MOLECULAR ANALYSIS OF GENETIC DIVERSITY IN Manihot Esculenta Crantz

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Introduction

Cassava (*Manihot esculenta Crantz*) is one of the most important food crops of the tropics along with rice, maize and sugarcane (plate 1). It ranks as the fourth major source of carbohydrates in the tropics (FAO, 1995). Unlike many other crops, cassava can be grown with minimal inputs and it is able to yield reasonably well under unfavorable conditions such as low soil fertility, acidic soil or drought. It is a staple crop for more than 500 million people, despite the low attention historically received in research because it is considered as a subsistence crop. Cassava is also an industrial crop for starch, flour and animal feed (Charrier et al., 1998).Cassava is an out breeding species originated in the American continent (Roger, 1972). Presently it is cultivated in all the tropical regions of the world and it is produced in more than 80 countries covering an area of 16 million hectares, producing 153.6 million tones of tubers. . This is realized by the cultivation of improved varieties and Cassava, which is believed to have originated in Latin America, has a number of attributes that have made it an attractive crop for small farmers, with limited resources in marginal agricultural areas. Importance of Cassava includes - it is one of the most efficient carbohydrate producing crop, it is tolerant for low soil fertility and drought, it has the ability to recover from the damage caused by most pests and diseases, the roots can be left in the ground for long period as a food reserve and thus provide an insurance against famine, the crop is well adapted to traditional mixed cropping agricultural system and subsistence cultivation in which farmers seek to minimize the risk of total crop failure. Cassava is apparently emerging from its obscurity in the tropics and is marching northward and southward to fill new roles in temperate climates. In the tropics, cassava is the single most important source of calories in diet (CIAT, 1992). The Roots are the principle edible portion of the plant and the typical ranges of composition are given; water 62-65%, total carbohydrate 32-35 %, protein 0.7-2.6%, fat 0.2-0.5%, fiber 0.8-1.3% and ash 0.3-1.3% (Kay, 1987). The leaves of cassava can also be consumed which is rich in protein content (Hillocks,

1997).The crop is principally used as human food, either fresh (boiled, baked, fried or pounded) or in numerous processed forms (Lancaster et al., 1982). As the fresh tubers deteriorate rapidly they are often preserved in the form of sun-dried chips and consumed after cooking or being ground into flour. The principle form in which cassava is eaten in West Africa is as a fermented meal known as gari. A substantial industrial outlet for cassava is in the manufacture of starch for use in the foodstuff, textile and paper industries, in the manufacture of plywood and veneer, adhesives and in the oil drilling industries. Starch is also a raw material for producing many derived sugar products, such as glucose, fructose, maltodextrins and mannitol. Minor industrial applications include use in the manufacture of explosives, dyes, drugs, chemicals, and linoleum and in the coagulation of rubber latex (Balagopalan et al., 1988).Increasing quantities of dried cassava roots in the form of pellets are being used for livestock feeding. These pellets are used as a carbohydrate source in animal feed rations, particularly for pigs. The cassava flour is used in the preparation of bread, biscuit, confectionary and in the products such as macaroni, spaghetti

and rice substitutes, also as an adulterant of cereal flour and in the production of adhesives. It is also used for the preparation of pudding and in infant and invalid foods. Carbon from cassava peel can be used as an adsorbent in the removal of dyes and metal ions from aqueous solution (Pereira et al., 2001).But vast areas are cultivated as rain-fed crop by poor farmers, mainly for edible purposes predominance. Where the cooking quality gets Cassava Mosaic Virus is a major disease of the crop for which there is no control measure. So identifying the resistant varieties is important (Thresh and Otim - Nape, 1994). There is a need for diverse types of improved varieties suited to different agro climatic regions. Variation is the essence of any plant-breeding programme. The collections are maintained in a continuous vegetative phase by cyclic pruning or periodic renewal of the entire collection every 2-4 years (Cabral et al., 2000). Most attempts to conserve the diversity of crop plants were concerned with their incorporation in ex situ collection. Since cassava is a highly heterozygous, cross - pollinated crop, reasonably high variation is noticed among the indigenous stock themselves (Valle, 1990). Collection and conservation of germplasm is gaining importance all over the world with an aim to utilize these genetic resources in the ongoing as well as future plant improvement programs. Genetic resources form the backbone of plant breeding programs. The number of accessions collected as "genetic resources" and assembled in gene banks need to be evaluated in a single location. Vegetative

propagation of the crop and the fact that the same variety is known differently at different locations, and different clones have been erroneously called by the same name in different locations have caused duplications among the collections and detailed morphological studies have revealed similar clones in the germplasm (Hamrick, 1993).However, some of the genetically similar and vice versa. Morphological duplicates may not be more sophisticated

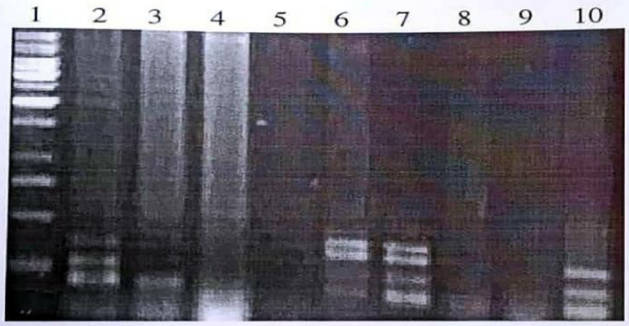
Biochemical and molecular tools by way of isozyme analysis and DNA analysis are now available to confirm the genetic identity of the duplicates. (Gater et al., 1979; Aguirre et al., 1999; Fuentes et al., 1999; Santha Pillai et al., 1999). Isozymes are different molecular forms of enzymes, which exhibit the same substrate specificity. The present concept of the term emphasizes the existence, within a single species, of two or more form of an enzyme. Random Amplified Polymorphic DNA (RAPD) denotes utilizing the character of Polymerase Chain Reaction (PCR) of DNA using arbitrarily selected primers. They generate reproducible fingerprints from any DNA template without the need for prior knowledge of the sequences or construction of clone banks; species that have not been studied with molecular tools can be immediately mapped or fingerprinted. These fingerprints contain DNA fragment polymorphisms that can be used for varietal identification and parentage determination : to study the population structure and genetics; to find the genetic diversity (Beeching et al., 1995; Carlose et al., 1998 Carvalho et al., 1998a, 1998c); as markers to construct genetic maps from segregating populations and as characters to study phylogenetic relationships (Schaal et al., 1995; Carvalho et al., 1998b).The study was formulated with the following objectives. To standardize the protocol for the extraction of total DNA from cassava leaves. To find the distribution of genetic variability within nine accessions of cassava varieties using RAPD analysis.

Materials and methods

The experimental procedure adopted for the study was conducted in four phases as follows: Phase I includes the Sample collection, Phase II includes Standardization of the protocol for extraction of DNA and Phase III includes RAPD analysis of cassava samples. Nine accessions of cassava were used in the study. They were Kalpaka, Sree Harsha, Vellayani Hraswa, Ullichuvala, Sree Prakash, Sree Vijaya, MNGA, Sree Sahya,H- 198. Cassava leaf samples are obtained from the germplasm collection maintained at Centre Tuber Crops Institute, Trivandrum. Sample collected for the study belongs to different varieties of Manihot

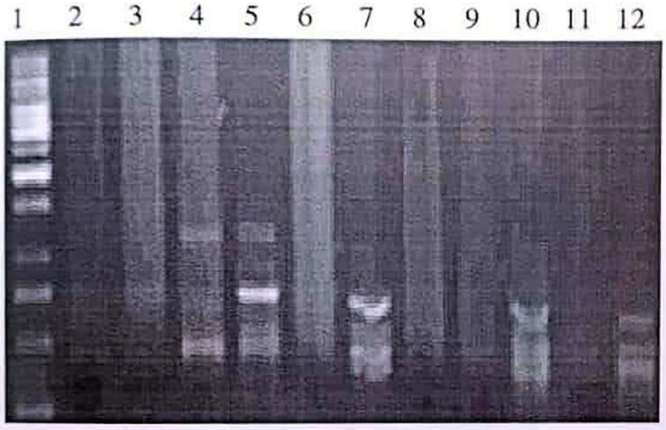
esculenta Crantz. To find the genetic diversity between these species following experiments were done.DNA was extracted from the fresh young leaves. RAPD analysis was carried out with nine accessions of cassava to check for genetic diversity using two primers from Operon Primer, Kit Q.For 500 The extracted DNA was amplified in a DNA Thermal Cycler T (MJ Research USA). The reaction mixture consisted of 2.5 ml reaction buffer (10 x Tris-HCI, 100 mM), 2.1 ml operon primer, 0.5 ml dNTPs (100 mM), 1 ml Dy nazyme II DNA polymerase (2m/ ml) and 4 ml of sample DNA (10ng/ml) The total reaction volume Nwas made upto 25 ml using sterile distilled water. The following 2 primers were used for RAPD analysis. OPQ-01 (5' GGGACGATGG3') and OPQ-09 (5'GGCTAACCGA3').The PCR amplification reactions were performed in the following steps: Denaturation at 92°C for 1 minute, Primer annealing at 36°C for 1 minute, Polymerization at 72°C for 2 minutes. These steps are repeated for 45 cycles. An initial heating at 94°C for 5 minutes was given at the beginning of the reaction and a final step of extension at 72°C for 7 minutes at the end of the reaction. Fox 500 ml Amplified D NA fragments were separated on 1% agarose gel. DNA digested with Hind III was used as molecular weight marker. The image of the gels were captured with a digital camera and analyzed for the presence and absence of bands by using the software 'AAB-ID Advanced' (Advanced American Biotechnology).

Different genetically verified Cassava crops



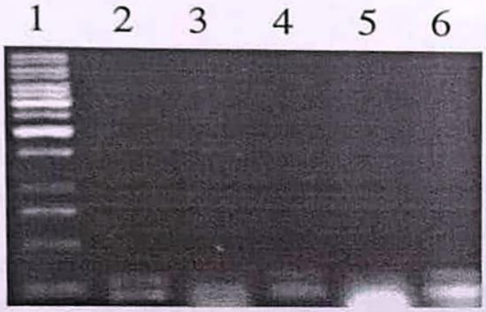
Lane 1. Marker
Lane 2. Kalpaka
Lane 3. Sree Harsha
Lane 4. Vellayani Hraswa
Lane 5. Ullichuvala
Lane 6. Sree Prakash
Lane 7. Sree Vijaya
Lane 8. Ullichuvala
Lane 9. MNGA
Lane 10. Sree Sahya

RAPD banding pattern of different varieties of Cassava using OPQ – 01 primer



Lane 1. Marker
Lane 2. Kalpaka
Lane 3. Sree Harsha
Lane 4. Vellayani Hraswa
Lane 5. Ullichuvala
Lane 6. Sree Prakash
Lane 7. Sree Vijaya
Lane 8. Ullichuvala
Lane 9. MNGA
Lane 10. Sree Sahya
Lane 11. H 198
Lane 12. Sree Sahya

RAPD banding pattern of different varieties of Cassava using OPQ – 09 primer



Lane 1. Marker
Lane 2. H 198
Lane 3. Ullichuvala
Lane 4. Sree Sahya
Lane 5. Vellayani Hraswa
Lane 6. MNGA

RAPD banding pattern of different varieties of Cassava using OPQ – 09 primer

Results and discussion

The extracted DNA was subjected to RAPD analysis using 2 primers (OPQ - 01 and OPQ - 09) were used .Both primers produced clear bands in same varieties. When tried with OPQ-1 Sree Prakash, Sree Vijaya showed similarities. The varities which got bands using OPQ 01 are Kalpaka, Harsha,SreePrakash,. Sree Vijaya, Ullichuvala, MNGA, and Sree Sahya. The varities which got bands using OPQ-9 are Vellayani Hraswa Ullichuvala, Sree Vijaya and H-198.Vellayani Hraswa is the latest variety of cassava. It is an early maturing variety with five months duration coupled with high yield and excellent quality. Apparently it is similar to Ullichuvala. It is a popular land race with 6 to 8 months duration with high yield and very high starch content(<30 %) .This study could find that inspite of morphological similarities these two varieties are genectally different.RAPD banding pattern showed high variability with in different cassava accessions studied. RAPD technic can be effectively used for studying genetic diversity of cassava varieties.

Summary and Conclusion

Manihot Esculenta Crantz of Euphorbiaceae family is one of the most important food crops of the tropics. Cassava is a subsistence crop, which provides high calorific value throughout the developing world. In India, cassava occupies 0.26 million hectare producing 5.868 million tones annually. Due to vegetative propagation and hybridization between different varieties that co-exist within a single field, a large variety of cassava is now available. In the present study, genetic diversity between groups of cassava cultivars was studied using RAPD analysis. Cassava mosaic disease (CMD) are the most important problem of this crop in India leading to 16-18 percent yield loss. In the present study, RAPD analysis was used for the identification of genetic variability of cassava. Dellapota method was used for extraction of DNA from the Cassava leaves. RAPD banding pattern showed high variability within different cassava varieties. From this study, it can be concluded that RAPD analysis is a better tool for identification of released varieties of Cassava.

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