddi, Dodda ragi and this is because farmers don't have acceptable alternatives to their local land races (Fig 3).

Table 3 Preference of farmers for the existing land races / varieties of finger millet in different locations

Sl. No.	Name of the variety	Year of Release	No. of farmers preferred	Percent
1	Indaf 5	1978	64	28.07
2	Indaf 6	-	3	1.32
3	Indaf 7	-	7	3.07
4	Indaf 8	1986	12	5.26
5	Indaf 9	1988	15	6.58
6	Indaf 11	-	1	0.44
7	HR 911	1986	43	18.86
8	PR 202	-	3	1.32
9	BPL 11	-	2	0.88
10	RH 5	-	2	0.88
11	Ratna	-	2	0.88
12	MR 1	1198	2	0.88
13	GPU 28	1998	49	21.49
14	Kaddimuruku Ragi	Local	3	1.32
15	Gutte Ragi	Local	1	0.44
16	Hynu Ragi	Local	10	4.39
17	Doddaballapur Ragi	Local	5	2.19
18	Karikaddi	Local	2	0.88
19	Dodda Ragi	Local	2	0.88

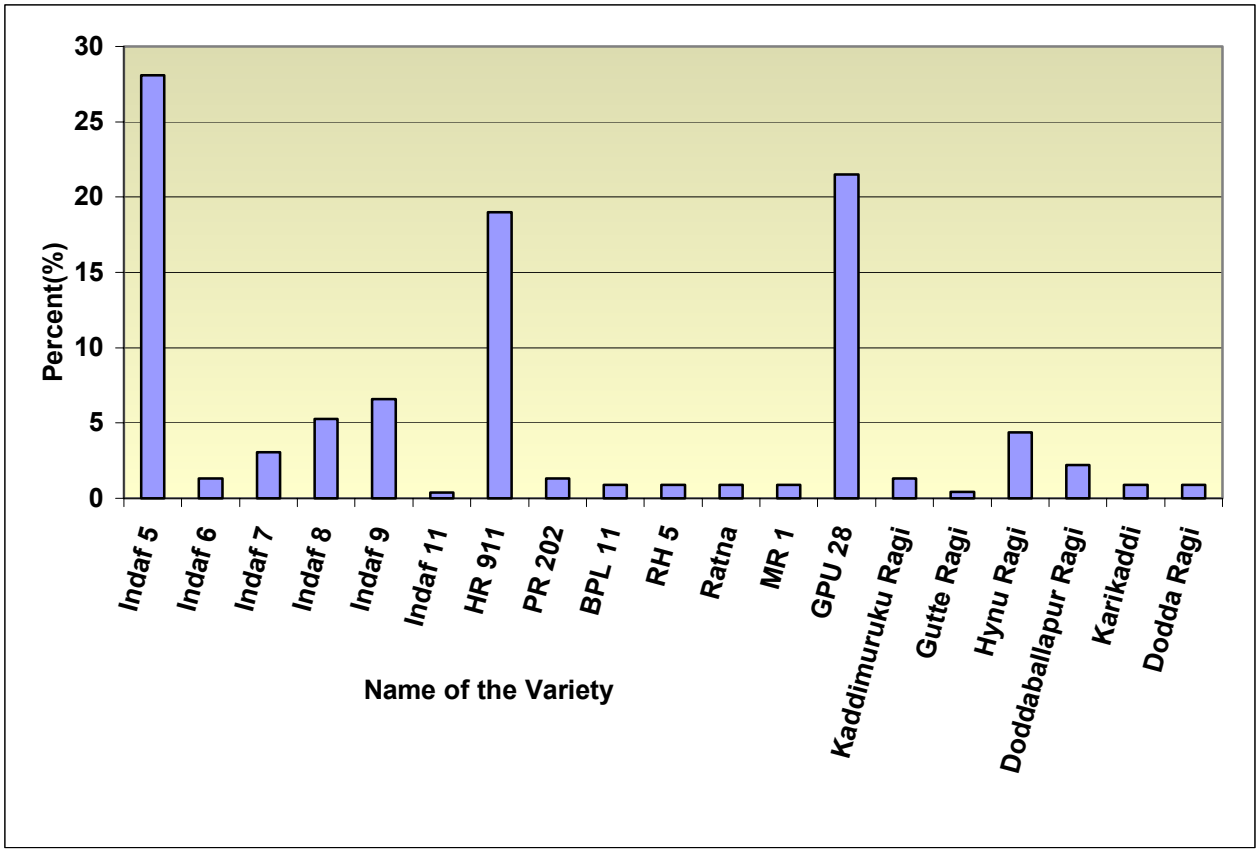

Fig.3 Percent of farmer's preference for local land races and existing varieties of finger millet

Farmers preferred traits in finger millet variety

Farmers preferred more than one trait while selecting the RILs / varieties of finger millet (Table 4). During the course of final evaluation of RILs / varieties enrolled in FPVS in finger millet, majority of the farmers preferred higher seed yield and fodder yield has their selection criteria (Fig. 4). It is a clear sign of farmers not only growing finger millet for seed purpose and also for fodder purpose for livestock's feeding has their additional source of income. The order of preference of traits as follows high seed yield, good fodder yield, disease resistance, drought tolerance, medium compact ear head, red grain color, more tillers *etc*.

Critical evaluation of RILs / varieties by the farmers during Farmers participatory varietal selection in Finger millet

During the course of Farmer's Participatory Varietal Selection in finger millet in different locations of Karnataka, we can able to identify how the farmers will assess the RILs/varieties critically. Majority of the farmers preferred yield and fodder as the imperative criteria in selection and also disease resistance, which are usually breeding objectives in centralized plant breeding system. Some of erudite farmers in finger millet

cultivation evaluated RIL's/varities with the minor traits which are not considered by scientists in crop breeding objectives. Those traits considered by farmers have added advantage while developing varieties in finger millet improvement.

Following are the traits considered by farmers in selecting RIL's / varieties

1. Medium compact ear head

Some of the farmers preferred medium compact ear head has their one of the selection criteria because, when harvesting is deferred for some time if rain occurs seed yield loss is reduced. The RIL's / varieties having medium compact ear head, if rainfall occurs water will not directly descend on the seeds because of compact ear head.

Table 4 Farmers preferred traits in a finger millet variety in all locations *Kharif* 2003

Sl. No.	Traits	No. of farmers preferred						Total	Percent
		GKVK	Hatna	Alugona	Jakkasandra	Aradeshalli	Shettigere		
1	Medium grain size	3	0	0	0	0	0	3	0.792
2	Red grain color	11	1	0	0	3	0	15	3.957
3	Big ear size	1	1	2	0	2	0	6	1.583
4	Medium compact ear head	8	1	2	4	0	2	17	4.485
5	Uniform maturity	1	2	1	1	1	0	6	1.583
6	Drought tolerance	7	2	1	10	6	8	34	8.971
7	Disease resistance	7	2	1	10	6	16	42	11.082
8	More tillering	1	4	0	1	1	1	8	2.111
9	Duration	0	1	0	2	0	7	10	2.639
10	Plant height	0	1	1	0	0	0	2	0.528
11	Dust	0	0	0	0	2	1	3	0.792
12	High seed yield	22	8	7	28	28	32	125	32.983
13	Good fodder yield	18	12	8	26	12	32	108	28.496

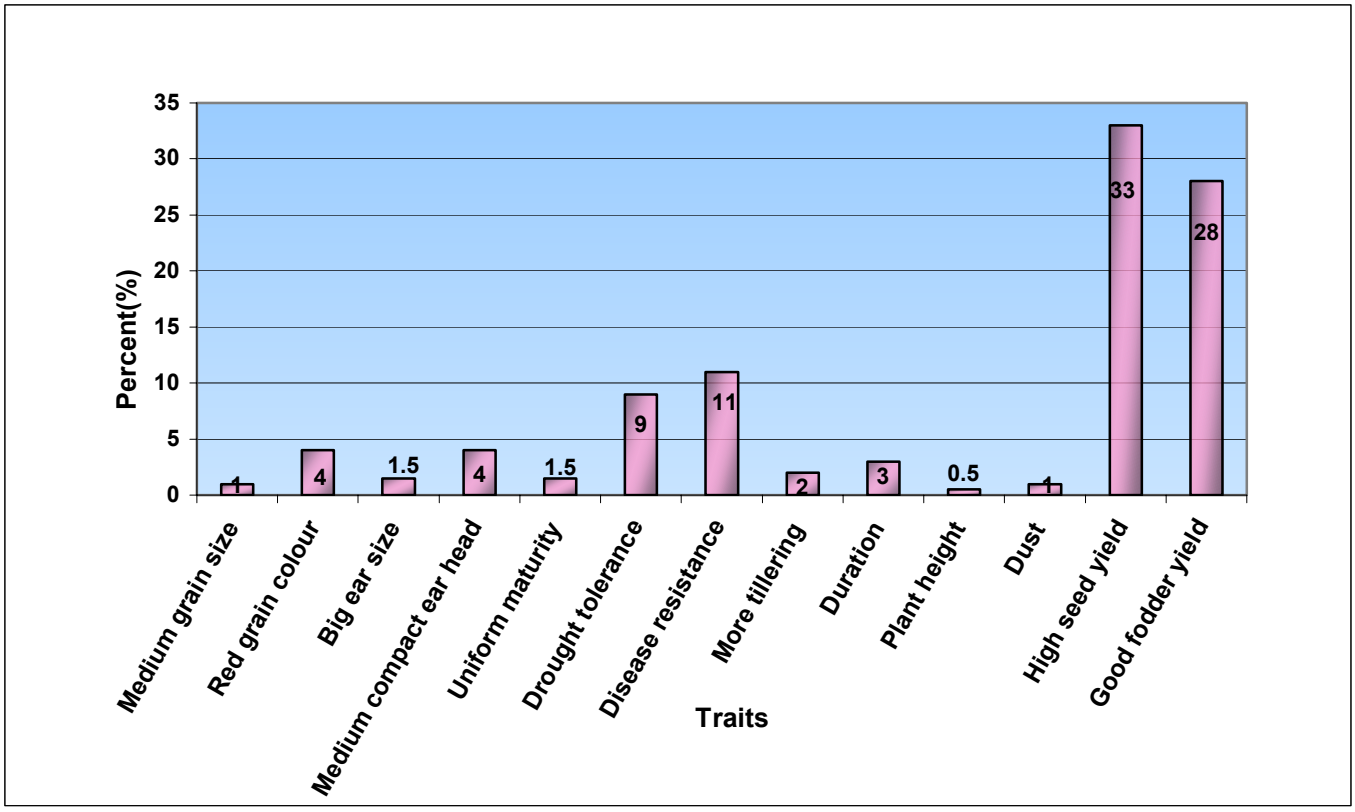

Fig 4 Graph showing % of farmers preferred traits in finger millet

2. Arrangement of the seeds on the ear head

The seeds should arrange on ear head intimately without living space. It will add to increase in seed yield (Fig 5).

3. Less dust after threshing of finger millet

After threshing of ear heads of finger millet if it gives less dust it gives more yield and more dust less yield.

4. Thickness of the stem for fodder purpose

The RIL's/ varities having less stem thickness are more preferred by the livestock's. Less stem thickness RILs / varieties in finger millet is vulnerable to lodging. It can be overcome by selecting RILs / varieties having less stem thickness having medium plant height (80 – 85 cms).

5. Size of the seeds

Few farmers compared size of the seeds while selecting the RILs / varieties. Farmers preferred bold sized seeds.

Fig 5 Farmers looking for arrangement of seeds on ear head

Farmer's preference for new and existing genotypes of finger millet

Location: GKVK
On station farmers managed participatory research trials

Twenty-five farmers were participated in participatory varietal selection programme. Information about new RILs / varieties of finger millet were solicited from farmers. It as found that 16 farmers chosen ML 181 has their Ist preferred genotype because of its good grain yield, more number of tillers, medium compact ear head and multiple disease resistance. And also five and three farmers preferred ML 181 as preferred genotype has their II and III preference. Four farmers selected ML 371 as Ist preferred genotype and only three farmers preferred ML 531 as Ist preferred genotype. The genotype ML 171 and GPU 28 are selected as its Ist preference. Even though GPU 28 as not performed well, farmers have selected because of they are growing since from two years.

Location – Hatna and Alugona, Tumkur District

Eight RILs including two varieties Indaf 5 and GPU 28 were included in both the locations. A total 22 farmers in Hatna and 14 farmers Algona village participated participatory evaluation. In Hatna viilage 10 farmers preferred GPU 28 has their Ist preference followed by ML 531and ML 298 by four farmers. Where as Algona village five farmers preferred ML 531 has their Ist preference followed GPU 28 by four farmers and Ml 264 by three farmers. Majority of the farmers in both the locations selected GPU

28 and ML 531has their Ist preference because of high yield, medium compact ear head and resistance neck and finger blast disease.

Location – Ardeshahalli, Bangalore rural district

A total of 30 RILs / varieties were included in FPVS. A total of 30 farmers were involved actively in FPVS in finger millet. Here about 11 RILs / varieties were chosen by the farmers has their Ist preference. It indicates farmers had a basket of new RILs / varieties as acceptable alternatives to their growing varieties. The genotype ML 31 was preferred by 8 farmers has their Ist preference, ML 193 by 7 farmers and ML 531 by five farmers. All these genotypes having ideal characters like high yield, good fodder yield, resistant finger and neck blast.

Location: Jakkasandra, Bangalore Rural District

Thirty RILs / varieties were enrolled in farmers participatory varietal selection. About 28 farmers participated in FPVS. The genotype ML 426 was selected by 18 farmers has their Ist preference and also eight farmers selected has their IInd preference and none of the farmers selected has their IIIrd preference. The ideal characteristics of ML 426 are High yield, moderately compact ear head, more tillers, resistance to finger and neck blast, moderate height and good fodder yield. About five farmers preferred ML 193 has their Ist preference followed by genotype ML 280 by three farmers. The RILs / varities ML 316 and GPU 28 was preferred by only one farmer.

Location: Shettigere, Bangalore Rural District

Total 32 farmers were participated in participatory varietal selection programme. Information about new RILs / varieties of finger millet were solicited from farmers. It as found that 10 farmers chosen ML 553 has their Ist preferred genotype because of its good grain yield, more number of tillers, blast disease resistance. And also three and eight farmers selected ML 553 as preferred genotype has their II and III preference. Where as seven farmers selected ML 434 and ML 531 as Ist preferred genotype.

A new vision for Future line of strategy

Farmer's participatory varietal selection in finger millet is acting as a catalyst between farmers and scientists to give impetus to farmers to adopt more novel varieties of finger millet. Preliminary evaluation of elite RILs / varieties of finger millet by farmers in different locations of Karnataka during *Kharif* 2003 as given top six RILs / varieties. These Six RILs / varieties will put it in a mother baby trial (Snap, 1999) in farmers field to finally come out with one or two elite RILs / varieties in finger millet. And also look

into dissemination rate of selected elite RILs / varieties by farmers in finger millet growing area of Karnataka.

Fig 6. Farmers in participatory varietal selection in on station scientists managed trials GKVK, Bangalore, *Kharif* 2003

Fig 7. Farmers in participatory varietal selection in Hatna and Alugona village, Tumkur District, *Kharif* 2003

Fig 8. Farmers in participatory varietal selection in Aradeshahalli village, Bangalore Rural District, *Kharif* 2003

Fig 9. Farmers in participatory varietal selection in Jakkasandra village, Bangalore Rural District, *Kharif* 2003

Fig 10. Farmers in participatory varietal selection in Shettigere village, Bangalore Rural District, *Kharif* 2003

References

1. Gowda, B. T. S., Halaswamy, B. H., Seetharam, A., Virk, D. S. and Witcombe, J. R., Curr. Sci., 2000, 366 – 368.
2. Joshi, A. and Witcombe, J. R., *Exp. Agric*., 1996, 32, 461-477.
3. Seetharam, A. and Halaswamy, B. H., *Hand Book Of Small Millets Varieties*, 2003, pp. 183.
4. Snapp, S., *The Newsletter of the Soil Fertility Network for Mize-Based Cropping Systems in Malawi and Zimbabwe*, January 1999 issue. CIMMYT, Zimbabwe.
5. Sthapit, B. R., Joshi, K. D. and Witcombe, J. R., *Exp. Agric*., 1996, 32, 479-496.
6. Witcombe, J. R., Parr, L. B. and Atlin, G. N., *Proceedings of a DFID Plant Sciences Research Programme / IRRI Conference, IRRI, Los Banos, Laguna, Phillipines*, 12 – 15 March 2002, 1 – 4.

Objective 3:
Field screening of finger millet (*Eleusine coracana* (L) Gaertn.) recombinant inbred lines (RIL's) against blast disease in different locations of southern districts of Karnataka and molecular characterization of different isolates of *Pyricularia grisea* by using RAPD technique

A. Introduction

Finger millet (*Eleusine coracana* (L) Gaertn.) commonly known as ragi is one of the most important and largely grown millet in Karnataka. It is widely consumed as the staple food in the rural community of southern Karnataka and the straw is also used as cattle fodder.

Even though, finger millet is known to be one of the hardiest crops, it is affected by good number of diseases such as blast, foot rot, smut, streak and mottling virus (Govindu and Shivanandappa, 1967). Among these, blast caused by the fungus *Pyricularia grisea* sacc. is the most devastating disease affecting different aerial parts of the plant at all stages of its growth starting from seedling to grain formation. Yield loss due to blast may be around 28 per cent (Vishwanath *et. al.,* 1997), but under favorable conditions it may go higher to 80-90 per cent. In spite of a great deal of research on the pathogen and the disease, blast still remains a serious constraint to ragi production in areas with conducive environments where susceptible cultivars are grown. Since finger millet is predominantly grown as rain fed crop by small farmers, the disease management by chemical means is found to be economically unaffordable. Hence, it would be useful for the disease to be managed with the inherent capacity of the plant. Growing resistant varieties is not only economical for minimizing the losses caused by the disease, but it is also an environmentally friendly method.

Blast of ragi is caused by the fungus *Pyricularia grisea* (Cooke) Sacc. (formerly *Pyricularia oryzae* Cavara.), anamorph of *Magnaporthe grisea* (Hebert) Barr; Rossman *et al*., 1990 is a heterothallic, filamentous fungus (Fig.1), pathogenic to almost 40 plant species in 30 genera of Poaceae including *Eleusine*. The fungus produces lesions on leaves (Fig. 2), necks (Fig. 3) and fingers (Fig. 4) and discolors the grains. The leaf spots are typically elliptical. The center of the spot is usually gray or whitish and the margin is usually brown or reddish brown. Both shape and color of the spots, however,

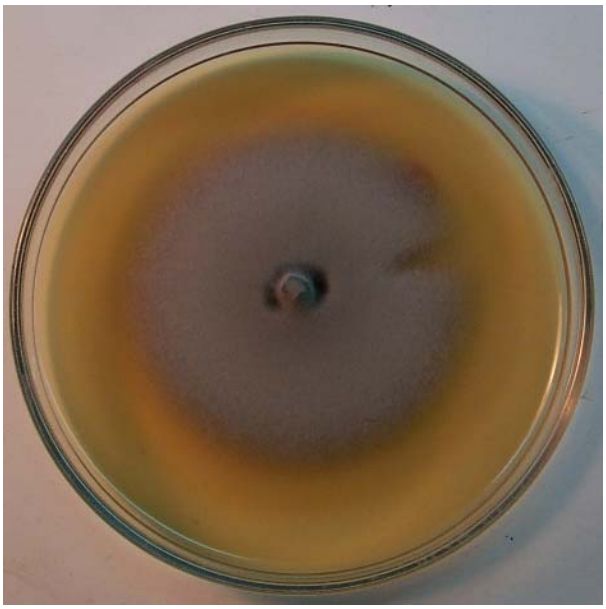

Fig. 1. A culture of *Pyricularia grisea*, the blast pathogen of finger millet.

Fig. 2. Acute leaf blast symptoms in finger millet crop.

Fig. 3. Distinct neck blast symptoms in finger millet panicles compared to the healthy one at right side of the photograph.

Fig. 4. Infected ear head of a standing ragi crop shows complete distortion of the fingers.

vary depending upon environmental conditions and the susceptibility of the host. Numerous lesions on a leaf may coalesce and cause complete drying of leaf or death of the plant. On resistant varieties only minute brown specks of pinhead size may develop.

Though resistance breeding is a high priority area for research now, single major gene resistance often breaks down within few years in a disease conducive environment. This is because of the differing population structure of the blast pathogen. The pathogen apparently evolves very rapidly in response to the selection pressure. This may be due to both diversity (pre-existing variation) and variability (novel variation) in the pathogen population (Sridhar and Singh, 2001). The desirability of combining multiple genes conferring resistance breeding has long been recognized for different crops. However, a

sound scheme for pyramiding resistance genes can be developed only after the genes to be used have been well characterized (Sridhar *et al.,* 1999).

In this context investigations were undertaken to search for RIL's that possess field resistance against blast of finger millet as well as identification and confirmation of molecular variation of the pathogen in different locations of southern districts of Karnataka. The following objectives were studied

1. Collection of blast diseased specimens from finger millet grown at different locations of Bangalore district.
2. Isolation, purification and long-term preservation of monosporic blast isolates collected from different locations.
3. Extraction of DNA from blast isolates.
4. Molecular characterization of different isolates by using RAPD technique.
5. Field evaluation of finger millet genotypes against blast disease at different locations.
6. Reaction of elite cultivars to various blast isolates obtained from different plant parts by artificial inoculation and assessment of pathogenic relationships.

B. Materials and methods

The present investigations were conducted during Khariff, 2003 at different locations of southern districts of Karnataka.

B.1. Plant materials used for artificial inoculation studies

Seeds of different ragi cultivars and recombinant inbred lines (RILs) used in this study were obtained from the MAS Laboratory, Department of GPB, UAS, Bangalore, India.

B.2. Collection of blast diseased specimens

Finger millet plants showing typical symptoms of leaf blast were collected in paper bags and stored in refrigerator at 4^{o}C for further studies. The plants were tagged for future collection of neck, finger and seed blast specimens. The samples were collected from different locations of Bangalore district viz. Aradeshahalli, GKVK, Suradevapura, Rajanakunta and Singhanayakanahalli separated by 25 km from each other. A set of 174 isolates of *P. grisea* obtained from diseased leaf, neck, finger and seed samples collected from different test locations were used for characterization (Table 1).

B.3. Media used

Ragi leaf extract sucrose agar (RLESA)

Healthy ragi leaves - 100 gms
Sucrose - 20 gms
Agar - 20 gms
Distilled water - 1000 ml
The pH was adjusted to 6.8 – 7.2 before autoclaving.

Yeast extract sucrose agar (YESA)

Yeast extract - 2 gms
Sucrose - 20 gms
Agar - 20 gms

Distilled water - 1000 ml
The pH was adjusted to 6.8 – 7.2 before autoclaving.

Yeast extract sucrose broth (YESB)

Yeast extract - 2 gms
Sucrose - 20 gms
Distilled water - 1000 ml
The pH was adjusted to 6.8 – 7.2 before autoclaving.

B.4. Isolation of mono-conidial isolates of *Pyricularia grisea*

Tissues with young sporulating lesions were cut into approximately 2 x 2 mm bits (In case of the discolored seeds, the infected seeds were taken as a whole). The cut bits were thoroughly surface sterilized with 0.01% sodium hypochlorite (NaOCl) and washed twice with sterilized distilled water. The lesion bits were placed over a sterilized moist cotton pad previously kept in the inside surface of the bottom portion of a Petri dish. The Petri dishes were closed with their lids and incubated for 24 hr at 25 ± 1^0C in an incubator (REMI, India). After incubation the lesion bits were transferred to another moist cotton pad sticked on the inside surface of the upper lid of a Petri dish. The bottom portion of the Petri dish contained 20 ml of ragi leaf extract agar (RLESA). This assembly was incubated at 25 ± 1^0C for 48 hr in an incubator under fluorescent light. During incubation, the newly formed spores drop on the surface of the medium and initiate germination. At the end of the incubation, the dishes were carefully observed under microscope and single germinating sporelings were picked up with the help of a thinly drawn glass needle and transferred to a fresh Petri dish containing RLESA medium. These dishes were incubated at 25 ± 1^0C for 4-5 days in an incubator. The identity of the fungal cultures developing from the single spores was established based on spore morphology (Ou 1985).

B.5. Storage of fungal isolates

The fungus was grown on yeast extract sucrose agar (YESA) slants in test tubes (15 x 150 mm) for 5-6 days at 25 ± 1^0C in an incubator. Sterilized filter paper (Whatmann No.3) bits (0.5 cm^2) were spread over the fungal mat under aseptic conditions. The slants were again incubated at 25 ± 1^0C for about another 5-6 days by which time the paper discs were fully colonized by the fungus. The paper bits were transferred to sterilized coin envelopes, dried in a desiccators under vacuum for three to four days at room temperature and stored at -20^0C in the presence of silica gel.

Table 1: Isolates of *Pyricularia grisea* obtained from different locations in Kharif 2003.

Sl. No.	Location	Farm category	Ecosystem	Ragi genotypes	No. of isolates				
					Tissue				
					Leaf	Neck	Finger	Seed	Total
1	Aradeshahalli	Farmers' field	IM	RILs	30	-	-	-	30
2	Aradeshahalli	Farmers' field	IM	RILs	-	30	-	-	30
3	Aradeshahalli	Farmers' field	IM	RILs	-	-	30	-	30
4	Aradeshahalli	Farmers' field	IM	RILs	-	-	-	30	30
5	Suradevapura	Farmers' field	RU	Indaf-5	10	-	-	-	10
6	Singhanayaknahalli	Farmers' field	RU	GPU 28	10	-	-	-	10
7	Rajankunta	Farmers' field	RU	Indaf-9	8	-	-	-	08
8	GKVK	Experimental plots	IM	GPU 28	6	-	-	-	06
9	GKVK	Experimental plots	IM	Mutated lines	10	-	-	-	10
10	GKVK	Nursery beds	IM	Mutated lines	10	-	-	-	10
								Total	**174**

IM: Irrigated Mediumland, RU: Rain-fed Upland

B.6. Artificial inoculation studies for host-pathogen interactions

B.6.1. Growing of ragi cultivars

A set of six elite ragi cultivars (Table 2) was used to assess the pathogenic relationship between the isolates obtained from different plant parts and from various locations. Another set of 300 RILs and four parents (Table 3) was inoculated separately with two isolates obtained from B-57 and B-77 lines in net house. Plants were grown in plastic cups (8 x 11 cm) filled with a mixture of field soil and farmyard manure at the ratio of 3: 1 from thickly sown seeds. The soil was fertilized with ammonium sulphate and single super phosphate (100 kg N and 40 kg P_2O_5 per hectare) as basal dose. No potassium fertilizer was added. The cups were numbered and arranged in galvanized iron trays (31 x 26 x 7 cm). The trays containing the cups were maintained in a net house under natural photoperiodic conditions [24-30^{0}C, R. H. 85 ± 15 % and total incident light energy measured with a solarimeter (OSK 76, Ogawa Seiki, Japan) averaging 475 W. m^2 with a range of 285-583 W. m^2]. The soil was maintained under field capacity level by irrigating with water as and when necessary. Ten days after germination, 15 seedlings per cup were maintained by thinning.

B.6.2. Preparation of inoculum

The fungal isolates were revived from filter paper pieces on YESA slants and mycelium from 10-day-old growth was removed and transferred aseptically to a test tube (2 x 15 cm) with 10 ml of sterile distilled water. The mycelial mass containing spores was gently disintegrated uniformly with a glass rod. The resulting suspension of mycelia and conidia was used to inoculate Petri dishes (10 x 10 cm) containing 20 ml of RLESA. The dishes were incubated for a week at 25±1^{0}C under alternate light (14 hr light energy (total) 8.4 W.m^{-2}, Philips cool day light fluorescent tubes TL-40 W/54) and dark (10 hr) regime in a BOD incubator (REMI, India). The plates were flooded with 10-15 ml of sterile water and fungal growth containing mycelium and spores was gently removed by scrapping with a sterile glass slide, and the suspension was transferred to 100 ml beaker. The fungal mass was gently disintegrated with a glass rod and the suspension was filtered through muslin cloth. The spore concentration was adjusted to approximately 5 x 10^4 conidia/ml. One or two drops of Tween 20 (0.02%) was added to the suspension just before inoculation.

Table 2. List of ragi cultivars used.

Sl. No.	Cultivars used
1	IE 2912
2	Indaf-5
3	GPU 28
4	Indaf-9
5	IE 1012
6	IE 2885

B.6.3. Inoculation of plants

Plants were inoculated 21 days after sowing at 4-5 leaf stage [prophyll or incomplete leaf was counted as the first leaf (Yoshida, 1981)]. Trays were placed in growth chambers (31 x 26 x 50 cm) and covered with polythene bags for preventing cross contaminations. Each isolate was replicated three times for eliminating errors. Differentials were sprayed uniformly with spore suspension by a mist sprayer through a hole previously made in the polythene bag till run-off stage. A total of 150 ml spore suspension per fungal isolate was sprayed. A negative control was always maintained by spraying only water. The inoculated trays were kept in the dew chamber for 36 hr at 25 ± 1^{0}C and 100% relative humidity. After incubation the trays were uncovered, transferred to net house and kept under mist created by a sprinkler for 6-7 days.

B.6.4. Disease scoring

Disease reaction of the inoculated plants were scored 10-14 days after inoculation by using a 0 – 5 scale (0 = no visual symptoms; 1 = brown specks smaller than 0.5 mm in diameter; 2 = brown specks about 0.5-1 mm in diameter; 3 = roundish to elliptical lesions about 1-3 mm in diameter with gray centers and brown margins; 4 = typical spindle- shaped blast lesions, 3 mm or longer with little or no coalescence of lesions; 5 = same as 4 but half of or more leaf killed by coalescence of lesions) (Mackill and Bonman 1992). Plants rated 1 – 3 were considered resistant, and those rated 4 – 5 were considered susceptible.

Table 3. List of finger millet RILs used for artificial inoculation studies

Sl. No.	RIL No.	Sl. No.	RIL No.	Sl. No.	RIL No.	Sl. No.	RIL No.
1	ML2	42	ML106	83	ML199	124	ML281
2	ML4	43	ML109	84	ML203	125	ML282
3	ML5	44	ML110	85	ML204	126	ML284
4	ML6	45	ML111	86	ML208	127	ML285
5	ML7	46	ML113	87	ML209	128	ML287
6	ML8	47	ML114	88	ML210	129	ML288
7	ML9	48	ML118	89	ML211	130	ML289
8	ML10	49	ML122	90	ML212	131	ML293
9	ML11	50	ML124	91	ML213	132	ML296
10	ML13	51	ML125	92	ML215	133	ML297
11	ML15	52	ML127	93	ML218	134	ML298
12	ML19	53	ML128	94	ML220	135	ML299
13	ML25	54	ML131	95	ML222	136	ML300
14	ML31	55	ML140	96	ML223	137	ML301
15	ML33	56	ML143	97	ML224	138	ML302
16	ML37	57	ML145	98	ML226	139	ML305
17	ML39	58	ML146	99	ML230	140	ML306
18	ML40	59	ML148	100	ML231	141	ML307
19	ML41	60	ML149	101	ML232	142	ML311
20	ML42	61	ML153	102	ML234	143	ML314
21	ML43	62	ML154	103	ML243	144	ML315
22	ML50	63	ML155	104	ML244	145	ML316
23	ML52	64	ML157	105	ML247	146	ML318
24	ML55	65	ML161	106	ML249	147	ML320
25	ML58	66	ML164	107	ML250	148	ML322
26	ML59	67	ML168	108	ML252	149	ML324
27	ML61	68	ML169	109	ML253	150	ML325
28	ML63	69	ML170	110	ML255	151	ML326
29	ML64	70	ML171	111	ML257	152	ML329
30	ML67	71	ML172	112	ML258	153	ML330
31	ML68	72	ML179	113	ML262	154	ML331
32	ML73	73	ML181	114	ML264	155	ML334
33	ML75	74	ML182	115	ML265	156	ML335
34	ML80	75	ML184	116	ML267	157	ML336
35	ML81	76	ML187	117	ML270	158	ML338
36	ML85	77	ML189	118	ML271	159	ML341
37	ML89	78	ML191	119	ML273	160	ML342
38	ML93	79	ML194	120	ML276	161	ML343
39	ML98	80	ML195	121	ML278	162	ML345
40	ML100	81	ML197	122	ML279	163	ML348
41	ML103	82	ML198	123	ML280	164	ML349

Table 3 contd…

Table 3 contd…

Sl. No.	RIL No.	Sl. No.	RIL No.	Sl. No.	RIL No.	Sl. No.	RIL No.
165	ML350	200	ML420	235	ML484	270	ML553
166	ML352	201	ML426	236	ML485	271	ML556
167	ML353	202	ML427	237	ML486	272	ML557
168	ML356	203	ML430	238	ML489	273	ML559
169	ML357	204	ML433	239	ML493	274	ML562
170	ML358	205	ML434	240	ML494	275	ML563
171	ML359	206	ML436	241	ML495	276	ML564
172	ML360	207	ML438	242	ML496	277	ML568
173	ML361	208	ML439	243	ML498	278	ML570
174	ML368	209	ML441	244	ML499	279	ML572
175	ML370	210	ML444	245	ML500	280	ML573
176	ML371	211	ML447	246	ML501	281	ML574
177	ML373	212	ML449	247	ML505	282	ML576
178	ML376	213	ML452	248	ML507	283	ML578
179	ML378	214	ML454	249	ML510	284	ML579
180	ML379	215	ML455	250	ML513	285	ML580
181	ML381	216	ML456	251	ML516	286	ML581
182	ML383	217	ML457	252	ML521	287	ML586
183	ML387	218	ML458	253	ML522	288	ML587
184	ML389	219	ML459	254	ML523	289	ML588
185	ML390	220	ML461	255	ML527	290	ML591
186	ML391	221	ML462	256	ML529	291	ML592
187	ML392	222	ML463	257	ML530	292	ML593
188	ML398	223	ML465	258	ML531	293	ML594
189	ML399	224	ML467	259	ML534	294	ML596
190	ML400	225	ML468	260	ML536	295	ML597
191	ML401	226	ML469	261	ML537	296	ML598
192	ML405	227	ML470	262	ML538	297	ML600
193	ML407	228	ML471	263	ML540	298	ML601
194	ML409	229	ML472	264	ML541	299	ML604
195	ML411	230	ML473	265	ML542	300	ML609
196	ML412	231	ML477	266	ML543	301	GPU-28
197	ML415	232	ML479	267	ML545	302	IE-1012
198	ML416	233	ML480	268	ML547	303	Indaf-5
199	ML417	234	ML483	269	ML549	304	Indaf-9

B.7. Field Screening of finger millets RILs for leaf, neck and finger blast disease incidence at different locations

A set of 27 selected recombinant inbred lines (RIL's) viz., ML 31, ML 32, ML 177, ML 181, ML 197, ML 203, ML 208, ML 224, ML 255, ML 264, ML 265, ML 284, ML 280, ML 297, ML 298, ML 300, ML 302, ML 330, ML 322, ML 343, ML 371, ML 392, ML 426, ML 430, ML 434, ML 531, ML 553, three parents IE 1012, Indaf–5 and GPU 28 and a susceptible

check ML 349 were used to evaluate for leaf, neck and finger blast under field condition at different locations namely Aradeshahalli, Jakkasandra, Shettigere and AICSMP (GKVK) in Bangalore district. Another set of eight selected RILs viz. ML 264, ML 531, ML 371, ML 181, ML 224, ML 298, ML 330, ML 349 and two parents indaf-5 and GPU 28 were used at two different locations namely Hatna and Alugona (Tumkur district). The experiments were undertaken in farmers' field for farmers participatory varietal selection programme (FPVSP). The seeds were sown in a randomized complete black design (RCBD) during *Kharif* 2003-04 with two replications having plot size of 3 x 2.25 M^2. Susceptible check ML 349 was used in each replication.

Field screening of all the 300 RILs and four parents was performed at three different locations namely Shettigere, Hebbal and GKVK in Bangalore district for leaf, neck and finger blast disease. The seeds were sown in single row replicated lines.

The plants were raised according to the recommended package of practices (Anon, 1995) in all the locations. Leaf blast (LB) severity was recorded at 45 to 50 days old crop by using 0 to 5 scale (Anon, 1996) and the incidence of neck blast (NB) was recorded by counting the number of peduncles infected in a total number of plants. Neck and finger blast (FB) observations were recorded at the time of grain maturity. Further percent disease index (PDI) was calculated using the formula given by Wheeler (1969).

B.8. DNA extraction from blast fungus

B.8.1. Culturing of the fungus

Aliquots of 50 ml of YESB were dispensed in 100-ml Erlenmeyer flasks and were sterilized by autoclaving. The flasks were inoculated with 3-5 small blocks (3 x 3 mm^2 size) of mycelia removed from 4-day-old cultures under aseptic conditions. The flasks were incubated by shaking (100-120 rpm) at 25 ± 1^0C in an environmental shaker (REMI, India) for 10-12 days. At the end of the incubation, the mycelial mat was harvested by filtering through a sterilized Whatmann No. 3 filter paper. The mycelial mats were transferred to sterilized butter papers (150 x 100 mm size) and freeze dried for 16 hr at -50^0C, 50-75 *atm* in a freeze drier (Vertis, USA). Freeze-dried mycelia were used directly for DNA extraction or they were stored by placing them inside sterilized (twice by autoclaving) coin envelopes (90 x 50 mm size) at -20^0C in a freezer until use.

B.8.2. DNA extraction

Genomic DNA was extracted following the procedure of Murray and Thompson (1980) for plant DNA with modifications for mini-scale preparation as described by Scott *et al.* (1993). The frozen mycelium was ground in a mortar with a pestle in liquid nitrogen to a fine powder. DNA isolation was done on a microfuge tube scale because it allows for the handling of more samples compared to large-scale DNA preparations.

Reagents

Extraction buffer (50 mM Tris-HCL; 150 mM NaCl; and 100 mM EDTA)
10% sodium dodecyl sulfate (SDS)
5M NaCl

10% CTAB in 0.7M NaCl solution
24:1 chloroform isoamyl solution
2-propanol
70% ethanol
TE (10 mM Tris-HCl, pH 7.5-8.0; 1 mM EDTA)
TBE (89 mM Tris, pH 7.8; 89 mM boric acid; and 2 mM EDTA)

Extraction

A portion of approximately 20-25 mg of pulverized mycelium was placed in a 2-ml Eppendorf tube and the powder was suspended in 750 µl of extraction buffer. The contents were vortexed until evenly suspended. To this, 75 µl of 10% SDS was added and the mixture was shaken gently at 37^0C for 1 hr. Then, 112.5 µl of 5 M NaCl was added to this mixture and again the contents were mixed thoroughly but gently. To this 97.5 µl of CTAB/NaCl solution was added, mixed well and the contents were incubated at 65^0C for 10-20 min in a water bath (Tempo, India). This step will remove cell wall debris, denatured proteins, and polysaccharides complexed to CTAB, while retaining the nucleic acids in solution. Equal volume of (1000 µl) of 24:1 chloroform: isoamyl alcohol was added to the mixture. Care was taken to leave some space in the tubes for mixing. The tubes were mixed vigorously in a shaker for 5 min. This was done by arranging the tubes in an Eppendorf tube rack and placing another Eppendorf tube rack on top of the tubes. The two racks were secured with rubber bands and are then placed on their sides while shaking. After incubation, the mixture was centrifuged at 10,000 g for 12 min in a micro centrifuge (Kubota, Japan). CTAB-protein/polysaccharide complexes formed a white interface after centrifugation. The aqueous, viscous supernatant was transferred to a fresh tube and 0.6 volume (675 µl) cold 2-propanol was added to precipitate the nucleic acid. The tubes were left inside -20^0C freezer for at least an hour. The tubes were centrifuged at 10,000 g for 12 min. The supernatant was removed with a micropipette instead of pouring because of the jelly-like consistency of the pellet. The pellet was washed with 500 µl of 70% EtOH and dried completely. The pellet was dissolved in 100 µl of 1 X TE at 65^0C in a water-bath. The yield of DNA varied from 10 to 100 µg per sample. DNA samples were stored in a deep freezer (-20^0C).

B.8.3. Reprecipitation of DNA

Whenever necessary, the DNA was re-precipitated for obtaining pure preparations. An aliquot of 3 M sodium acetate solution (pH 5.2) amounting to one-tenth the volume of the DNA solution was added to the DNA stock. The tubes were flicked gently for mixing thoroughly the contents and centrifuged for 5 min at 10,000 g. The clear supernatant liquid was transferred to another Eppendorf tube. To this two volumes (of the original DNA solution) of chilled absolute alcohol were added and the contents were mixed gently by flicking the tubes and were aged in a refrigerator for about 2 hr. The tubes were centrifuged at 10,000 g for 15 min and the supernatant liquid was discarded by decanting. The pellet remaining in the tubes was air-dried for about 4 hr at room temperature and then dissolved in 50 µl of sterilized distilled water.

B.9. DNA quality/ quantity check

The concentration and condition of the DNA was assessed by a mini-gel electrophoresis. Aliquots of 1µl of the DNA solution was mixed with 2 µl sterilized nanopure water and 3 µl of 10 X loading dye [0.4% bromophenol blue, 0.4% xylene cyanole FF and 50% glycerol (Sigma,

USA)] and the mixture was loaded in a gel (10 x 14 cm size) containing 0.7% agarose (Life Technologies GIBCO BRL, USA) and 0.5 X Tris-borate buffer (89 mM Tris, pH 7.8; 89 mM boric acid; and 2 mM EDTA) along with 1kb DNA ladder (Life Technologies, GIBCO BRL, USA) and DNA quantitation standards of 50 ng and 100 ng/µl (Life Technologies, GIBCO BRL, USA). Gels (14 x 10 cm size) were run for 1 hr at 100 Volts, stained with ethidium bromide (1 µg/ml), and then photographed using a gel documentation system (Imago) equipped with Imacomm soft ware. Intact high molecular weight genomic DNA is resolved as a single band. Any DNA appearing as a smear due to degradation was discarded and prepared fresh again. Relative concentration of DNA present in the samples was approximately derived by visual comparison with DNA quantitation standards.

B.10. Fingerprinting of *P. grisea* isolates

DNA samples from *P. grisea* isolates were fingerprinted by using randomly amplified polymorphic DNA (RAPD) technique with OPA-13 primer (5'CAG CAC CCA C 3') obtained from Operon Technologies Inc., USA.

B.10.1. Polymerase chain reaction (PCR)

Amplification was performed in a 20 µl cocktail containing 50pmoles of the primer, 100 ng of genomic DNA, 185 µm of dNTPs (mixture of dATP, dGTP, dCTP and dTTP, Bangalore Genei, India), approximately 1 unit of *Taq* polymerase (Bangalore Genei, India) in 10X PCR buffer (10 mM Tris-HCl, pH 8.3, 500 mM KCl, 1.5 mM $MgCl_2$, and 0.01 mg/ml gelatin). The reaction mixture was overlaid with one drop of mineral oil, initially denatured for 5 min at 95^0C, and, then, subjected to 34 cycles of 1 min denaturation at 94^0C, 1 min annealing at 36^0C, 2 min extension at 72^0C; and a final extension for 5 min at 72^0C using a model Mastercycler Gradient (Eppendorf, Germany).

B.10.2. Electrophoresis and visualization of PCR products

To visualize the DNA fragments, PCR products were mixed with 5 µl of 5 X loading dye. Aliquot of 10 µl of this mixture was loaded in a gel (15 x 14 cm) containing 1.4% agarose and 0.5 X Tris-borate (TBE) buffer (For 5 X TBE, 89 mM Tris, pH 7.8; 89 mM boric acid; and 2 mM EDTA). A solution of 10 µl of 1kb ladder (0.05µg/µl) was loaded along with the samples for marking the bands. The samples were electrophoresed for four hours at 90 V. The gel was stained with ethidium bromide (1µg/ml) and the image was photographed using a gel documentation system equipped with Imacomm software.

To ensure that only reproducible bands were scored, PCR was done at least twice for each DNA sample and those that were consistently amplified were scored. Resolved bands ranging in size from 320 bp to approximately 3 kb were scored regardless of intensity.

To reduce the possibility of cross contamination and to find variation in amplification reaction, master mixes of reaction were always freshly prepared and used. In each set of amplification, a positive control with DNA of a standard isolate and a negative control without template DNA were included with reaction mixture.

B.10.3. Scoring and analysis of fingerprints

Data were scored for computer analysis on the basis of the presence or absence of the amplified products. The fingerprints were manually scored by taking 1 at presence and 0 at absence of bands for each isolate. Pair-wise comparisons of isolates, based on the presence or

absence of unique and shared polymorphic products, were used to generate similarity coefficients (Jaccard, 1908). The data was then cluster analyzed and a dendrogram was drawn by UPGMA (unweighted pair-group method with arithmetical averages) using a computer programme Statistica showing linkage distance between isolates.

C. Results and Discussion

Information on pathogen population structure such as the type of variants present in a location and the amount and distribution of variation should assist plant breeders and farmers in the development and strategic deployment of resistant cultivars. Monitoring changes in population structure over time and space would also contribute to an understanding of the process that drive genetic changes in pathogen population and hopefully lead to a more effective disease management programme.

C.1. Molecular analysis of pathogen population

A total of 174 isolates of *P. grisea* was obtained from different parts of Southern Karnataka and molecularly characterized by using an RAPD primer, OPA-13 (Fig. 5, Fig. 6) for inferring a complete and comprehensive analysis of the overall population structure. It included a sub set of isolates numbering 120 of which, 30 each was originated from different parts viz. leaf, neck, finger and seed of 30 different recombinant inbred lines (RILs) and another 44 isolates were obtained from the leaf tissues of some other widely grown ragi cultivars grown in farmers' fields and experimental plots.

The fingerprints obtained were analyzed by UPGMA and a dendrogram was constructed showing the linkage distance between the groups (Fig. 7). At 100% similarity level, a total of 71 haplotypes were formed out of which 41 haplotypes contained group of isolates, where as 30 isolates did not come under any group and were called as lone isolates (Table 4). The same figures continued for 95% similarity level. However, when the similarity levels further decreased, the number of groups and lone isolates were also decreased (Fig. 8) and at 75% similarity level, three major clusters were formed.

There is no information regarding the possible natural variability of blast pathogen analyzed by using RAPD markers in ragi. But in case of rice, by using OPA 10 and OPA 13 primers in RAPD-based DNA fingerprinting, Sharma *et al.* (2002) grouped 250 isolates of *P. grisea* collected from North-Western Himalayan region into 25 lineages. However, Viji *et al.*, 2000 established a high degree of genetic variation among isolates collected from finger millet grown at a single location by using grh pKE 2-2, an RFLP based probe. Hence, the result revealed the fact that OPA-13 primer may be universal in fingerprinting the blast isolates originating from rice as well as non-rice hosts.

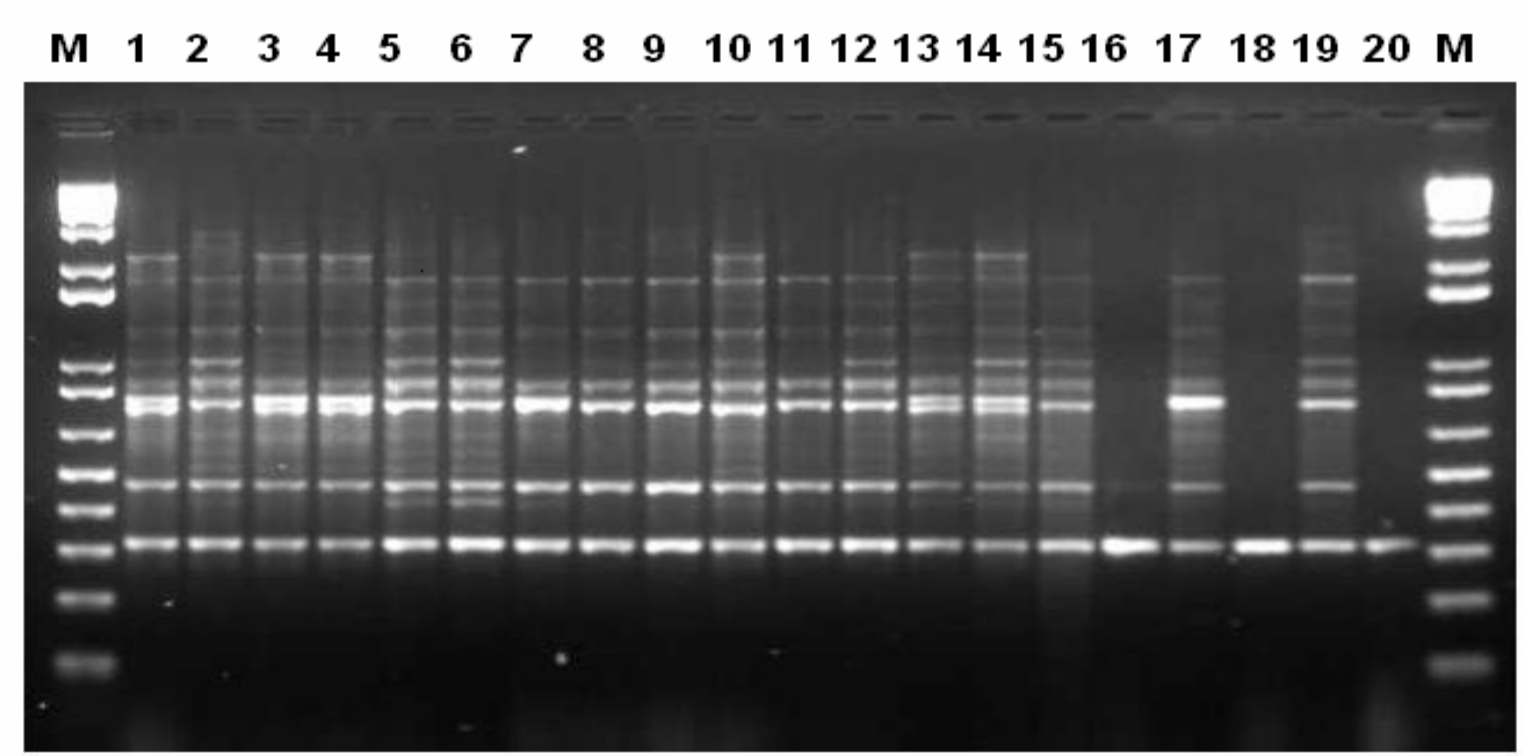

Fig. 5. ***OPA-13*** **based polymerase chain reaction fingerprint patterns from genomic DNA of** ***Pyricularia grisea*** **isolates obtained from ragi genotypes grown at the farmers' fields of Singhanayakahalli and experimental plots of GKVK, Bangalore. Lanes 1 to 20 contain the fingerprints of the following isolates. 1. Singha-1, 2. Singha-2, 3. Singha-3, 4. Singha-4, 5. Singha-5, 6. Singha-6, 7. Singha-7, 8. Singha-8, 9. Singha-9, 10. Singha-10, 11. M-1, 12. M-2, 13. M-3, 14. M-4, 15. M-5, 16. M-6, 17. M-7, 18. M-8, 19. M-9, 20. M-10. The DNA molecular size marker, 1-kb ladder (Life Technologies, GIBCO BRL, USA) is on the lanes labeled M on both the sides of the gel.**

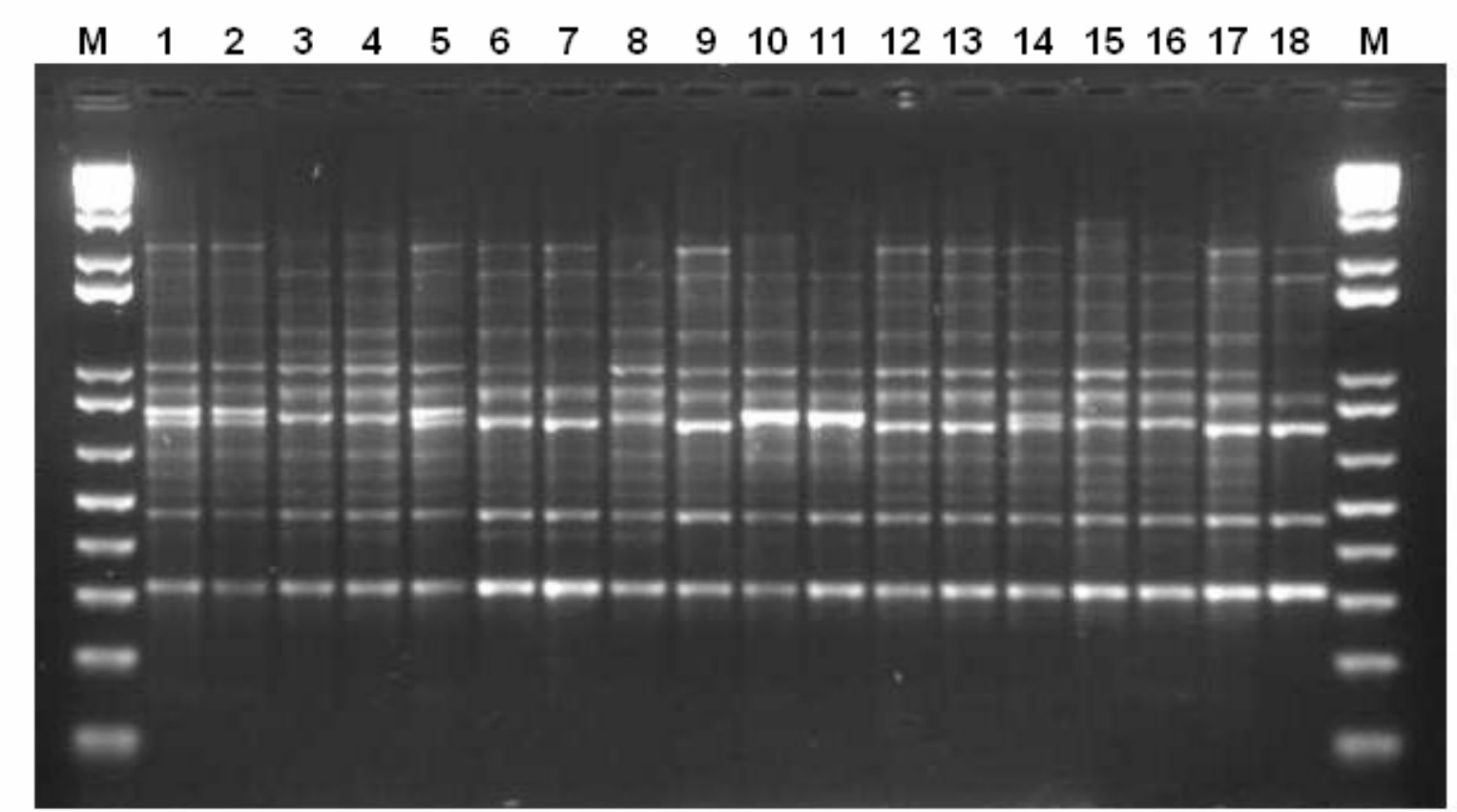

Fig. 6. *OPA-13* based polymerase chain reaction fingerprint patterns from genomic DNA of *Pyricularia grisea* isolates obtained from ragi genotypes grown at the farmers' fields of Rajankunta and Suradevanapura, Bangalore. Lanes 1 to 18 contain the fingerprints of the following isolates. 1. Rajan-1, 2. Rajan-2, 3. Rajan-3, 4. Rajan-4, 5. Rajan-5, 6. Rajan-6, 7. Rajan-9, 8. Rajan-10, 9. Suradev-1, 10. Suradev-2, 11. Suradev-3, 12. Suradev-4, 13. Suradev-5, 14. Suradev-6, 15. Suradev-7, 16. Suradev-8, 17. Suradev-9, 18. Suradev-10. The DNA molecular size marker, 1-kb ladder (Life Technologies, GIBCO BRL, USA) is on the lanes labeled M on both the sides of the gel.

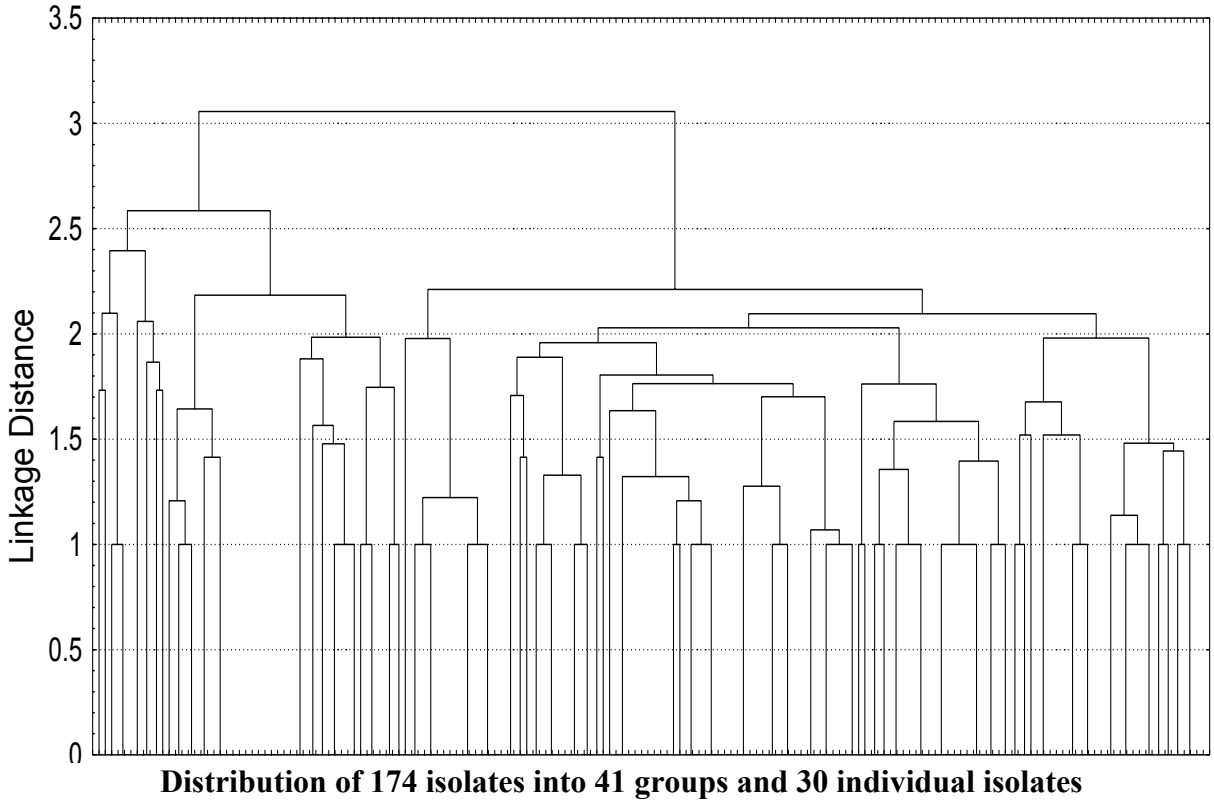

Fig. 7. Dendrogram constructed with OPA-13 primer based PCR fingerprint data for a collection of 174 isolates of *P. grisea* from different parts of Southern Karnataka. The individual isolates are identified based on the name of the gel image and lane number.

Table 4. Distribution of *Pyricularia grisea* isolates into different haplotypes in the pathogen population collected from southern Karnataka during *Kharif*, 2003.

Group	Isolate No.	No. of isolates
1	GPU28-2, GPU28-3, GPU28-4	3
2	AF-4, AL-15	2
3	AF-13, AF-28	2
4	AF-1, GPU28-6	2
5	AS-21, AS-30, AF-17	3
6	AF-8, AF-10, AF-11, AL-17, AL-18, AL-21, AL-22, GPU28-5, NM-7, NM-8, M-6, M-8, M-10	13
7	AL-3, AL-30	2
8	AF-2, AF-9, AF-13, AF-14	4
9	Singha-7, Singha-8, Singha-9	3
10	AL-20, AL-23	2
11	AS-6, AS-7	2
12	AS-12, AS-14	2
13	AS-9, AS-10, M-2, M-5, Singha-5, Singha-6, Singha-10	7
14	Suradev-7, Suradev-8, Rajan-3, Rajan-4	4
15	AN-11, AN-13	2
16	AN-27, AN-28	2
17	AS-5, AS-8, AS-11, AS-13	4
18	M-3, M-4	2
19	Singha-3, Singha-4	2
20	AN-1, AN-2, AN-3, AN-4, AN-5, AN-6, AN-8, AN-12, AN-14	9
21	AN-7, AN-9, AN-10	3
22	Al-26, AL-28, AL-29, NM-2, NM-4	5
23	AF-18, AF-19, AF-21, AF-23, AF-25	5
24	AF-22, AF-24	2
25	AS-26, AS-27, AS-29, AL-27	4
26	AN-21, AN-23	2
27	AL-5, AL-7, AL-8, AL-9, AL-10	5
28	AF-26, AF-27	2
29	AS-17, AL-16, AL-19, AL-24	4
30	AN-25, NM-3, NM-5, NM-6	4
31	AL-1, AL-2, AL-11, AL-12, AL-13, AL-14	6
32	Suradev-4, Suradev-5, Rajan-9	3
33	AN-19, AN-20	2
34	AS-18, AS-19, AS-20, AS-22, AS-23	5
35	AS-24, AS-25	2
36	AF-3, AF-5, AF-6, AF-12	4
37	AL-4, AL-6	2
38	AN-24, AN-26, AN-29, AN-30	4
39	Suradev-2, Suradev-3	2
40	AS-1, AS-2	2
41	Suradev-6, Rajan-1, Rajan-2, Rajan-5	4
42	AS-28	1
43	AF-20	1

Table 4 contd…

Table 4 contd…

Group	Isolate No.	No. of isolates
44	AN-15	1
45	GPU28-1	1
46	M-9	1
47	Singha-1	1
48	AF-29	1
49	AF-15	1
50	NM-9	1
51	NM-1	1
52	M-1	1
53	Suradev-10	1
54	Singha-2	1
55	AF-7	1
56	M-7	1
57	AN-18	1
58	AL-25	1
59	AS-15	1
60	AN-16	1
61	AS-4	1
62	Rajan-10	1
63	Suradev-9	1
64	Suradev-1	1
65	NM-10	1
66	AN-22	1
67	Rajan-6	1
68	AS-16	1
69	AF-16	1
70	AN-17	1
71	AS-3	1
	Total	**174**

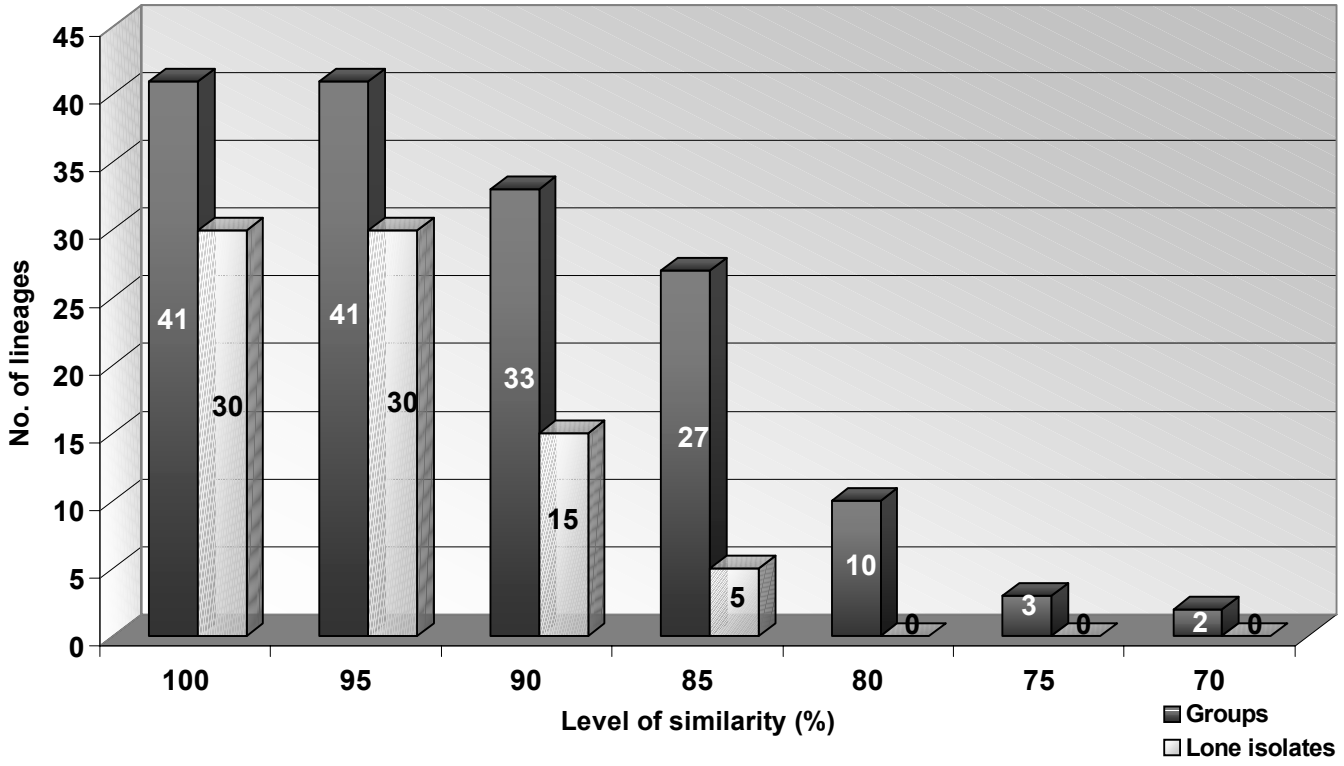

Fig. 8. Total number of lineages containing groups and lone isolates at different levels of similarity formed by genetic analysis of 174 *P. grisea* isolates using OPA-13 primer.

The overall analysis showed a genetic relationship between isolates originated from different plant parts and a spatial relationship was also noticed among isolates originated from different locations. Isolates obtained from finger, neck, seed and leaf parts of 30 RILs numbering 120 were found to be distributed in 15, 13, 15 and 12 haplotypic groups, respectively. However, the 44 isolates obtained from other traditional cultivars grown in different locations were distributed in 27 haplotypic groups (Fig. 9). The extent of variation among the isolates was calculated, as the proportion of total number of haplotypic groups formed to the total number of isolates studied and was found to be 41%. However, the proportion was 46% in the case of the subpopulation originated from RILs and as high as 61% in the second subpopulation consists of isolates obtained from traditional ragi cultivars (Fig. 10).

In a comprehensive study regarding the genetic relationship between leaf and neck blast symptoms in rice, Ou, 1972 reported that there may be some differences in genes conferring resistance to leaf and neck blast development and it is not the different races causing leaf and neck blast separately. Xia *et al*. (1993) analyzed 113 isolates from a rice cultivar Newbonnet grown in two commercial fields of Arkansas, USA through RFLP technique using MGR586 probe and found seven distinct fingerprint groups (A to G) in the population. But they did not find any distinct group causing only neck blast or leaf blast either. However, there is no report suggesting the above facts in case of finger millet as a host for blast. Hence this study suggesting that there may not be any specific isolate infecting a particular aerial part of the plant.

There has been no effort in analyzing the pathogen population occurring in different genotypes in India. The result presented in this study clearly brings out the population structures of the pathogen harbored by improved genomorphs (RILs) and traditional cultivars. In addition, the analysis presented in this study revealed that the traditional ragi cultivars were infected by a higher number of lineages of the pathogen in contrast to the improved genomorphs, which were infected, by comparatively lesser number of lineages of the blast pathogen during Kharif, 2003.

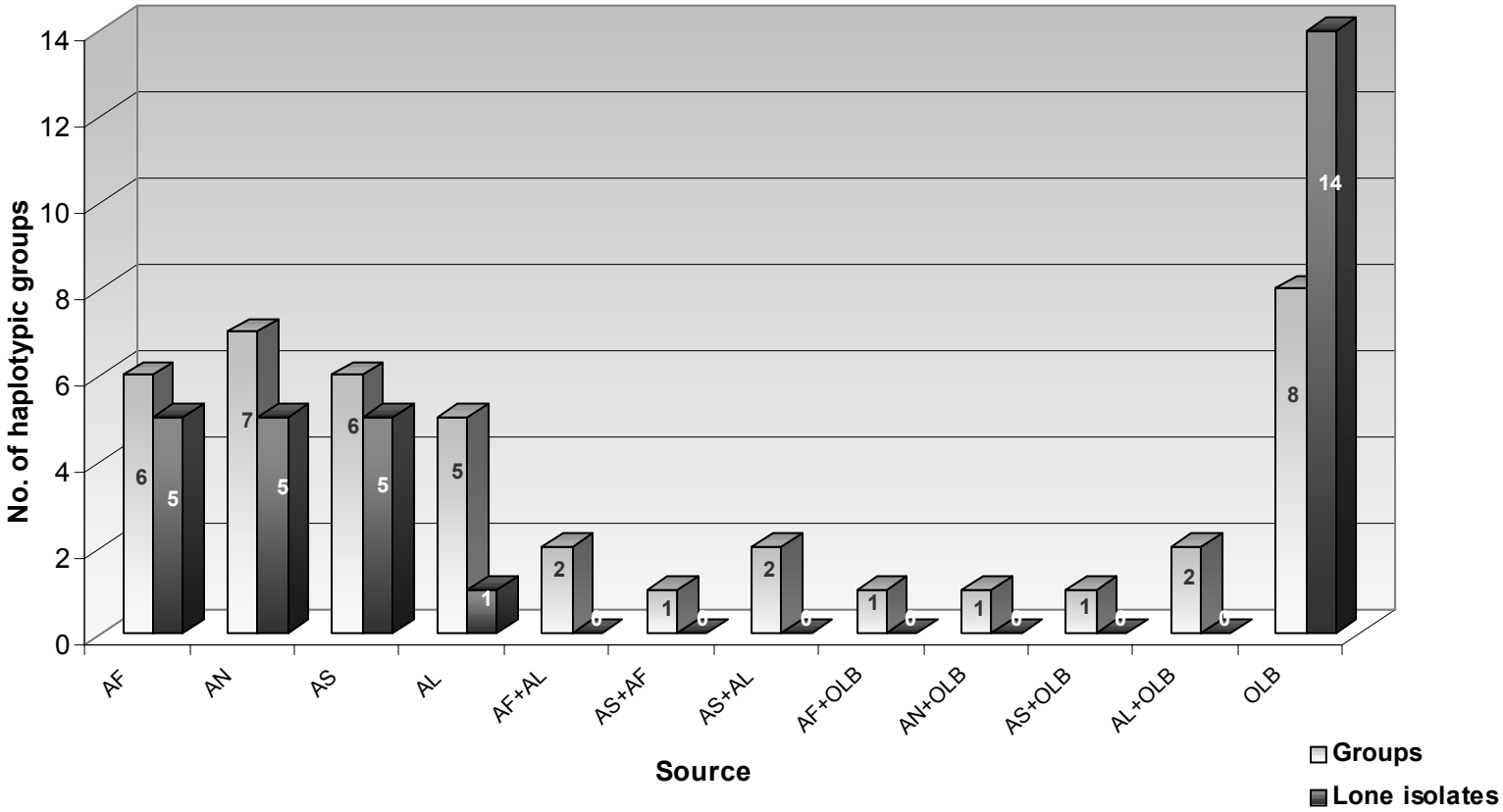

Fig. 9. Number of haplotypic groups formed by genomic analysis of blast isolates originating from various sources.

AF: Aradeshnahalli Finger Blast, AN: Aradeshnahalli Neck Blast, AS: Aradeshnahalli Seed Blast, AL: Aradeshnahalli Leaf Blast, OLB: Other Leaf Blast (collected from Suradevanapura, Singhanayakahalli, Rajankunta and GKVK)

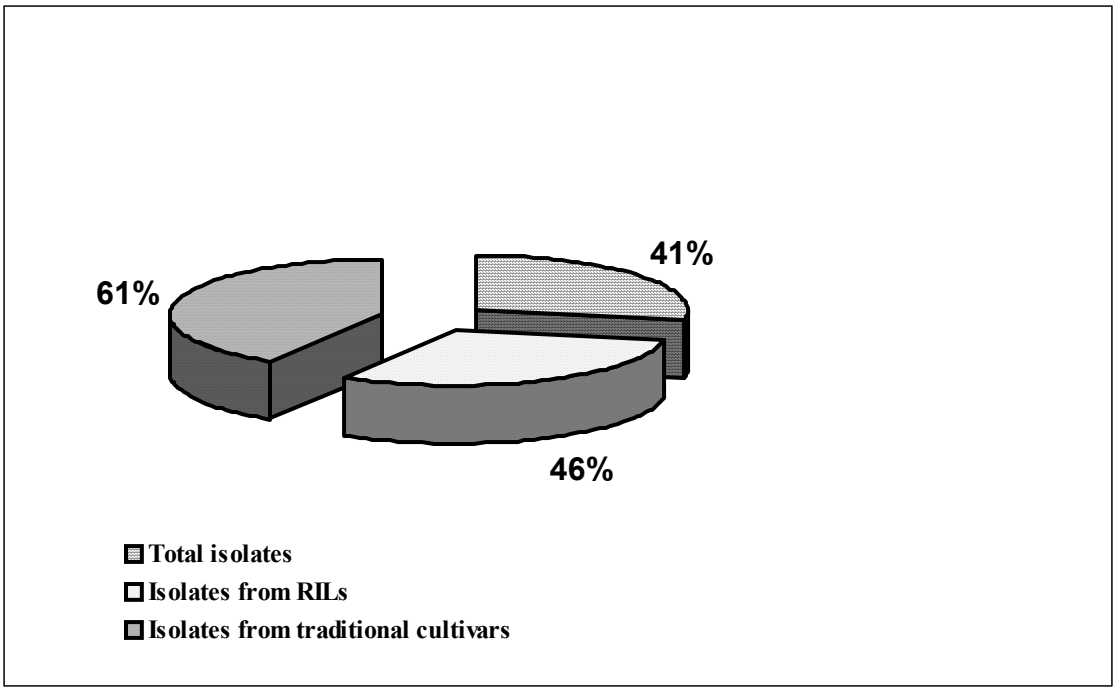

Fig. 10. Proportion of number of haplotypic groups formed to the number of blast isolates studied.

C.2. Virulence analysis of type isolates of *P. grisea*

Studies on the virulence of ragi blast pathogen populations have led to the investigations on genetics of host resistance. This may result in the selection of differential ragi cultivars for identifying the races of *P. grisea*. This study was also aimed to assess the reactions of different isolates obtained from various aerial parts of the plant to leaf tissue of the ragi cultivars used. A higher degree leaf blast symptoms were observed in all the cultivars and some differential reactions were also noticed among cultivars to a particular blast isolate (Fig. 11, Fig. 12). Artificial inoculation studies have revealed the fact that almost all the 12 isolates inoculated could able to infect the six cultivars used (Fig. 13). However, in an average, the cultivar IE 1012 showed moderately resistance reaction to all the isolates studied and the cultivars Indaf-5 and Indaf-9 were found to be highly susceptible to all the isolates in controlled environment (Table 5 and Fig. 14).

There is no information available for any attempt made to develop a tentative set of differentials for assessing the racial differentiation among ragi blast pathogen population. However, in case of rice crop, so many site specific and International sets of differentials have been developed (Atkins *et al*., 1967, Ling and Ou, 1969, Ou, 1972; Bonman *et al*., 1986). Hence, it is needed for developing a set of ragi differentials to actively and successfully assess the racial differentiation in the pathogen population prevailing in a particular geographical region. For successful resistance breeding

strategies, a proper know-how of the prevailing pathogen population and its dynamics is necessary and for developing a suitable set of differentials, genetic studies of the cultivars as well as the knowledge of resistance gene(s) involved is a priority.

In the present study, the results shows that the cultivar IE 1012 can be taken as a differential host and Indaf-5 and Indaf-9 can be used as susceptible controls in the differential set. More work should be done for identifying at least ten differential host cultivars for race identification. Representative isolates originated from neck, finger and seed tissues also infected the leaf tissues of all the six cultivars suggesting the fact that there may not be any specific isolate or group of isolates involved in development of blast symptoms in leaf, neck, finger and seed parts of the plant.

C.3. Field screening of finger millet recombinant inbred lines (RILs) and different parents

Regular field observations of the disease severity and incidence in different lines and cultivars help the breeders to select the better genotypes for the farmers. In Farmers' Participatory Varietal Selection (FPVS) trials, the high performing genotypes can easily be chosen and accepted. Therefore, the field screenings or trials by taking the selected and improved genotypes should be given priority in a resistance-breeding programme.

C.3.1. Occurrence of leaf, neck and finger blast disease in 8 RILs and two parents of finger millet at two different locations

Results of this study (Table 6) clearly reveal that among all the genotypes studied, no lines showed immune response to leaf blast severity. Lowest leaf blast severity of 3.35% was noticed in ML 264 at Hatna and 3.00% in ML 181 at Alugona. In comparison, ML 349 showed highest leaf blast severity at both the locations figuring 12.30 and 10.20%, respectively.

For neck blast disease incidence, ML 298 showed 3.30% at Hatna and ML 224 showed 3.03% at Alugona. However, ML 181 also showed a lower neck blast severity at both the locations. In comparison, ML 349 again showed a high level of neck blast incidence at both the locations figuring 53.33 and 51.67%, respectively. Interestingly, the parent GPU 28 showed immune response to the neck blast at both the locations.

For finger blast disease severity, the result showed that ML 181 showed as low as 4.33% at Hatna and ML 371 showed 3.33% finger blast severity at Alugona. In comparison, ML 349 again showed the highest degree of severity at both the locations figuring 71.67 and 64.67%, respectively. The parent GPU 28 showed immune response to finger blast at Alugona.

In the above results it can be easily inferred that, the line ML 181 has got the general resistance for all the three different types of blast diseases and in contrast, ML 349 has the proneness to leaf, neck and finger blast at both the locations. Also it can be seen that both the parents, Indaf-5 and GPU 28 are combating well against blast at all the three different stages.

Fig. 11. Photograph shows the susceptible reaction of the host after seven days of the inoculation with blast pathogen.

Fig. 12. Photograph shows the susceptible (left) as well as resistance (right) reaction of the differential hosts to the same blast isolate.

Fig. 13. Photographs showing reactions of six elite ragi cultivars to six different blast isolates originating from various sources.

(Isolate 1: AN-19, Isolate 2: AL-28, Isolate 3: M-1, Isolate 4: AF-18, Isolate 5: Singha-1, Isolate 6: Suradev-1)

Table 5. Overall reaction of six cultivars inoculated with twelve blast isolates in controlled environment conditions.

Sl. No.	Isolates	Parents					
		IE 2912	Indaf-5	GPU 28	Indaf-9	IE 1012	IE 2885
1	AN-19	4	4	3	5	3	4
2	AL-28	4	4	4	4	3	4
3	M-1	3	5	3	5	3	3
4	AF-18	5	3	4	5	3	3
5	Singa-1	3	5	4	5	4	4
6	Suradev-1	4	5	4	3	3	4
7	AL-4	4	4	3	4	2	4
8	AL-7	4	4	4	4	2	4
9	AL-12	4	4	4	4	3	4
10	AL-14	4	4	4	4	2	4
11	AS-21	4	4	4	4	3	4
12	AN-28	4	4	4	4	2	4
Mean		3.92	4.20	3.75	4.25	2.75	3.83
Reaction		S	HS	S	HS	MR	S

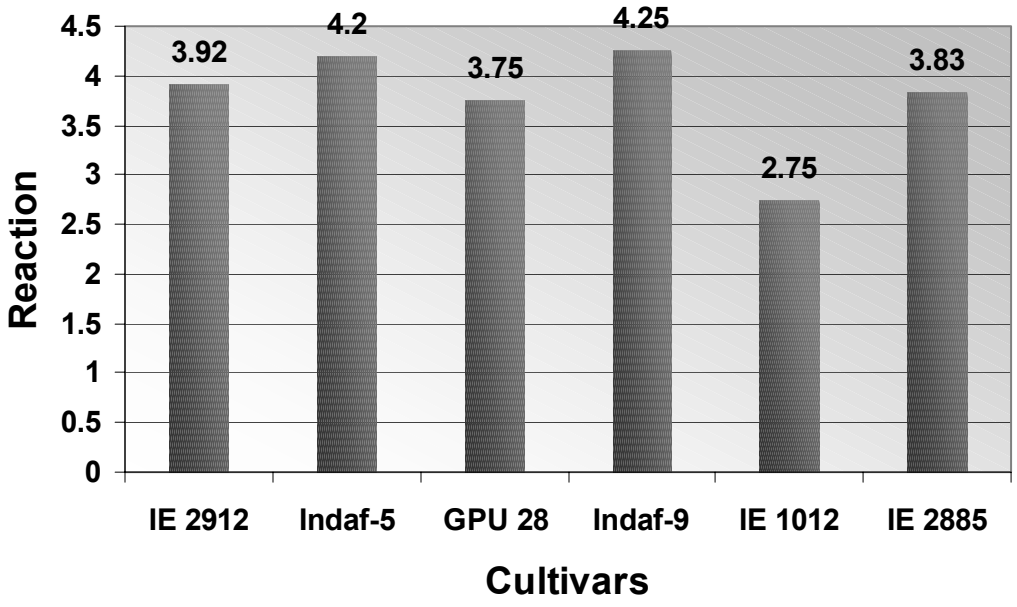

Fig. 14. Graphical presentation of the overall reaction of six elite ragi cultivars to twelve blast isolates in controlled environment conditions.

Table 6. Comparison of leaf blast, neck blast and finger blast (%) in 8 selected RILs and 2 parents of finger millet at two locations

Sl. No.	RILs	Leaf Blast (%)		Neck Blast (%)		Finger Blast (%)	
		Hatna	Alugona	Hatna	Alugona	Hatna	Alugona
1	264	3.35	4.10	8.56	11.50	17.00	17.67
2	531	6.50	6.10	10.16	13.00	25.00	22.33
3	371	4.58	4.08	13.87	3.67	6.33	3.33
4	181	3.55	3.00	4.43	3.10	4.33	14.67
5	224	8.50	10.00	4.10	3.03	17.67	14.00
6	298	8.20	7.60	3.30	8.83	13.67	10.67
7	330	6.30	7.20	22.83	24.27	21.00	24.33
8	349	12.3	10.20	53.33	51.67	71.67	64.67
9	Indaf-5	4.30	5.00	2.13	3.77	3.00	2.33
10	GPU 28	3.52	4.70	0.00	0.00	4.33	0.00
SEM ±							
CD@ 0.5%							

C.3.2. Occurrence of leaf, neck and finger blast disease in 26 RILs and four parents of finger millet at four different locations

Results of the study (Table 7) indicated that no RIL's were immune to leaf blast at different locations. In Ardeshnahalli, ML 553 and ML 302 showed a lowest leaf blast severity of 2.0%, which was followed by 2.3% in ML 203 and ML 343. In Jakkasandra, lowest leaf blast severity of 1.8% was noticed in ML 553 followed by 2.2% in ML 197 and ML 255. At Shettigere, lowest leaf blast severity of 1.0% was noticed in ML 531 and IE 1012 followed by 1.2% in ML 300, ML 371, ML 430 and Indaf-5. At All India Coordinated Small Millets Improvement Project (AICSMIP), lowest leaf blast severity of 1.0 per cent was noticed in IE 1012, followed by 1.3% in ML 32 and ML 430. Mean leaf blast severity across locations was 4.60% in ML 264, followed by ML 349 (4.60 %) and ML 298 (4.55 %). Highest leaf blast severity in experimental plots recorded was 7.0, 4.7, 4.2 and 4.7% respectively, in Aradeshahalli, Jakkasandra, Shettigere and AICSMP(GKVK) locations. However, due to failure of monsoon in all the locations the disease pressure was not high as expected.

Complete absence of NB was noticed in the RIL's i.e., ML 31, ML 197, ML 265, ML 300, ML 322, ML 343 and ML 434 at Aradeshahalli (Table 8). Among others, lowest neck blast incidence of 2.60% was recorded in ML 255, ML 302 and in IE 1012. At Jakkasandra, ML 197, ML 255, ML 280, ML 300, ML 302, ML 322, ML 434, ML 531 and GPU 28 have showed no incidence of neck blast. Whereas, among others, ML 426 had lowest neck blast incidence of 2.40% followed by ML 343 (2.50 %), ML 297 (3.10 %). In Shettigere, ML 197, ML 343, ML 426, ML 443, ML 531 and GPU 28 showed no neck blast disease incidence. Among others, lowest neck blast incidence of 1.20% was recorded in IE 1012 followed by 1.6 and 2.1% in Indaf-5 and ML 322, respectively. At AICSMIP (GKVK), ML 197, ML 265, ML 392, ML 426, ML 430 and ML 434 had showed immune response to neck blast. Among others, ML 171, ML 255 and ML 553

had 2.60% of disease incidence. Highest disease incidence was recorded in the experimental field was 60.10, 33.40, 47.20, and 35.60%, respectively at Aradeshahalli, Jakkasandra, Shettigere and AICSMP (GKVK) locations. Mean of neck blast disease seen that both the parents, Indaf-5 and GPU 28 are combating well against blast at all the three different stages.

Table 7: Comparative data of Leaf Blast Severity (%) in 26 RILs and four parents at different locations

Sl. No	RILs	Leaf Blast Severity (%)				
		Aradeshahalli	Jakkasandra	Shettigere	GKVK	Mean
1	Ml31	2.7	2.3	-	2.3	2.25
2	Ml32	3.0	2.7	-	1.3	2.33
3	ML171	3.3	3.0	2.5	2.3	2.77
4	ML181	4.3	2.8	2.2	2.3	2.90
5	ML197	3.0	2.2	2.4	2.0	2.40
6	ML203	2.3	2.0	2.3	3.3	2.47
7	ML208	2.7	2.6	2.2	2.0	2.37
8	ML224	6.3	3.8	3.0	3.3	4.10
9	ML255	2.7	2.2	2.0	2.0	2.22
10	ML264	6.7	4.7	3.6	4.0	4.75
11	ML265	3.3	3.2	3.2	3.0	3.17
12	ML280	3.3	3.0	2.1	2.7	2.77
13	ML297	4.3	2.4	2.5	2.7	2.97
14	ML298	5.7	3.8	4.0	4.7	4.55
15	ML300	4.7	3.7	1.2	2.0	2.90
16	ML302	2.0	1.8	2.5	2.7	2.25
17	ML330	5.7	3.1	1.5	1.7	3.00
18	ML322	4.7	4.2	2.2	2.0	3.27
19	ML343	2.3	2.0	2.5	2.3	2.27
20	ML371	4.0	3.7	1.2	1.7	2.65
21	ML392	4.0	3.8	2.2	2.7	2.65
22	ML426	2.7	2.0	4.2	3.0	2.97
23	ML430	2.7	3.7	1.2	1.3	2.22
24	ML434	3.7	3.5	2.5	2.7	3.10
25	ML531	4.3	3.7	1.0	2.0	2.75
26	ML553	2.0	1.8	2.0	2.7	2.12
27	IE 1012	3.7	3.1	1.0	1.0	2.20
28	Indaf-5	5.3	4.2	1.2	2.7	3.35
29	GPU-28	3.7	3.6	1.8	2.0	2.77
30	ML349	7.0	4.2	3.5	3.7	4.60
SEM+/-		0.1752	0.0961	0.1005	0.1318	
Cd at 0.5%		0.4972	0.2738	0.3087	0.3727	

Table 8. Comparative data of Neck Blast Incidence (%) in 26 RILs and four parents at different locations

Sl. No	RILs	Neck blast incidence (%)				
		Aradeshahalli	Jakkasandra	Shettigere	GKVK	Mean
1	Ml31	0.0	-	-	8.0	
2	Ml32	2.7	6.3	-	4.0	4.33
3	ML171	5.1	6.1	3.3	2.6	4.27
4	ML181	7.4	9.4	11.2	3.9	7.97
5	ML197	0.0	0.0	0.0	0.0	0.0
6	ML203	6.6	4.0	5.0	10.8	6.42
7	ML208	4.1	6.2	2.5	2.9	3.92
8	ML224	20.2	32.3	20.3	27.5	25.07
9	ML255	2.6	0.0	2.3	2.6	1.87
10	ML264	44.0	14.6	38.2	20.4	29.30
11	ML265	0.0	4.2	4.4	0.0	2.15
12	ML280	4.7	0.0	2.8	5.1	3.15
13	ML297	5.0	3.1	2.7	4.8	3.90
14	ML298	48.5	19.5	42.3	16.3	31.65
15	ML300	0.0	0.0	4.9	4.8	2.42
16	ML302	2.6	0.0	2.5	8.6	3.42
17	ML330	29.9	19.0	28.2	32.6	27.42
18	ML322	0.0	0.0	2.1	5.8	1.97
19	ML343	0.0	2.5	0.0	6.1	2.15
20	ML371	4.0	8.7	20.1	8.0	10.20
21	ML392	2.7	4.9	2.1	0.0	2.42
22	ML426	5.8	2.4	0.0	0.0	2.05
23	ML430	6.2	4.0	2.2	0.0	3.10
24	ML434	0.0	0.0.	0.0	0.0	
25	ML531	5.0	0.0	0.0	5.5	2.62
26	ML553	4.2	5.0	3.8	2.6	3.90
27	IE 1012	2.6	3.2	1.2	4.2	2.8
28	Indaf-5	8.3	11.3	1.6	6.1	6.82
29	GPU-28	4.8	0.0	0.0	4.5	2.32
30	ML349	60.1	33.4	47.2	35.6	44.07
SEM+/-		1.2146	0.4895	0.6606	0.4143	
Cd at 0.5%		3.4355	1.3845	1.8686	1.1717	

Disease incidence recorded in all the locations clearly revealed that ML 197 and ML 434 were having immune response and ML 349 which had an average disease incidence of 44.07% showed susceptible reaction to neck blast.

The finger blast severity was nil in ML 265 at Aradeshahalli (Table 9). Lowest finger blast severity of 1.30% was noticed in ML 322, which was followed by 2.0% in ML 208 and ML 392. In Jakkasandra ML 171, ML 208, ML 255, ML 280, ML 553 showed immune response to finger blast where as, among others ML 265 showed lowest finger blast severity of 2.0% and was followed by 2.30, 2.70 and 2.70% in ML 300, ML 392 and IE 1012, respectively. In contrast, the line ML 349 showed as high as 76.70% of disease severity. At Shettegere, no RILs showed immune response to finger blast and a lowest of 2.30% was noticed in ML 430 and Indaf-5. At AICSMIP (GKVK), ML 171, ML 255, ML 265, ML 300, ML 302, ML 343 and IE 1012 showed immune response to finger blast. Among others, lowest severity of 1.30% was noticed in ML 392 followed by 1.70% in ML 203, 2.0% in ML 434 and ML 553 and 3.0% in ML 280, ML 297 and ML 531. Highest finger blast severity of 80.0, 35.0, 85.0, and 86.00% was recorded in the plots at Aradeshahalli, Jakkasandra, Shettigere and AICSMP(GKVK), respectively.

In the present study, most of the purple colored recombinant inbred lines showed more susceptible reactions to neck and finger blast. The lines ML 31, ML 32, ML 171, Ml 181, ML 197, ML 203, ML 208, ML 255, ML 265, ML 280, ML 300, ML 302, ML 322, ML 343, ML 371, ML 392, ML 426, ML 430, ML 434, ML 531, ML 553 and parents IE 1012, Indaf -5 and GPU 28 were showing resistance to both neck and finger blast disease. This type of observations has not been generated in finger millet RIL's against blast diseases by any worker. However, a few reviews are available on other finger millet germplasm lines screened against blast disease (Vishwanath *et al.,* 1986: Ravikumar *et al.,* 1990 and Mantur *et al.,* 2001).

C.3.3. Occurrence of leaf, neck and finger blast disease in the entire population of RILs at three different locations

In order to see the field resistance of all the recombinant inbred lines (RILs) against blast disease, an experiment was conducted at three locations namely Shettigere, Hebbal and GKVK. The result after evaluation revealed that, all the RILs showed either highly resistance or resistance reaction to leaf blast at three locations (Table 10, Fig. 15). There were no lines showing immunity and susceptibility to leaf blast at these locations. Strikingly, a maximum number of RILs were showing immunity towards the neck blast disease incidence at all these locations was observed (Table 11, Fig. 16). A maximum of 42 lines showed moderate to high degree of susceptibility at GKVK followed by 38 at Shettigere and 33 at Hebbal. Similarly, maximum proportion of RILs showed immunity or resistance to finger blast disease at all the three locations (Table 12, Fig. 17). However, a maximum of 73 lines were showing susceptibility to finger blast at Shettigere followed by 49 at Hebbal and 35 at GKVK.

Table 9. Comparative data of Finger Blast Severity (%) in 26 RILs and four parents at different locations

Sl. No	RILs	Fingerblast Severity (%)				
		Aradeshahalli	Jakkasandra	Shettigere	GKVK	Mean
1	ML 31	7.2	-	-	8.5	
2	ML 32	3.8	5.0	-	7.3	4.02
3	ML 171	5.3	0.0	11.3	0.0	4.15
4	ML 181	9.7	5.7	3.0	5.3	5.92
5	ML 197	3.7	6.7	10.7	4.0	6.27
6	ML 203	2.3	8.3	11.3	1.7	5.9
7	ML 208	2.0	0.0	5.0	9.0	4.0
8	ML 224	58.3	26.7	48.3	40.7	43.5
9	ML 255	7.3	0.0	9.3	0.0	4.15
10	ML 264	56.7	35.0	45.7	36.3	43.42
11	ML 265	0.0	2.0	6.0	0.0	2.00
12	ML 280	4.0	0.0	9.3	3.0	4.07
13	ML 297	3.7	4.0	9.7	3.0	5.10
14	ML 298	70.0	31.7	63.5	66.7	57.97
15	ML 300	6.3	2.3	4.0	0.0	3.15
16	ML 302	6.3	6.0	4.3	0.0	4.15
17	ML 330	41.0	15.7	15.7	38.7	27.6
18	ML 322	1.3	9.7	4.7	3.3	4.75
19	ML 343	3.0	3.7	7.0	0.0	3.42
20	ML 371	10.7	4.0	7.0	7.0	7.17
21	ML 392	2.0	2.7	10.7	1.3	4.17
22	ML 426	5.0	5.0	10.3	8.7	7.25
23	ML 430	6.0	5.3	2.3	4.3	4.47
24	ML 434	4.3	3.0	9.7	2.0	4.75
25	ML 531	6.7	4.7	4.7	3.0	4.78
26	ML 553	4.3	0.0	8.7	2.0	3.75
27	IE 1012	3.7	2.7	10.0	0.0	4.10
28	Indaf-5	13.0	10.3	2.3	6.0	7.9
29	GPU-28	3.0	10.3	6.3	10.7	7.9
30	ML 349	80.0	76.7	85.0	86.00	81.92
SEM +/-		1.3489	0.8903	08868	1.2601	
Cd at 0.5%		3.8158	2.5181	2.5082	3.7648	

C.3.4. Evaluation of the entire population of 300 RILs and four parents by artificial inoculation with two different blast isolates in controlled environment

With a view to evaluate the proportion of lines showing differential reaction to different isolates in controlled environmental condition, the whole population of 300 RILs and four parents were artificially inoculated with two different blast isolates namely B57 and B77 in *Kharif* 2003. The results obtained clearly shows that for both the isolates the proportions of differential reactions for leaf blast in the entire population were almost similar (Fig. 18). Unlike the occurrence of natural leaf blast under field conditions, artificial inoculation studies for two isolates generated some paradoxical results showing a high proportion of susceptibility in the population of RILs. There were 105 lines showing resistance and 199 lines showing susceptibility to both the isolates.

Table 10. Field resistance of 300 RILs and four parents in different locations against leaf blast

Reaction (%)	Shettigere	Hebbal	GKVK
I (0.0)	0	0	0
HR (0.1-5)	300	302	297
R (5.1-10)	4	2	7
MS(10.1-25)	0	0	0
S (>25)	0	0	0

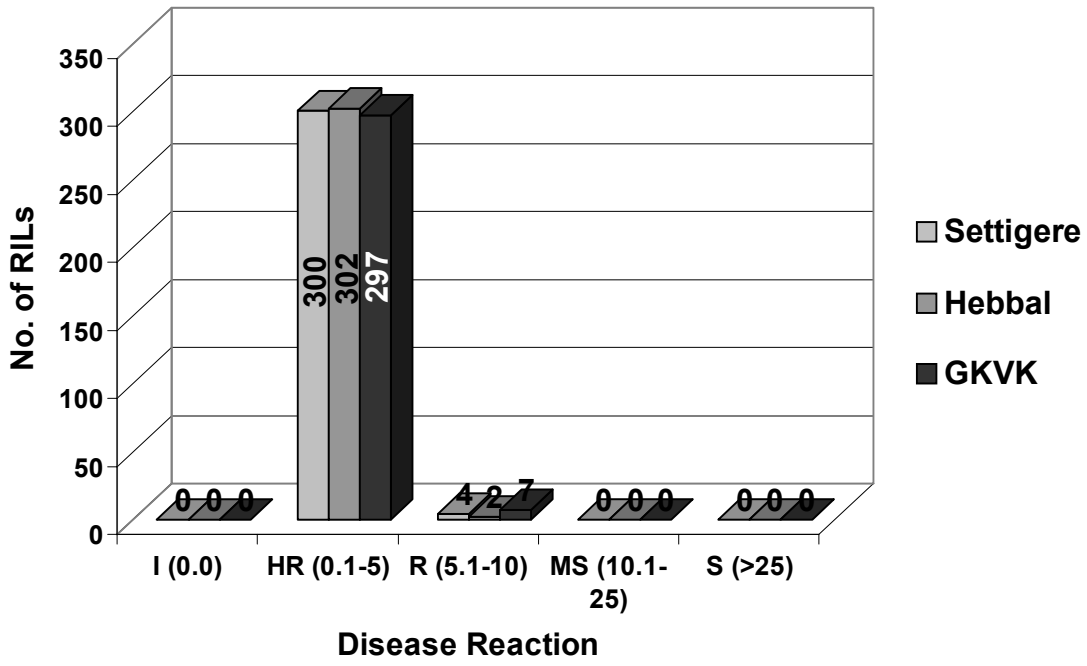

Fig. 15. Field resistance of 300 RILs and four parents in different locations against leaf blast

I: Immune; HR: Highly Resistance; R: Resistance; MS: Moderately Susceptible; S: Susceptible

Table 11. Field resistance of 300 RILs and four parents in different locations against neck blast

Reaction (%)	Shettigere	Hebbal	GKVK
I (0.0)	135	204	134
HR (0.1-5)	97	42	70
R (5.1-10)	34	25	58
MS(10.1-25)	31	18	31
S (>25)	7	15	11

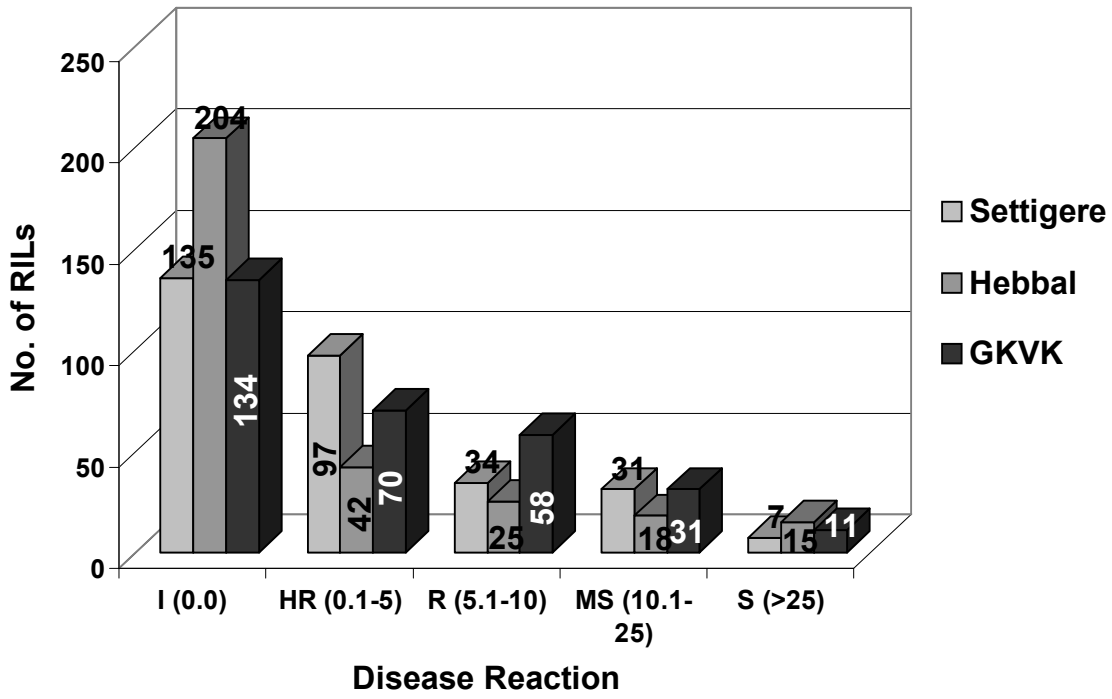

Fig. 16. Field resistance of 300 RILs and four parents in different locations against neck blast

I: Immune; HR: Highly Resistance; R: Resistance; MS: Moderately Susceptible; S: Susceptible

Table 12. Field resistance of 300 RILs and four parents in different locations against finger blast

Reaction (%)	Shettigere	Hebbal	GKVK
I (0.0)	46	102	48
HR (0.1-5)	126	122	194
R (5.1-10)	55	32	27
MS(10.1-25)	47	22	23
S (>25)	26	27	12

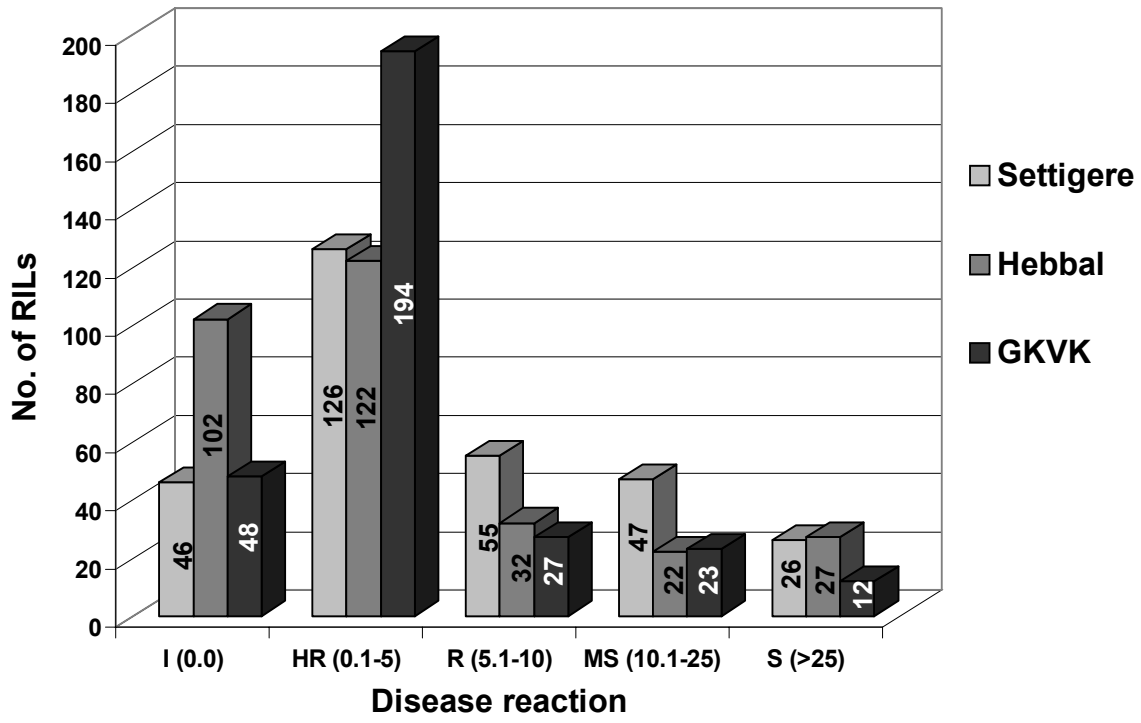

Fig.17. Field resistance of 300 RILs and four parents in different locations against finger blast

I: Immune; HR: Highly Resistance; R: Resistance; MS: Moderately Susceptible; S: Susceptible

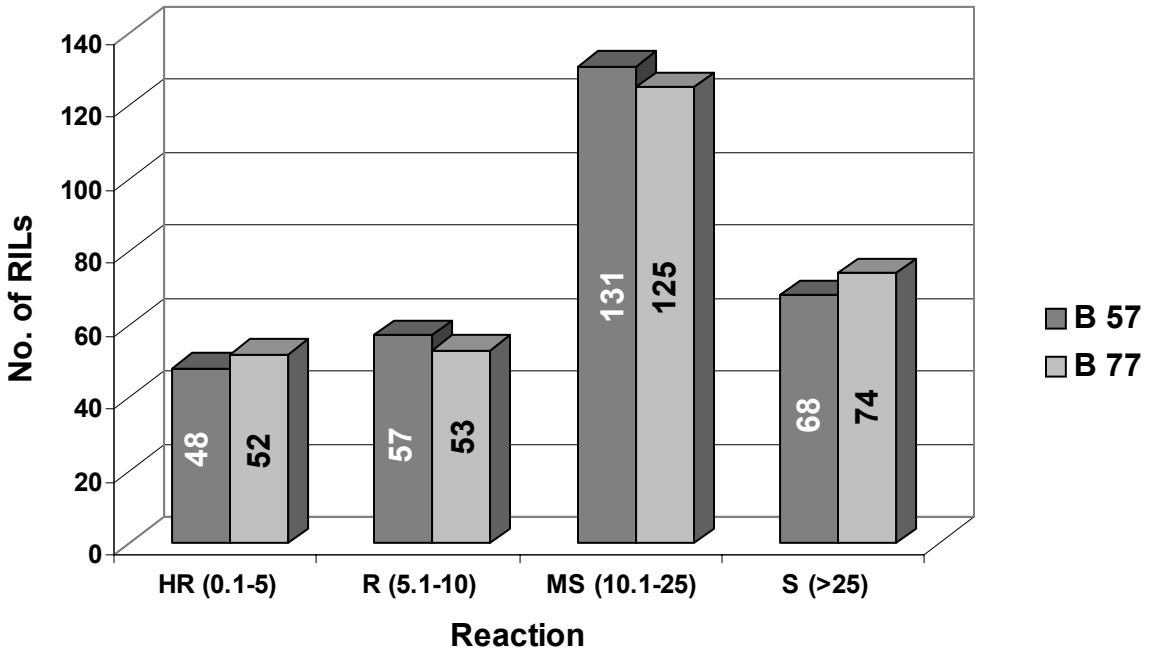

Fig. 18. Artificially inoculated 300 RILs and four parents with isolates B57 and B 77 showing differential leaf blast reaction

References

Anonymous, 1995. Package of practices for high yielding varieties, UAS, Bangalore.

Anonymous, 1996. Annual report for 1995-96. All India Coordinated Small Millets Improvement Project, UAS, ICAR, Bangalore – 65.

Atkins, J.G.; Robert, A.L.; Adair, C.R.; Goto, K.; Kozaka, T.; Yanagida, R.; Yamada, M. and Matsumoto, S. 1967. An International set of rice varieties for differentiating races of *Pyricularia oryzae*. Phytopathology 57: 297-301.

Bonman, J.M.; Vergel de Dios, T.I. and Khin, M.M. 1986. Physiologic specialization of *Pyricularia oryzae* in the Philippines. Plant Dis. 70: 767-769.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44: 223-270.

Ling, K.C. and Ou, S.H. 1969. Standardization of the international race numbers of *Pyricularia oryzae*. Phytopathology 59: 339-342.

Mackill, D.J. and Bonman, J.M. 1992. Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology 82: 746-749.

Mantur, S.G., Vishwanath, S. and Anilkumar, T.B., 2001. Evaluation of finger millet genotypes for resistance to blast. *Indian Phytopath.* 54(3): 38-41.

Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. Nucl. Acids Res. 8: 4321-4325.

Ou, S.H. 1972. Rice Diseases. Commonwealth Mycol. Inst., Kew, Surrey, England.

Ou, S.H. 1985. Rice diseases, 2nd ed. Commonwealth Agric. Bureaux., p. 380, Central Sales. Farnham Royal, Slough, U.K.

Ravikumar, R.L., Seetharam, A. and Shankare Gowda, B.T., 1990. Identification of sources of stable resistance to finger millet (*Eleusine coracana* (L) Gaertn.) blast. *SABRAO J.,* 22:117-121

Rossman, A.Y.; Howard, R.J. and Valent, B. 1990. *Pyricularia grisea*, the correct name of the rice blast disease fungus. Mycologia 82: 509-512.

Scott, R.P.; Zeigler, R.S. and Nelson, R.J. 1993. A procedure for miniscale preparation of *Pyricularia grisea* DNA. Int. Rice Res. Notes 18(1): 47-48.

Sharma, T.R.; Chauhan, R.S.; Singh, B.M; Paul, R.; Sagar, V. and Rathour, R. 2002. RAPD and pathotype analyses of *Magnaporthe grisea* populations from the north-western Himalayan region of India. J. Phytopathol. 150: 649-656.

Sridhar, R. and Singh, U.D. 2001. Genetic and pathogenic diversity of the rice blast pathogen. In: Major Fungal Diseases of Rice, Recent Advances (Sreenivasaprasad, S. and Johnson, R. eds.), pp. 1-7, Kluwer Academic Publishers, Netherlands.

Sridhar, R.; Singh, U.D.; Agrawal, P.K.; Reddy, J.N.; Chandrawanshi, S.S.; Sanger, R.B.S.; Bhatt, J.C.; Rathaiah, Y. and Row, K.V.S.R.K. 1999. Usefulness of blast resistance genes and their combinations in different blast-endemic locations in India. Intern. Rice Res. Notes 24(2): 22-24.

Viji, G.; Gnanamanickam, S. and Levy, M. 2000. DNA polymorphisms of isolates of *Magnaporthe grisea* from India that are pathogenic to finger millet and rice. Mycol. Res. 104: 161-167.

Vishwanath, S., Sanne Gowda, S., Seetharam, A. and Shankare Gowda, B.T., 1986. Reaction to blast disease of released and pre-released varieties of finger millet from different states. *Millet Newsl.,* 5: 31

Wheeler, B.E.J., 1969. *An introduction to plant disease*. John Wiley and Sons Ltd., London, 301pp.

Xia, J.Q., Correll, J.C., Lee, F.N., Marchetii, M.A. and Rhodes, D.D. 1993. DNA fingerprinting to examine microgeographic variation in the *Magnaporthe grisea* (*Pyricularia grisea*) populations in two rice fields in Arkansas. Phytopathology 83: 1029-1035.

Yoshida, S. 1981. Fundamentals of Rice Crop Science, p. 269, Int. Rice Res. Inst. Los Banos, Philippines.

1. Molecular mechanism of finger millet genotypes in responses to infested by *Magnaporthe grisea* isolate tosa #50

1.1 Project design

Somasehkhara *et. al* (1992) previously found that Finger millet genotype IE1012 is free from neck and finger blast infections as well as highly resistant to three other fungal and two viral diseases. We further found that genotype IE1012 and another genotype INDAF-5 showed a quantitative difference in disease density when 4-5-week seedlings were inoculated with ***Magnaporthe grisea*** isolate strain tosa #50. For example, IE1012 showed fewer lesions, smaller lesions, less chlorosis surrounding the lesions on leaves and most importantly, no infection of leaf sheaths.

To understand the molecular mechanism underlying this quantitative difference in the fungal infection responses, we used microarrays to detect ***Magnaporthe grisea*** infection responding genes from both ***Magnaporthe grisea*** and finger millet. The Magnaporthe/Rice Long Oligonucletide Chip (G4137A, 60mer) from Agilent Company consists of 7,415 rice genes and 13,666 Magnaporthe genes. Genotypes IE1012 and INDAF-5 were grown in the growth chamber, ~4-week plants were inoculated with *Magnaporthe grisea* strain tosa #50. Samples (leaves and shoots) were collected from infected plants at the time points of 45hr, 72hr and 4days after inoculation, as well as from healthy plants growing under the same conditions. Total RNA was isolated from these samples with a Qiagen plant mini kit and purified using the DNA-free System from Ambion Company. RNA samples were first checked for purity using denaturing gel electrophoresis, then labeled with an Agilent Fluorescent Direct Labeling Kit and hybridized to the Agilent chips for 17 hours following Agilent's recommended protocol. The experimental design is showed in Table 1:

Table 1. Experimental design for Chip hybridization experiments

Array	Cy-3 labeled RNA sample	Cy-5 labeled RNA sample	Type
1	Fingermillet IE1012, 45hr –healthy	Fingermillet IE1012, 45hr –infected	Differential
2	Fingermillet INDAF-5, 45hr -healthy	Fingermillet INDAF-5, 45hr – infected	Differential
3	Fingermillet IE1012, 72hr –healthy	Fingermillet IE1012, 72hr – infected	Differential
4	Fingermillet INDAF-5, 72hr -healthy	Fingermillet INDAF-5, 72hr – infected	Differential
5	Fingermillet IE1012, 4d –healthy	Fingermillet IE1012, 4d – infected	Differential
6	Fingermillet INDAF-5, 4d –healthy	Fingermillet INDAF-5, 4d – infected	Differential

1.2 Results

To analyze Agilent chip data for which no replicate is yet available, except for one experiment (two chips were hybridized with IE1012 RNA at 45hr), we set up our standard to define genes showing significant expression changes in infected samples

relative to healthy samples, including: (1) the fluorescence intensity for a given gene in disease samples should undergo at least 3-fold change relative to that in healthy samples, and (2) the p-value of log ratio (red channel vs. green channel) for that gene should not be bigger than 0.05. Based on these two criteria, we found many finger millet and *Magnaporthe* genes could be involved in the fungal-host interaction at the time of 45hr, 72hr and 4days after inoculation, respectively (Tables 2, 3).

Table 2. Finger millet response genes during fungi-host interaction

Genotype: IE1012	45 hr	72 hr	4 d
Significantly up-regulated	364	159	1
Significantly down-regulated	2	5	133
Non-Changed	6778	6980	7010
Total	7144	7144	7144

Genotypel: INDAF-5	45hr	72hr	4d
Significantly up-regulated	239	157	1
Significantly down-regulated	2	0	217
Non-Changed	6903	6897	6926
Total	7144	7144	7144

Table 3. Expressed *Magnaporthe grisea* genes during fungi-host interaction

Genotype: IE1012	45hr	72hr	4d
Significantly expressed	1341	885	1
Non-expressed	12325	12781	13665
Total	13666	13666	13666

Genotype: INDAF-5	45hr	72hr	4d
Significantly expressed	1035	874	2
Non-expressed	12631	12792	13664
Total	13666	13666	13666

1.2.1 Responses of blast developing processes in IE1012 and INDAF-5 genotypes

Based on the chip data analysis, we found that IE1012 and INDAF-5 have somewhat different mechanisms involved during infection. More host and fungal genes are responding to infection by 45hr through 72hr after inoculation. As far as the difference in genotypes, more host and fungal genes are detected to be up-regulated or expressed in IE1012 than in INDAF-5, which we will discuss in more detail.

1.2.1.1 Host responses

A total of 414 unique finger millet genes are detected to be up-regulated in the host-fungus interaction at the studied time points in both genotypes (appendix 1, 414 genes). Among them, 126 genes are commonly up-regulated in both genotypes. In detail, at 45hr after inoculation, 364 host genes are up-regulated in IE1012 but only 239 such genes in INDAF-5. In both IE1012 and INDAF-5, 201 host genes are commonly up-regulated. A total of 163 genes are detected as specifically up-regulated in IE1012 while only 38 genes are specifically up-regulated in INDAF-5, which could account for their differential resistance to isolate tosa#50. These genes and their functional descriptions are attached as an appendix in this report (appendix 2, 163 genes, appendix3, 38 genes). At 72hr after inoculation, 159 host genes are up-regulated in IE1012 and 157 such genes in INDAF-5. A total of 137 genes are commonly up-regulated in both genotypes, while 22 genes are specifically up-regulated in IE1012, and 20 genes specifically up-regulated in INDAF-5.

A total of 315 unique finger millet genes are down-regulated at the studied time points in both genotypes, most of which are detected at 4days after inoculation.

1.2.1.2 Fungal responses

At 45hr after inoculation, 1,341 *Magnaporthe grisea* genes are expressed in IE1012 during disease development, while only 1,035 genes are expressed in INDAF-5. One thousand and three genes were commonly expressed in both genotypes. At 72hr after inoculation, 885 *Magnaporthe grisea* genes are expressed in IE1012 during disease development, and 874 genes are expressed in INDAF-5. In both genotypes, 831 genes are commonly expressed. It will be interesting to see if differences are more dramatic at later times during sheath and finger development where strong resistance is observed. Also whether sporulation is restricted in IE1012 lesions found on leaves thus producing a lower inoculum for secondary cycles of infection on the sheath and fingers.

1.2.2 Homolog identification in finger millet EST data set against finger millet infection response genes

We formatted 197 finger millet TCs (XXXX) provided by Dr. Katrien Devos at the University of Georgia as a blastable database. We then used 414 genes that are upregulated in the host-fungal interaction as a query to search the above database using the blastn program. Six homologs were found that are 36 to 52 bp identical over the 60 bp rice oligonucleotide representing each spot on the chip (Table 4).

Table 4. Possible homologs between finger millet and rice

Finger Millet (FM) TC	FM length	FM start	FM end	Rice Feature (RF)	RF length	RF start	RF end	E value	Identity
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Contig41	405	336	375	A_97_P28647	60	1	40	4.00E-18	40/40

2. Large scale screening of Single Feature Polymorphisms (SFPs) with NimbleGen high density chip

2.1 Project design and pilot study

A subset of the rice full length cDNA collection showing high amino acid identity with Arabidopsis genes was used to design an oligonucleotide chip with features of 24 bp long. Data analysis from a pilot of genotyping arrays fabricated with NimbleGen Technology showed that 3,709 Nipponbare (reference genome) features had hybridization intensity values significantly higher than corresponding *Oryza Rufipogon* features at the False Discovery Rate of (FDR) 7.0 %.

2.1.1 Properties of features that are putative SFPs

Since microsatellites are highly abundant, repeat-containing regions found in plants, the 156,777 non putative SFPs and 3,709 putative SFPs were searched for ones that contain stretches of polynucleotides (N3-6). The results showed that the occurrence of longer polynucleotides in putative SFPs is much more frequent than in other non-SFP features, demonstrating that the particular class of sequences with nucleotide repeats would show higher rates of SFPs (Table 5), and thus more likely to be useful markers.

Table 5. Properties of features that are SFPs

Polynucleotides	3N	4N	5N	6N
Putative SFPs (3709 features, %)	67.8	25.0	6.47	1.35
Non-SFPs (156777 features, %)	62.7	18.2	1.51	0.12

2.2 SFPs validation with PCR and traditional sequencing

Initially, 12 pairs of primers were designed based on Nipponbare genomic sequences (where the cDNAs are mapped to) to validate the 12 putative SFPs with the most extreme hybridization intensity changes. Only seven out of 12 were amplified successfully from both Nipponbare and Rufipogon genomes, of which six were confirmed to be correctly ordered within the gene homologs in Rufipogon. The remaining five were difficult to amplify even from the reference genome. Most of these regions turned out to be in areas with low sequence quality in the genomic DNA sequence of the BACs of origin. Sequence comparisons of the successfully amplified 7 homologs in Rufipogon confirmed 3 putative SFPs, 3 false positives and 1 wrong product (should be amplified from chromosome 3, but it was turned out from chromosome 12). Since this conclusion is based on single pass sequence data, more PCR and sequencing must be done.

Primers were then designed from the exonic regions of thirteen putative SFP-containing genes to validate an additional 26 SFPs which were randomly selected from the 3,709 putative ones. Twelve homologs in Rufipogon were successfully amplified and gel extracted for sequencing. However, sequence comparisons only confirmed 2 SFPs in one homolog. One is deletion of 1 base pair, and another is single base polymorphism. Whole sequence alignments did find other SNPs, small deletions and insertions (up to 3 bases) in non-feature regions of other cloned homologs, thus excluding the possibility of template contamination by the reference genome.

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Based on the above facts, we will refine our chip design with regard to the following. First, we will switch to various sequence sources rather than only full-length cDNAs, such as sequences from intergenic, untranslated, intronic and CDS regions in rice which was released by TIGR at the end of April, 2004. This diversity of sequence sources could reveal the preference of SNP occurrence in rice genome. Secondly, instead of only choosing *O.Rufipogon* as the target genome, we will try some indica cultivars such as GLA4, a typical indica variety, in which 9,056 SNPs were found in 2.3MB of chromosome 4 and their collinear sequence from Nipponbare (Han, et al, 2003; Feng, et al, 2002). We will also try some wild rice varieties, which are genetically distant to japonica. Finally, we will refine the target DNA preparation process, and raise the hybridization stringency to achieve the better specificity. Once these methods are established, we will employ the growing number of ESTs generated from UGA for probe design. We have obtained the putative, diploid parents of *E. coracana* and will use these as well as the lines being employed to develop maps of Eleusine in this project to discover SFPs.

References

1. Somasehkhara *et. al,* 1992. Trop. Agric (Trinidad) 69:293-295

2. Nasu, S. *et. al* 2002. Search for and Analysis of Single Nucleotide Polymorphisms (SNPs) in rice (Oryza sativa, Oryza, rufipogon) and Establishment of SNP Markers. DNA Res. 9: 163-171

3. Han, B. *et. al* 2003. Genome-wide Intraspecific DNA-sequence Variations in Rice. Current Opinion in Plant Biology, 6: 134-138

4. Feng, Q. *et. al* 2002. Sequence and Analysis of Rice Chromosome 4. Nature, 420: 316-320

Objective4:
Development of SSR markers and other markers

DEVELOPMENT OF HIGH YIELDING, DISEASE RESISTANT AND DROUGHT TOLERANT FINGER MILLET (*Eleusine coracana* Gaertn.) GENOTYPES

Report Year 2 (May 1st 2003 – April 30th 2004)
Katrien M. Devos (JIC/UGA)

Research and training progress report

1. Development of microsatellite markers for finger millet

As stated in the first year report, the development of STMP microsatellites has been discontinued. In a new approach, three random genomic libraries were constructed of DNA from the finger millet accession PI 321125. The DNA was digested separately with *Hin*dIII, *Pst*I and *Sal*I, and fragments in the range 1 – 1.5 kb were cloned in the pcr4TOPO (Invitrogen) vector. A total of 18,431 clones (3072 HindIII, 6144 SalI and 9,216 PstI clones) were gridded in duplicate on a nylon membrane. The membrane was hybridized to the oligonucleotides $(AG)_{15}$ + $(AC)_{15}$, $(AAG)_{10}$ + $(AAC)_{10}$ + $ATC)_{10}$, $(AGC)_{10}$ + $(AGG)_{10}$ and (CCG)10. Three hundred and eighty four positive clones were sequenced. Thresholds for definition of a SSR were 10 and 7 repeat units for 2 and 3 bp repeats, respectively. The results of the SSR analysis are given in Table 1.

Table 1: SSR analysis

	SSR	%SSR (Hybr.)[a]	%SSR (Seq)[b]	% false positives
*Hin*dIII	$(AG)_{15}/(AC)_{15}$	0.42%	0.26%	38%
	$(AAC)_{10}/(AAG)_{10}/(ATC)_{10}$	0.88%	0.07%	93%
	$(AGC)_{10}/(AGG)_{10}$	0.20%	-	100%
	$(CCG)_{10}$	0.46%	-	100%
	Average	1.95%	0.33%	
*Sal*I	$(AG)_{15}/(AC)_{15}$	1.29%	0.49%	62%
	$(AAC)_{10}/(AAG)_{10}/(ATC)_{10}$	0.23%	0.08%	64%
	$(AGC)_{10}/(AGG)_{10}$	0.47%	0.11%	76%
	$(CCG)_{10}$	0.65%	0.02%	98%
	Average	2.64%	0.70%	
*Pst*I	$(AG)_{15}/(AC)_{15}$	0.89%	0.56%	37%
	$(AAC)_{10}/(AAG)_{10}/(ATC)_{10}$	0.26%	0.12%	54%
	$(AGC)_{10}/(AGG)_{10}$	0.18%	0.03%	82%
	$(CCG)_{10}$	0.49%	0.02%	96%
	Average	1.82%	0.74%	

[a] The percentage of SSRs is calculated based on the number of positive signals following colony hybridization of 18,431 clones.

[b] The percentage of useful SSRs identified after sequence analysis. It should be noted that SSRs below the threshold values of 10 and 7 repeats for di- and tri-nucleotide repeats were excluded. Also excluded were SSRs that were located within repeat DNA.

The restriction enzymes *Sal*I and *Pst*I are methylation sensitive and selection of fragments in the 1.5 kb range following digestion with these enzymes was expected to enrich for genic regions. Overall, the *Pst*I and *Sal*I fragments appeared to contain a higher number of SSRs compared to *Hin*dIII fragments (Table 1; %SSRs following hybridization). The exceptions were repeats of the type $(AAC)_{10}/(AAG)_{10}/(ATC)_{10,}$ which were present in significantly higher proportion in the *Hin*dIII library (Table 1). Sequence analysis identified a (CAA) repeat within a DNA repeat which was represented multiple times in the library. Eleven different, but highly homologous, elements were sequenced of this repeat family. This CAA repeat may account for the higher amount of $(AAC)_{10}/(AAG)_{10}/(ATC)_{10}$ SSRs detected by hybridization in the *Hin*dIII library. These elements were not suitable for the design of locus-specific primers. The highest success rate in SSR identification was obtained in the screens for $(AG)_n$ and $(AC)_n$ microsatellites in the *Sal*I and *Pst*I libraries.

Primers were designed against a total of 111 SSR sequences. Seventy sequences gave consistent amplification against a sample of eight finger millet varieties, including the parents of the mapping populations IE 1012 x Indaf-5 and Okhale-1 x MD-20. IE 1012, Indaf-5 and Okhale-1 are cultivated *E. coracana* subsp. *coracana* lines, MD-20 is a wild *E. coracana* subsp. *africana* accession. Approximately 33% and 88 % of the SSRs were polymorphic in IE 1012 X Indaf-5 and Okhale-1 X MD-20, respectively. All results were obtained after separation of the SSRs on 6% denaturing polyacrylamide and visualization of the fragments by silverstaining. It should be noted that the observed differences, in particular in the IE 1012 X Indaf-5 cross, were often very small (probably representing 1 repeat unit) and difficult to score reliably on a silverstained gel. We are therefore exploring the possibility of conducting the mapping on a ABI3730xl.

Genotype analysis on the ABI3730 requires the use of fluorescently labelled primers. To reduce the cost, we are testing the use of M13 tailed forward SSR primers in combination with a fluorescently labelled universal M13 primer and a reverse SSR primer. A first test provided clear separation of the alleles. However, we had to dilute the PCR products 50 times before loading them on the ABI3730xl. We are therefore further optimizing the PCR conditions before conducting the mapping experiments.

2. QTL mapping of blast and drought resistance

No further work has been conducted on the mapping of the IE 1012 x Indaf-5 population.

3. Distribution of SSR markers to collaborating Institutes and Data management

The SSR markers are available to interested parties upon request and signing of an MTA form.

4. Comparative mapping in the Okhale-1 x MD-20 population

Fifteen hundred cDNAs from leaf tissue from Okhale-1 and MD-20, from root tissue from MD-20 and from inflorescences from IE 1012 have been sequenced. Five hundred sequences have been analyzed so far for redundancy, organellar sequences and homology to rice. About half of the sequences were unique, and about 50% of these (25% of the

total sequences analyzed) showed homology to rice and could be used for comparative analyses. It should be noted that we only screened against the 'nr' section of Genbank. As more rice genomic sequence is annotated and moved from the 'httg' section to the 'nr' section, we expect that the percentage of finger millet sequences that show homology to rice will increase. Primers were made against all sequences for which a rice orthologue had been identified. Where possible, primers were designed across putative introns, identified on the basis of the alignment of the finger millet EST with the rice genomic sequence. A total of 107 primer pairs have been designed, 77 of which have been tested for variation between Okhale-1 and MD-20.

A comparison of different mapping methods (RFLP, SSCP, CAPS) has shown that the clearest signals and highest levels of variation were obtained with SSCP. Therefore, amplification products obtained using Okhale-1 and MD-20 as DNA templates were separated on MDE-gels (Cambrex Bio Science) and visualized by silverstaining. Forty-two (55%) of the 77 primer pairs tested revealed scorable variation between Okhale-1 and MD-20. So far, 27 ESTs have been mapped.

The Okhale-1 X MD-20 genetic map now consists of 13 linkage groups containing 8 or more markers, five linkage groups containing 6 or 7 markers, and 10 linkage groups containing 4 markers or less. Some 35 markers remain unlinked. We aim to finish the genetic map by the end of 2004.

Problems and constraints

1. Due to the move of Katrien M. Devos to UGA and the delay in hiring a new staff member until March 2003, progress on the project was slow during the months October 2003 – March 2004.
2. Because UASB does not recognize UGA as a full partner, the recombinant inbred population that will be used for QTL mapping of blast and drought resistance has not been transferred to UGA. The parents of the mapping population, IE 1012 and Indaf-5, have been obtained from ICRISAT, Patancheru and are in the process of being screened for polymorphisms with SSR markers. However, no mapping will be carried out at UGA in the RILs.
3. Because the QTL mapping is no longer an objective for UGA, we will focus on the development of additional SSR markers. Based on our SSR study described above, we will focus only on dinucleotide repeats in gene-enriched libraries.

Staff issues

Nicholas Bird's employment at the John Innes Centre terminated on 14th September 2003. Tyler Eaton started employment at UGA on March 1st. A master's student at Purdue University, Sujata Ramakrishnan, constructed the finger millet SSR libraries and conducted the hybridization experiments during a 2-month summer project (summer 2003).

1. Molecular mechanism of finger millet genotypes in responses to infested by *Magnaporthe grisea* isolate tosa #50

1.1 Project design

Somasehkhara *et. al* (1992) previously found that Finger millet genotype IE1012 is free from neck and finger blast infections as well as highly resistant to three other fungal and two viral diseases. We further found that genotype IE1012 and another genotype INDAF-5 showed a quantitative difference in disease density when 4-5-week seedlings were inoculated with ***Magnaporthe grisea*** isolate strain tosa #50. For example, IE1012 showed fewer lesions, smaller lesions, less chlorosis surrounding the lesions on leaves and most importantly, no infection of leaf sheaths.

To understand the molecular mechanism underlying this quantitative difference in the fungal infection responses, we used microarrays to detect ***Magnaporthe grisea*** infection responding genes from both ***Magnaporthe grisea*** and finger millet. The Magnaporthe/Rice Long Oligonucletide Chip (G4137A, 60mer) from Agilent Company consists of 7,415 rice genes and 13,666 Magnaporthe genes. Genotypes IE1012 and INDAF-5 were grown in the growth chamber, ~4-week plants were inoculated with *Magnaporthe grisea* strain tosa #50. Samples (leaves and shoots) were collected from infected plants at the time points of 45hr, 72hr and 4days after inoculation, as well as from healthy plants growing under the same conditions. Total RNA was isolated from these samples with a Qiagen plant mini kit and purified using the DNA-free System from Ambion Company. RNA samples were first checked for purity using denaturing gel electrophoresis, then labeled with an Agilent Fluorescent Direct Labeling Kit and hybridized to the Agilent chips for 17 hours following Agilent's recommended protocol. The experimental design is showed in Table 1:

Table 1. Experimental design for Chip hybridization experiments

Array	Cy-3 labeled RNA sample	Cy-5 labeled RNA sample	Type
1	Fingermillet IE1012, 45hr –healthy	Fingermillet IE1012, 45hr –infected	Differential
2	Fingermillet INDAF-5, 45hr -healthy	Fingermillet INDAF-5, 45hr – infected	Differential
3	Fingermillet IE1012, 72hr –healthy	Fingermillet IE1012, 72hr – infected	Differential
4	Fingermillet INDAF-5, 72hr -healthy	Fingermillet INDAF-5, 72hr – infected	Differential
5	Fingermillet IE1012, 4d –healthy	Fingermillet IE1012, 4d – infected	Differential
6	Fingermillet INDAF-5, 4d –healthy	Fingermillet INDAF-5, 4d – infected	Differential

1.2 Results

To analyze Agilent chip data for which no replicate is yet available, except for one experiment (two chips were hybridized with IE1012 RNA at 45hr), we set up our standard to define genes showing significant expression changes in infected samples relative to healthy samples, including: (1) the fluorescence intensity for a given gene in disease samples should undergo at least 3-fold change relative to that in healthy samples, and (2) the p-value of log ratio (red channel vs. green channel) for that gene should not be bigger than 0.05. Based on these two criteria, we found many finger millet and Magnaporthe genes could be involved in the fungal-host interaction at the time of 45hr, 72hr and 4days after inoculation, respectively (Tables 2, 3).

Table 2. Finger millet response genes during fungi-host interaction

Genotype: IE1012	45 hr	72 hr	4 d
Significantly up-regulated	364	159	1
Significantly down-regulated	2	5	133
Non-Changed	6778	6980	7010
Total	7144	7144	7144

Genotypel: INDAF-5	45hr	72hr	4d
Significantly up-regulated	239	157	1
Significantly down-regulated	2	0	217
Non-Changed	6903	6897	6926
Total	7144	7144	7144

Table 3. Expressed *Magnaporthe grisea* genes during fungi-host interaction

Genotype: IE1012	45hr	72hr	4d
Significantly expressed	1341	885	1
Non-expressed	12325	12781	13665
Total	13666	13666	13666

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The failure of this pilot array can be explained as follows. First, NimbleGen technology is still in a dynamic stage of development. For example, its probe selection algorithms picked up many unfavorable probes with dramatic different TMs and chains of polynucleotides. These features could introduce a lot of noise for which even the most sophisticated analytical tools are unable to correct. In addition, their hybridization protocol needs to be refined to achieve the best hybridization specificity and their method of reading the feature from raw images doesn't give statistical criteria as Affymetrix does. Second, *O. rufipogon* is reported to be genetically more closely related to japonica (Nipponbare is a typical japonica) than to *indica* subspecies (Nasu, *et. al*, 2002). In the putative intergenic regions, the frequencies of polymorphisms between japonica cultivars and *O. rufipogon* were about 0.33% to 0.34%, while such frequencies between japonica and indica can be up to 0.68% to 0.70% (Nasu, *et. al*, 2002). Third, selecting those full-length cDNAs showing high amino acid identity with Arabidopsis as candidate sequence sets as features made the SFP candidate pool smaller. Since these genes could be very conserved for the plants, the frequencies of SNPs could be even less. Finally, Nasu *et al* also found that the SNPs are not evenly distributed at the both chromosome and sub-chromosome level, for example, SNP-rich regions were found in chromosome 1,4,5,7, and 11, and the SNP-rich regions detected in chromosomes 1,4,5 and 7 fell in putative intergenic regions, but that detected in chromosome 11 was in transcribed regions. In our

pilot chip design, features were picked up evenly along the full length cDNAs, many of them were selected from protein coding regions.

Based on the above facts, we will refine our chip design with regard to the following. First, we will switch to various sequence sources rather than only full-length cDNAs, such as sequences from intergenic, untranslated, intronic and CDS regions in rice which was released by TIGR at the end of April, 2004. This diversity of sequence sources could reveal the preference of SNP occurrence in rice genome. Secondly, instead of only choosing *O.Rufipogon* as the target genome, we will try some indica cultivars such as GLA4, a typical indica variety, in which 9,056 SNPs were found in 2.3MB of chromosome 4 and their collinear sequence from Nipponbare (Han, et al, 2003; Feng, et al, 2002). We will also try some wild rice varieties, which are genetically distant to japonica. Finally, we will refine the target DNA preparation process, and raise the hybridization stringency to achieve the better specificity. Once these methods are established, we will employ the growing number of ESTs generated from UGA for probe design. We have obtained the putative, diploid parents of *E. coracana* and will use these as well as the lines being employed to develop maps of Eleusine in this project to discover SFPs.

References

1. Somasehkhara *et. al,* 1992. Trop. Agric (Trinidad) 69:293-295

2. Nasu, S. *et. al* 2002. Search for and Analysis of Single Nucleotide Polymorphisms (SNPs) in rice (Oryza sativa, Oryza, rufipogon) and Establishment of SNP Markers. DNA Res. 9: 163-171

3. Han, B. *et. al* 2003. Genome-wide Intraspecific DNA-sequence Variations in Rice. Current Opinion in Plant Biology, 6: 134-138

4. Feng, Q. *et. al* 2002. Sequence and Analysis of Rice Chromosome 4. Nature, 420: 316-320

Objective 5:
Phenotyping and Map development

Mapping of finger millet genome using recombinant inbred line (RIL) population

A. Field experiment: The plant material consisted of three hundred recombinant inbred lines (RILs) derived from the cross involving IE 1012 and Indaf 5 as parents. Field evaluation for different growth and yield characters was carried out during *kharif*, 2003 at Hebbal, Bangalore. The experiment was laid out in a randomized complete block design with two replications and the following observations were recorded.

1. Flowering characters: Days to 1st flowering
   Days to 50 % flowering
   Days to maturity

2. Pigmentation characters: Pigmentation of ear head
   Pigmentation of leaf base
   Pigmentation of node
   Pigmentation of internode
3. Ear head characters: Open
   Open top curved
   Semi compact
   Compact
4. Plant height
5. Number of tillers
6. Number of earheads
7. Number of fingers
8. Finger length
9. Grain yield

Overall view of the experimental plot

B. Lab Work:

Objectives:
1. To identify different kind (RAPD, RM, ISSR, AFLP,STS, EST, Candidate Genes) of polymorphic primers between two parents.
2. To screen the polymorphic primers for the entire mapping population and scoring of the marker data.
3. Construction of the basic linkage map.

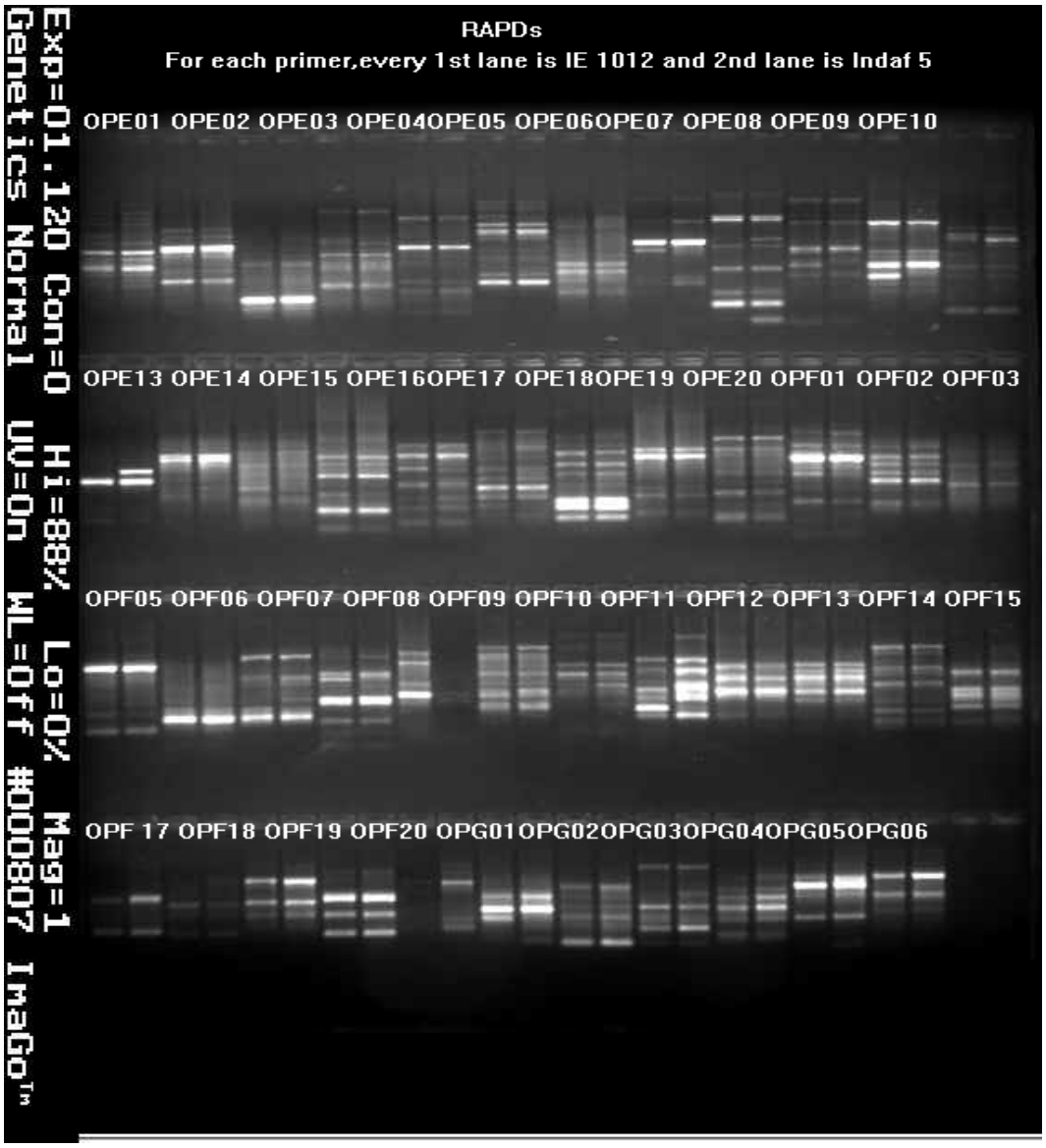
Plant material: The plant material consists of a subset of 250 recombinant inbred lines, derived from IE1012 x Indaf 5 cross.

Methodology: The leaf samples of 300 RILs and their parents were collected from the field at 30 days after sowing. Plant DNA extraction of all 250 RILs and their parents was carried out by CTAB method and DNA quantification was done by spectrophotometric method.

PART I

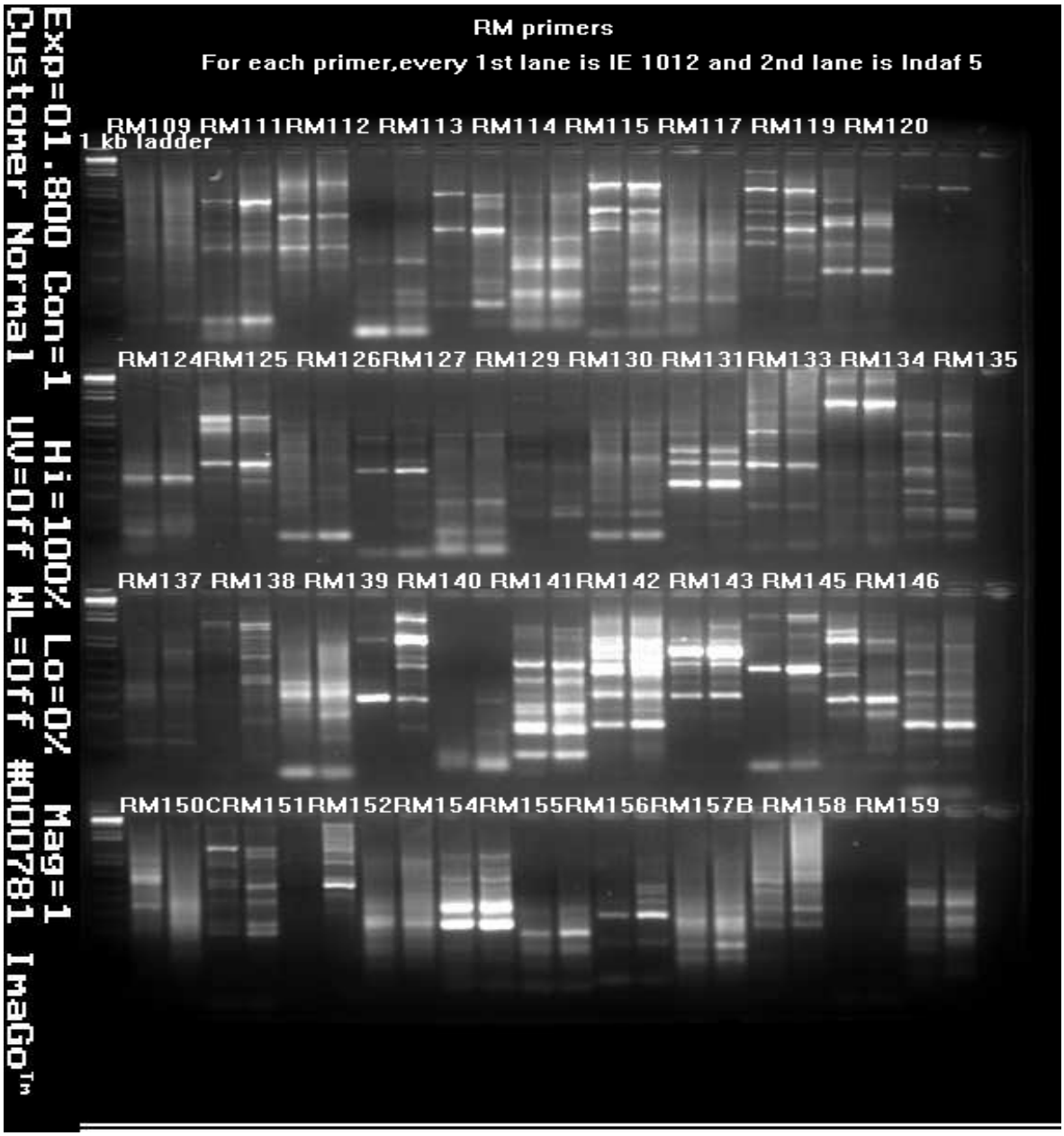
Initial survey of the parents for identifying RAPD, rice microsatellite (RM) and resistant gene analog (RGA) primers was done and information on the number of primers used, number of polymorphic primers identified is given below.

1. RAPDs: RAPD primers were tried at 40^0C annealing temperature using 1.6 % agarose gel. Out of 109 RAPD primers screened, 40 primers were found to be repetitively polymorphic, indicating 36.69 % polymorphism between the parents.



Gel picture showing amplified products of the parents with different RAPD primers.

2. RMs: RM primers were tried at 58^0C annealing temperature using 3 % agarose gel. Out of 219 RM Primers screened, 190 primers were found to be polymorphic at various levels, indicating 36.69 % polymorphism between the parents. Unlike in rice, most of the RMs used in this study for finger millet amplified multiple bands. Of these 190 polymorphic primers, 64 primers amplified single band each at lower level (between 100-250 bp length) and this band was found to vary in its repetitive length in two parents.

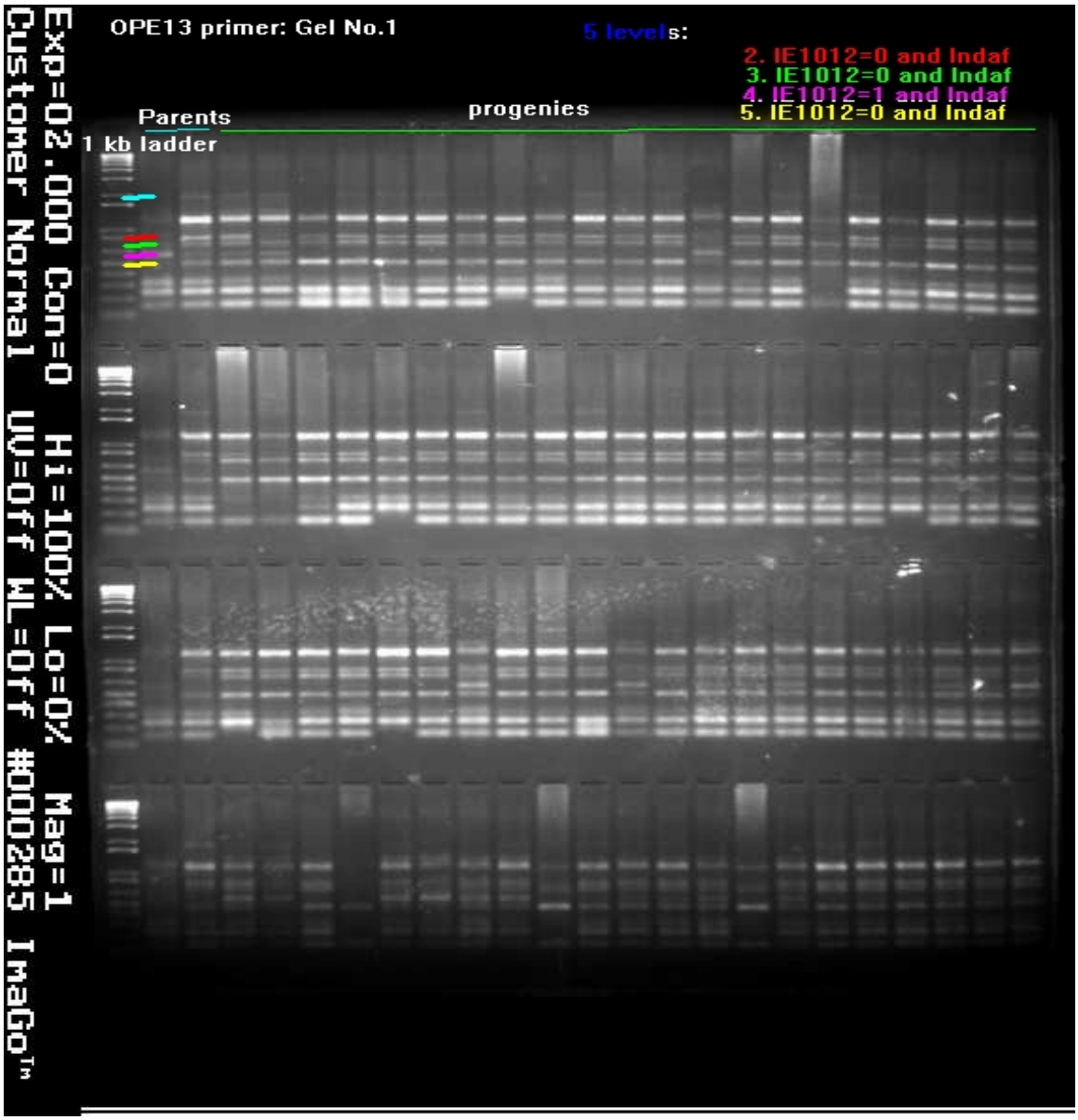


Gel picture of the amplified products of the two parents with different RM primers.

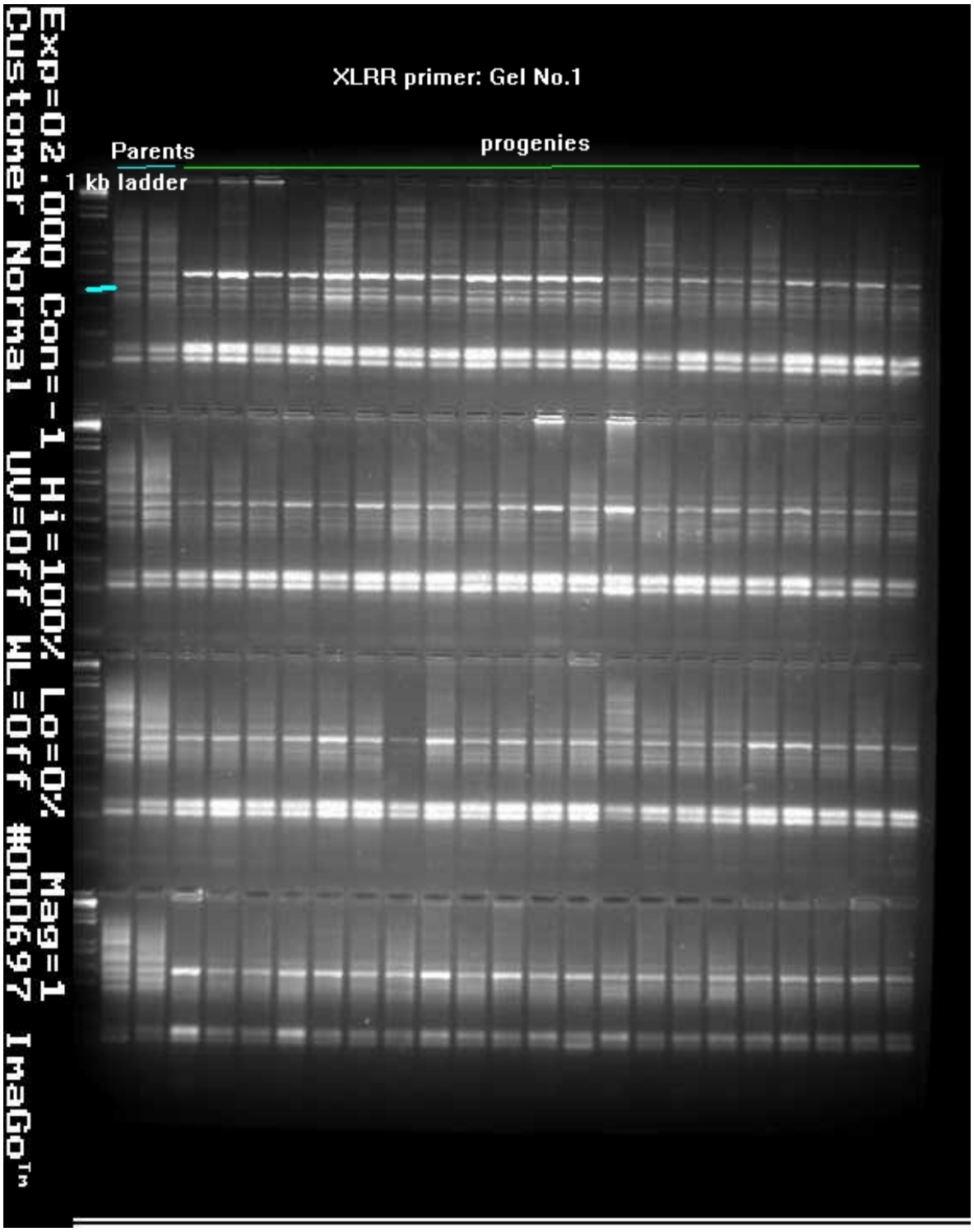
3. Resistant Gene Analogs (RGAs): RGA primers were tried at 45^0C annealing temperature using 3.5 % agarose gel. Three RGA primers were screened and all were found to be polymorphic between the parents.

PART II

The polymorphic primers identified were used for screening all the 250 RILs. At present, the population has been screened for 9 RAPD polymorphic levels and 8 RGA (Resistant Gene Analogue) polymorphic levels.



Gel picture showing amplified products of different RILs and parents with OPE 09 RAPD primer.



Gel picture showing amplification of different RILs and their parents with XLRR primer.

Objective 6.
Development of introgression lines-part include

Crossing program:

Among these 300 recombinant inbred lines, 30 elite lines tolerant to blast, drought and were selected and were involved in the crossing program wherein the lines were backcrossed to both the parents with the objective of developing QTL introgressed lines

Backcross breeding for QTL introgressed lines BC-1

View of crossing program using 30 elite lines for development of QTL introgressed line