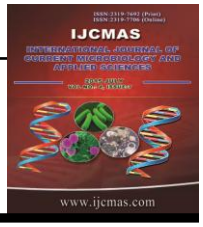
Int.J.Curr.Microbiol.App.Sci (2015) 4(8): 671-694

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 8 (2015) pp. 671-694**
http://www.ijcmas.com



Original Research Article

Assessment of Genetic Diversity among Wheat Varieties in Aurangabad Using RAPD Analysis

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A B S T R A C T

Assessment of genetic diversity among wheat varieties in Aurangabad using RAPD analysis under invitro condition was studied at MGM College of Agricultural Biotechnology & MGM-Institute of Biosciences and Technology, Aurangabad. The Genetic diversity of eleven different wheat (*Triticum aseitivium*) varieties (Mrugnayani chandoshi, Hathi sharbati, Mini sharbati, 147 Best loose, Loose lokwan grade 1, Loose sharbati grade 1, Standard 147, Agni sharbati, Narmada 496, Vishnu padma chandoshi, HD 2189)was assessed using the RAPD primers and PCR- Polymerase chain reaction. Electrophoretic analysis of amplified products revealed higher incidence of polymerase in 11 varieties. Pair wise comparisons of unique and shared polymorphic amplification products were used to generate Jaccards coefficients. In the present investigation of molecular marker analysis in wheat was carried out using RAPD primer for assessment of parental variability. It was carried out using following points: DNA isolation was carried out of different (11) wheat variety using CTAB DNA extraction method and it was confirmed using Agarose gel electrophoresis fig 1. DNA purification was carried out by treating the DNA suspended in TE buffer with RNase A to remove the RNA contamination from the DNA and it was checked using agarose gel electrophoresis Quantification of purified DNA was carried out by two methods: UV spectrophotometer by using 260 and 280 ratio and Eye ball estimation using standard DNA. RAPD amplification was done using PCR with primer 3 universal random primer i.e. RPI 3 was used for study and 26 RAPD amplification bands were generated. Among all eleven wheat 147 best loose and HD 2189 variety gave more amplification than other. Polymorphic and Monomorphic banding pattern was studied. Polymorphic pattern was obtained. Polymorphic present was calculated in 11 wheat varieties using primer three and it was found to be 80.76 % RAPD. These were employed to construct the phenograms using an unweighted pair group method with arithmetical averages (UPGMA). Analysis of RAPD data appears to be helpful in determining the genetic relationships among the genotypes.

K e y w o r d s

Silver nanoparticles, Antibacterial, Zone of inhibition, Chemical synthesis, Sodium borohydride

Introduction

Wheat (*Triticum aestivum*) is an important cereal food crop in the world. Most of the wheat varieties/genotypes are related with each other. All the wheat varieties share

same gene pool in India due to transfer of dwarfing gene into cultivated varieties and later these varieties acquitted with the different resistance genes. The use of random amplified polymorphic DNAs as a molecular marker for diversity assessment is a reliable and important tool in modern era. Now a day it has become necessary to assess the diversity among different genotypes to know the extent of similarity and dissimilarity at genetic level. Wheat is the most important food grain of the temperate zones - both north and south. World acreage in wheat is estimated at near 500 million with near 60 million acres in the United States. Production in the United States was 1,524,340,000 bushels in 1967.

Wheat classification

Kingdom: Plantae-Plants
Subkingdom: tracheobionta-Vascularplants
Super division: Spermatophyta-Seedplants
Division: Magnoliophyta-Floweringplants
Class: Liliopsida-Monocotyledons
Subclass: Commelinidae
Order: Cyperales
Family: Poaceae-Grassfamily
Genus: *Triticum*-wheat
Species: *Triticum aestivum* - common wheat

The information at genetic and molecular level infers about the variation in characters among genotypes. An attempt was made to know the relatedness among eighteen bread wheat varieties to detect the variations among the wheat genotypes during 2005-06. Standard protocols were used for the isolation of DNA and RAPD analysis. It is stable diet for more than one third of world's population and contributes more calories and proteins to the world's diet than any other cereal crops. Nowadays in Egypt there is an urgent need to increase the productivity level of wheat to reduce the food gap resulting from population increase. The breeders have to develop a new set of varieties with higher production.

The true knowledge of gene action of various Durum wheat traits is useful in making decision with regard to appropriate breeding system effects were played the major role in controlling the genetic variation in the days to flowering, one thousand grain weight and grain yield per plant. It was reported that the importance of both additive and dominance gene effects in the inheritance of one thousand grain weight.

However the dominance gene effects were more than in magnitude favorable condition. Conventional breeding has accomplished a remarkable success in development of high yielding varieties. However, use of other non conventional approaches may further accelerate the progress of such a breeding program. Wheat (*Triticum aestivum* L.) is an important cereal crop widely cultivated in India and world providing ample food calories and proteins to the human population. It is the second most important cereal crop after rice grown under diverse agro climatic conditions. Wheat is important winter cereal crop contributing about 32% of the total food grain production in India. It is staple food crops in at least 43 countries. Wheat (*Triticum* sp) is one of the most important cereal crops which constitute a very important source of food to a vast population. Bread wheat is an important cereal crop in global agricultural economy is cultivated in a range of mega environments of the world. It is most widely grown and consumed food crop of the world cultivated on larger area and produce more tonnage of food than any other cereals.

Triticum aestivum, common bread wheat, contains 3 different but genetically related genomes (A, B and D) with a total genomic

size of 1.7x1010 base pairs, which is about 500 times larger than that of rice1 illustrating the complex nature of wheat genome. Wheat has been a food crop for mankind since the beginning of agriculture. Carbonized grains dating to at least as early as 6750 B.C. have been found in Iraq and many other findings in Eastern Mediterranean countries are nearly as old. The Middle East is probably the area of origin, and wheat apparently spread throughout Europe not later than the Stone Age. Wheat is essentially a cool season crop that thrives best at preharvest temperatures averaging around 60 F. The minimum frost-free growing season is about 100 days. In continental United States wheat is grown in every state although production in New England is minor. From 15 to 20 or more inches of precipitation are necessary for annual cropping. In some areas with not more than 10 to 15 inches of precipitation wheat is grown once in 2 years, with the land kept free of vegetation one of the years to accumulate moisture in the soil. The wheat plant is an annual grass. It is mainly grown as a winter annual in milder climates, with seeding in the fall and harvest from June through August depending on the length of the winter. In areas with rigorous winter climates it is mainly spring seeded. Planting is as early as soil can be worked, and harvest is in late summer and early fall. In early growth stages the wheat plant consists of a much compressed stem or crown and numerous narrowly linear or linear-lanceolate leaves. Leaves are mainly near glabrous. Buds in the leaf axils below the soil surface grow into lateral branches termed tillers. From both the main crown and the tillers, elongated stems develop later and terminate in a spike or head in which the flowers, and finally the seed or grain, develop. In fall-seeded wheat the plant usually remains in the rosette stage throughout the fall and winter, sending up the elongated stems in late spring. In spring-seeded wheat the rosette period may be short, and tillering is usually much less than in fall plantings. During late fall and early spring, fall-seeded wheat can be lightly pastured without greatly reducing grain yields, and this is frequently done. The pasturage at this stage is nutritious and highly palatable. Stems of wheat reach from 18 inches to 4 or more feet in height depending on kind and growing conditions. The spike or head may be from less than 2 inches to 4 or 5 inches long. Both stems and spikes from the latest- formed tillers are usually somewhat smaller than those of the earlyformed tillers. Wheat is an annual plant belongs to trip tritiace subfamily poodiaceae of family pooaceae. Wheat is having seven pair of chromosome. The different species of *Triticum* are grouped into diploid and tetraploid and hexaploid. Out of 50 wild species 3 species are being cultivated in India *Triticum* dicoccum it is also called as emmer wheat, *Triticum* durum as macaroni wheat and *Triticum* aestivium as bread wheat. About 87 % of total wheat production is of bread wheat 12% of durum wheat and very less i.e. 1 % of dicoccum wheat.

Wheat is having therapeutic value. It is having wider adaptability ie tropical subtropical as well as temperate zone. It can tolerate severe cold as well as snow and resume growth with grain setting in a warm weathers in spring. Wheat is naturally self pollinated cro0p which is usually grown to a height of about 3 feet and completing life cycle within 120-130 days. Inflorescence of wheat is made up of spikelets enclosed by outer lemma and palea, spike takes several days to complete flowering and opening of the flower stars from the lower spikelet and continued towards top, it requires seldom 20 minutes. Another dehiscence takes placed within two to three minutes.

Wheat has grown since pre historic time so long ago that the origin of wheat is still a matter of speculation. All available records and evidence reveal its origin from south western Asia, where wild forms of wheat were cultivated as early as 10000-15000 B.C. In world wheat occupies an average area of 215.26 million ha with total production of 584.76 million tones with productivity of 2715 kg/ha. Maximum area under wheat is China followed by India, Russia, and USA. In production China ranks first. In India, during 2010-11 area under cultivation was 27.50 ha with the annual production of 80.58 metric tons. In other words about only fifth of global production of wheat comes from India and now occupies second position in wheat Gujarat, Haryana Madhya Pradesh

Punjab and Uttar Pradesh are major wheat growing states in country. Through the maximum acreage and production of wheat is in UP but Punjab gives highest average yield followed by Haryana. In Maharashtra it occupied an area of 10.21 million ha with production of 14.83 metric tons with an average production of 14052 q/ha. While in Vidharba it occupies 2.81 million ha area with a production of 3.51 metric ton having productivity of 13.387 q/ha. Wheat is manually used as a human food. It is nutritious, concentrated, easily stored and transported and processed into various types of food. It is good source of protein minerals vitamins (Riboflavin, Thiamine), sugars and fats. Protein in the wheat varies from 7-24 %, wheat protein contains glutin which is responsible for providing framework for spongy texture of bread and bakery products. It is mostly consumed in the form of chapatti puri paratha upma and suji or suji halwa prepared from atta maida or suji of aesitivium wheat. Besides mainly bakery products such as biscuits, bread, cake and pastries. Durum wheat is used for making instant food such as macaroni, semolina, noodles, vermicelli, spegathii and other pasta products. Common wheat is seriously damaged by different diseases most dangerous disease of leaf rust, stem rust, fusarium head blight and powdery mildew of these fungal disease specially leaf rust is often the most serious disease which can reduce total yield by about 1% for each 1% increase of infection.

RAPD marker analysis provides virtually unlimited no of markers to compare individual genotypes and considering easy handling and cheaper cost per assay, it is possible to carry out large scale training for breeding population and genetic resources. The present study aims to determine the types of gene action effects controlling morphological traits, yield and its component as well as estimating heterosis, heritability, inbreeding depression and genotypic variability coefficient of the studied traits. Moreover the study aims to detect the genetic variation of the wheat genotypes under the study using RAPD PCR marker technique. RAPD is PCR based technology. Arbitrarily primed PCR and RAPD are essentially same techniques most molecular biologists like more frequently

RAPD'S: RAPD technique does not require any previous knowledge of target genome and is relatively simple and rapid to carry out genetic analysis of bio diversity and study of relationship among species at different level the technique has been applied to identify cultivars and revealing phylogenetic relationship among them. RAPD technique has great potential in finding DNA marker for breeding programs and in their use OPA, OPB AND OPC primers used in RAPD reaction for analysis of genetic diversity in various crop i.e. wheat, sorghum, chili, tomato and cotton. These primers are universal primers.

Different showed variations in their ability to detect polymorphism.

Application of RAPD markers

The RAPD is that is the fast technique, easy to perform and comparatively cheap. It is immediately applicable to the analysis of most of organism because universal sets of primers are used without any need for prior sequence information.

·0 Analysis and individual specific genotype
·1 RAPD used for genetic identity to study closely related species.
·2 It is used for gene mapping
·3 Used for fingerprinting
·4 It is used for gene tagging
·5 Preparation of genetic map
·6 RAPD marker technology is widely used to find marker related to target gene.
Taking the advantage of evolutionary background of wheat and the available molecular tools, an attempt has been made to reveal the genetic variability of 11 accessions. The effort were made to the diversity analysis in the wheat cultivars with the following
objectives
·7 Analysis of genetic diversity using PCR based molecular marker.

ISSR marker and agronomical characterization and 25 cotton germ plasm 45 ISSR primers and 40 RAPD primers were used to amplified the germ plasm. (Dongare *et al.,* 2003) 19 scorable ISSR markers generated 90 marker while 21 reproducible RAPD primer generated 150 marker of which markers from ISSR and markers from RAPD were scored as polymorphic. Dendrogram were developed from ISSR and RAPD analysis by using NTSYS PC software.

Genetic diversity was identified in Korean tomato cultivars by RAPD marker with the usage of molecular marker for cultivar identification and protection of plant breeders and intellectual property rights (Man kyu Huh *et al.,* 2011).

Ten bread wheat varieties were assessed using RAPD marker and genetic diversity of wheat using RAPD was studied (Esra Ayodgyan *et al.,* 2012).

Genetic diversity among cucumber varieties available in Karnataka was studied using RAPD analysis to extracting the DNA (Asif Ali Khan *et al.,* 2010)

DNA analysis of musk Rose with RAPD was carried. The genetics of different rose varieties were compared by RAPD (Karla Fredrick *et al.,* 2012). All varieties were found to be extremely similar if not identical except for "BREMO".

Materials and Methods

Plant material

Experimental material consists of varieties of *Triticum* aesitivium all those materials collected from local market are as given below:-

Methods

Plant samples: - Eleven varieties from local market was collected information regarding their common name was also collected as per table no 1. Seed of wheat were grown in 11 different pots under shade condition where sufficient amount of light is available. Seeds were germinated to form seedlings within 6-7 days.

Description of different varieties: These plants were grown to good heights to form mature leaves. Different variety showed

variation in their heights and leaves color. Some showed dark green leaves, some showed light green in color, some showed medium range, within two weeks plants were grown to a good height and were ready for extraction of DNA.

DNA extraction: The plant genomic DNA was extracted by following method.

Reagent for DNA extraction buffer

A) DNA extraction buffer
200 mM Tris HCl
250 mM NaCl
25 mM EDTA
0.5% SDS
2% beta mercaptoethanol
All these chemicals are added to distilled water to make up the volume to 100 ml.
B) Chloroform: Isoamyl alcohol (24:1)
C) 70% ethanol
D) TE buffer pH-8
E) RNase A stock (10mg/ml)

DNA isolation

Total genomic DNA of each variety was extracted from all eleven varieties of wheat. Extraction of DNA was performed using CTAB extraction method (Deepak Kumar *et al.,* 2012).To obtain the clean DNA sample the extraction procedure included the addition, per each 50µl DNA sample resuspended in TE buffer. 0.5 µl of RNase A was added to the sample and were incubated at 37 0 c for half an hour for purification.

Agarose gel electrophoresis: The quality of DNA checked by quality of wheat genomic DNA USING 0.8% of agarose gels.

Procedure for gel electrophoresis

1X electrophoresis buffer was prepared. An appropriate amount of agarose gel was weighed to the appropriate volume of 1 X electrophoresis buffer in conical flask solution was heated in a microwave oven flask was rotated occasionally until the agarose was dissolved.

Allowed the gel to cool near about 55-60 o c and ETBR was added and mixed properly. With the help of comb wells were prepared on the molten agarose. Gel was poured on assembly and allowed to fix with comb. Wells were prepared after 20-30 minutes after removing the comb.

Care to be taken of interference of air bubbles fill the assembly with buffer. One volume of sample with five volume of loading dye was loaded on the gel and the sample was applied on the wells in the gel. Electrode was connected and current (1-10 volts/cm) was supplied until the dye has migrated and appropriate distance in gel.

Analysis of gel

DNA purification

Purification of DNA is essential to removed RNA, proteins and polysaccharides which are consider to be the major containments in DNA based PCR. Inclusion of SDS in DNA extraction buffer helps elimination of polysaccharides RNA was removes by RNase. Sterile distilled water (500 µl) was added and mixed. RNAse A (0.5 µl) added and kept at room temperature for 30 minutes. Then it was treated with chloroform: isoamyl alcohol until it turns milky. Centrifuged at 10000 rpm for 10 min at 40 C. Aqueous layer was collected and one tenth volume of 3 M Sodium acetate was added slowly and mixed equal volume of ice chilled isopropanol added fallowed by centrifugation of 10000 rpm for 15 minutes at 4oC. Pellet obtained washed with ethanol was suspended in 50 or 100 µl of TE.

DNA quantification

DNA obtained after extraction was confirmed by running it on 0.8% agarose gel containing Et Br (10µl) in electrophoresis system. Five micro lit of genomic DNA with Five micro lit loading dye in each well. After completion of 5 cm run the gel was observed under UV light and the DNA yield and quality was confirmed.

Spectrometric analysis

The ratio between reading at 260 nm and 280 nm provided and estimated for the purity of nucleic acid any sample showing the ratio below 1.8 or above 2 was further subjected to purification.

Measurement of DNA concentration

The concentration of DNA was estimated by the measurement of the UV radiation observed by nucleic acid basis. The spectrophotometer meter was calibrated using 1000 µl of TE in quartz cuvette at 260 and 280 nm 10 µl of DNA sample was added to 1990 µl to TE, mixed well and absorbance was taken. Te concentration of the DNA in the sample was estimated by multiplying OD at 260 nm with dilution factor and coefficient of DNA (50).

Eye ball estimation

A particular amount of DNA with its capability to intercalate a particular quantity of Et Br there for under UV radiation the inflorescence intensity reflects the amount of DNA present in each spot depending upon the intensity of the film the DNA amount was quantified as compare with standard DNA electrophoresis is technique of separation of charged molecules under the influence of electric field so that they migrate in direction wearing the opposite charge.

DNA sample 2 µl was loaded on gel with dye for electrophoresis alone with standard DNA and electrophoresis was carried out estimation was done by comparing the result with standard DNA.

Dilution of DNA

DNA sample 50 µland diluted with 500 µl sterile distilled water.

PCR based amplification with RAPD primers

DNA from each variety used to amplify with universal primer each contain in a volume of (final concentration) dNTPs (10 mM),PCR assay buffer (10 X), Mgcl2 (25 mM), primers (5 p mol), Taq DNA polymerase (3 U /µl), DNA (60 ng) and sterile water to make the volume

The reaction was carried out in thermo cycler using an initial cycle of denaturation at 94 oC per 2 min. Second denaturation at 94oC at 30 sec annealing at 37oC for 30 seconds extension for 5 minutes at 72oC. and final extension at 72o C with 35 cycles repeats.

The fragments obtained were thin analyzed by electrophoresis in gel with 1.5% agarose gel carried out at 100 V for 3 hrs. Gels were then visualized under UV light. The profiles were then obtained and analysis was carried out.

Clustering

The molecular weight marker gene ruler 100bp DNA ladder was used as a standard and to determine the size of polymorphic fragments. After electrophoresis, gel was visualized under UV transillumination and was photographed using gel doc system. DNA fragment was done using inbuilt software and by scoring photographs.

Individual bands with lanes were assigned to a particular molecular weight comparing with DNA molecular weight marker. Total no of bands within each; lanes and number of polymorphic bands were noted.

Data scoring and analysis

RAPD amplified bands were scored as present (1) and absent (0) for each primer population combination.

The data entry was in a binary data matrix as discrete variable with Jaccards coefficient similarity was calculated and dendrogram was generated based on similarity coefficient by using paired group method. Most efficient primers were selected on the extent of polymorphism.

Similarity coefficient

Selected genotype\e was compared with the rest of genotypes for similarity coefficient. Greater the value of coefficient compared variety will be more similar to selected variety.In addition to this the value of co efficient also signifies about the extent of similarity between two varieties.
Nxy = No of bands common in sample a and b
Nt = total no of bands present in all samples.
N2 =no of bands not present in samples a or b but found in either samples

The similarity matrix was subjected to generate a dendrogram using software program NTSYS PC version 2.1, Exter software.

Analysis of result using NTSYS software

Generated excel data sheet was given as an input in dissimilarity or similarity matrix. Genetic distance was calculated using J & C coefficient results are then computed and output file was generated. The output file is then used as an input for cluster analysis SAHN tool. Thus, after computing second output file gets generated which was given as an input for tree plot generation. Thus, a clustered tree plot is obtained and analysis was carried out

Result and Discussion

In the present investigation of molecular marker analysis in wheat was carried out using RAPD primer for assessment of parental variability. It was carried out using following points:

DNA isolation was carried out of different (11) wheat variety using CTAB DNA extraction method and it was confirmed using Agarose gel electrophoresis (Fig. 1).

DNA purification was carried out by treating the DNA suspended in TE buffer with RNase A to remove the RNA contamination from the DNA and it was checked using agarose gel electrophoresis (Fig. 2).

Quantification of purified DNA was carried out by two methods. UV spectrophotometer by using 260 and 280 ratio. Eye ball estimation using standard DNA. Gel picture for quantification is given in (Fig. 3).

RAPD amplification was done using PCR with primer 3 universal random primer i.e. RPI 3 was used for study and 26 RAPD amplification bands were generated. Among all eleven wheat 147 best loose and HD 2189 variety gave more amplification than other. Polymorphic and Monomorphic banding pattern was studied. Polymorphic pattern was obtained by calculating the polymorphic percentage it is given by the following formula

$$\text{Polymorphic percentage (\%)} = \frac{\text{polymorphic bands} \times 100}{\text{Total no of bands}}$$

21polymorphic bands were present

Polymorphic present was calculated in 11 wheat variety using primer three and it was found to be 80.76 % RAPD

Analysis of wheat variety

The genetic relationship between wheat genotype was determined on the basis of Jaccards pairwise similarity coefficient values. The value of similarity coefficient ranged from 0.088 to 9999.00. The variety Hati sharbati and Mrugnayani chandoshi showing highly similarity where value is 0.088 and high similarity between Agni shrbati and HD 2189 whose value is 9999.00

The value of similarity coefficient is 0.088 is found between Mini sharbati Mrugnayani chandoshi, Hati sharbati and 147 best loose.

Cluster analysis

A dendrogram was generated by UPGMA cluster analysis based on Jaccards similarity coefficient. The dendrogram is shown in figure: 4 and cluster analysis on the basis of coefficient value the accessions could be divided into two groups A and B i.e. A and B In which cluster A consists of 6 genotypes and B consists of 5 genotypes.

The cluster A is divided into two sub clusters i.e AI and A2. The cluster A1 consists 2 genotypes A2 consists 4 genotypes n which varieties Mrugnayani chandoshi and Mini sharbati ,Hati sharbati ,Agni sharbati are closely related to each other. related and variety. The cluster B is also divided in B1 And B2.the cluster B1consist of subgroup B1a and B2a in that B1 consist 2 genotypesB2 is consist ofB2a and B2b. In B2a consist of subgroupB2a1 and B2a2 having 2 genotypes B2b asolitary cluster having single genotype Which 147 best loose lokewan grade 1 and HD 2189

In the present investigation Random Amplified Polymorphism DNA (RAPD) markers were used to study the DNA fingerprinting of 11 wheat varieties. One single primer was used having accession no. AM773310, Amplification of genomic DNA of 11 genotypes, using RAPD primers yielded 26 fragments that could be scored of which 21were polymorphic. Percentage of polymorphism was calculated for eleven varieties using primer 3 which was found to be 80.76%.

The variety 4 & 11(147 best loose and HD2189) showed high amplification. The dendrogram obtained from unweighted pair wise group method for Arithmetic mean of cluster analysis using Jaccards Similarity matrix through NTSYS software revealed two clusters. The highest similarity coefficient similarity was found between genotype Mrugnayani chandoshi Mini sharbati and Vishnu padma chandoshi and Narmada496 and the genotypes having less similarity coefficient was between HD 2189 and Agni sharbati.

Single primers were showed polymorphic bands primer1(80.76 %)primer 3 this could be attribute the selection of primers, amplification protocol used genotypes of the Groundnut accessions and were found to be best suites for finger printing of wheat germ plasm and assessing genetic diversity. Present investigation revealved that RAPD marker is potentially rapid, simple reliable and effective method of detecting polymorphism for assessing genetic diversity between genotype and these help in selection of parent for hybridization.

RAPD technique is useful in areas of genetic diversity and DNA fingerprinting analysis. As the need to protect proprietary germ plasm as it is likely to increase in the future, RAPD will have an important role in securing a plant variety right by virtue of its unique efficiency is distinguishing closely related germ plasm.

II) RAPD analysis with multiple primers

Among RAPD marker primer 5 produce maximum number of bands i.e. 66 in all wheat variety followed by primer 1 and primer 3 generated maximum number of bands 52 and 26 while RAPD marker primer 4 and primer 8 generated minimum number of bands in the genomic pool.
In primer 1 Agni sharbati and Narmada 496 variety gave more amplification than other variety 37 polymorphic bands were present. The polymorphic percentage of primer 1, primer 3,primer 4,primer 5,primer 8 is 71.15%, 80.76%, 76.92%, 15.15%, 12.5% respectively according to the table of RAPD analysis.

Distance matrix analysis: similarity based on Jaccards coefficient. The genetic relationship between wheat genotypes was determined on the basic of Jaccards coefficient values. The value of similarity coefficient ranged from 0.056 to 1.155.The variety HD-2189 and Agni sharbati represents lowest average similarity coefficient value is 1.155.The variety loose lokwan grade 1 and 147 best loose showing the highly similarity its value 0.056 and high similarity between Mrugnayani chandosi and loose sharbati grade 1 whose value is 0.116.

Cluster analysis

A dendrogram was generated by UPGMA cluster analysis based on 1 Jaccards similarity coefficient. The cluster analysis on the basis of coefficient value the accessions could be divided into two group A and B i.e. A and B in which cluster a consist of 10 genotypes and B consists of 1 genotypes.

The cluster A divided into two sub cluster i.e A1 and A2.The cluster A1 consists of sub groups.A1a and A1b in that A1 has 9 genotypes and A1a consist of 7 genotypes which Mrugnayani chandoshi and loose sharbati grade 1 are closely related with Hathi sharbati,Mini sharbati,standard,147 best loose and loose lokwan grade 1.

In which the A1b Narmada 496.Vishnu padma chandosi these two variety are closely related with each other. The cluster B is also having single genotype HD-2189.

RAPD markers were used to study the DNA fingerprinting of 11 wheat variety. Five primers were used having its accession no.(AM765819), (AM773310), (AM773769), (AM773770), (AM773773), Amplification of genomic DNA of 11 genotypes using RAPD primers yielded 173 fragments that could be scored of which 80 were polymorphic bands. Percentage of polymorphism was calculated for eleven wheat varieties using the 5 primers which was found to be 46.24%.

The dendrogram obtained from unweighted pair wise group method for arithmetic mean of cluster analysis using Jaccards similarity matrix through NTSYS software revealed two clusters. The highest similarity coefficient similarity was found between genotypes Mrugnayani chandosi,loose sharbati grade 1 and Narmada 496.Vishnu padma chandosi. The genotypes having less similarity coefficient was HD 2189 and Agni sharbati.

Five primers were showed polymorphic bands primer 1(71.05%), primer 3(80,76%),

primer 4(76.925), primer 5 (15.15%) and primer 8(12.5%) this could be attributed to the selection of primers amplification protocol used the genotypes wheat and were found to be best suited for fingerprinting of wheat germ-plasm and assessing genetic diversity.

RAPD marker is simple rapid reliable and effective method of detecting polymorphism for assessing genetic diversity between genotypes and these help in the selection of parent for hybridization. RAPD technique is useful in area of genetic diversity and DNA fingerprinting analysis as the need to protect proprietary germ plasm as it is likely to increase in the future RAPD will have an important role in securing a plant variety right by virtue of its unique efficiency in distinguishing closely related germ plasm.

Table 1

Sr no	Name of variety
1	Mrugnayani chandoshi
2	Hathi sharbati
3	Mini sharbati
4	147 Best loose
5	Loose lokwan grade 1
6	Loose sharbati grade 1
7	Standard 147
8	Agni sharbati
9	Narmada 496
10	Vishnu padma chandoshi
11	HD 2189

Analysis of wheat variety

SR NO	PRIMER	ACCESSION NO	TOTAL BANDS	MONOMORPHIC BANDS	POLYMORPHIC BANDS	PERCENTAGE
1	PRI 3	AM773310	26	5	21	80.76 %

RAPD analysis table

Sr no	Primer	Accession no	Total bands	Monomorphic bands	Polymorphic bands	% of polymorphism
1	Primer 1	AM 765819	52	15	37	71.15
2	Primer 3	AM773310	26	5	21	80.76
3	Primer 4	AM 773769	13	3	10	76.92
4	Primer 5	AM773770	66	56	10	15.15
5	Primer 8	AM773773	16	14	2	12.5

Image of DNA isolation

RNA contamination
Pure DNA Band

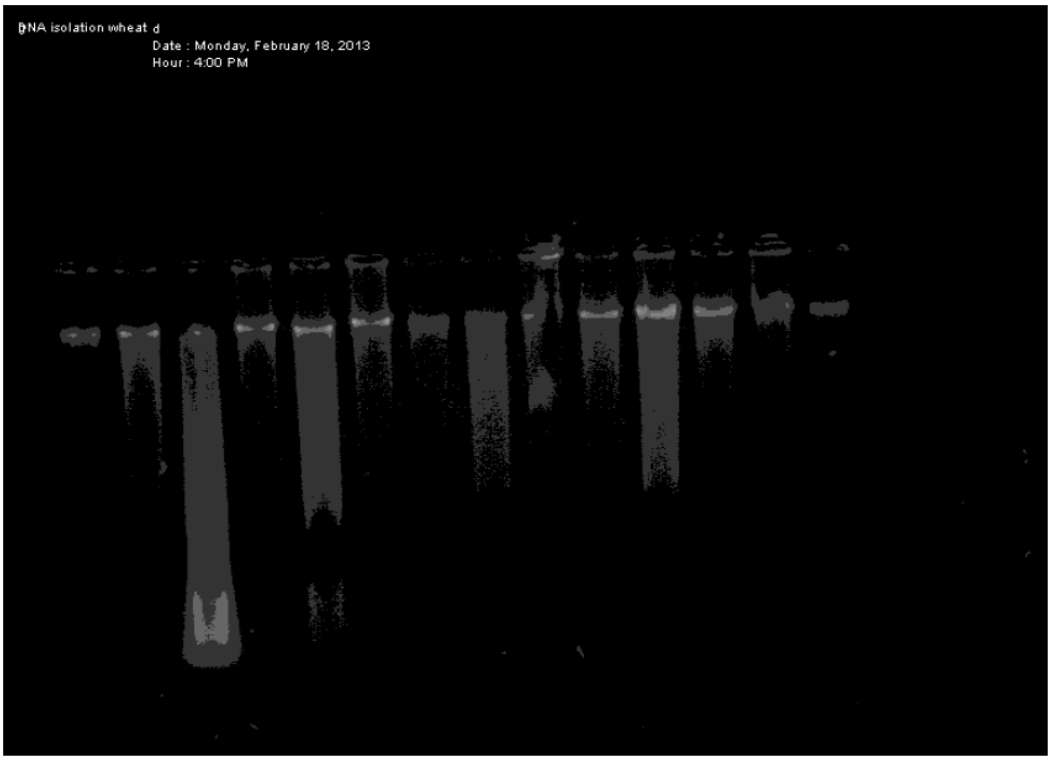

Fig.1 DNA isolation

Bigger circle from right indicates the contamination Of RNA
Smaller circle indicates the pure DNA band.

Image of DNA purification
RNA band
DNA band

Fig.2 DNA purification

The smaller band indicates the presence of pure DNA band
The bigger circle indicates the presence of contamination of RNA.

Image of DNA Quantification
Pure DNA bands
Standard DNA

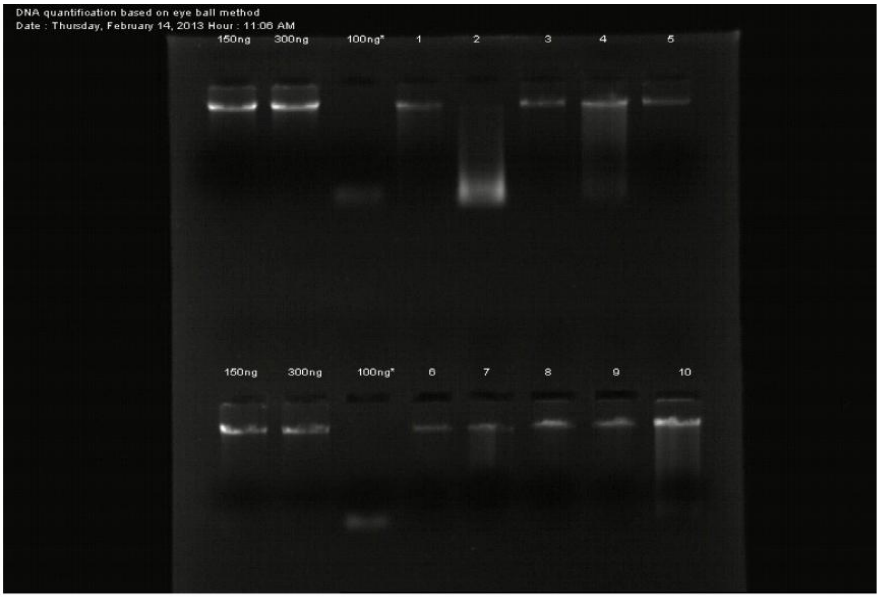

Fig. DNA quantification

The upper circle indicates the presence of standard DNA
The lower circle indicates the presence of pure DNA bands.

RAPD Analysis

DNA LADDER
Monomorphic band

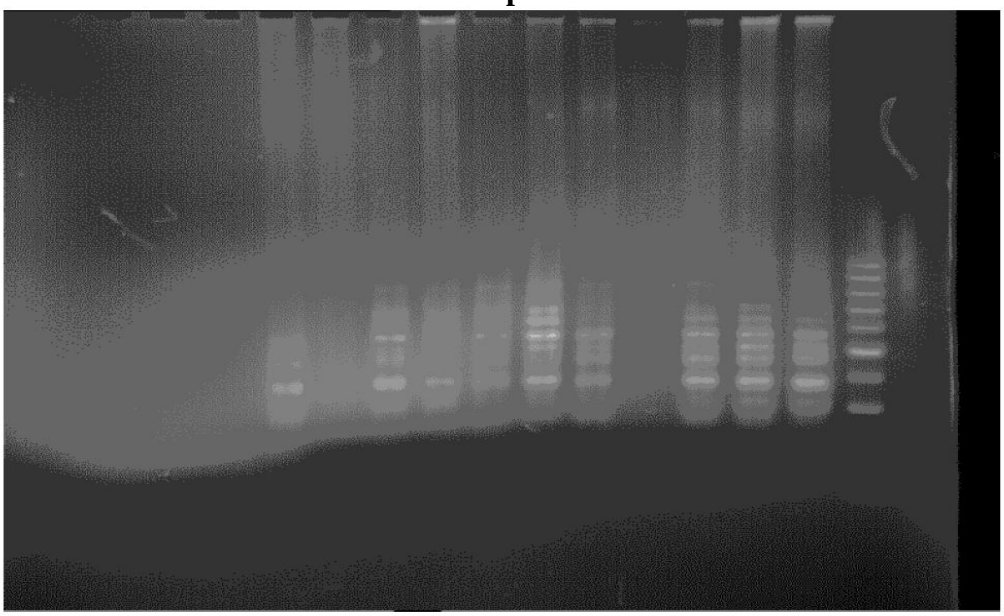

Fig. RAPD analysis

The right side circle indicates the presence of DNA ladder
The next circle indicates the presence of monomorphic DNA bands.

Report generate

Primer 3 Report 1
Simgend: NTSYSpc 2.21o, (C) 1986-2011, Applied Biostatistics Inc.
Date & time: 03/01/2013 3:55:28 PM

Input parameters
Read input from file: C:\Documents and Settings\MGMIBT\Desktop\vikas and sachin d.xlsx
Compute by: cols
Save results in output file: C:\Documents and Settings\MGMIBT\Desktop\vikas n sachin output.NTS
Coefficient: J&C
Reading spreadsheet: Sheet1
Comments:
Matrix type = 1, size = 12 by 11, missing value code = "none" (rectangular)
Genetic distance matrix (11 by 11) saved in file: C:\Documents and Settings\MGMIBT\Desktop\vikas n sachin output.NTS
Ending date & time: 03/01/2013 3:55:28 PM
Report 2
SAHN: NTSYSpc 2.21o, (C) 1986-2011, Applied Biostatistics Inc.
Date & time: 03/01/2013 4:01:07 PM

Input parameters

Read input from file: C:\Documents and Settings\MGMIBT\Desktop\vikas n sachin output.NTS
Save result tree in output file: C:\Documents and Settings\MGMIBT\Desktop\vikas n sachin output 2.NTS
Clustering method: UPGMA
In case of ties: find all tied trees
Max. no. tied trees: 25
Comments:
SIMGEND[2.21o]: input=C:\Documents and Settings\MGMIBT\Desktop\vikas and sachin d.xlsx, coeff=J&C, dir=cols
Matrix type = 2, size = 11 by 11, missing value code = "none" (dissimilarity)
Results will be stored in file: C:\Documents and Settings\MGMIBT\Desktop\vikas n sachin output 2.NTS
Searching for all tied trees
Solution tree number 1
Sorting tree nodes. ..
none needed.
Solution tree number 2
Sorting tree nodes. ..
done.
Solution tree number 3
Sorting tree nodes. ..
done.
Solution tree number 4
Sorting tree nodes. ..
done.
Solution tree number 5
Sorting tree nodes. ..
done.
Solution tree number 6
Sorting tree nodes. ..
done.
Solution tree number 7
Sorting tree nodes. ..
none needed.
Solution tree number 8
Sorting tree nodes. ..
done.
Solution tree number 9
Sorting tree nodes. ..
done.
Solution tree number 10
Sorting tree nodes. ..
done.
Solution tree number 11
Sorting tree nodes. ..
done.

Solution tree number 12
Sorting tree nodes. ..
done.
Solution tree number 13
Sorting tree nodes. ..
done.
Solution tree number 14
Sorting tree nodes. ..
done.
Solution tree number 15
Sorting tree nodes. ..
done.
A total of 15 tied trees were found
written to file: C:\Documents and Settings\MGMIBT\Desktop\vikas n sachin output 2.NTS
(note: some trees may be duplicates)
Report 3
matrix(11objects)savedinfile:C:\Documentsand Settings\MGMIBT\Desktop\vikas n sachin output 2.NTS
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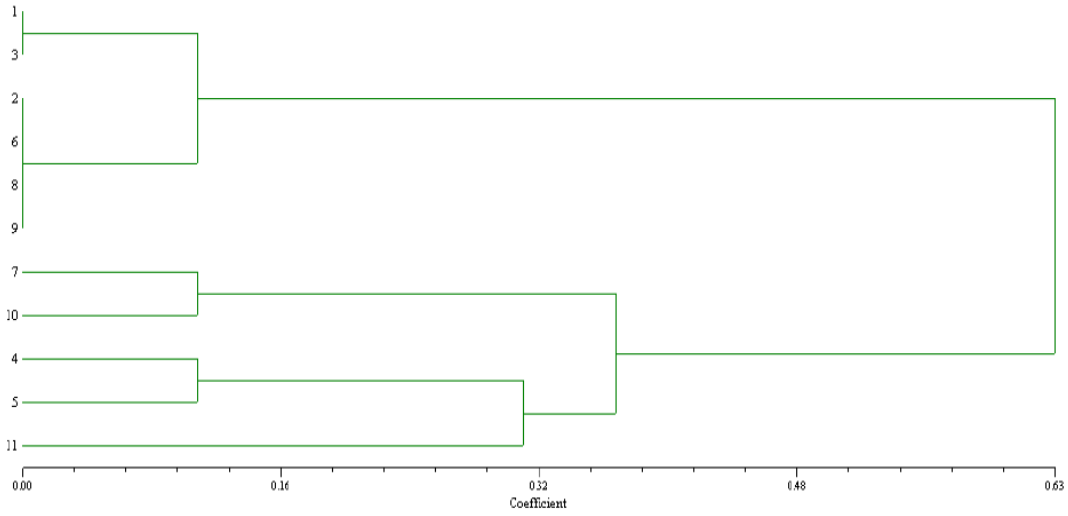
matrix(11objects)savedinfile:C:\Document and Settings\MGMIBT\Desktop\vikas n sachin output 2.NTS

Ending date & time: 03/01/2013 4:01:07 PM



A

B1

Tree generation with primer 3 using 11 wheat varieties (UPGMA method)

B2

B

Fig. Tree generation with primer 3 using 11 wheat varieties (UPGMA method)

Distance matrix

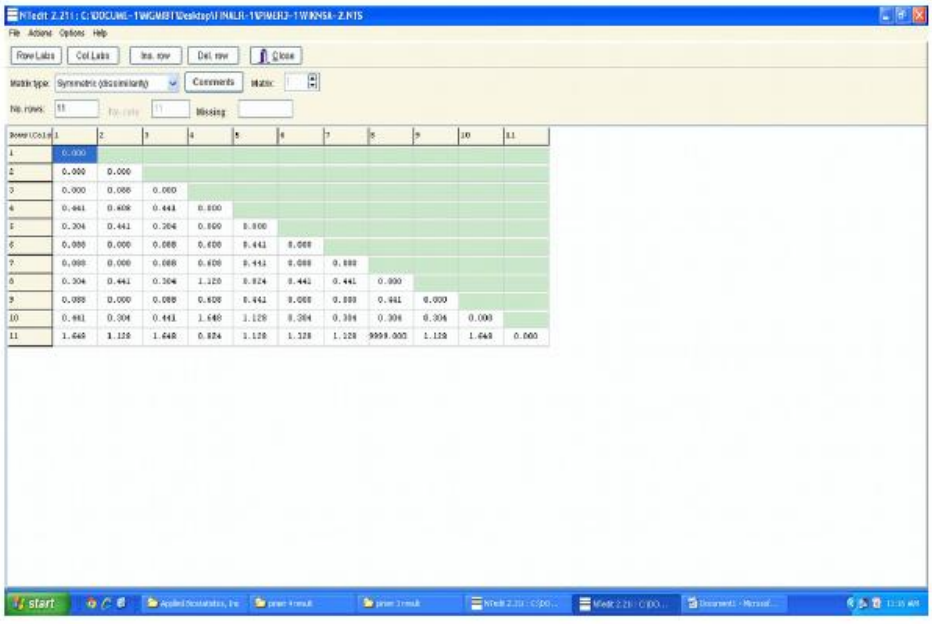


Fig. Distance Matrix table

Result for primer 1

Polymorphic band
DNA ladder
Monomorphic band

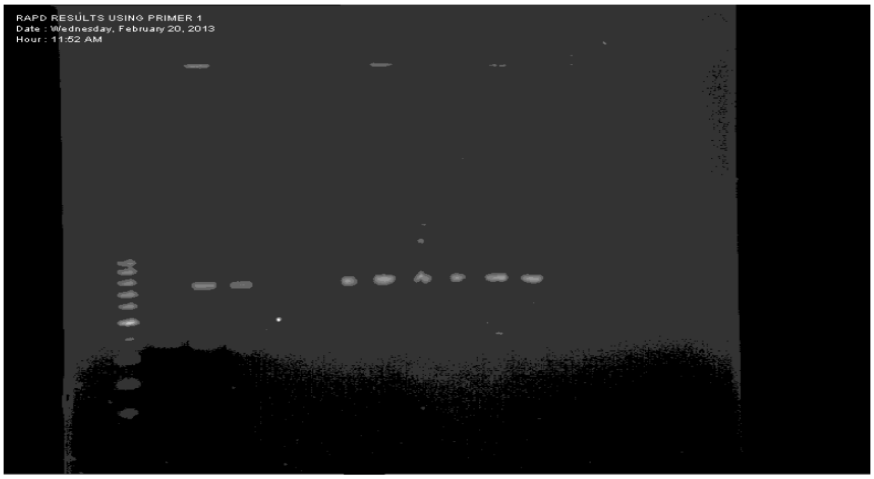


Fig.RAPD using primer 1
The first cicle of right side indicates the presence of monomorphic bands
The centre circle indicates the presence of polymorphic band
The next circle indicates the presence of DNA ladder.

Result for Primer 3

Polymorphic Band
DNA Ladder
Monomorphic band

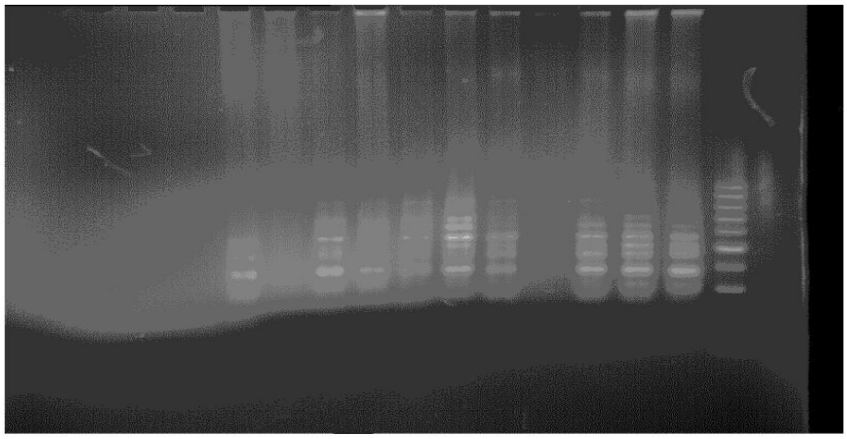


Fig.RAPD using primer 3
The right side band indicates the presence of DNA ladder
The centre circle indicates the presence of Polymorphic band
The arrow indicates the presence of Monomorphic band.

Result for Primer 4

Polymorphic DNA
Monomorphic DNA
DNA LADDER

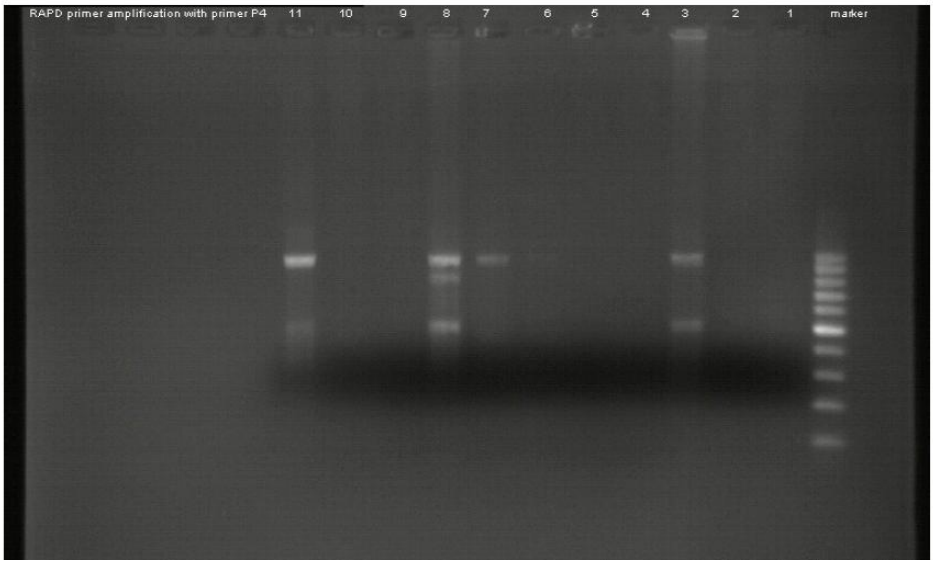


Fig.RAPD using primer 4
The right circle indicated the presence of DNA ladder
The middle circle indicates the presence of polymorphic band.
The next circle indicates the presence of monomorphic band.

Result for Primer 5

Polymorphic band
Monomorphic band
DNA Ladder

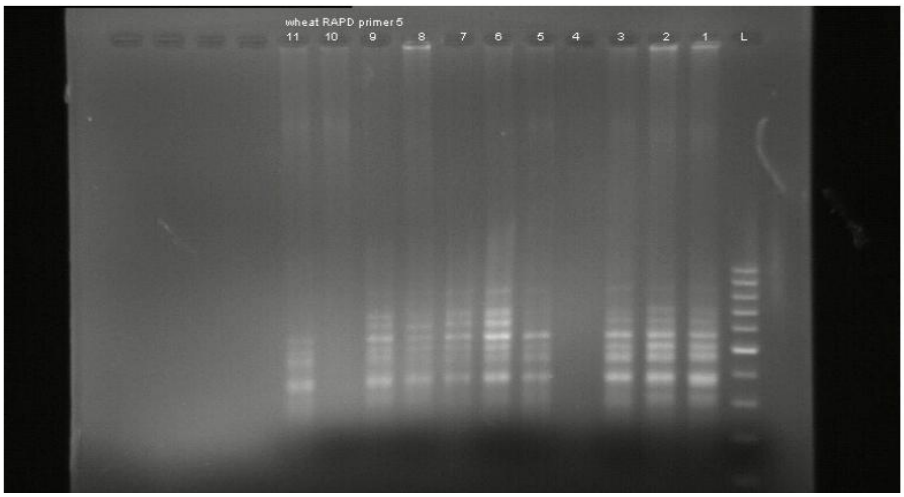


Fig.RAPD using primer 5

The arrow represents the DNA ladder
The first circle indicates the presence of polymorphic band
The second circle indicates the presence of monomorphic band.

Result for Primer 8

Polymorphic band
Monomorphic band
DNA ladder

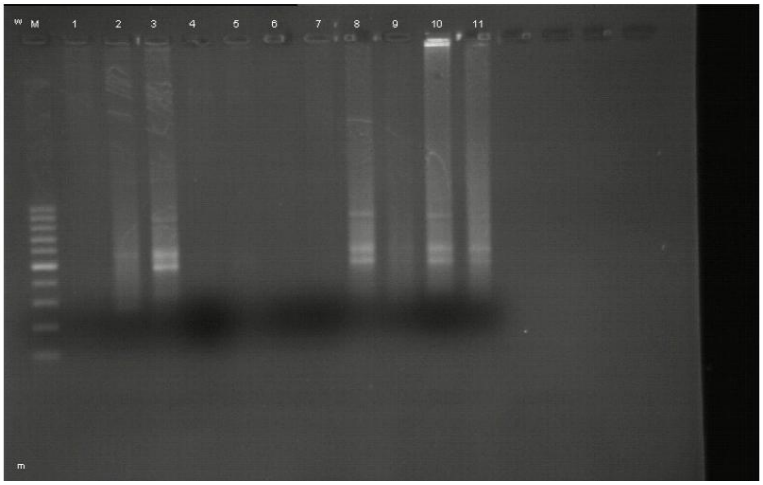


Fig.RAPD using primer 8

The arrow indicates the presence of DNA ladder
The Centre circle indicates the presence of Polymorphic band
The right circle indicates the presence of Monomorphic band.

Report 1

Simgend: NTSYSpc 2.21o, (C) 1986-2011, Applied Biostatistics Inc.

Date & time: 03/08/2013 10:27:35 AM
Input parameters
Read input from file: C:\Documents and Settings\MGMIBT\My Documents\excel file of p1-p4.xlsx
Compute by: cols
Save results in output file: C:\Documents and Settings\MGMIBT\My Documents\file of p1-p4 output.NTS
Coefficient: J&C
Reading spreadsheet: Sheet1
Comments:
Matrix type = 1, size = 56 by 11, missing value code = "none" (rectangular)
Genetic distance matrix (11 by 11) saved in file: C:\Documents and Settings\MGMIBT\My Documents\file of p1-p4 output.NTS

Ending date & time: 03/08/2013 10:27:35 AM

Report 2

SAHN: NTSYSpc 2.21o, (C) 1986-2011, Applied Biostatistics Inc.
Date & time: 03/08/2013 10:29:58 AM
Input parameters
Read input from file: C:\Documents and Settings\MGMIBT\My Documents\fileof p1-p4 output.NTS
Save result tree in output file: C:\Documents and Settings\MGMIBT\My Documents\file of p1-p5 output 2.NTS
Clustering method: UPGMA
In case of ties: find all tied trees
Max. no. tied trees: 25
Comments:
SIMGEND[2.21o]: input=C:\Documents and Settings\MGMIBT\My Documents\excel file of p1-p4.xlsx, coeff=J&C, dir=cols
Matrix type = 2, size = 11 by 11, missing value code = "none" (dissimilarity)
Results will be stored in file: C:\Documents and Settings\MGMIBT\My Documents\file of p1-p5 output 2.NTS
Searching for all tied trees
Solution tree number 1
Sorting tree nodes. ..
none needed.
Solution tree number 2
Sorting tree nodes. ..
done.
A total of 2 tied trees were found
written to file: C:\Documents and Settings\MGMIBT\My Documents\file of p1p5 output 2.NTS
(note: some trees may be duplicates)
matrix (11 objects) saved in file: C:\Documents and Settings\MGMIBT\My Documents\file of p1-p5 output 2.NTS
matrix (11 objects) saved in file: C:\Documents and Settings\MGMIBT\My Documents\file of p1-p5 output 2.NTS
Ending date & time: 03/08/2013 10:29:58 AM

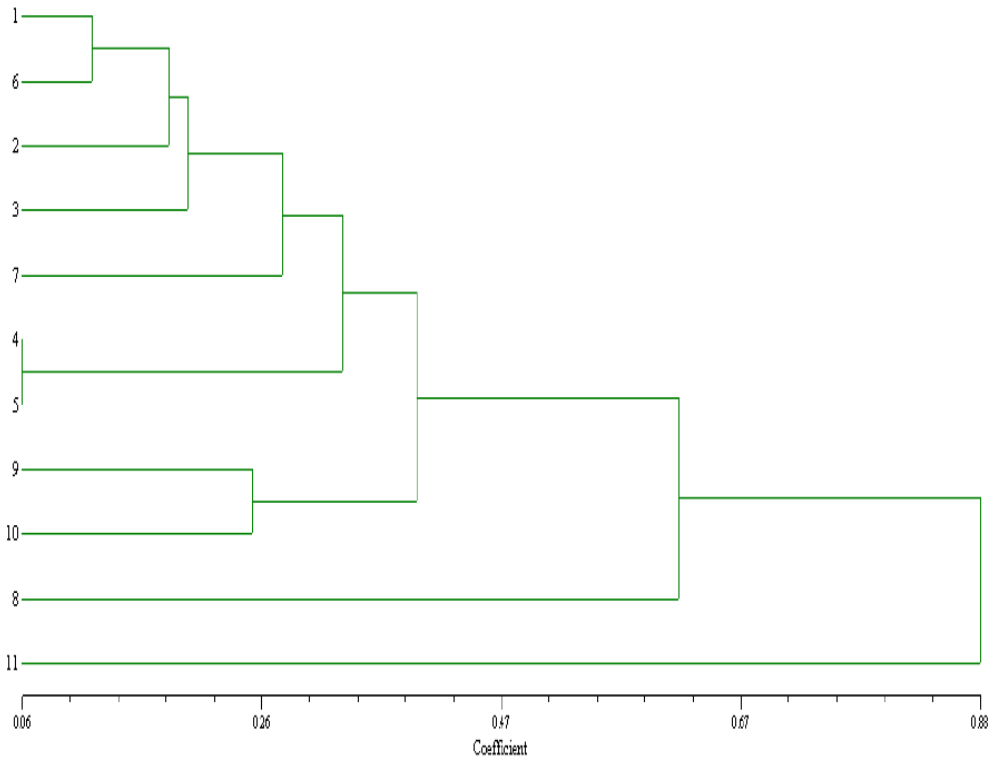
Report 3

Tree: NTSYSpc 2.21o, (C) 1986-2011, Applied Biostatistics Inc.
Input parameters
Read input from file: C:\Documents and Settings\MGMIBT\My Documents\ fileof
p1-p5 output 2.NTS
Comments:

SIMGEND[2.21o]: input=C:\Documents and Settings\MGMIBT\My Documents\excel file of p1-p4.xlsx, coeff=J&C,
dir=cols
SAHN[2.21o]: input=C:\Documents and Settings\MGMIBT\My Documents\fil of p1-p4 output.NTS,
method=UPGMA, tie=FIND
Solution tree number 1
Matrix type = 5, size = 11 by 2, missing value code = "none" (tree (dissimilarity))----- next dataset ----
Comments:
SIMGEND [2.21o]: input=C:\Documents and Settings\MGMIBT\My Documents\excel file of p1-p4.xlsx, coeff=J&C,
dir=cols
SAHN [2.21o]: input=C: \Documents and Settings\MGMIBT\My Documents\file of P1-p4 output.NTS,
Method=UPGMA, tie=FIND
Solution tree number 2
Matrix type = 5, size = 11 by 2, missing value code = "none" (tree (dissimilarity))
Ending date & time: 03/08/2013 10:31:01 AM

Final Tree generated

A1a.22
A1a.1
A1b
A2
B
A
A1
A1a



Distance matrix table generation by J & C co-efficient

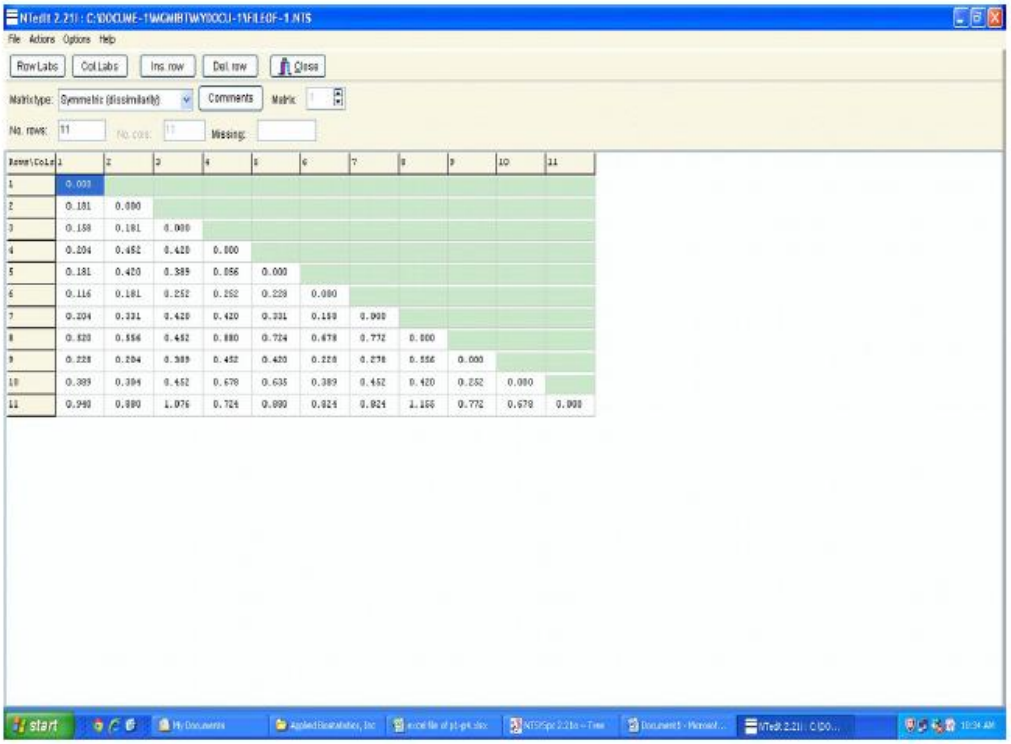


Fig. Distance Matrix table

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