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Research Article

Determination of molecular characterization of *Brassicaceae* family using RAPD molecular markers

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Abstract

The genus *Brassica* includes many important cultivated species worldwide. They play a vital role as vegetable, oilseed, condiment and forage crops, with some species presenting this whole range of use. Many parts of the plant are in edible forms, *Brassica* crops are used in the cuisine of many cultures and are a valuable source of minerals, vitamins, dietary fibres, and other possible salubrious factors such as anticancer compounds. The present investigation deals in estimating genetic variability using RAPD markers. The banding pattern by RAPD was variable depending upon the primer. The primers produced multiple band profiles with a number of amplified DNA fragments varying from 3 to 7. The genetic data collected during this work will guide the choice of genotypes to cross according to their lineage belonging or their level of diversity. Thus, the exploitation of genetic resources to broaden genetic variability is promising for the development of its use as a model in various genetic studies.

Keywords: RAPD, *Brassicaceae*, Mustard, Broccoli, Bok Choy.

Introduction

Brassica genus contains a large number of wild and cultivated species which includes six major crop species that have the potential to yield economically significant types of vegetables, oilseeds and mustards. The genus is *Brassica* is mainly characterized by diploid genomes with type species *B. rapa*, *B. nigra* and *B. oleracea* that have hybridised to form the amphidiploids *B. juncea L*. (AB), *B. napus L*. (AC) and *B. carinata Braun* (BC) (UN 1935). The polyploidisation event resulted in diploid condition of the species that took place 7.9–14.6 Mya (Lysak, 2005). The A and C genomes diverged less than 4 Mya (Inaba and Nishio, 2002). The Cross between and among *B. oleracea* and C genome relatives produce fertile or semi-fertile offspring (Kianian and Quiros, 1992; Go´mez-Campo, 1999), making it challenging with respect to taxonomy. Wild populations of genus *Brassica* also exist for most of the species, where in the C genome is mainly seen in a number of related species which have distinct eco-geographic centres of diversity, mostly of Mediterranean origin (Snogerup *et al*., 1990).

DNA polymorphisms have been detected by RAPDs (Welsh and McClelland 1990; Lankhorst *et al*., 1991; Martin *et al*., 1992; Carlson *et al.*, 1991). The generated polymorphism behaves as a genetic marker for the species which can be used for the construction of the genetic maps within the species

(Williams *et al*., 1990). The genome specific markers for Brassica were identified by Quiros et al. (1991) using RAPDs.

Characterization of plant with the use of molecular markers is an ideal way to conserve plant genetic resources. Molecular characterization helps to determine the breeding behaviour of species, individual reproductive success and the existence of gene flow, that is, the movement of alleles within and between populations of the same or related species, and its consequences. Molecular data improve or even allow the elucidation of phylogeny, and provide the basic knowledge for understanding taxonomy, domestication and evolution of plants .Information from molecular markers or DNA sequences offers a good basis for better conservation approaches. Modern molecular techniques have been developed in order to meet the demands of the horticulture industry. Population genetics, genetic linkage map and marker assisted selection techniques have significantly simplified the breeding procedures and overcome some of the agronomic, abiotic and biotic problems, which otherwise would not be achievable through conventional breeding methods. The development and remarkable achievements with molecular biotechnology in ornamental plants made during the three decades have been reviewed. Identifications of variety, cultivar or genotype by molecular markers have been attempted since the early 1980's. These 'markers' of genetic variation are generally independent of environmental factors and more numerous than phenotypic characters, thereby providing a clearer indication of the underlying variation in the genome of an organism.

Quantification of plant material using DNA markers has already become a very valuable tool in plant breeding and gene bank management. In the present study the genetic diversity of *Hibiscus* varieties and species cultivated in Mauritius was characterized using both morphological markers and the RAPD technique.

The molecular approach for identification of plant species or varieties seems to be more effective than traditional morphological markers because it allows direct access to the hereditary material and makes it possible to understand the relationships between plants (Paterson *et al.*, 1991). Random amplified polymorphic DNA (RAPD) technique has been widely used in many plant species for varieties analysis, population studies and genetic linkage mapping (Williams *et al.*, 1990; Yu *et al.*, 1993; Rout *et al.*, 2003). Optimization of the RAPD analysis depends on selection of primers. Although, the RAPD method uses arbitrary primer sequences, many of these primers must be screened in order to select primers that provide useful amplification products.

Materials and Methods

Sample collection

Leaves samples belonging to *Brassicaceae* family, namely *Brassica oleracea* (Broccoli, Cauliflower, Cabbage, Brussels sprouts and Red Cabbage), *Raphanus sativus* (radish) and *Brassica rapa* (Bok Choy) were collected from Lalbagh Botanical Garden, Bangalore and from local market. Young leaves were used as the source for genomic DNA which were stored in sterile zip lock bags and maintained at -20°C until further used.

Isolation of DNA

0.5g of fresh young leaf samples of *Brassica oleracea* (Broccoli, Cauliflower, Cabbage, Brussels sprouts and Red Cabbage), *Raphanus sativus* (radish) and *Brassica rapa* (Bok Choy) were collected and grinded in mortar with 3ml of STE buffer and 300µl of SDS (20%). The contents were then transferred to new microfuge tubes and add 200 µl of 8M LiCl, 0.2% β mercaptoethanol and 1.5% of PVP was added to each vials. The vials were then Incubate in water bath at 60˚C for 45 minutes. Equal amount of chloroform: isoamyl alcohol (24:1) solution was added to the vials and centrifuge at 13,000 rpm for 15 min in room temperature. Aqueous layer was carefully transferred into to a new microfuge tube and then 1ml of ice cold ethanol was added and the vials were incubate at 4˚C in refrigerator for 15 – 30 min and then centrifuge at 10000 rpm for 10 min in

room temperature. 1ml of 70% ethanol was added to the vials containing pellet, this step was carried out until there is no sticky nature. The vials are the centrifuge at 10000 rpm for 10 min in room temperature and the pellet is rinsed with 1ml of 70% ethanol and centrifuge the tube at 10000 rpm for 10 min in room temperature (Doyle and Doyle, 1990). The traces of ethanol were removed and the vials with pellet were air dried and re-dissolved in TE buffer. The qualitative analysis of the DNA was carried out by Gel electrophoresis and the quantitative analysis was tested using NanoDrop at 260nm.

RAPD-PCR

RAPD-PCR was performed using primer OPD- 10 and OPD- 20 mix in micro centrifuge tubes containing 150 mM of each dNTP, 10 pmol primer, 25 ng template DNA, 2.5µl 10X reaction buffer, 1U/µl of Taq polymerase.

Cycling conditions

The RAPD-PCR was carried out at the following conditions, where in the initiation step was carried out at 94^0C for 5 min, de-naturation at 94^0C for 1 min, annealing at 37^0C for 1 min and elongation at 72^0C for 1 min (36cycles), the final elongation was carried out at 72^0C for 5 min, and subsequent cooling down at 4^0C.

Results

Qualitative estimation of DNA

The qualitative analysis of DNA by gel electrophoresis is shown in following pictures. The obtained DNA showed sharp single bands on 1.0% agarose gel without any degradation or RNA contamination (Figure-1).

DNA Quantification using NanoDrop spectrophotometer

DNA quantification was done using Nanodrop ND 1000. The graphs represent the DNA quantity in ng/ul (Figure 2-3). Also it shows the 260/280 and 260/230 ratios which represent the purity of DNA. All the samples showed good amount and purity of DNA.

PCR- Random Amplification of Polymorphic DNA

The samples were amplified with two arbitary primers namely OPA- 10 (Figure 4 & 5) and OPD-20 (Figure 6 & 7). The amplified product was run on a 1 % agarose gel. After which the bands were analysed and used further for dendrogram analysis.

Discussion

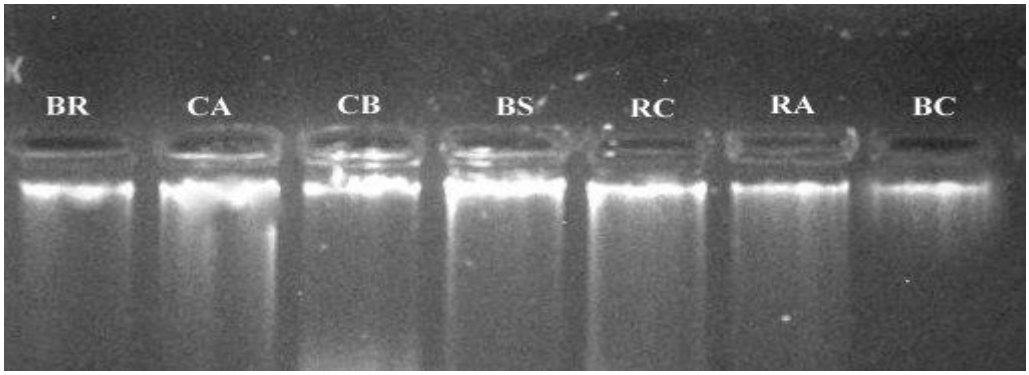
RAPD can be suitable and efficient tool for genetic characterization of many plant species (Ghosh *et al*., 2009), therefore in the present study the genetic diversity of the members of *Brassicaceae* family was determined using RAPD. Similarly Lazoro *et al.* (1998) evaluated the genetic diversity in 29 populations of wild taxa of the *Brassica oleracea* L. group and two cultivars, using RAPDs as molecular markers.

Sanchita Saha *et al.* (2008) studied the genetic diversity and relationship among nine *Brassica* varieties of four different species using Random Amplified Polymorphic DNA (RAPD) markers and their observations are relatively similar to the findings of the present investigation.

In the present study genetic diversity analysis among the *Brassicaceae* family members was performed using RAPD markers. The primers produced multiple band profiles with a number of amplified DNA fragments varying from 3 to 7. Lazaro (1998) analyzed genotypes of *Brassica* spp. including wild variety, exotic, Indian and mutants using RAPD primers and grouped the genotypes into four clusters.

In the present study OPD-10 and OPD-20 were used to analyse the diversity among *Brassicaceae* family using RAPD. Similarly researchers carried out RAPD analysis using random primers such as GM10, GM12, GM37 and GM100 for detecting genetic diversity of five mustard varieties as the

Figure 1. DNA samples run on 1% agarose gel



BR-Broccoli, CA-Cauliflower, CB-Cabbage, BS-Brussels Sprouts, RC-Red Cabbage, RA-Radish, BC-Bok Choy.

Figure 2. Quantification of DNA using NanoDrop Spectrophotometer for Radish

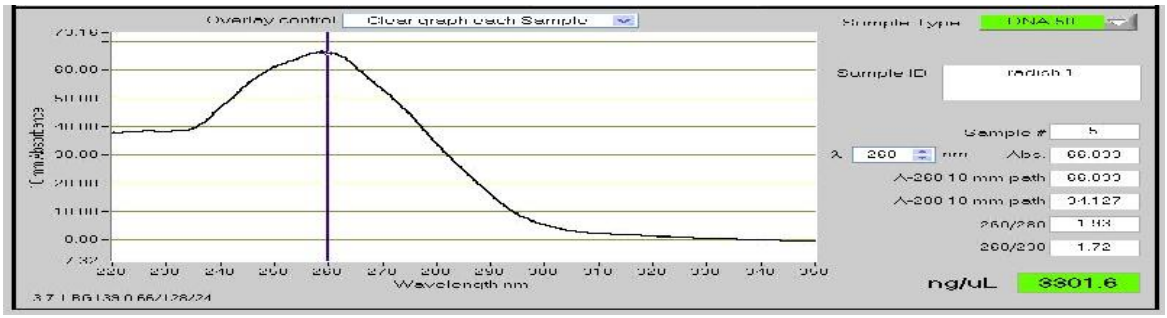


Figure 3. Quantification of DNA using NanoDrop Spectrophotometer for Red Cabbage

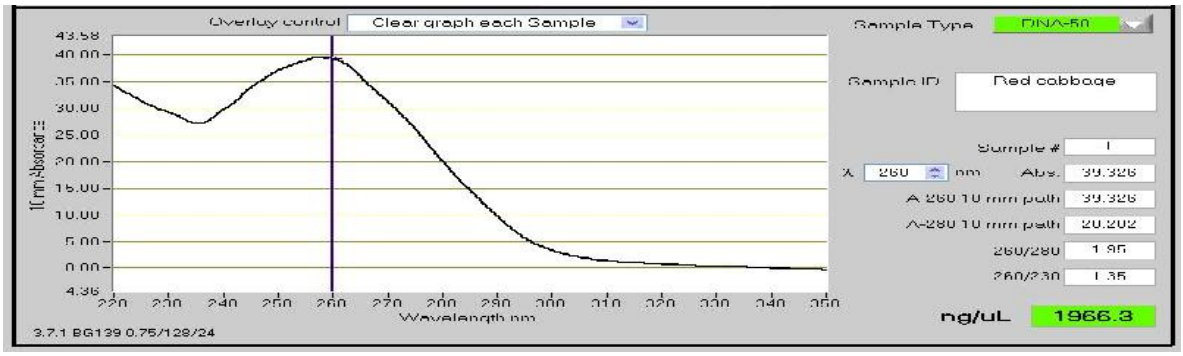
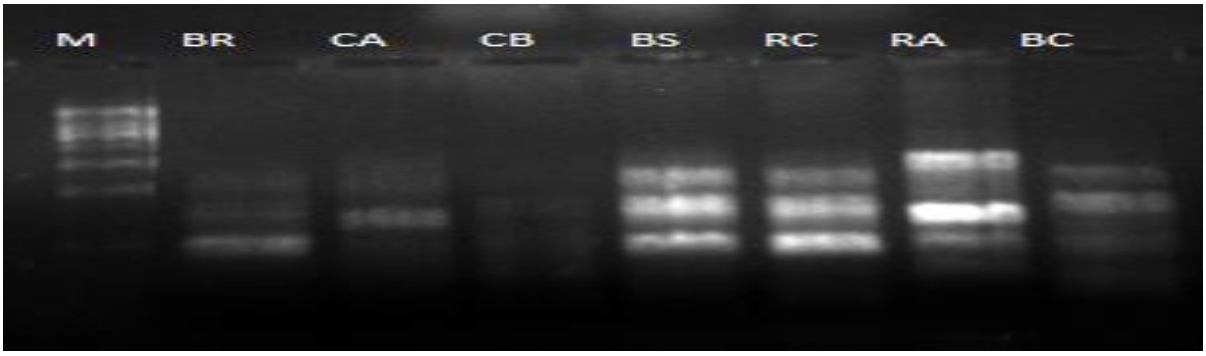


Figure 4. DNA Amplification using primer OPD-10



BR-Broccoli, CA-Cauliflower, CB-Cabbage, BS-Brussels Sprouts, RC-Red Cabbage, RA-Radish, BC-Bok Choy.

Figure 5. Dendrogram Analysis for OPD-10

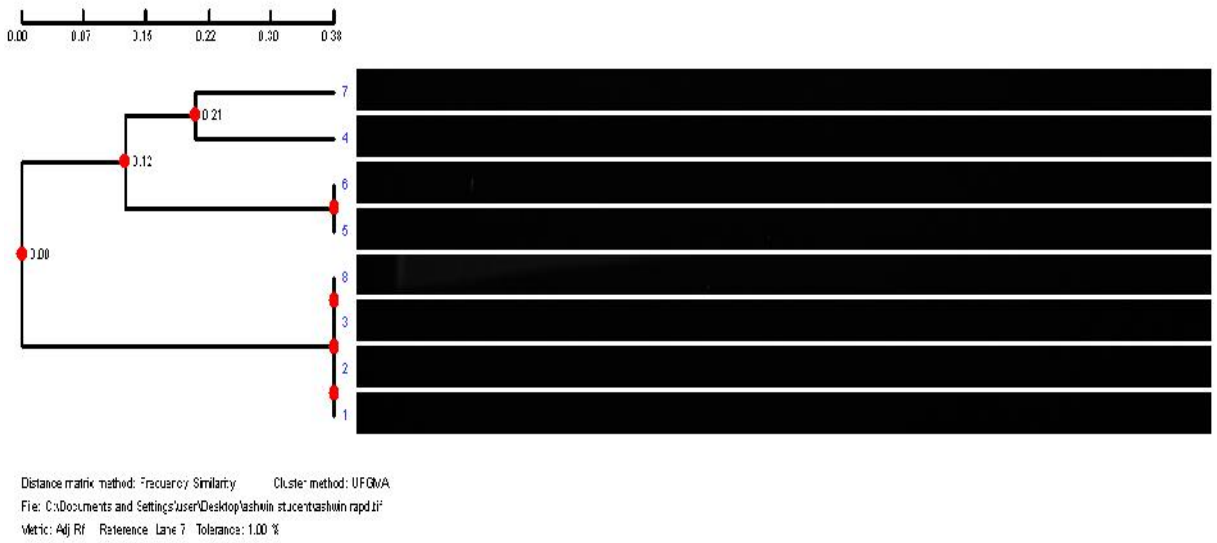
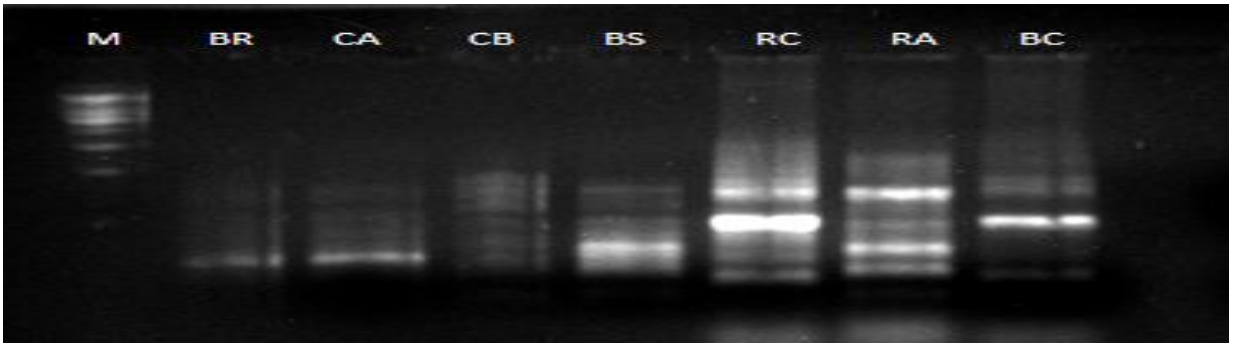
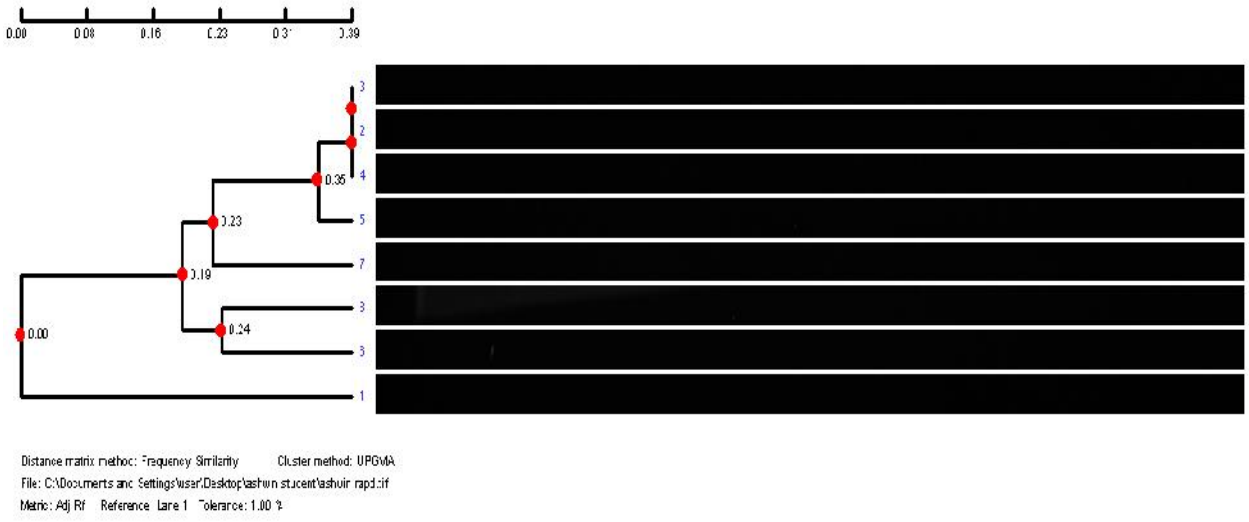


Figure 6. DNA Amplification using primer OPD-20



BR-Broccoli, CA-Cauliflower, CB-Cabbage, BS-Brussels Sprouts, RC-Red Cabbage, RA-Radish, BC-Bok Choy.

Figure 7. Dendrogram Analysis for OPD-20



detection of genetic diversity is essential for proper use in the genetic improvement of the mustard, thus suggesting that the present investigation could also be helpful in determining the improvement for the members of *Brassicaceae* family.

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