Indian J. Pl. Genet. Resources 12(2): 232-236, 1999

OPTIMIZATION OF PCR CONDITIONS FOR GENETIC CHARACTERIZATION OF KENYAN BANANAS AND PLANTAINS (*Musa* ssp.)

J. M. ONGUSO, K. V. BHAT[1], E. M. KAHANGI, D. W. NDIRITU[2], F. N. WACHIRA[3], S. CHADHA[1], Institute for Biotechnology Research, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, Nairobi; [1]N.R.C. on DNA Fingerprinting, National Bureau of Plant Genetic Resources, New Delhi 110 012; [2]Botany Department, Kenyatta University, P.O. Box 43844, Nairobi [3]Tea Research Foundation, P.O. Box 820, Kericho

Optimization of the reaction conditions for Polymerase Chain Reaction (PCR) should be done before the actual Random Amplified Polymorphic DNA (RAPD) analysis is carried out to get repeatable results. Optimizations of concentration was carried out the reaction components; template DNA and Taq DNA Polymerase. The optimum concentrations were determined by the ability to form amplification products with the highest consistency. The significance of optimization is discussed and the results compared with similar published ones.

Key words : *Musa*, optimization, banana, plantain, PCR

Bananas and plantains (*Musa* ssp) are very important crops in Kenya as they contribute a part of the staple diet for the Kenyan population. They are grown almost all the areas around the households in the country and are regarded as one of the most valuable crops for implementing the drive for increased food production in order to meet food requirements in Kenya.

Bananas and plantains are also a major staple food for millions of other people in the tropical world. They rank as the fourth most important global food commodity after rice, wheat and milk in terms of the gross value of production (Vulysteke, 1993). Only 15 per cent of total world production of 76 metric tonnes goes to the banana export, while the rest grown by small scale holders in the developing world, is sold for local consumption in the urban and rural markets.

Spontaneous somatic mutations in bananas and plantains are of great agricultural significance in giving rise to several clonal forms of important cultivars. Due to the extensive proliferation of numerous mutants, however, the identification of original parental forms available in India, Hawaii and East Africa has not been visible (Stover and Simmonds, 1989). Positive identification of even the most widely cultivated clones is difficult or impossible due to the multiple vernacular names of each of the clones and the relative lack of morphological and genetic variation between them (Jarret and Litz, 1986).

Strict adherence to Simmonds and Shepherds (1955) system of *Musa* classification permits the grouping of cultivars based upon the relative dosage effects of *Musa acuminata* (A-genome) and *Musa balbisiana* (B. genome) on plant and fruit morphology. However, the subjective nature of

the scoring process makes this procedure prone to error (Bhat *et al.*, 1995 b). Environmental effects on gene expression makes direct comparison of clones growing in different locations impossible (Swennen, 1990).

Cytoplasmic genetic effects on morphology may be an additional factor contributing to ambiguities encountered in the results of key derived genomic classification. The extent to which cytoplasmic genetic effects confound efforts to differentiate clones based upon their morphological characteristics, especially within the hybrid group AB, ABB and AAB has not been systematically examined (Bhat *et al.*, 1995a).

This experiment was carried out with the objective of optimizing the reaction conditions for the polymerase chain reaction (PCR) to enable the genetic characterization of Kenyan bananas and plantains by Random Amplified Polymorphic DNA (RAPD) techniques.

MATERIALS AND METHODS

Plant materials were originally collected from farmers' fields throughout the banana growing regions in Kenya and maintained at the Kenya Agricultural Research Station - Kisii for germplasm conservation and research. Suckers were requested and grown at the Jomo Kenyatta University of Agriculture and Technology demonstration field to serve as a source of leaf tissue for DNA isolation. The same accessions were also maintained *in vitro* at the Institute for Biotechnology Research Laboratory, Jomo Kenyatta University. They were as shown below (Table 1). The laboratory work was done at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. Young unfurled leaves were collected from healthy plants of each cultivar. Five 10g samples were collected from randomly selected plants of each cultivar. They were transported in a cooler box to India where they were kept at –80°C and taken out for DNA extraction as required.

Table 1. *Musa* germplasm analyzed for RAPD genetic markets

Serial No.	Cultivar/species	Utilization
1.	Kimuga	Cooking
2.	Ngombe	Cooking
3.	Pekera	Dessert
4.	Mokoya	Beer
5.	Ntobe	Cooking
6.	Nusu Ngombe	Cooking
7.	Spambia	Roasting
8.	Murure	Cooking
9.	Sialamule	Cooking
10.	Matumbo	Cooking
11.	Manyatta	Dessert
12.	Sibusi	Cooking
13.	Bukamba	Cooking
14.	Nyar Sausett	Dessert
15.	Kibuzi	Cooking
16.	Mfupi	Cooking
17.	Gitigi	Beer
18.	Muraru	Cooking
19.	Mtama	Cooking
20.	Horn plantain	Roasting

DNA extraction, purification and estimation of DNA concentration by fluorimetry were done using the published methods by Bhat *et al.*, (1995a). Optimization of reaction conditions should precede the actual RAPD analysis to get repeatable results. The following optimizations were done: Mg2+ ion concentration, primer concentration template DNA concentration and taq polymerase concentration. Experiments with the following ranges of three components were set :

Experiment I : Mg2+ ion concentration, i.e. 0, 1, 2, 3, and 4 uM. This was repeated five times. The other components were used at the optimum concentrations.

Experiment II : Template DNA concentration i.e. 0, 5, 10, 15, and 20 ng/ml per master mix. This was repeated five times.

Experiment III : The experiment was set up with different concentrations of primer OPC-15, i.e. 0, 0.55, 0.60, 0.65 and 0.70 uM of 10-mer primer OPC 15 (Operon technologies, Alameda, CA). This was repeated five times.

Experiment IV : The experiment was set up with different concentrations of taq DNA polymerase i.e. 0, 0.70, 0.75, 0.80 and 0.85U. The experiment was repeated five times.

RAPD analysis

Polymerase chain reaction (PCR) was carried out in a thermal cycler (Perkin Elmer/Cetus 9600 USA) : 1 minute denaturation at 94°C, 1 minute primer annealing at 35°C and 1 minute primer extension at 72°C for 40 cycles followed by a further extension at 72°C for 8 minutes. Amplification products were mixed with a loading buffer containing bromophenol blue, electrophoresed on 1.8 per cent agarose (metaphor) gels in IXTAE (Tris Acetate) buffer. The gels were stained in ethidium bromide, bands visualized on a transilluminator and photographed.

RESULTS AND DISCUSSION

There was no amplification in all the samples without $Mg2^{+}$ ions. The amplification with 2 uM Mg2+ was better than that with 1 uM, 3uM Mg2+ ion concentration gave better amplification than 1 uM, and 2 uM Mg2+ ion concentration. There was no difference in amplification with 4 uM, and 3 uM as shown in the Fig 1. This showed that Mg2+ is essential for the polymerase chain reaction to proceed. The results showed that 3uM of Mg2+ gave more informative amplification than the others. This was consistent with other published work such as Bhat *et al.*, (1995a) and Howell *et al.*, (1994). Using a higher concentration would not improve the results and would lead to wastage of the chemical.

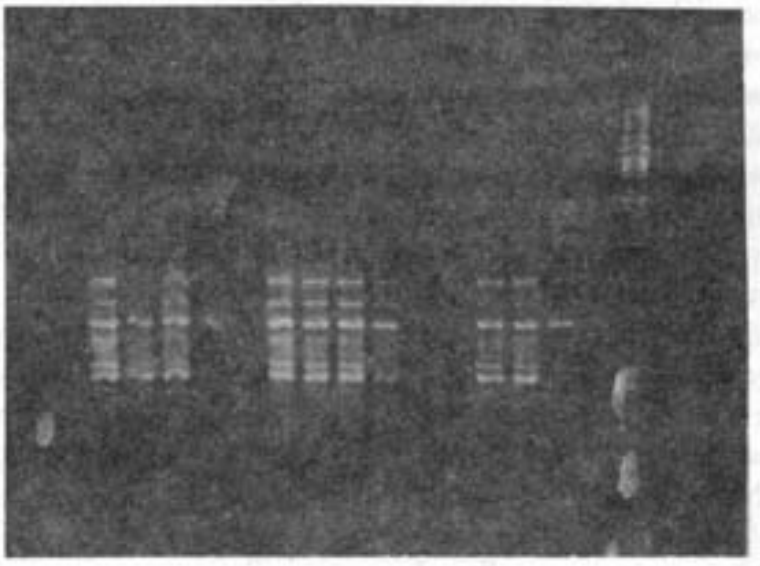

Fig. 1. Amplification pattern with different concentrations of Mg2+ ion using primer OPC-15. There was no amplification in the samples where no template DNA was added. The amplification with 10 ng/ml of template DNA was better than that with 5 ng/ml. There was no significant difference between the amplification with 10 ng/ml, 15 ng/ml, and 20 ng/ml of template DNA concentration. It is therefore, better to use 10 ng/ml template DNA for economy of the samples and better results. Little amounts of crude DNA are sufficient for RAPD analysis (Erlich and Arnheim, 1992; Wachira, 1996).

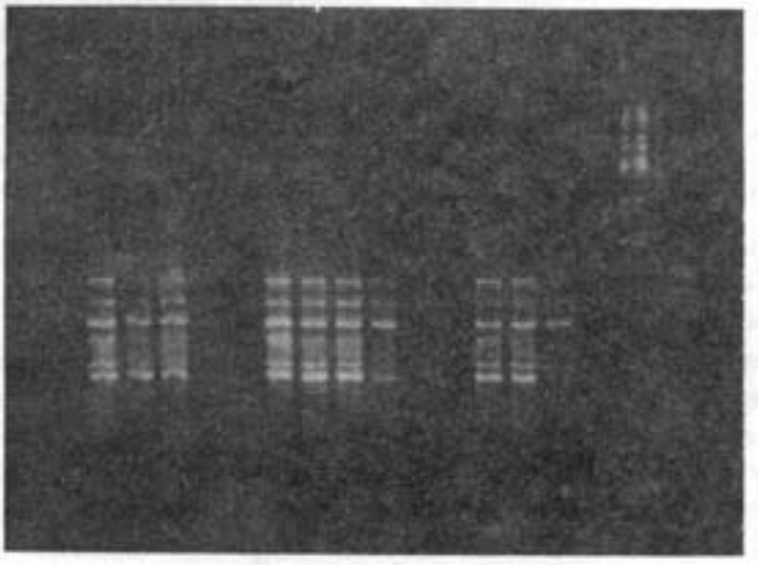

Fig. 2. Amplification pattern with different concentrations of template DNA using primer opc-15. For template DNA, there was no amplification in the samples where no template DNA was added. The amplification with 10 ng/ml of template DNA was better than that with 5 ng/ml. There was no significant difference between the amplification with 10 ng/ml, 15 ng/ml, and 20 ng/ml of template DNA concentration. It is therefore, better to use 10 ng/ml template DNA for economy of the samples and better results. Little amounts of crude DNA are sufficient for RAPD analysis (Erlich and Arnheim, 1992; Wachira, 1996).

There was no amplification in samples without primer. This showed that a primer is important for the polymerase chain reaction to proceed. L0.60 uM of 10-mer primer gave more informative amplification that 0.55 uM 0.65 uM gave better amplification than 0.60 and 0.55. There was no significant difference between 0.65 uM, and 0.70 uM of the primer. It is better to use 0.65 uM for the PCR reaction for banana and plantains.

There was no amplification in all the samples without taq DNA polymerase. This showed that taq DNA polymerase is essential for the reaction to take place. Amplification with 0.75 U gave more consistent results than 0.70U. There was no distinct difference between 0.75 U, 0.80 U and 0.85 U. It is therefore, better to use 0.75 U of taq DNA polymerase for all amplification in banana and plantains to reduce wastage of the chemical which is quite expensive. This concentration is comparable with what has been used by Fashima *et al.*, 1999 and Wachira, 1996.

ACKNOWLEDGEMENTS

The authors are grateful to the Kenya Agricultural Biotechnology Platform for financing the research work, Jomo Kenyatta University for granting study leave and the Director, National Bureau of Plant Genetic Resources (NBPGR), New Delhi for allowing use of their excellent facilities.

REFERENCES

Bhat, K.V., R.L. Jarret and R. S. Rana. 1995a. DNA profiling of banana and plantain cultivars using RAPD and RFLP markers. *Electrophoresis* **16**: 1736-1745.

Bhat, K.V., S.R. Bhat, K.P.S. Chandel, S. Lakhanpaul and S. Ali. 1995b. DNA finger printing of Musa cultivars with

oligonuclotide probes specific for simple sequence repeat motifs. *Genet. Anal.* **12**: 45-51.

Erlich, H.A. and N. Arnheim. 1992. Genetic analysis using polymerase chain reaction. *Ann. Rev. Genet.* **26**: 479-506.

Fashima, T., G.L. Sun, A. Beharar, T. Krngman, A. Bellies and E. Novo. 1999. Polymorphisms of wild wheat populations, *Triticum dicoccoides* in Israel. *Euphytica* **99**: 137-142.

Hovel, E.C., H.T. Newbury, R.L. Swennen, L.A. Withers and B. V. Ford-Lloyd. 1994. The use of RAPD for identifying and classifying *Musa* germplasm. *Genome* **37**: 328-332.

Jarret, R.L. and R.E. Litz. 1986. Determining the inter specific origins of clones within the 'saba' cooking banana complex. *Hort. Science* **21**(6): 1433-1435.