Pak-US Science and Technology Co-operative Research Project

SSR Markers for DNA Fingerprinting and Diversity Analysis of Sugarcane Cultivars Resistant and Susceptible to Red Rot

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INTRODUCTION

Plants are marvelously productive under ideal growing conditions, but ideal growing conditions rarely occurs.

More typically, plant encounters abiotic stresses such as water shortage, lack of nutrients, or the biotic stresses in the form of plant pathogens, insect pests or weeds

FOOD LOSSES OF THE WORLD

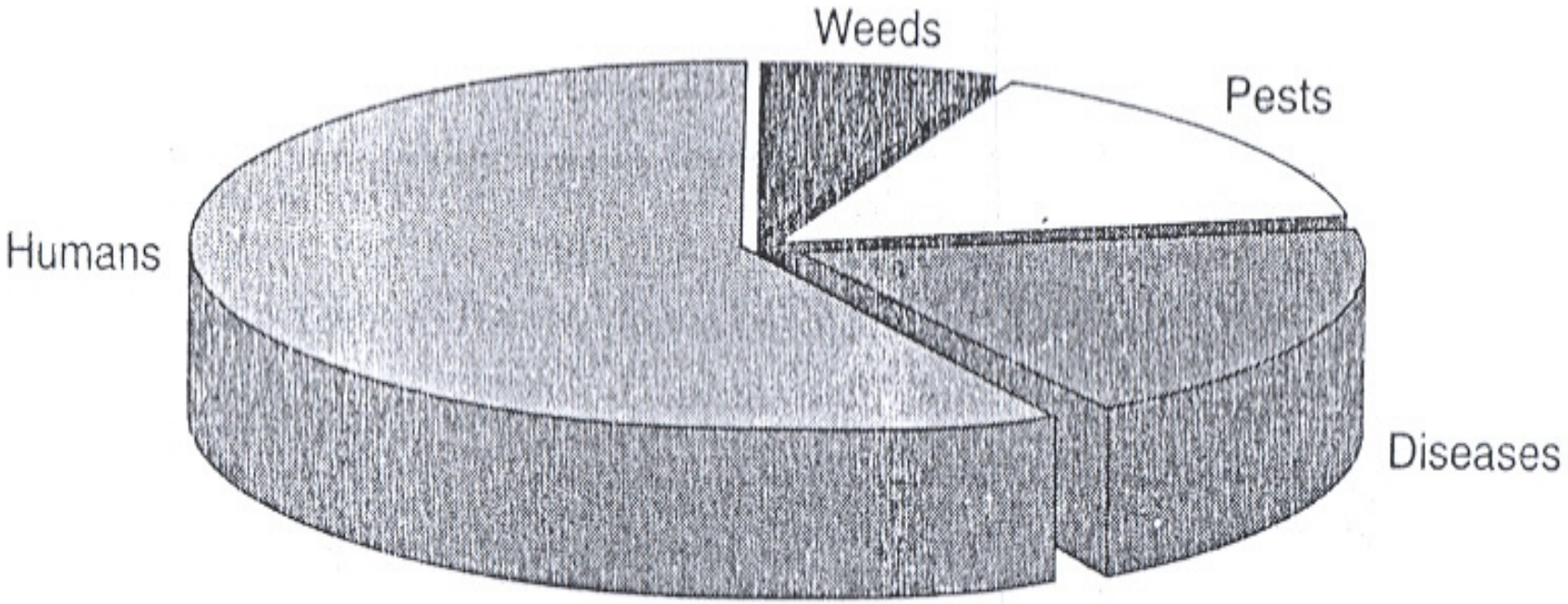


Figure 1 The relative proportions of total world crop production lost to weeds, pests and diseases. The figure shows that of the total potential yield, only 63% is available for human consumption. The remainder is lost in more or less equal proportions to weeds, pests and diseases.

SIGNIFICANCE OF MOLECULAR MARKERS

DNA profiling markers being wholly genetic are free from environmental modulations and have been looked upon as tools for a large number of applications ranging from localization of a gene to improvement of plant varieties by marker-assisted selection (Fracaro *et al.*, 2005).

OBJECTIVES

- The goal of this research was to reduce the adverse impact of diseases.
- Identify germplasm with resistance to the major diseases affecting sugarcane.
- Identify molecular markers that are linked to gene for disease resistance.
- Priority was given to finding markers for red rot disease.

APPROACH

- To identify and develop germplasm with resistance to the major diseases affecting sugarcane in Pakistan, highly domesticated and wild clones of sugarcane or near relatives were evaluated for resistance to major sugarcane diseases following either natural infection or artificial inoculation.

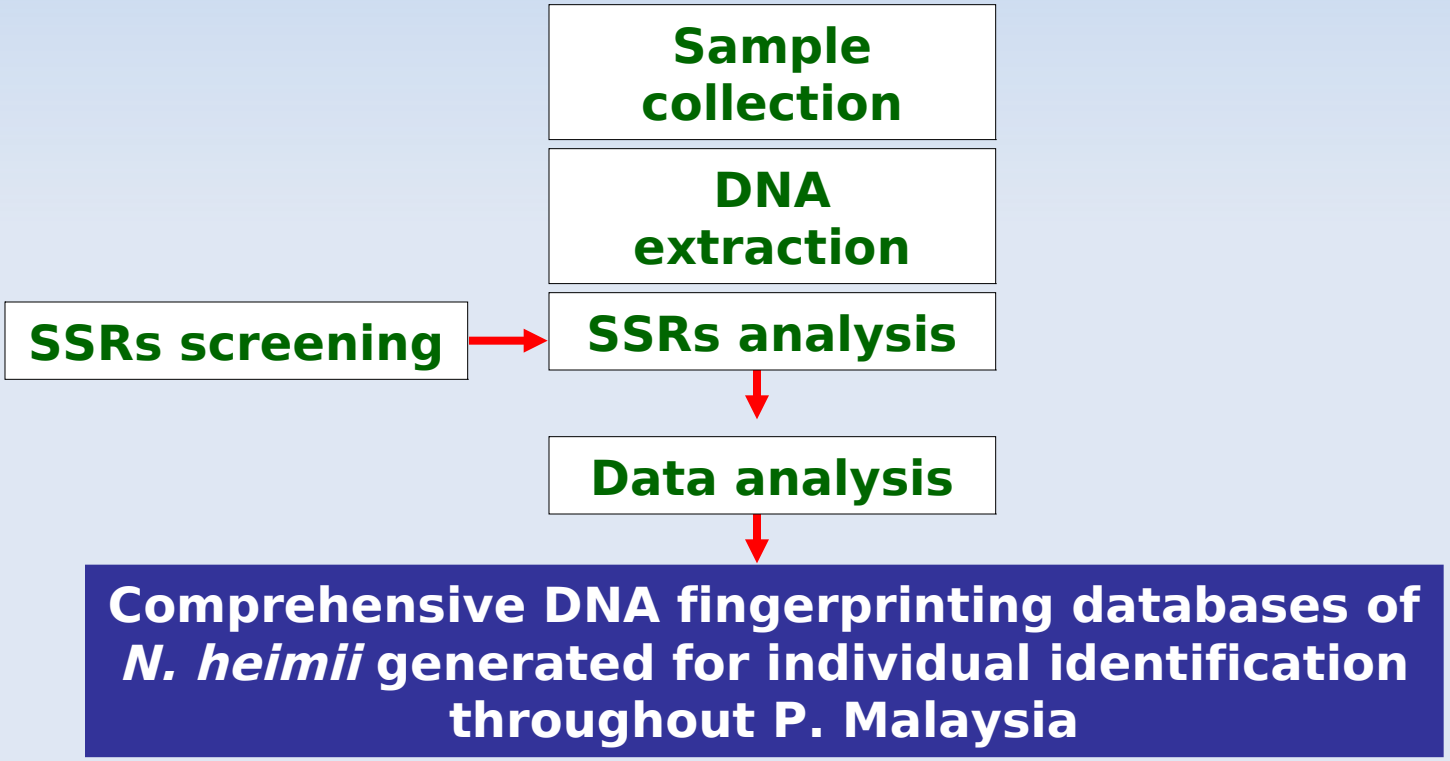
- To identify molecular markers that are linked to genes for disease resistance, polymerase chain reaction (PCR) based methods such as SSR were used to identify genetic markers clearly linked to the resistant genes

MATERIALS AND METHODS

- Different known and characteristically studied genotypes of *Saccharum* spp. Hybrids were cultivated in the field of School of Biological Sciences (SBS), Punjab University, Lahore.

- Grouping of sugarcane cultivars was made on the basis of their resistance and susceptibility against red rot

Methodology



Genotyping procedure

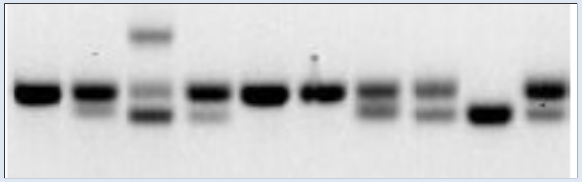
PCR

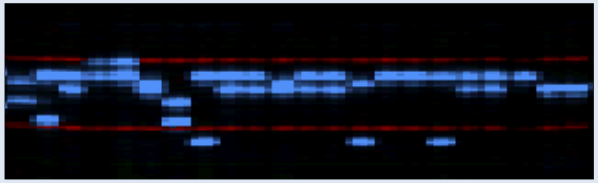
Electrophoresis

- Agarose
- PAGE
- Denaturing PAGE
- Capillary

Visualization

- Silver staining
- SybrGreen staining
- Autoradio-graphy
- Fluorescent dyes





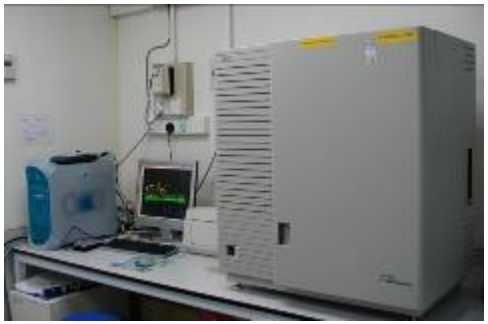
- **The use of fluorescently labeled primers, combine with automated electrophoresis system greatly simplified the analysis of microsatellite allele sizes**

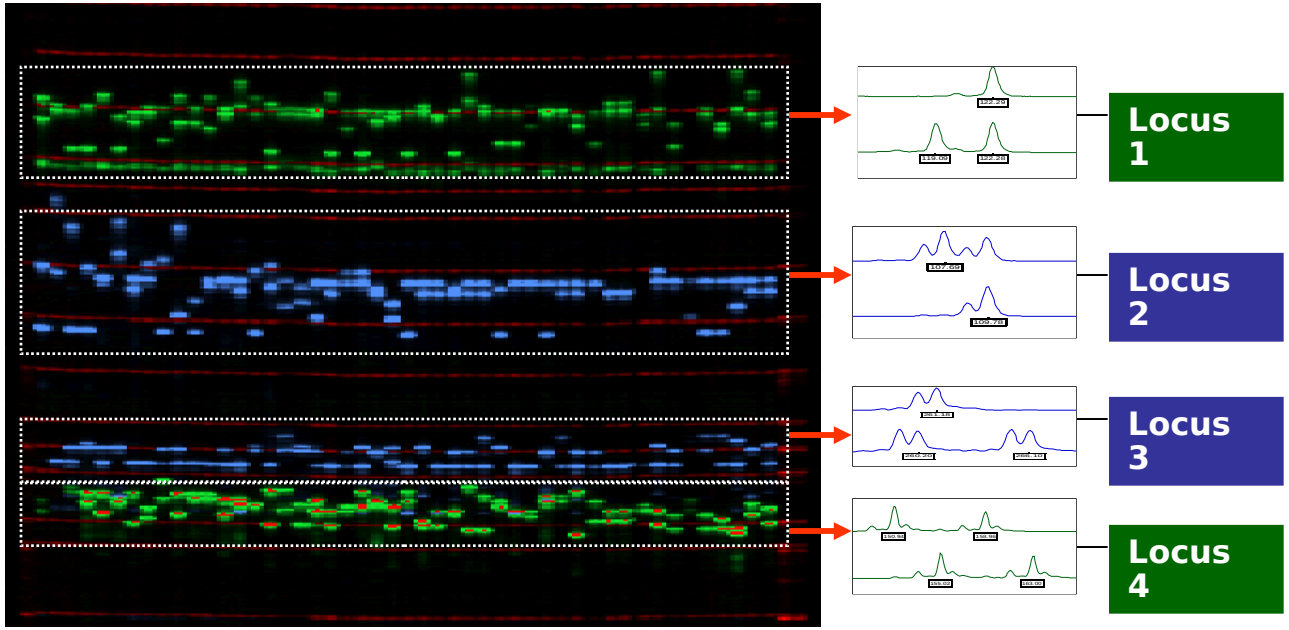
Primer1

Primer2

Primer3

Primer4





Genotypes Reaction to Red Rot

Highly Resistant
HSF-240, CPF-237, NSG-555 & CSSG-676

Resistant
NSG-311, HOSG-315, CPSG-437, CPSG-3481, CPF-246, CP77-400, SPSG-79, NSG-59 & CPD-334

Moderately Resistant
SPF-213, CPD-335 & SPSG-2453

Moderately Susceptible
CPD-346

Susceptible
CO-1148, HSF-242, SPSG-394 & SPF-234

MATERIALS AND METHODS

DNA extraction

- **PCR** amplification and capillary electrophoresis

- SSR fragment analysis were conducted following a high throughput sugarcane genotyping procedure of Pan *et al*. (2007)

MATERIALS AND METHODS

- Simple sequence repeats (SSRs) or microsatellites are short DNA repeats found in genome of the most eukaryotic organism.

- These SSRs provide co-dominant Mendalian markers,much more powerful than dominant markers and can be used to determine population genetic structure, kinship, reproductive mode, mapping of useful genes and genetic mapping etc.

- These dinucleotide and trinucleotide sequences can be used to design primer for the amplification of the these sequences.

MATERIALS AND METHODS

- International Sugarcane Micro-satellite Consortium (ISMC) has designed 221 micro-satellite markers based on the sugarcane genomic DNA sequences.

- In 2006 Pan *et al* used these 221 micro-satellite markers to investigate germplasm evaluation and fingerprinting of US cultivars and out of these 67 markers showed robust PCR product.

- 67 markers were selected 21 highly polymorphic micro-satellite markers to genotype 20 sugarcane cultivars grown in Pakistan

MATERIALS AND METHODS

- The presence of a particular SSR allele was given a score of “1” or “A” and its absence a score of “0” or “C”.

- The overall scorings for the 144 SSR alleles in an affixed sequence order that were amplifiable from all the 21 SSR markers for particular variety constituted its genotyping file as was described by Pan *et al.* (2007).

RESULTS

- Based on these 21 markers,a clear differentiation between resistant and susceptible cultivars to red rot was established.
- This information is vital for future breeding programme

Table II. Primer sequence, annealing temperature and number of alleles amplified

No.	SSR Name*	Repeat Motif*	Forward Primer Sequence (5' to 3') Reverse Primer Sequence (5' to 3')	Annealing Temp	# Alleles Amplified
1	mSSCIR74	(CGC)9	ACGCAACGCAAAACAACG	54	5
2	SMC31CUQ	(TC)10 (AC)22	CATGCCAACTTCCAATACAGACT	62	11
3	mSSCIR66*	(GT)43GC (GT)6	ACTAAAATGGCAAGGGTGGT	48	4
4	SMC278CS	(TG)19 (AG)25	CATGCCAACTTCCAAACAGACT	64	8
5	SMC24DUQ	(TG)13	CGCAACGACATATACACTTCGG	64	6

RESULTS

- The resulting genotyping files of all 20 Pakistan varieties were aligned using the Multiple Sequence Alignment program of the DNAMAN® software (Lynnon Biosoft, Vaudreuil, Quebec, Canada) to generate a homology tree.

- The tree allowed the identification of those sequences that were unique for a particular cultivar as well as an indication of how many cultivars could be identified by a particular SSR marker.

SSR POLYMORHIC POTENTIAL

- The 21 SSR markers amplified a total of 144 alleles, of which 135 (93.8 %) were polymorphic. The numbers of alleles produced by each marker varied from 3 to 11, with an average of 6.85 alleles.

- Markers SMC31CUQ and SMC597 CS produced the largest number of 11 alleles, while marker SMC36BUQ produced the least number of 3 alleles.

RESULTS

- Number of cultivars distinguishable by any SSR marker varied from zero to 20. SMC36BUQ did not generate unique binary sequence for any cultivar, so no cultivar could be identified using this primer.

- In contrast, markers SMC31CUQ, mSSCIR3 and SMC597CS produced 20 different allelic binary sequence combinations, with each sequence being unique for a particular cultivar.

Polymorphism Information Content (PIC)

- The PIC value shows the ability of a SSR marker to detect polymorphism within a given population that is usually related to the usefulness of that marker in fingerprinting sugarcane cultivars. In this study, the PIC values of the 21 SSR markers varied from 0.4 to 0.84 with a mean of 0.69 (Table III).

Table III. Polymorphism content values (PIC) of marker, resolving power of marker and number of cultivars distinguished by each marker pair

# Cultivars distinguished	PIC	# Cultivars distinguished
mSSCIR74	0.48	5
SMC31CUQ	**0.80**	**20**
SMC703BS	0.46	11
SMC851MS	0.80	10

Table III. Polymorphism content values (PIC) of marker, resolving power of marker and number of cultivars distinguished by each marker pair

# Cultivars distinguished	PIC	# Cultivars distinguished
SMC336BS	0.81	12
SMC22DUQ	0.47	5
SMC1604SA	0.70	9
mSSCIR3	**0.64**	**20**

Table III. Polymorphism content values (PIC) of marker, resolving power of marker and number of cultivars distinguished by each marker pair

# Cultivars distinguished	PIC	# Cultivars distinguished
SMC597CS	**0.84**	**20**
SMC119CG	0.80	6
SMC486CG	0.61	2

Resolving Power (RP)

- Resolving power (RP) depended upon the distribution of the alleles within genotypes. The RP values ranged from 0.0 to 5.1 with a mean value of 2.37 (Table IV).

- In the current study, markers with higher RP values were more informative and could identify more cultivars

- Three markers, namely, SMC31CUQ, mSSCIR3, and SMC597CS, had RP values greater than 4.9 and could distinguish all the 20 Pakistan sugarcane cultivars.

Table IV. Resolving Power value (RP) of Marker and Number of Cultivars Distinguished by Each Marker Pair

SSR Name	RP	# Cultivars distinguished
mSSCIR74	1.0	5
SMC31CUQ	**5.1**	**20**
mSSCIR66	0.6	6
mSSCIR43	3.9	16

Table IV. Resolving Power value (RP) of Marker and Number of Cultivars Distinguished by Each Marker Pair

SSR Name	RP	# Cultivars distinguished
SMC1604SA	2.8	9
mSSCIR3	**4.9**	**20**
SMC334BS	3.1	7

Table IV. Resolving Power value (RP) of Marker and Number of Cultivars Distinguished by Each Marker Pair

SSR Name	RP	# Cultivars distinguished
SMC597CS	**5.0**	**20**
SMC119CG	2.3	6
SMC486CG	1.0	2

CONCLUSIONS

- Three SSR markers, namely, SMC31CUQ, mSSCIR3, and SMC597CS, were identified that could discriminate among these 20 Pakistan cultivars.

- It is possible to use these three SSR markers for precise, efficient and reliable screening of sugarcane genotypes in for RED ROT DISEASE in Pakistan

QUESTIONS ???