

Progress Report of the McKnight Foundation funded Project

IVth Year

2005-2006

Title of the Project

**Development of High Yielding, Disease Resistant,
Drought Tolerant Finger Millet (*Eleusine
coracana* Gaertn.) Genotypes**

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Title of the Project: Development of High Yielding, Disease Resistant, Drought Tolerant Finger Millet (*Eleusine coracana* Gaertn.)

Executive summary:

Farmer participatory varietal identification and dissemination was further carried out to popularize and spread three genotypes earlier selected for cultivation in farmers field under rainfed situation. These genotypes that are blast resistant and drought tolerant were successfully grown in drought prone dry region of Karnataka. Mother-baby trials was a successful attempt to disseminate the new genotype to hundreds of farmers through the farm demonstrations and trial on farmers fields. The seeds were disseminated by farmers themselves from Village to village. These lines are in the third year of state multilocation trials conducted by the Millet coordinator. The initial assessment of the spread of varieties is satisfactory through participatory selection. New SSR markers were developed at UAS, Bangalore as well as at UGA. 95 New SSR markers were developed at UAS. SSR primers were shared to the collaborators from UGA.). A preliminary map was developed and comparative analysis with other cereals is reported by UGA group. Co-linearity between finger millet and rice was reported. Six finger millet homoeologous groups corresponded to a single rice chromosome, and three to two rice chromosomes. In most cases, marker orders were completely collinear between finger millet and rice. A new population for identification of QTLs was considered and parental polymorphism was tested at UAS, Bangalore. A managed moisture stress experiment to quantify the water required used during severe drought stress was carried out and genotype ML 181 the most drought tolerant genotype both at Vegetative stage and Reproductive stage with 4.5 tons per hectare grain yield. Identification of *Eleusine coracana* blast fungal elicitor-responsive genes by differential display identified, Diacylglycerol Kinase, Calcium dependent protein Kinase and new proteins in finger millet upon blast inoculation. Microarray technology was used for discovering Single Feature Polymorphisms (SFPs) that could be used for DNA-based markers for genetic mapping. Primers were designed for 20 genes from conserved regions among grass orthologs/paralogs. The sequences for primers, ESTs and other sequences have been deposited in Gen Bank. Publications on map development, mapping population evaluation for blast disease, root phenotyping and participatory evaluation have been submitted for publication.

Objective 1: Farmer's Participatory Varietal Selection

Background information:

- Based on Rural Appraisal Surveys (RAS) in the past three years we have identified the major finger millet growing areas and marginal farming communities in rainfed conditions of Southern Karnataka. Broadly three constraints were identified viz., higher grain production, non-powdery quality fodder and good grain quality.
- Considering the above constraints, we have evaluated 30 elite genotypes that could satisfy the farmers preferred attributes. Six promising genotypes were identified among these elite lines based on the farmers' participatory yield trials conducted in multiplications during wet season, 2003, under rainfed conditions and during dry season, 2004, under controlled stress situation.
- Further, Mother Baby trials were conducted in five farmers' field representing different agro climatic conditions during wet season, 2004. Three promising genotypes viz., ML 31, ML 322 and ML 365 were selected as the most preferred genotypes based on grain yield, fodder yield, fodder quality, cooking quality and nutrition parameters. ML 181 was more suitable for rainfed conditions. GPU 28, ruling high yielding variety, was used as a check for all the attributes.

Year 2005

- Mother Baby trials were conducted for the pre selected three promising genotypes and the seeds were distributed to several farmers in the same villages and other villages.
- In continuation of this we have distributed seeds of three elite genotypes (ML 31, ML 322 and ML 365) through farmer distributaries to 49 farmers (Table 1 and Fig. 1) spread over different parts of Karnataka during Wet season 2005. Performance of these lines has been documented for all the traits under consideration.
- Additionally, these genotypes are being evaluated in the second year of state multilocation trials and are performing consistently well during wet season in both rainfed and drought prone areas.

Results:

Dissemination

The seeds of elite genotypes distributed by farmers were ML 31, ML 322, ML 365 and **ML 426**. Maximum varietal spread of 5 kilo meter distance from a baby Village Narugnahalli in Tumkur District was recorded (Fig 2).

From Hullenhalli village of Tumkur District, one of the contact farmers distributed 90 kgs of seeds to five different farmers of same village. The new genotype ML 426 spread quickly and was grown in 11.25 ha. (it is to say that seeds are being dissemination because of our participatory efforts quickly, rather than the formal system)

The seeds of the new varieties spread to several villages by participatory effort of the farming community even before it was released by the formal system.

It is clearly evident from the farmers' participation that, they actively participated during *Kharif* 2004 for evaluation and selection of elite genotypes and selected the same genotypes in following season, *Kharif* 2005. We enquired farmers in Tumkur District and they are satisfied with the consistence performance of elite genotypes and showed positive interest in continuing to grow the same genotypes in their fields. One of the farmer from Singanayakanahalli village of Devanahalli Taluk, Bangalore district, got a bumper harvest from these elite genotypes and he sown ML 365 in an area of 3 ha. From this genotype a good seed and fodder yield (Figure) was obtained.

Performance of elite genotypes in new areas

In *Wet season* 2005, we made foray into new finger millet-growing areas to popularize the elite genotypes of finger millet by distributing the seeds in Tiptur Taluk, Chikkanayakanhalli Taluk and Pavagada Taluk of Tumkur District as well as in Devanahalli Taluk of Bangalore Rural district (Fig 1). In these areas farmers harvested a good crop and satisfied with the performance of new genotypes with respect plant height, compact ear head, number of tillers, color of the seeds, resistance to neck and finger blast, drought tolerance, high seed and straw yield and stiff straw (preferred by milching animals) Majority of the farmers harvested an average of 3.5 – 4.0 tons / ha under semi drought situation.

New Genotypes in State trial conducted by the All India Co-ordinated Millet Project (AICMP):

Three genotypes, ML322, ML 365, ML31 are in the second year of the state multilocation trials, which is the formal procedure to release variety by the government authority. These genotypes recorded on an average 5.60 tons/ha yield and the highest yield was recorded ML322 genotype (5.6 tons/ha) under rainfed conditions. Among these three genotypes, two genotypes will be promoted to farm trials during *Kharif* 2006.

Table 1. Distribution of seeds to farmers during Wet Season, 2005

Sl. No.	Location	No. of farmers
1	Narugahalli, Tumkur District	8
2	T. G. Palya, Tumkur District	6
3	Halugona, Chikkanayakanahalli Taluk, Tumkur District	15
4	Nonavinakere, Tiptur Taluk, Tumkur District	7
5	Konehalli, Tiptur Taluk, Tumkur District	2
6	Kondapura, Pavagada Taluk, Tumkur District	2
7	Singanayakanahalli, Bangalore Rural District	1
8	Nelloore, Devanahalli Taluk Bangalore Rural District	4
9	Chennarayapattana, Devanahalli Taluk Bangalore Rural District	4
Total		49

Objective 2: Screening of selected promising genotypes for drought tolerance

The selected subset of genotypes from 30 genotypes, that are in farmers trials were subjected to managed stress experiment to assess their drought tolerance.

Based on the findings of drought screening, ML 181 genotype was found to be superior in grain and straw yield under water limited environments. This genotype showed good tolerance to drought with good rooting ability and quick rejuvenation after moisture stress released. In general, scheduling irrigation at 0.8 IW/CPE ratio throughout the cropping season was found to be the best for getting an optimum yield under stress. The genotype ML 181 performed very well under severe stress and generated upto 4.5 tons per hectare grain yield. The details are shown in table 2 and 3 below.

Table 2. Effect of Phenophosed irrigation schedules on growth and yield component of finger millet cultivars.

Irrigation schedules	10-40 DAS	41-70 DAS	71-harvest DAS	Plant height (cm) at harvest	Number of tillers per hill at harvest	Total dry matter at harvest per hill (g)	Number of ear per hill	Number of finger per ear	Total grain yield per hill (g)
I ₁	0.6	0.6	0.6	61.56	4.18	60.12	2.65	8.56	8.02
I ₂	0.8	0.8	0.8	78.87	4.42	68.91	2.85	8.88	12.93
I ₃	0.6	0.8	0.8	76.44	3.65	61.67	2.72	8.52	9.88
I ₄	0.8	0.6	0.8	74.56	3.65	58.71	2.83	8.31	8.02
I ₅	0.8	0.8	0.6	75.37	3.70	65.70	2.96	8.41	8.76
		SEM±		1.5600	0.1164	1.2344	0.0970	0.0660	0.1023
		CD at 5%		4.6210	0.3444	3.6533	NS	0.1952	0.3029
Cultivars									
C ₁	MASGPB	181		71.43	4.10	69.37	3.52	8.54	12.38
C ₂	MASGPB	224		79.36	3.82	62.41	2.77	9.42	8.42
C ₃	MASGPB	316		79.91	3.88	66.52	2.63	9.64	9.74
C ₄	Indaf	5		65.72	3.86	53.79	2.30	6.54	7.55
		SEM±		1.3910	0.1041	1.1041	0.0867	0.0590	0.0915
		CD at 5%		4.6221	NS	3.2676	0.2568	0.1746	0.3029
Interaction									
		SEM±		3.1230	0.2328	2.4688	0.1939	0.1319	0.2047
		CD at 5%		NS	NS	NS	NS	NS	NS

DAS: Days after sowing;

IW/CPE: Irrigation Water: Cumulative Pan Evaporimeter

NS: Non-Significant

Table 3. Grain yield (g ha⁻¹) and straw yield (g ha⁻¹) of Finger millet cultivars as influenced by irrigation schedules

Irrigation (DAS)	schedule			Grain yield (g ha ⁻¹)					Straw yield (g ha ⁻¹)				
	10-40	41-70	71-harvest	C1	C2	C3	C4	Mean	C1	C2	C3	C4	Mean
I ₁	0.6	0.6	0.6	32.28	24.40	28.68	20.16	26.38	78.53	68.59	83.62	53.96	71.17
I ₂	0.8	0.8	0.8	56.19	40.50	43.07	31.98	43.12	116.44	95.16	92.24	92.24	99.02
I ₃	0.6	0.8	0.8	37.03	25.73	35.48	37.98	31.55	97.96	93.71	85.54	72.86	87.52
I ₄	0.8	0.6	0.8	36.00	23.73	25.96	21.07	26.68	92.40	82.39	76.72	68.46	79.99
I ₅	0.8	0.8	0.6	38.80	26.31	27.27	22.28	28.89	98.26	82.82	82.80	62.74	81.66
Mean				40.40	28.11	32.09	24.69		96.71	84.53	84.19	70.05	
				SEM±		CD at 5%			SEM±		CD at 5%		
Cultivar (C)				0.5911		1.7463			1.3515		3.0528		
Irrigation Schedules (I)				0.6609		1.9558			1.1533		3.4132		
Interaction (CXI)				1.3217		3.9116			2.3066		6.8263		

C₁: MASGPB 181
C₂: MASGPB 224
C₃: MASGPB 316
C₄: Indaf 5

DAS: Days after sowing
IW/CPE: Irrigation water: cumulative pan Evaporimeter

Objective 3: Molecular Mapping of Finger millet

Development of SSR markers and mapping of Finger millet

Introduction

We continued previous years' work on the development of DNA markers in finger millet and mapping both at UGA and at UAS. At UAS, progeny analysis of the mapping population with 300 recombinant inbred lines (RILs) between IE 1012 Indaf5 was conducted. In the previous year, we identified polymorphic primers (RAPDs and RGAs) between the two parents for progeny analysis. We made an effort to map rice microsatellite markers but found multiple bands. However, we designed new SSR markers and also obtained finger millet SSRs from the University of Georgia. Large skewness was observed in the population. Therefore we chose a new RIL population that was derived from GPU28 and Indaf9 which we had previously developed. At UGA a map of finger millet was completed in an intersubspecific F₂ population generated from a cross between subsp. *africana* acc. MD-20 and subsp. *coracana* cv. Okhale-1. A comparative analysis between the genomes of finger millet and rice was carried out.

Research carried out at UGA

1. Development of markers for finger millet

An earlier analysis of the presence of simple sequence repeats in small (~1,500 bp) insert *Pst*I, *Sal*I and *Hind*III libraries had demonstrated that the frequency of dinucleotide repeats in libraries generated using methylation-sensitive restriction enzymes (*Pst*I and *Sal*I) is about twice that present in libraries constructed with *Hind*III. In total, 99 SSRs had been generated from these three libraries, 31 of which were placed on the genetic map. To generate a further set of SSRs, two new genomic libraries were constructed. One library was constructed using DNA of *E. coracana* subsp. *africana* acc. MD-20 restricted with *Pst*I and size selected to the range 1.5 – 2.0 kb. For the second library, genomic DNA of *E. coracana* subsp. *coracana* cv. Okhale-1 restricted with *Sal*I and size selected to the range 1.5 – 1.8 kb was used. A total of 18,432 clones, 15,360 *Sal*I Okhale-1 and 3,072 *Pst*I MD-20 clones, were double-gridded on high-density filters. The colonies were screened with the dinucleotide repeats (AC)₁₅ and (AG)₁₅ and the trinucleotide repeat (GAA)₁₀. The probes were labeled using the AlkPhos Direct Labelling and Detection System (Amersham). Seven hundred positive clones were identified and, from these, 384 that displayed a strong hybridization signal on X-ray film were selected for sequencing. Eighty percent of the sequenced clones contained an SSR above the threshold of ≥ 10 and ≥ 7 repeat units for dinucleotide and trinucleotide repeats, respectively. To date, 60% of the sequences have been analyzed resulting in the design of 143 pairs of primers. No primers were designed for about 25% of the sequences that contained an SSR, either because the SSR was too close to the end of the clone or the SSR was located within repeated DNA. The first 50 primer sets are currently being tested for amplification in Okhale-1 and MD-20.

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

Aliquots (enough for 500 reactions) of sixty-two SSR primer sets developed at UGA have been sent to Dr. Shailaja Hittalmani. The full set of SSRs has also been provided to Dr. R.K. Varshney at ICRISAT, Patancheru.

3. Generation of ESTs

All finger millet ESTs generated within the McKnight project have been submitted to GenBank. Accession numbers are DY625711 – DY625766, DY632421 and EB684283 - EB684316 for Okhale-1 leaf ESTs; DY684794 – DY684907 for MD-20 leaf ESTs; EB086105 – EB086294 for MD-20 root ESTs; and EB187296 – EB187503 for IE 1012 inflorescence ESTs.

4. Comparative genome analysis

Finger millet – rice comparative maps

Checking of the mapping data has been completed, and the genetic maps have been constructed. Maps were initially generated at LOD 11 and consisted of 18 major linkage groups (LGs) with seven or more markers and 10 minor LGs with 2 to 5 markers. Three point and multipoint analyses demonstrated that two of the major LGs were part of the same chromosome. The number of minor LGs was reduced from 10 to eight. The final maps are shown in Figure 3. The A genome is covered by 15 linkage groups consisting of 130 markers and totaling 714 cM. The B genome is covered by nine linkage groups consisting of 152 markers and spanning 787 cM. There is no evidence of major rearrangements between the A and B genome linkage groups. A paper describing the finger millet genetic map was submitted to TAG on April 23, 2006.

Colinearity between finger millet and rice is also highly conserved. Six finger millet homoeologous groups correspond to a single rice chromosome, and three finger millet homoeologous groups correspond to two rice chromosomes (Figure 3). In most cases, marker orders are completely colinear between finger millet and rice. A paper reporting on the finger millet – rice comparative map is in preparation.

Finger millet – tef comparative maps

All 133 primer sets against ESTs that had been mapped in tef and obtained from Mark Sorrells, Cornell University, were screened for amplification potential and polymorphism levels in Okhale-1 and MD-20. Thirty markers that showed clear polymorphisms have been analyzed for segregation in the MD-20 x Okhale-1 mapping population, but have not yet been integrated into the genetic map. We will also attempt to map some further markers that show putative polymorphisms.

Please note that Figure 3 is confidential – Please do not display on the McKnight website.

Research carried out at UAS, Bangalore

Mapping effort in new population at UAS, Bangalore

The new mapping population derived from a subset of GPU28 and Indaf9 cross consisted of one hundred and fifty genotypes having wide phenotypic variation, which was estimated from the previous field evaluation studies. SSR primers obtained from Dr. Devos, USA and the new markers developed and designed at UAS, Bangalore were used for parental polymorphism.

Results and Discussion

There was not satisfactory genotypic variation in the RIL population of IE1012 and Indaf5 (Fig. 5 and 6). The allelic distribution was highly skewed towards one parent and we made this inference after analyzing three SSR markers. In case of SSR16 (Fig. 5), which amplified 225 bp band from IE1012 parent, out of 252 genotypes, only 10 genotypes (3.98 %) showed IE1012 banding pattern, 237 genotypes (94.43 %) showed Indaf5 pattern, 4 genotypes showed heterozygous bands (1.59 %), while one genotype did not amplify. Similarly, SSR66 amplified 220bp length band from IE1012 parent, wherein 13 genotypes (5.17 %) amplified IE1012 type band and 238 genotypes (94.82 %) showed Indaf5 pattern, again showing biased allele distribution (Fig. 6).

Due to above reasons, we shifted to another new RIL population developed by crossing GPU28 and Indaf9 varieties and presently we are working with this population. The population consists of 150 genotypes with high phenotypic variation. Initially, we screened RAPD markers for parental polymorphism and some of which, that were polymorphic were used in the progeny analysis. Figure 6 shows the amplification of GPU28/Indaf9 parents and their RIL population using the RAPD marker OPE13, which amplified a 500bp length polymorphic band. The SSR markers were initially run on agarose gel (3.5 %) and the left out samples were run on PAGE gels (7 %). Out of 62 SSRs, 9 were polymorphic on agarose gel (Fig. 7) and 12 were polymorphic on PAGE gel. But the resolution was high in the latter one (Fig. 8). In order to find out the population distribution, a sub set of 82 RILs was screened using polymorphic primer, SSR on agarose gel. Out of 82 genotypes, GPU28 type band was amplified in 51 genotypes (62.19 %) and Indaf9 type band was observed in 31 genotypes (31.80 %) (Fig. 9). From these results, in both RAPD and SSR marker analyses, considerable genotypic variation was observed in the population, hence we initiated marker work in the new population and we plan to detect QTLs mapping in this population.

Objective 4: Identification of *Eleusine corocana* Geartn., Blast Fungal Elicitor-Responsive Genes by Differential Display and development of EST markers

In this study an attempt has been made to identify the genes underlying Blast responsive pathway.

The differential display polymerase chain reaction strategy developed by Liang and Pardee¹ allows the comparison of gene expression between any two comparative cell types. Differential display reverse transcription PCR is a procedure to identify the induction and repression in gene expression analysis. In order to study molecular interactions that occur between Ragi and Blast fungus upon infection, we isolated fungal elicitor –responsive genes from ragi genotypes, viz., **IE-1012** (Blast resistant variety) and **Indaf-9** (Blast Susceptible variety) adapted to upland conditions were grown for 22 days duration in plastic pots at 100% Field capacity. Microbial suspension of *Magnaporthe Grisea* of 10⁻¹ dilution, was sprayed to one set of both plant varieties, while the other set of plants were maintained as controls for disease. Leaf samples from both diseased and control plant types at different stages of infection, viz., 24 hours, 48 hours, 72 hours and 1-week were harvested.

For the initial experiment, 24hrs Blast control and infected (stress) plants of both IE1012 and Indaf-9 were harvested. Total RNA was extracted and purified from the harvested samples using Lithium chloride precipitation method. The integrity of the extracted total RNA was observed on 1.2% formaldehyde agarose gel. 1µL (~1-5µg concentration) of the extracted RNA was used as template for cDNA construction. Different poly (A) tailed mRNA sub-populations from the total RNA extract were pooled by the use of combinations of single penultimate based anchored primers H-T11G (2µM/µL) 5'-AAGCTTTTTTTTTTTG-3' (Gen Hunter Corporation, USA). The second strand synthesis and PCR was performed using arbitrary primers shown in the table below:

1. H-AP1 (2µM)- 5'-AAGCTTGATTGCC-3'
2. H-AP2 (2µM)- 5'-AAGCTTCGACTGT-3'
3. H-AP3 (2µM)- 5'-AAGCTTTGGTCAG-3'
4. H-AP4 (2µM)- 5'-AAGCTTCTCAACG-3'
5. H-AP5 (2µM)- 5'-AAGCTTAGTAGGC-3'
6. H-AP6 (2µM)- 5'-AAGCTTGCACCAT-3'
7. H-AP7 (2µM)- 5'-AAGCTTAACGAGG-3'
8. H-AP8 (2µM)- 5'-AAGCTTTTACCGC-3'
9. H-AP9 (2µM)- 5'-AAGCTTCATTCCG-3'
10. H-AP10 (2µM)- 5'-AAGCTTCCACGTA-3'
11. H-AP11 (2µM)- 5'-AAGCTTCGGGTAA-3'
12. H-AP12 (2µM)- 5'-AAGCTTGAGTGCT-3'
13. H-AP13 (2µM)- 5'-AAGCTTCGGCATA-3'
14. H-AP14 (2µM)- 5'-AAGCTTGGAGCTT-3'
15. H-AP15 (2µM)- 5'-AAGCTTACGCAAC-3'
16. H-AP16 (2µM)- 5'-AAGCTTTAGAGCG-3'

The reaction temperatures and number of cycles were followed as per the company indications. The amplified cDNA products were resolved on 6% sequencing gel. The cDNA bands were then eluted and purified using Gel Extraction Gel Elution Kit (Sigma-Aldrich) as per the company indications. After elution and purification, these bands were re-amplified with the same set of primer combination used for the differential display under the same PCR conditions. Most of the cDNA products identified ranged between 400-500bp. Automated sequencing analysis (Bangalore Genei Pvt Ltd, India) of the purified samples and their further sequence homology search against NCBI –BLAST database program was carried out and two key candidate genes Di Acyl glycerol Kinase, Calcium dependent protein Kinase were detected.

Objective 5: Evaluation of Microarray Technology for Discovery of SFPs in Rice/Finger Millet and Design of New Primers for Marker Development.

Progress:

- Conducted in silico analysis on newer versions of 93-11 and Nipponbare genomes;
- Optimized genome target labeling method using random priming method and evaluating amount of labeled target to add to hybridization to obtain saturation of probes under stringent and relaxed hybridization conditions;
- Evaluated data normalization methods to determine if SFPs found by hybridization method are found with in silico method;
- Contacted Partek Inc. software company to encourage collaboration on development of software for statistical evaluation of genotyping oligonucleotide arrays;
- Designed primers for mapping based on upregulated genes found on Agilent microarray during blast infection of finger millet;
- Prepared a physical map of 329 of the 414 upregulated genes using the rice chromosome pseudomolecules v.3.;
- Compared the location of these markers with those previously associated with blast and other disease resistances in rice;
- Ordered SRAP primers for evaluation by project collaborators and by the Leong Lab using Beckman Genetic Analyzer.
- Ordered primer pairs from Pearl Millet project of Michael Gale which are based on conserved sequences in Pearl Millet at intron junctions. These have recently been distributed to project members Shailaja Hittalmani and Katrien Devos as well as Mark Sorrells for use in tef.
- Ordered a subset of primers that were designed by Shulan Tian based on multiple sequence alignments of infection-related, upregulated genes she identified on Agilent rice oligonucleotide arrays using finger millet mRNA probes. These have been distributed to project members and Mark Sorrells.

As part of the McKnight Foundation's CCRP and the USDA-NRI's Rice Coordinated Applied Genomics Program, we are trying to use oligonucleotide microarray technology as a means of rapidly discovering Single Feature Polymorphisms (SFPs) that can be used as DNA-based markers for genetic mapping. A test genome scan was conducted using the full genome sequences of rice varieties 93-11 (indica) and Nipponbare (japonica) compared with the Affymetrix rice gene array probe set to determine which probes are polymorphic. A total of 170,256 putative single feature polymorphisms were identified using high stringency criteria in the 93-11 genome, indicating that this approach should be fruitful. A random priming method for labeling of the genomic DNA target was optimized by varying temperature, time of incubation, DNA substrate, and ratio of biotinylated dCTP to dCTP. Test hybridizations were conducted with labeled genomes of the two rice genotypes 93-11 and Nipponbare. Analysis of this preliminary, un-replicated data using several background correction and data normalization methods showed that a percentage of the putative SFPs can be reliably

detected when both perfect match and mismatch and single copy probe data are used. Using the most stringent criteria as many as 4524 putative SFPs were detected with high assurance on arrays. Of these 3846 had no match in the 93-11 genome. Increasing signal to noise and insuring probe saturation have also been investigated and have shown that conditions described by Borevitz et al. (www.naturalvariation.org) do not lead to saturation of the probes on the rice array.

Discussion with Richard Michelmore, who has recently described new software tools for SFP detection on Affymetrix gene expression arrays in Arabidopsis, along with recent findings of Ed Buckler on application of this approach to discovery of SFPs from maize, has revealed that genome complexity reduction is essential to the success of this work in maize. Richard has recommended a similar strategy for rice. Finger millet, which is even more complex and tetraploid will certainly require genome simplification. Evaluating genome complexity reduction methods will be the subject of the final year's no cost extension.

Discussion with Partek software developers has spurred a mutual interest to work on software for rapid and stringent statistical analysis of genotyping arrays. Their software for gene expression array studies was highly touted in correspondence with Gene Tanimoto at Affymetrix. I have taken an online workshop to learn more about this software and was very impressed with its rapid ability to do complex multivariate statistical tests and present data for each of the 1.3 million probes in a spreadsheet format. I am currently evaluating the demo program for Affymetrix array data analysis and Partek is independently examining our data. This work sets the stage for application of this technology to the discovery of single feature polymorphisms that can be used for genetic mapping of quantitative resistance to blast and drought in finger millet as well as sheath blight resistance and milling quality and yield in rice germplasm of importance to the U.S. rice growers and industry.

Three hundred and twenty nine of the 414 gene chip sequences associated with upregulated genes were mapped in the Nipponbare pseudochromosomes v.3 of TIGR. These are listed in Appendix 1 and are shown graphically in Appendix 2. Some of the genes show an association with previously identified loci for disease resistance in rice and should be used for high priority markers for this project.

Thirty six of the 414 genes were screened for primer design based on multiple sequence alignment of grass genome and EST sequences, and primers were found for 20 genes in this first batch. For most of these 20 genes (>95%), one primer was designed from 60 mer region (chip sequence) and the other primer from conserved regions among grass orthologs/paralogs.

For each gene, multiple primers were recorded in the appended text file Appendix 3, and the preferred primer sets can be choose and tested for the final design. Once the design is choosen, the primers sequences can be mapped back to cDNA sequence to find the predicted size of the product. The primers have been synthesized by Proligo and have recently been sent to project participants for evaluation.

Publications and thesis supported by the McKnight Project: UAS, Bangalore

Title of the Thesis:

1. “Evaluation of Physico-chemical and functional properties of new Indo-African finger millet genotypes” Ms. Shashi B. K.
2. “Technological dimensions of finger millet and their impact on output – A study in Karnataka” Ms. Shoba N.
3. Generation of Mutants in finger millet for identification of blast resistance near isogenic lines and molecular investigations. (Ph. D in Progress). Mr. Keshava Murthy B.C.

List of finger millet research papers

1. Shashi, Sunanda Sharan, **Shailaja Hittalmani** and Shankar A.G., 2006. Standardization of protocols for wine making from Elite Indo-African finger millet genotypes. Journal of Food Science and Technology
2. Shashi, Sunanda Sharan, **Shailaja Hittalmani** and Shankar A.G., 2006. Evaluation of Protein nutritive factors of elite finger millet genotypes. Journal of Food Science and Technology
3. Shashi, Sunanda Sharan, **Shailaja Hittalmani** and Shankar A.G., 2006. Initial evaluation of Elite finger millet genotypes for micronutrients, bio-availability of iron and anti-nutritional factors. Journal of Food Science and Technology
4. Shashi, Sunanda Sharan, **Shailaja Hittalmani** and Shankar A.G., 2006. Functional properties, proximate composition and fiber content of elite finger millet genotypes. Journal of Food Science and Technology:00
5. SanathKumar V.B., Rudresh, N.S., Appaji Gowda, H.C and **Shailaja Hittalmani**, **2006**. Field evaluation of Recombinant Inbred Lines (RIL’s) of Finger millet (*Eleusine coracana* Gaertn) against blast disease (*Pyricularia grisea* Sacc.) at different locations of southern Karnataka. Environment and Ecology:00 Accepted for publication
6. **Shailaja Hittalmani**, Rudresh.N.S., Nagabhushan. K., Sanath Kumar V.B., Appaji Gouda, H.C., Hanamareddy, B.G and Keshavamurthy, B.C., 2006. Farmers Participatory Varietal selection in finger Millet at Southern Districts of Karnataka, India. Submitted publication (Indian Journal of Genetics and Plant Breeding)

Publication sent from UGA:

7. Dida MM, Srinivasachary, Ramakrishnan S, Bennetzen JL, Gale MD, **Devos KM**, 2006. The genetic map of finger millet, *Eleusine coracana*. Theor Appl Genet (submitted).

Possible Papers sent from University of Wisconsin:

8. A publication describing the Affymetrix microarray work and genome scan is anticipated.
9. A collaborative publication describing the gene expression study using the Agilent rice array, validation of these findings by real time PCR, tentative physical location of the regulated genes in the rice genome and syntenous regions in finger millet based on emerging maps from this project, and development of mapping markers based on these genes and their genetic location is anticipated.
10. Possible publications derived from exploratory work with SRAP markers and the brain storming strategy based on the Agilent array work may be anticipated.

List of finger millet Abstracts presented in seminars/ Symposium/ conferences (UAS)

1. Sanath Kumar V. B., **Shailaja Hittalmani**, Sirisha Chittala, Debasis Mishra, Rudresh, N.S, and Appaji Gowda, 2006. DNA characterization of Magnaporthe grisea isolated from finger millet genotypes. Biotechnology Workshop JAINS, February 2nd, 2006
2. Shashi B K, Sunanda Sharan, **Shailaja Hittalmani** and Shankar A G, 2005. Protein nutrition of elite finger millet genotypes, ML322, ML 365 and ML31. ICFOST on December 9, 2005
3. Keshava Murthy B C, Hanamareddy B, Rudresh N.S, Nagabhushana K, Appaji Gowda H.C, Girish T.N. and **Shailaja Hittalmani**, 2005. DNA marker analysis of chemically induced mutants of finger millet (*Eleusine coracana* L. Gaertn.). International conference on “Plant Genomics and Biotechnology: Challenges and Opportunities”. October 26-28, IGAU, Raipur, India
4. Nagabhushana K, Rudresh N.S, Appaji Gowda H.C. Keshava Murthy B.C, Sanath Kumar V. B. and **Shailaja Hittalmani**, 2005. Evaluation of recombinant inbred lines for yield traits and development of molecular markers in finger millet (*Eleusine coracana* Gaertn L.). International conference on “Plant Genomics and Biotechnology: Challenges and Opportunities”. October 26-28, IGAU, Raipur, India
5. **Shailaja Hittalmani**, Rudresh N.S, Nagabhushana K, Sanath Kumar V. B, Appaji Gowda H. C, Hanamareddy B.G. and Keshava Murthy B. C., 2005. Identification of genotype specific markers for elite genotypes of finger millet enrolled in farmer’s participatory varietal selection using RAPD markers. International conference on “Plant Genomics and Biotechnology: Challenges and Opportunities”. October 26-28, IGAU, Raipur, India

6. Sanath Kumar V.B, Rudresh N.S, Appaji Gowda H.C, Nagabhushana K. and **Shailaja Hittalmani, 2005**. Phenotypic evaluation of recombinant inbred lines of finger millet (*Eleusine coracana* L. Gaertn) against blast disease at different locations of southern Karnataka. International conference on "Plant Genomics and Biotechnology: Challenges and Opportunities". October 26-28, IGAU, Raipur, India
7. Shashi B K, Sunanda Sharan, **Shailaja Hittalmani** and Shankar A.G., 2005. Wine making from Indio-African finger millet genotypes. National seminar on recent advances in Biotechnology of fermented foods on July 16, 2005

University of Wisconsin

8. **Leong, S. A.**, Tian, S., and Splinter BonDurant, S., 2006. Discovery of Genomic DNA Polymorphisms using Oligonucleotide Arrays. **RTWG Meeting - 2006**

Review articles

UAS

1. Rudresh, N. S. and Shailaja Hittalmani, Review article on Farmers Participatory Approach in Crop improvement – Bridging the gap between farmers and scientists (Agricultural News), 2006.
2. Rudresh, N. S. and *Shailaja Hittalmani*, Review article on Farmers Participatory Approach in Crop improvement – Bridging the gap between farmers and scientists is prepared in local Language in Kannada, 2005. (English Translation).
3. Keshava Murthy, B.C., Hanamareddy, B., Rudresh, N.S., Nagabhushana, K. and **Shailaja Hittalmani**, 2006. DNA marker analysis of chemically induced mutants of finger millet (*Eleusine coracana* (L.) Gaertn), Indian Journal of Biotechnology (in press).
4. Questionnaires is prepared in local language Kannada and also in English for conducting Rural Appraisal Survey in Finger Millet, 2002-2005.
5. Questionnaire is prepared in local language Kannada and also in English for conducting Farmers Participatory varietal Selection in Finger Millet, 2002-2005.

Papers submitted for journals:

1. Quality preferences of Finger millet technology products: Application of Conjoint Analyses – **Indian Journal of Agricultural Economics**, Mumbai, India
2. Technical efficiency and resource use productivity in new Finger millet genotypes of rainfed ragi cultivation. – **Agricultural Economics Research Review**, New Delhi.
3. An inter-district comparison of risk in new Ragi variety developed and their production in Karnataka: **Mysore Journal of Agricultural Science**, UAS, Bangalore.

Staff at University of Agricultural Sciences, Bangalore

Scientists:

Dr. Lalith Achuth (Economist)
Dr. H V Nanjappa, (Agronomist)
Dr. Shivamurthy (Extension)
Dr. Mohan Rao (Breeder)
Dr. Sunanda Sharan (Food and Nutrition)

Research Associates

Mr. Appaji Gowda H.C. (Resigned in March, 2006)
Mr. Hanama Reddy B.G.
Dr. Sanath Kumar V.B. (February 2006)
Mr. Rudresh N.S.

Senior Research Fellows

Ms. Pavana Hiremath

Accounts

Mr. B.G. Rohith

Research Assistants

Field Labours

Staff issues at University of Gerogea

The stipend of Vinod Jakkula, a Graduate Student, was cost-shared between the McKnight grant and a departmental studentship until August 15th 2005. Dr. Mathews Dida, a visiting scientist from Maseno University, Kenya, was associated with the McKnight project until December 2005. His stipend was paid from a grant from the Kirkhouse Trust. Dr. Sam Ajanga, a visiting scientist from KARI, Kakamega, Kenya, joined the project for 1 year on April 20th 2006. His stipend is also paid from a grant from the Kirkhouse Trust.

Staff issues at WISC: Sally Leong, PI

Shulan Tian, Assistant Researcher supported by the McKnight Foundation
Sandra Splinter-BonDurant, Director, University of Wisconsin Gene Expression Center
Donald Meyer, Partek Inc.

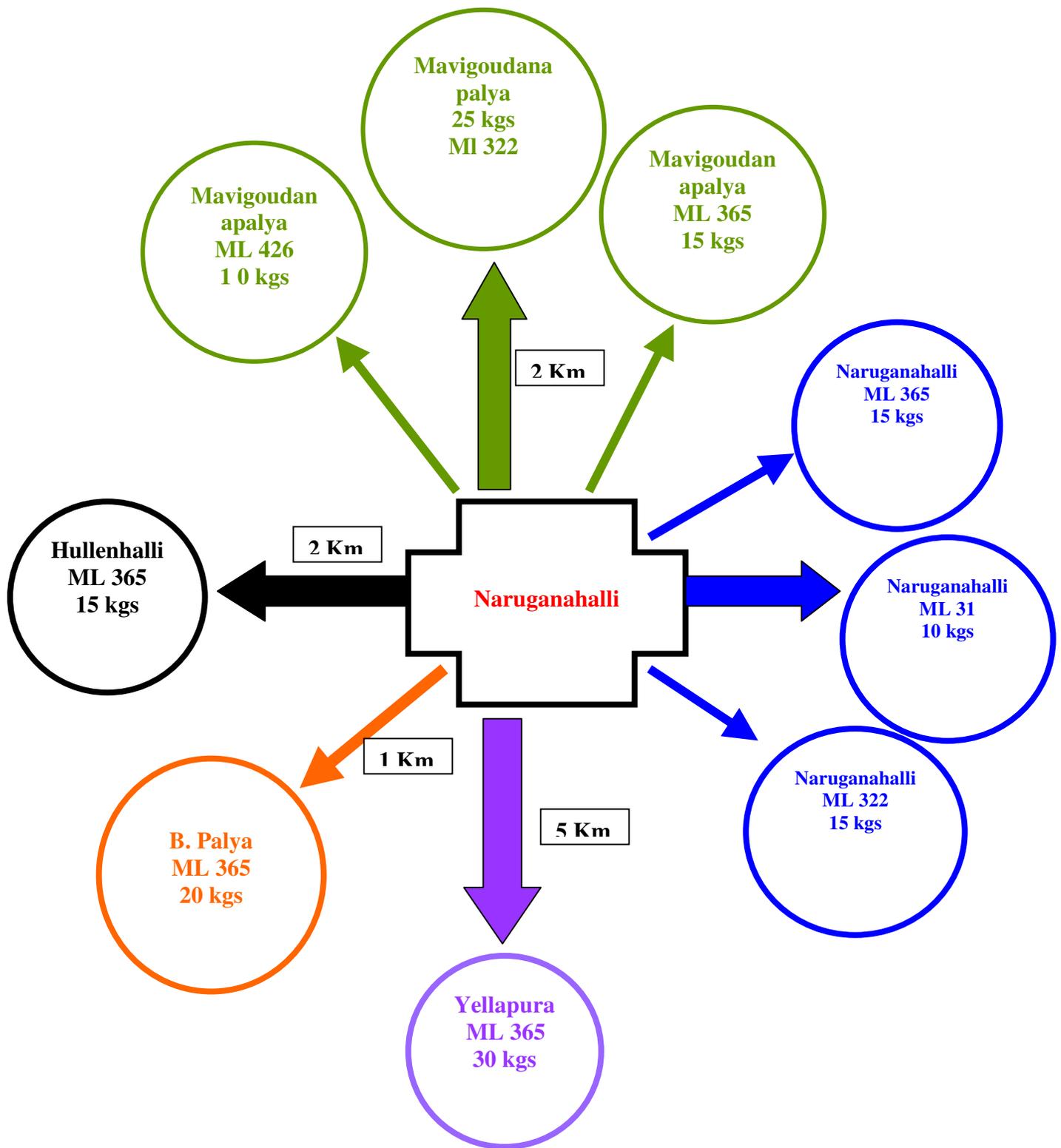


Fig 2. Dissemination of elite finger millet genotypes from Naruganahalli village farmers, Tumkur District



A good grain and fodder yield of new elite lines and Scientists collecting the information from farmers

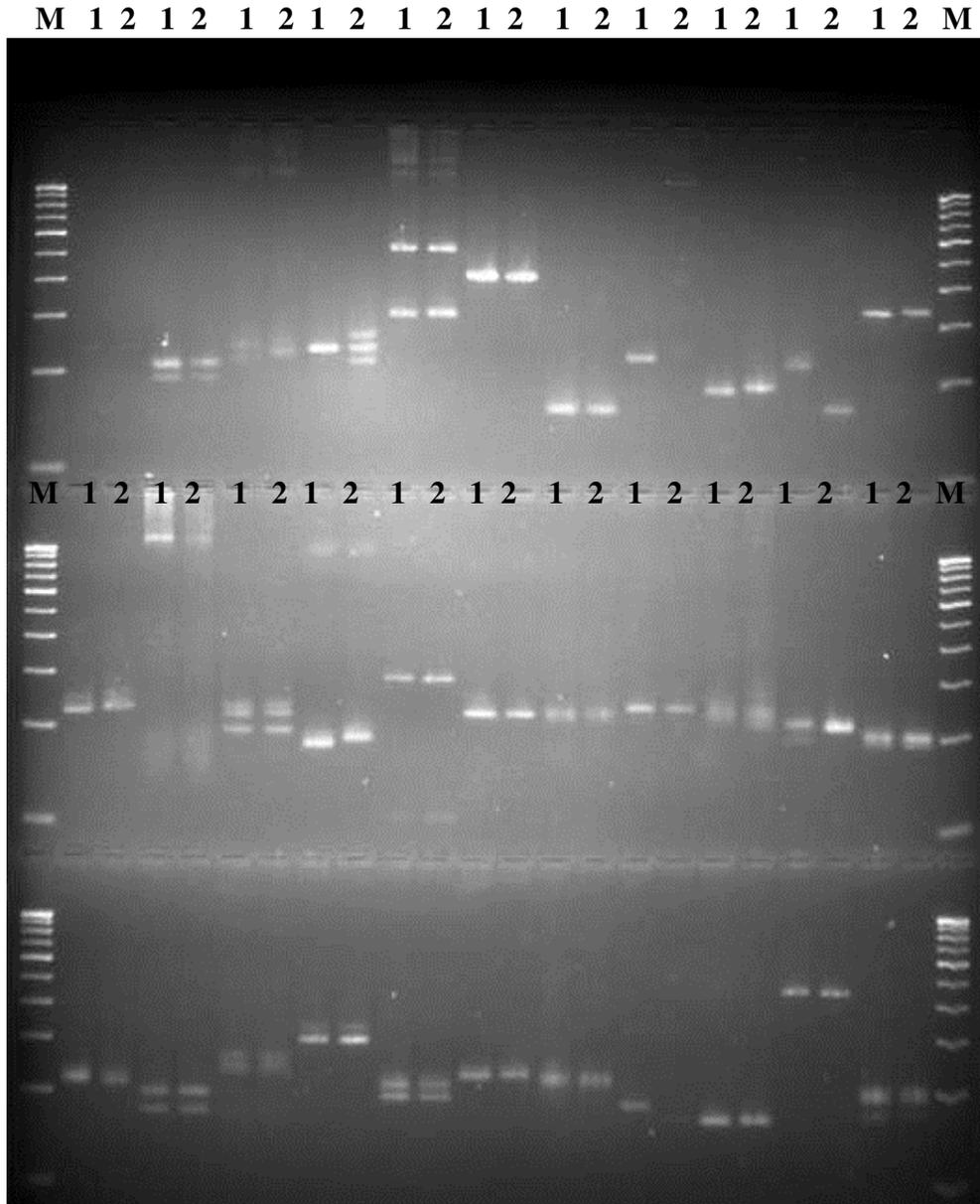


Figure 4: SSR markers used for parental polymorphism survey in IE1012 (1) and Indaf5 (2) parents.

M 1 2 -----Progenies-----M

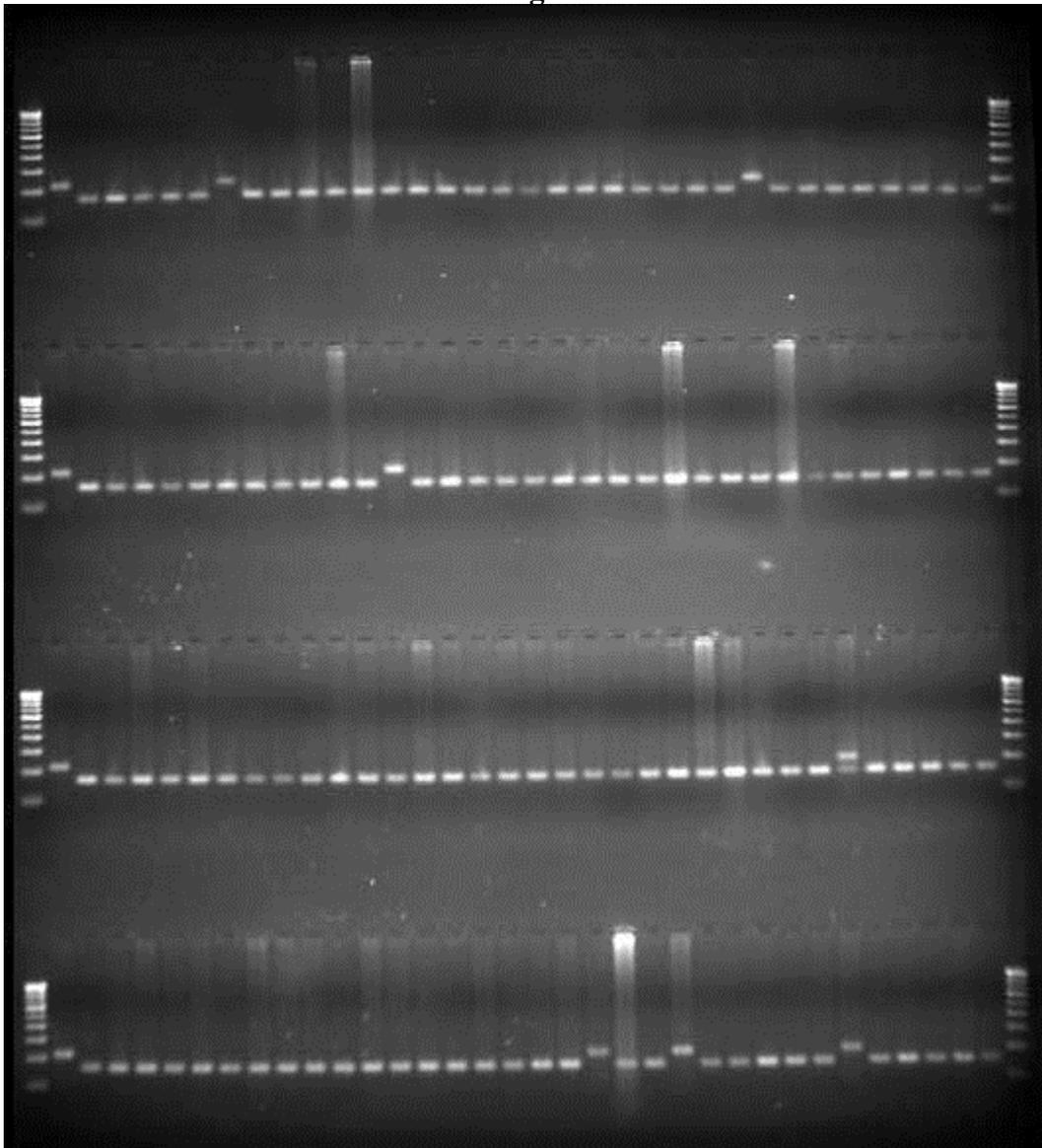


Figure 5: SSR16 used for screening of RIL population of IE1012 and Indaf5 cross.

M 1 2 -----Progenies-----M

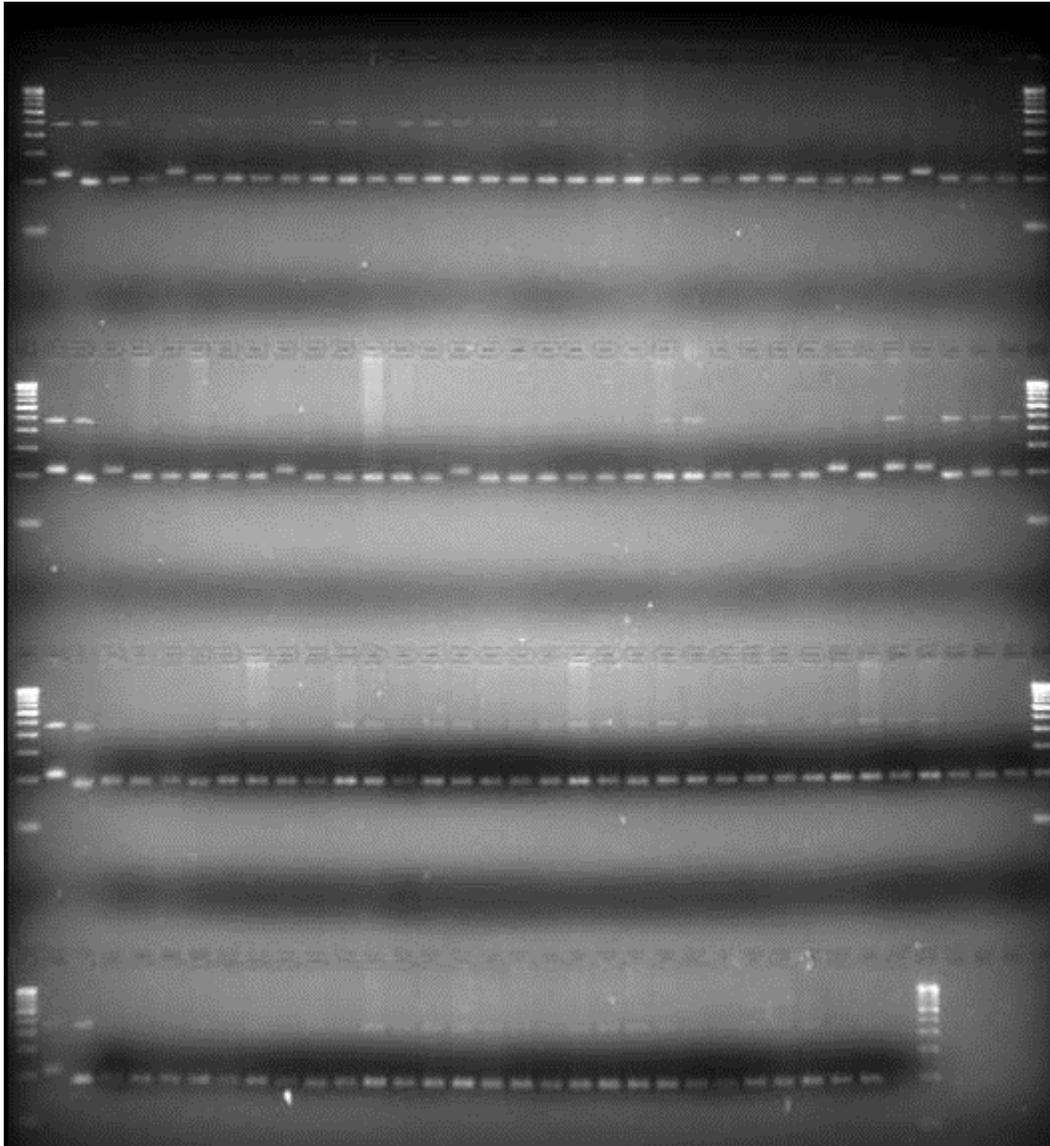


Figure 6: SSR66 used for screening of RIL population of IE1012 and Indaf5 cross.

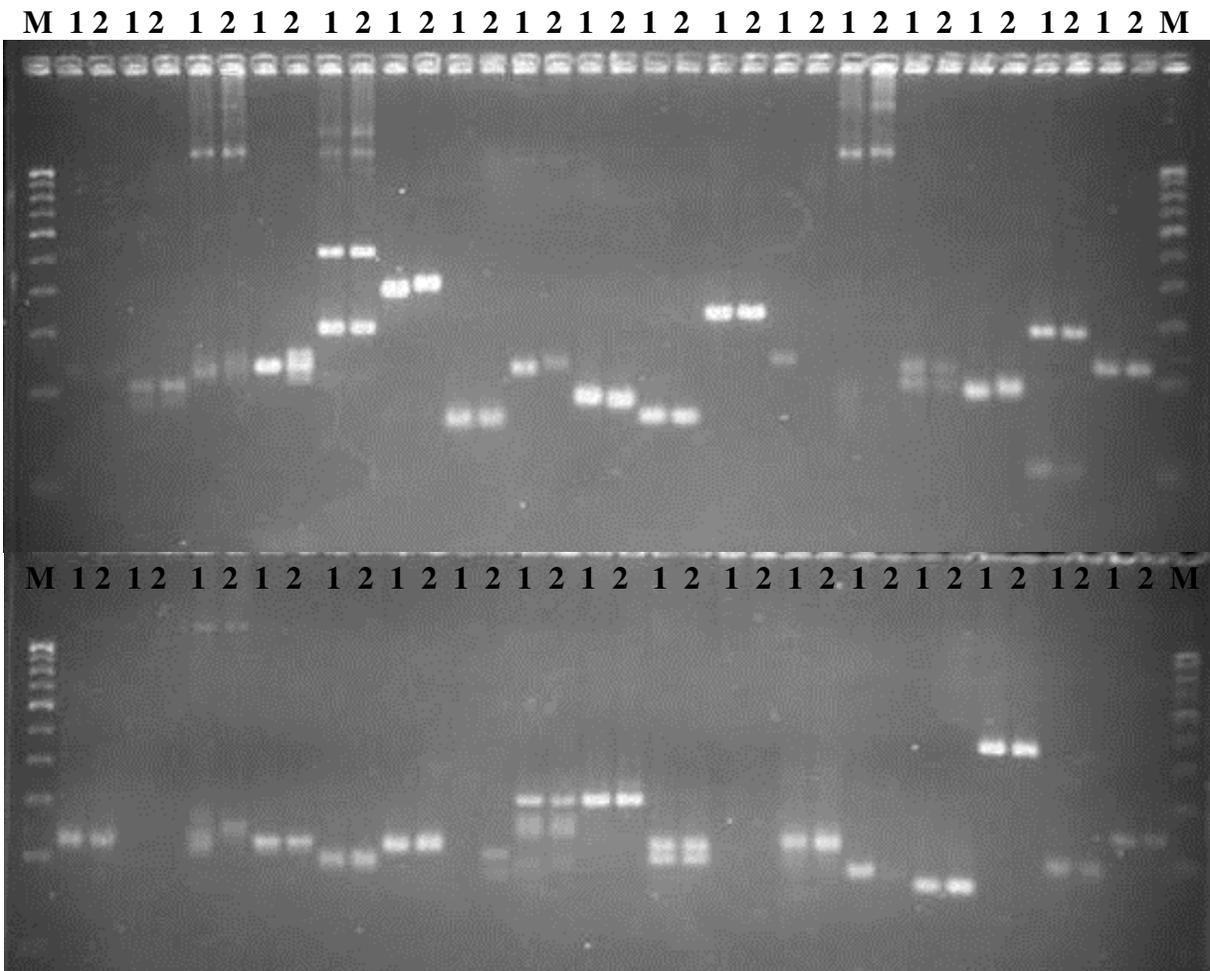


Figure 7: SSR markers used for parental polymorphism survey in GPU28 (1) and Indaf9 (2) parents on 3.5 % agarose gel.

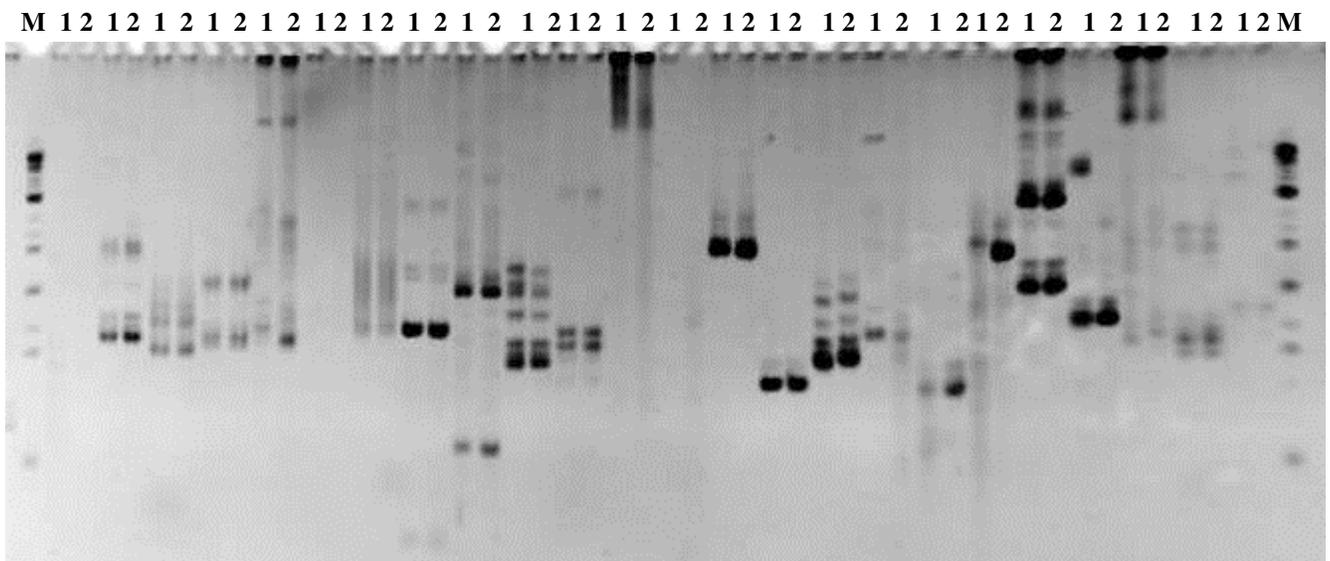


Figure 8: Finger millet SSR markers used for parental polymorphism survey in GPU28 (1) and Indaf9 (2) parents on 7 % PAGE gel.

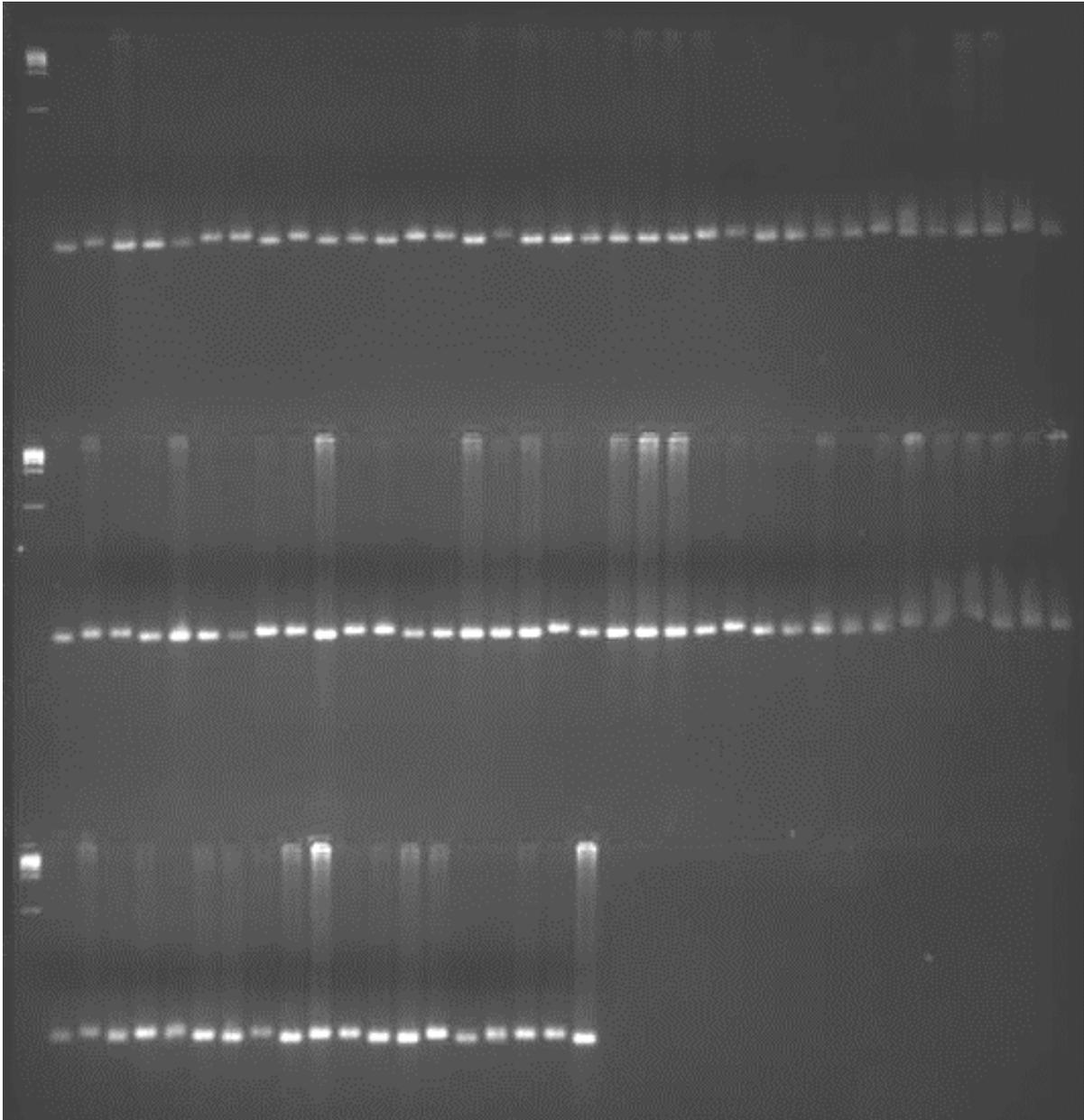


Figure 9: SSR16 used for progeny screening of RILs of GPU28/Indaf9 cross.

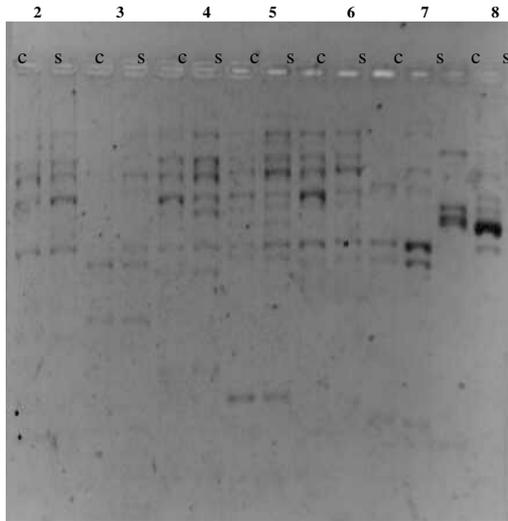
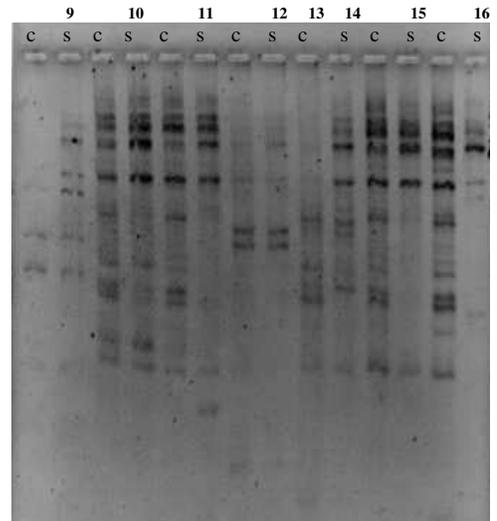


Fig. 10(A) Indicating the differential display pattern in IE-1012 for 24 hrs Blast control and stress with anchored primer 'H-T11G' and 'H-AP-2,3,4,5,6,7,8' respectively .
C=Control for Blast.S= Stress for B



Fig(B) Indicating the differential display pattern in IE1012 for Blast infection for 24hours with Anchored primer HT11G and HAP-9,10,11,12,13,14,15 arbitrary primers .
C=Control for Blast. S= Stress for Blast

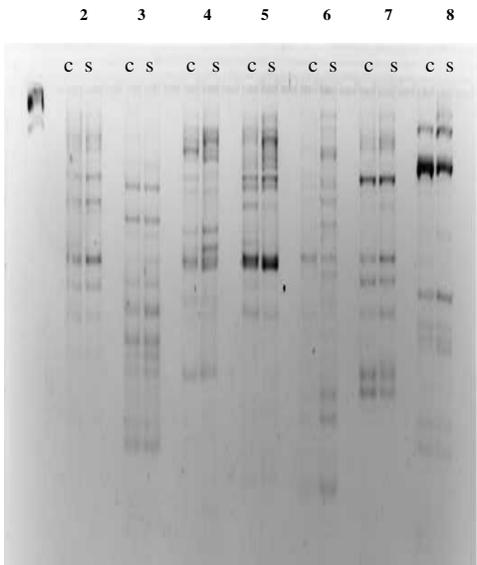


Fig (c). Differential display pattern for 24hrs Blast infection In Indaf-9. cDNA amplification with anchored primer 'H-T11G' and 'H-AP-2,3,4,5,6,7,8' respectively.

L= Ladder;
C=Control for Blast.
S= Stress for Blast

me1	TGAGTCCAAACCGGATA
me2	TGAGTCCAAACCGGAGC
me3	TGAGTCCAAACCGGAAT
me4	TGAGTCCAAACCGGACC
me5	TGAGTCCAAACCGGAAG
em1	GACTGCGTACGAATTAAT
em2	GACTGCGTACGAATTTGC
em3	GACTGCGTACGAATTGAC
em4	GACTGCGTACGAATTTGA
em5	GACTGCGTACGAATTAAC
em6	GACTGCGTACGAATTGCA

Table1 pearl millet polymorphic oligos

Xpsms75 CD725252 F: AAGAGGGCCTTGAAGTGTG b a a b c d a b 0.69 middle
Phosphopyruvate hydratase
R: CAGATCTTTCAGGCTGTCTCC

Xpsms76 CD725251 F: CAACCATGCTACTCTATCTGG b a a b a b a a 0.47 middle Xylose
isomerase
R: GCAATGTCTGTCATGAACTG

Xpsms77 CD725246 F: GGATGCTACCTTCTCCTTCAC b a b b a b ab ab 0.62 middle/ unknown
protein
R: AACCTTCTACAGCTTCGCTG bottom

Xpsms78 CD725244 F: GCGCGATCTTGAACCACTCG b a a a b b b 0.5 top Phosphogluconate
dehydrogenase
R: GCCATCTTCCTTGACCGCATC

Xpsms79 CD725227 F: GGTGACCTTGAGCGTTCTATC b a a b c d c c 0.72 middle/
Lactoylglutathione lyase
R: CTCATCAGCATAGCCCAAC bottom

Xpsms80 CD725216-1 F: GTACAAGGAGATCGAGAACG b ? c d e b f g 0.82 top Translationally
controlled tumor protein homolog

Xpsms81 CD725216-2 R: GACGGAAGGTGTCAACAATG

Xpsms82 CD725185 F: TGGGCTTGATCGCTCATCTG b a a b b b b 0.375 top Uridylate kinase
R: CAACCTCTGACATACGGAAGG

Xpsms83 CD725183 F: ACCAAGCTTCCAAGGATGAC a a a b c b d a 0.66 top elongation factor
1-BETA
R: CACGCTCTTCAGCAGCCTTC

Xpsms84 CD725157 F: AGTTGTTGTCTTGGAGAAGC b a a b a b a a 0.47 middle ribophorin I
homolog
R: GTAAGATGGAGAGCTCTTGG

Xpsms85 CD725154 F: TGGACTCATTCTACTTGCTCC b a a b b b b 0.375 top LMBR1 integral
membrane family protein
R: GGAGAGATTGCAATGTGTGG

Xpsms86 CD725153-1 F: CGTACAAGGAGATCGAGAAC a b c d e f g h 0.875 top translationally
controlled tumor protein homolog

Xpsms87 CD725153-2 R: AATGTGACATCAACAGCTC

Xpsms88 CD725147 F: AATGCACTAGTCCACCGTCC b a c d d e b d 0.75 bottomGlutaminyl-
Trna synthetase
R: CCTACACCACGCTTCCTC

Xpsms89 CD725143 F: AGGGACACGCGAATACAAGC ? a a ? b a c d 0.66 top soluble NSF attachment protein
R: CTTGAGAAGGAGAGTTGTCTTC

Xpsms90 CD725138 F: GAGAACCACTGCGAGTGAAC b ? c ? ? ? d e 0.75 top Pyruvate decarboxylase
R: ACTGACCAGCCAATTGATCC

Xpsms91 CD724997 F: AATGAGGCTTCCATTGCCAG b a b b c b b b 0.41 bottomSHOOT1 protein
R: TCCTCAAGATTTCAAGATCCG

Xpsms92 CD724986 F: TGGTGATGCTGCTGCTTTAG a a a a b a a c 0.41 middle Ubiquinone biosynthesis monooxygenase
R: CGACCGAGTACATCTTCTGG

Xpsms93 CD726638 F: TCTCACTTGAAATGGTACAGAG a a a a a b b 0.375 Pyrophosphate--fructose 6-phosphate 1-phosphotransferase
R: TCTGGATGACTACTACAGGAC subunit

Xpsms94 CD726569 F: TCCCGAAGTGCTGGAAGAGG a a b b ? a a a 0.41 coatomer delta subunit
R: CTTCCCAAGTGGTCAGAACG