

An approach to suggest the possibilities of interspecific hybridization using RAPD analysis of some commercial fruits and vegetables for improving the genetic characteristics.

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Abstract

RAPD (Random Amplified Polymorphic DNA) analysis is a PCR based molecular marker technique. Here, single short oligo nucleotide primers are arbitrarily selected to amplify a set of DNA segments distributed randomly through out the genome. RAPD was the first to become available and is most commonly and frequently used. Vegetables provide many essential vitamins and minerals. Additionally, higher intakes of vegetables are associated with healthier lives including lower risks of cancer and coronary heart disease. It is recommended that children eat five fruits and vegetables a day. Here different species and varieties of vegetables are used to know the diversity between them. The DNA is isolated and subjected for PCR amplification. The PCR done samples are run on agarose electrophoresis. The analysis is carried out using RAPD software.

Key words: RAPD, PCR, primers, Amplification, agarose electrophoresis.

be gleaned from a RAPD reaction. In recent years, RAPD is used to characterize, and trace, the phylogeny of diverse plant and animal species. Our major goal in this study was to establish and characterize an efficient callus induction and shoot regeneration protocol for cauliflower (*Brassica oleracea* var. Botrytis) we have further analyzed the somaclonal variation of regenerated plants at the DNA level by using the RAPD molecular markers. By PCR amplification, we were able to amplify 75 scoreable bands from 15 primers out of 40 arbitrary primers screened, where 35 of them were monomorphic and 40 polymorphic bands (53.3%) in four varieties studied. The absence of polymorphism among regenerated plants from the same variety indicated the conformity of the regeneration protocol. (Qin Y, Gao LH, *et al*, 2006) To study the genetic variation of *Cnidium monnieri* from different regions. METHODS: Random amplified polymorphic DNA technique was used to analyze genetic polymorphy of *Cnidium monnieri* from 9 different regions, and dendrogram was constructed by UPGMA. RESULTS: 20 random primers were used for polymorphic selection CONCLUSION: The smaller the geographic distances between two *Cnidium monnieri*, the smaller genetic differences. (Huang B, Nian H, *et al*, 2004) Five RAPD markers were identified to be relevant to percent regeneration in maize shoot-tip culture system. The information provided here should benefit to determine the

Introduction

Humans evolved from ancestral apes, and our genes are still reflecting a vegetation and small animal eating past. Evolving in Africa and then Asia, we ate a huge range of leaves, buds, flower buds, stems, gums, roots, tubers, and even pollen. The number of plant families we used as food was very much greater than the restricted range we eat today. Wild foods were carefully selected to avoid the plants or parts of plants with bitter and unpleasant taste, which likely contained toxic compounds. Today's plants are more palatable, and yet paradoxically, we eat very few plants as part of our daily diet.

The major contribution of plants to human health has always been thought to be the large

amounts of vitamin A, the folic acid vitamin, and the vitamin C they contained; as well as a good amount of some minerals. One of the newest campaigns in nutrition education is "5 a Day for Better Health." This slogan comes from the "Healthy People: National Health Promotion and Disease Prevention Objectives." This particular objective promotes the consumption of 5 or more fruits and vegetables a day. RAPD stands for random amplification of polymorphic DNA. It is a type of PCR reaction, but the segments of DNA that are amplified are random. The scientist performing RAPD creates several arbitrary, short primers (8-12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify. By resolving the resulting patterns, a semi-unique profile can

genetic mechanisms involved in the maize regeneration response related to shoot meristem culture pathway and benefit to select high regenerable germplasm by using marker-assisted selection. (Li W, Sun G, *et al*, 2004) Compared the differences of the RAPD results between pure Semen Cuscutae and 12 commercial samples, it comes into the conclusion that the existing other sample influences the RAPD results when its content is over 60% and the results is same as its content being under 60%. (Liu C, Yan Y, *et al*, 2000 May) Twenty primers for RAPD (among 60 tested) and seven for ISSR (among 10 tested) were chosen as creating polymorphic DNA bands differentiating the investigated genotypes for strawberry. Based on those identity markers, the genetic distance between genotypes was determined, and their relatedness was estimated. (Korbin M, Kuras A, *et al*, 2002)

Random amplified polymorphic DNA (RAPD) profiles were used to differentiate between several food and feed legume species. Template DNA was extracted from 63 seed meal samples representing 27 legume species Amplification was performed with commercial RAPD-PCR beads and six 10-mer primers. Three primers (1, 5, and 6) generated RAPD profiles with all 63 templates. Two of these primers produced identical profiles only for two species of the same genus. Identification of all 27 species in homogeneous food or feed samples such as meals was demonstrated by applying either primer 5 or primer 6 and primer 3 to differentiate between swordbeans and jackbeans. (Weder JK. 2002 Jul). Randomly amplified polymorphic DNA (RAPD) was used to determine the unique DNA profiles that are characteristic not only of the genus *Panax* but also of various *Panax* subgroups collected from five different countries. . RAPD results of OP-13B primer demonstrated that OP-13B primer could be used as a unique RAPD marker to differentiate *Panax* species. These results support that this approach could be applied to distinguish Korean Ginseng (*Panax ginseng*) from others at the molecular level. (Shim YH, Choi JH, *et al*, 2003) The present study was designed to distinguish *Aloe* species by

random amplified polymorphic DNA (RAPD) analysis. . By comparison of the characteristic bands of PCR products on agarose gel, it was possible to distinguish the 4 species. Thus, the botanical species of *Aloe* in commercial food products can be identified by RAPD analysis. (Shioda H, Satoh K, *et al*, 2003 Aug). The parental plants apomict and the five F1 families were analysed using RAPD molecular markers. Ninety-five oligonucleotides were assayed on the progenitors in order to search for male-specific bands. Our results indicate that pollination at anthesis allows the greatest potential for sexuality to be expressed in this facultative apomictic genotype. (Espinoza F, Pessino SC, 2002) comparing the genetic similarity among the pairs of seeds developing within a pod with that among (a) random pairs from the pool of all seeds, (b) random pairs from single-seeded pods, and (c) random pairs from two-seeded pods, using both randomly amplified polymorphic DNA (RAPD) and isozymes in five trees. . We found that the pairs of seeds developing within a pod are genetically more similar than any random pairs of seeds in a tree. Thus the formation of two-seeded pods appears to be associated with increased genetic relatedness among the developing seeds. (Mohana GS, Shaanker RU, *et al*, 2001)

The random amplified polymorphic DNA (RAPD) technique was applied to settle a lawsuit involving unauthorized commercialization of a patented strawberry variety of high economical relevance ('Marmolada'). The results were accepted as evidence in the court. This study confirms that the RAPD technique is especially suitable for identification of asexually reproduced plant varieties for forensic or agricultural purposes. (Congiu L, Chicca M, *et al*, 2000) We have studied genetic variability of nine wild growing soya populations of Primorye Territory using RAPD analysis. The level of *G. soja* genetic variability was considerably higher than that of *G. max*. We have analyzed phylogenetic relationships in the genus *Glycine* subgenus *Soja* using RAPD markers. Our data confirm validity of allocation *G. gracilis* in a rank of a species. (Nedoluzhko AV, Dorokhov DB, 2007) Using genomic

DNA from these vegetables and combinatory sets of nucleotide primers, we screened for random amplified polymorphic DNA (RAPD) fragments and found three cabbage-specific fragments. These rapid fragments based on sts sites CAI fragments are determined with these markers kale accessions are distinguished from cabbage accessions and identified kale juices experimentally spiked with different amounts of cabbage. (Etoh K, Nijima N, *et al*, 2003) The method of polymerase chain reaction with random primers (RAPD) was used to assess genetic variation and population differentiation in the rare endemic plant *Oxytropis chankaensis* Jurtz. (Fabaceae). . High variation along with the low degree of differentiation characteristic of two most geographically remote populations of *O. chankaensis* can have several explanations, among which a polyploid origin of the species seems to be most important. (Artiukova EV, Kholina AB, *et al*, 2004) An interspecific hybrid between leek (*Allium ampeloprasum* L.) and garlic (*Allium sativum* L.) was produced by hybridization using a fertile garlic clone as a pollen donor and an ovary culture. The hybridity was confirmed by chromosome observation ($2n = 3x = 24$) and randomly amplified polymorphic DNA (RAPD) analysis. The results of the study suggest the possibility of direct use of an interspecific hybrid between *A. ampeloprasum* and *A. sativum* as a new crop. (Yanagino T, Sugawara E, *et al*, 2003). The use of random amplified polymorphic DNA (RAPD) markers for evaluating seed purity in a commercial F1-hybrid cabbage (*Brassica oleracea* var. *capitata*) cultivar is demonstrated. Hence showing that RAPD analysis can be used for seed purity testing of commercial hybrid cabbage seeds. (Crockett PA, Bhalla PL, *et al*, 2000)

Materials and Methods

Materials Used

-mercapto ethanol, Chloroform:Isoamyl alcohol (24:1), 5M Sodium Chloride, Ice-cold ethanol, 70% ethanol, 7.5M Ammonium acetate, Agarose, 1X TAE buffer, primer.

DNA Isolation from Fruits and Vegetables

1. The method used for DNA isolation is CTAB method.
2. One gram of the fruits and vegetable sample of orange, lemon, citrus, cabbage, cauliflower, and tomato were weighed and used for DNA isolation.
3. Each of the weighed fruit and vegetable samples was taken in pestle and mortar and ground well.
4. 10ml of DNA extraction buffer & 10 l of -mercaptoethanol was added into five centrifuge tubes, which contain orange, lemon, citrus, cabbage, cauliflower, and tomato. The tubes were inverted several times and incubated for one hour at 60^oc with intermittent shaking at 10min interval.
5. The tubes were cooled at room temperature and 10ml of chloroform isoamylalcohol (24:1) was added and mixed gently by inverting the tube.
6. The tubes were centrifuged at 5000rpm for 15min, & supernatant was transferred to the fresh tubes.
7. 5M Nacl (1/4th of total volume of supernatant) was added to the supernatant and mixed well.
8. To this double the volume of cold ethanol was added and incubated at -20^oc for over night.
9. Next day tubes were subjected to centrifugation at 10000rpm for 10min.
10. Supernatant was discarded and pellet was washed with 3ml of 70% alcohol and centrifuged again at 5000rpm for 5min.
11. Supernatant was discarded and ethanol was removed completely by blotting the tubes and was dried at 37^oc.
12. The dried pellet was suspended in 300 l of TE buffer. (10mM Tris HCL and 1Mm EDTA) pH 8.

Purification of DNA

1. 300µl of 7.5 M Ammonium acetate & 500µl of Ethanol was added into 5

centrifuge tubes and incubated at -20^oC for over night.

2. Tubes were centrifuged at 8000rpm for 5min.
3. The supernatant was discarded and pellet was washed with 100µl of 70% alcohol and centrifuged again at 5000rpm for 5minutes.
4. The supernatant was discarded again and ethanol was removed completely by blotting the tubes were dried at 37^oc.
5. Dried pellet was dissolved in 100µl of TE buffer.

Quantification of the DNA samples

1. 10µl of DNA sample was made up to 3ml using TE buffer and used for quantification.
2. Using 3ml of TE buffer as blank, the UV-Visible spectrophotometer was set to 1005 transmittance and 0.00 Absorbance.
3. The Absorbance of the DNA sample in TE buffer was measured at 260nm and the O.D value obtained was noted.
4. The concentration of the DNA sample was calculated using the formula.

$$\text{DNA } \mu\text{g}/\mu\text{l} = (\text{O.D at } 260 \times 50 \times \text{Dilution factor}) / 1000$$

$$\text{Dilution factor} = (3000\mu\text{l}) / 10\mu\text{l} = 300\mu\text{l}$$

PCR Amplification of DNA using random primers

The following components were added to 0.5ml PCR tube.

DNA sample (75ng)	-	2.5µl
Primers (10µM)	-	2µl
Taq buffer (10x)	-	2.5µl
dNTPS (10mM)	-	1.5µl
Taq polymerase (30µl)	-	0.5µl
Mgcl2 (25mM)	-	1.0µl
Sterile water	-	14.5µl
Total	-	25µl

Results and Discussion

Isolation of DNA

One gram of vegetable samples was used for isolation of DNA using 10ml of extraction

buffer. Then the samples were purified using Ammonium acetate. DNA was purified and quantified. The yield of DNA varied from 1.755 7.410 g/ l.

A. Quantification of DNA

The DNA samples were quantified by using spectrophotometer at 260nm by using the standard formula.

Table 1: DNA quantification of different samples

Sample	O.D at 260nm	Concentration in g/ l
Cabbage	0.043	1.937
Cauliflower	0.063	1.323
Kohlrabi	0.064	0.096
Orange	0.014	5.952
Lemon	0.149	0.555
Citrus	0.283	0.291

2. Standardization of PCR Amplification

The PCR was carried out by using HB cycler Automated PCR.

PCR Reaction mixture: The 25µl of total reaction mixture was contained 75ng of DNA with 10mM dNTPs, random primers (10µM), 30µl of Taq polymerase. The total reaction was made up to 25µl by using sterile water. The PCR reactions were standardized by screening with different sets of random primers, The banding pattern obtained from the primers namely A1, A2, A3, A4, A5 was used for analysis

PCR Reaction condition: The PCR mixture was subjected to initial denaturation of 94^oc for 2mintues. The reaction was subjected to the 45 cycles of initial denaturation of 94^oc for 1mintute, 37^oc for 1min 30sec and 72^oc for 2min annealing with a final extension of 72^oc for 10min.

3. Agarose gel electrophoresis

The electrophoresis was carried out on 0.24% gel with 7.0µl of ethidium bromide. Then 2.0µl of loading dye was added to the sample.

4. Statistical Analysis

The gels were scored by using binary coding method and uploaded to the software. Presence of band is noted as '1' and absence of the band is considered as '0'. The data was

uploaded to the software “statistic” where the analysis was done band on the clustering method.

The binary scoring of primer A4 with all samples are shown were horizontally marked as number of samples and vertically marked as number of wells loaded.

Table 2 : Binary scoring of A4 primer with all samples.

	A4					
	1	2	3	4	5	6
1	0	0	1	1	1	1
2	0	0	1	1	1	1
3	1	1	0	1	1	1
4	1	1	1	0	1	1
5	1	1	1	0	1	1
6	1	1	1	0	1	1
7	0	0	0	0	0	0
8	0	0	0	0	0	0

The analysis resulted in the formation of two major groups having all the fruit samples in one group and vegetables in the other group this result are on par with,

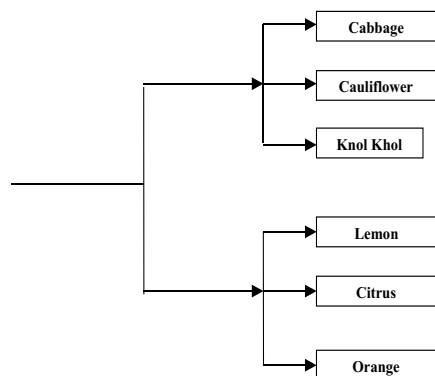


Fig: 1 - Phylogenetic analysis for samples

Herewith mentioned above the tree shows phylogenetic analysis for Vegetables. Suggest that there is a highest possibilities for hybriding between cabbage & Cauliflower when, compare to that of hybriding between cabbage and Knol Khol since genetic relationship between cabbage & cauliflower is much closer when compare to Knol Khol. With respective fruit samples there is a highest possibilities for hybriding between lemon & citrus and compare to lemon and orange, since the genetic relationship between lemon and citrus much closer when compare to orange. The dendrogram analysis of fruits and vegetables suggested that possibilities of

interspecific hybridization for improved qualitative and quantitative characters like pomato. (Hybrid of potato and tomato)

Reference

- Qin Y, Gao LH, *et al*, 2007 Dec, Shoot differentiation, regeneration of cauliflower and analysis of somaclonal variation by RAPD, *Hereditas*, **143**(2006),91- 8.
- Huang.B,Nian.H, *et al*, 2004. Jul, Analysis on intraspecific variation of *Cnidium monnieri* by RAPD],*Zhong Yao Cai*. **27**(7), 469-71.
- Li W, Sun G, *et al*, 2004 Feb, Inheritance of plant regeneration from maize (*Zea mays* L.) shoots meristem cultures derived from germinated seeds and the identification of associated RAPD and SSR markers. *Theor Appl Genet*. **108**(4), 681-7.
- Liu C, Yan Y, *et al*, 2000 May, Influences of counterfeits on RAPD results of *Semen Cuscutae*, *Zhong Yao Cai*. **23**(5), 256-8.
- Korbin M, Kuras A, *et al*, 2002. Fruit plant germplasm characterisation using molecular markers generated in RAPD and ISSR-PCR., : *Cell Mol Biol Lett*. **7**(2B), 785-94
- Weder JK. 2002 Jul, Identification of plant food raw material by RAPD-PCR: legumes. *J Agric Food Chem*. **50**(16): 4456-63
- Shim YH, Choi JH, *et al*, 2003 Aug, Molecular differentiation of *Panax* species by RAPD analysis. *Arch Pharm Res*. **26**(8):601-5.
- Shioda H, Satoh K, *et al*, 2003 Aug, Identification of Aloe species by random amplified polymorphic DNA (RAPD) analysis, *Shokuhin Eiseigaku Zasshi*. **44**(4): 203-7.
- Espinoza F, Pessino SC, *et al*, 2002 Feb, Effect of pollination timing on the rate of apomictic reproduction revealed by RAPD markers in *paspalum notatum*. : *Ann Bot (Lond)*. (2): 165-170.
- Mohana GS, Shaanker RU, *et al*, 2001 Jul, Genetic relatedness among developing seeds and intra fruit seed abortion in *Dalbergia sissoo* (Fabaceae). *Am J Bot*. **88**(7): 1181-1188.
- Subramanian V, Gurtu S, *et al*, 2000 Aug, Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. *Genome*. **43**(4): 656-60.
- Congiu L, Chicca M, *et al*, 2000 Feb, The use of random amplified polymorphic DNA (RAPD) markers to identify strawberry varieties: a forensic application. *Mol Ecol*. **9**(2): 229-32.
- Bhat KV, Jarret RL, *et al*, 1995 Sep, DNA profiling of banana and plantain cultivars using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. *Electrophoresis*. **16**(9):1736-45.
- Nedoluzhko AV, Dorokhov DB, 2007 May-Jun, Investigation of genetically modified soybean biosafety in the center of the origin and diversity of Russian Far East [Article in Russian], **41**(3): 72-81.

- Etoh K, Nijima N, *et al*, 2003, DNA-based identification of Brassica vegetable species for the juice industry, *J Nutr Sci Vitaminol(Tokyo)*, Oct;**49**(5):357-64
- Brandolini V, Tedeschi P, *et al*, 2005 Feb 9, Chemical and genomic combined approach applied to the characterization and identification of Italian *Allium sativum* L, *J Agri Food Chem*, **53**(3):678-83.
- Artiukova EV, Kholina AB, Kozyrenko MM, Zhuralev IuN, 2004 Jul, Analysis of genetic variation in rare endemic species *Oxytropis chankaensis* Jurtz. (Fabaceae) using RAPD markers, *Genetika*, **40**(7): 877-84.
- Souframanien J, Gopalakrishna T, 2004 Nov, A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers, *Theor Appl Genet*, **109**(8): 1687-93.
- Aguado V, Vitas AI, García-Jalón I, 2004 Feb 1, Characterization of *Listeria monocytogenes* and *Listeria innocua* from a vegetable processing plant by RAPD and REA, *Int J Food Microbiol*, **90**(3):341-7.
- Yanagino T, Sugawara E, Watanabe M, Takahata Y, 2003 Jun, Production and characterization of an interspecific hybrid between leek and garlic, *Theor Appl Genet*, **107**(1):1-5.
- Amadou HI, Bebeli PJ, Kaltsikes PJ, 2001 Dec, Genetic diversity in Bambara groundnut (*Vigna subterranea* L.) germplasm revealed by RAPD markers, *Genome*, **44**(6): 995-9.
- Lakhanpaul S, Chadha S, Bhat KV, 2000, Random amplified polymorphic DNA (RAPD) analysis in Indian mung bean (*Vigna radiata* (L.) Wilczek) cultivars, *Genetica*, **109**(3):227-34.
- Cheghamirza K, Koveza O, Kononov F, Gostimsky S, 2002, Identification of RAPD markers and their use for molecular mapping in pea (*Pisum sativum* L), *Cell Mol Bio Lett*, **7**(4):1158. 24
- Chen WS, Chiu CC, *et al*, 1998 Dec, Gene transfer via pollen-tube pathway for anti-fusarium wilt in watermelon, *Biochem Mol Bio Int*, **46**(6):1201-9.
- Voigt K, Wöstemeyer J, 1995 Nov, The combination of Gilbert/Maxam chemical sequencing and the dideoxynucleotide chain termination approach facilitates the construction of species specific PCR-primers based on diagnostic RAPD bands, *Microbiol Res*, **150**(4): 373-7. .
- Kazan K, Manners JM, Cameron DF, 1993 Feb, Inheritance of random amplified polymorphic DNA markers in an interspecific cross in the genus *Stylosanthes*, *Genome*, **36**(1):50-6.

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