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*Short Communication*

# Application of Hosaka and DJ Kim Whatmann paper protocols for rapid isolation of cowpea DNA

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**The traditional liquid nitrogen DNA extraction method is expensive and tedious. There is, therefore, the need for cheaper and faster methods of DNA extraction for efficient application of marker assisted selection (MAS) for breeding for striga resistance in cowpea to be able to handle large number of samples at a time. Two DNA extraction methods; Hosaka (2004) and DJ Kim Whatmann paper (unpublished) were tested on cowpea line IT06K-5-83 under different conditions. The results revealed that both methods with modifications can work with cowpea. However, Hosaka (2004) method seems to be more promising than the other method for DNA extraction in cowpea because it gives better and more consistent DNA band.**

**Key words:** Striga resistance, DNA extraction methods, marker assisted selection, cowpea DNA, rapid isolation.

## INTRODUCTION

The application of marker assisted selection in cowpea can accelerate the breeding process for striga resistance. In molecular marker studies, extracting DNA from a large number of plant accessions is difficult in plants that have high levels of polysaccharides and secondary metabolites (Pandey et al., 1996). A simple, rapid and effective DNA extraction method is highly desirable. Several procedures to achieve these goals have been reported (Doyle and Doyle, 1989; Edwards et al., 1991), but most require large amount of plant tissues to be ground in liquid nitrogen. The traditional liquid nitrogen DNA extraction method is expensive and tedious. There is, therefore, the need for faster methods of DNA extraction for efficient application of marker assisted selection (MAS) for breeding for striga resistance in cowpea to be able to handle large number of samples at a time. The objectives of this paper are therefore to identify a fast and reliable as well as reproducible method of DNA extraction in cowpea.

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## MATERIALS AND METHODS

Basically two DNA extraction methods viz: DJ Kim Whatmann paper (unpublished) and Hosaka (2004) DNA extraction methods were tested under different conditions and a final comparison was made. Initially DJ Kim method was tested using full disc paper as shown in Figure 1. Cowpea sample was taken from the line IT06K-5-63 and ordinary Whatmann filter paper (P1) was tested versus glass fibre (GF/C) paper (P2). The same genotype was used for all treatments. Washing of the PCR inhibitors with 70% ethanol was done as $W_0$; no washing at all, $W_{10}$; washing with 70% ethanol and vortexing for 10 min, $W_{20}$; washing with 70% ethanol and vortexing for 20 min, $W_{30}$; washing with 70% ethanol and vortexing for 30 min. The treatments were arranged in factorial combinations as shown in Figure 1.

### DJ Kim Whatmann paper (unpublished) protocol

1. The plant sample (leaf tissue) was crushed on Whatmann paper.
2. A sample disc was taken from the dried spot.
3. The disc was placed in PCR amplification tube.
4. 200 µl of FTA purification Reagent was added to PCR tube.
5. The mixture was Incubated for 5 min at room temperature (the tube was given moderate manual mixing to disrupt the debris and aid in washing).

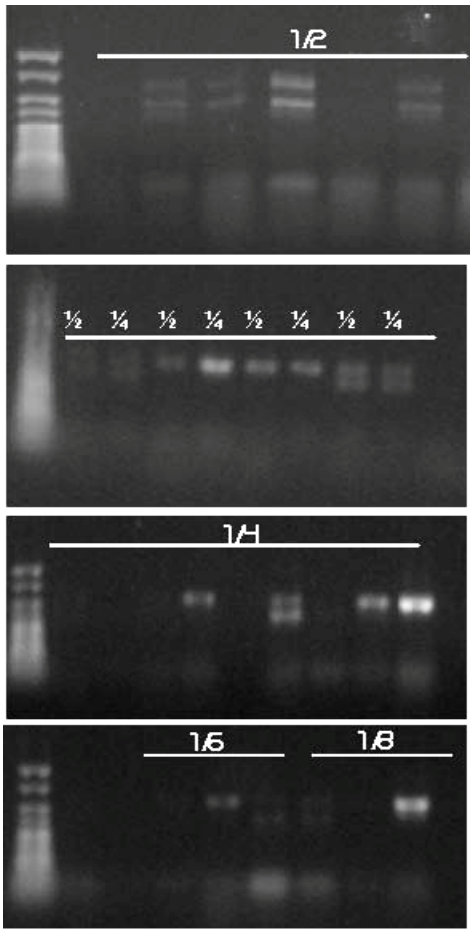


**Figure 1.** Testing of disc size using DJ Kim Whatmann paper DNA extraction method.

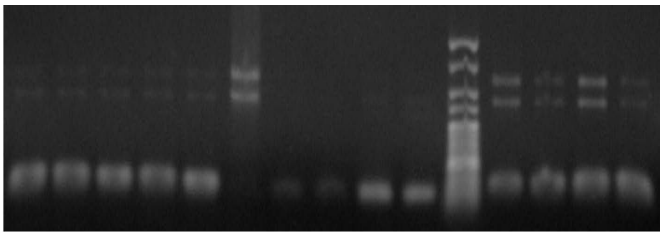

**Figure 2.** Testing of Hosaka extraction potocol (testing 2 4 and 6 leaves and dilutions of 20 and 50 times).

6. All used FTA purification Reagent was removed and discarded using a pipette.
7. 200 µl of TE Buffer (10 mM Tris-HCL, 0.1 mM EDTA, PH 8.0) was added.
8. The mixture was Incubated for 5 min at room temperature.
9. All used TE buffer was removed and discarded with a pipette.
10. Steps 8 - 9 were repeated once for a total of 2 washes with TE buffer.
11. All the liquid was removed before performing analysis. The disc was allowed to dry.

**Hosaka (2004) protocol**

1. A piece of fresh leaf (5 x 5 mm) was placed in a plastic bag.
2. 500 µl of EB was added (100 mM Tris-Cl buffer pH.0, 50 mM EDTA pH 8.0, 500 mM NaCl, 1,25% SDS and 0.2% 2-mercaptoethanol).
3. It was crushed by pressing a pestle or by rotating a bottle over the plastic bag.
4. 100 µl of the solution was collected into a 1.5 ml tube containing 32 µl of PA (5 M potassium acetate) and mix by pipetting up and down several times.
5. The tube was spinned at maximum speed for 10 s. 10 µl of the clear solution was collected into 1.5 ml tube containing 990 µl of sterile water.
6. The sample was used for PCR amplification. All the extraction steps were carried out at room temperature.

## RESULTS AND DISCUSSION

The DJ Kim Whatmann paper (unpublished) method indicated result with the use of half (1/2) disc, quarter disc (1/4), one sixth disc (1/6) and one eight disc (1/8); DNA bands were obtained (Figure 1). The results indicated better DNA bands with half disc and quarter discs, however brighter and better bands were obtained with quarter disc. No bands were obtained with full disc.

The results from Hosaka (2004) quick DNA extraction method indicated that better bands were obtained with normal PCR tubes when loaded in the small gel tank. Clearer bands were obtained with 2 and 6 leaf discs with a final dilution of 20 times, with 190 µl distilled water (Figure 2). Both Hosaka (2004) and DJ Kim Whatmann paper (unpublished) DNA extraction methods do not require dehydration of sample and expensive laboratory materials during the procedure in contrast to other DNA extraction methods proposed by Fulton et al. (1995), and Tai and Tanksley (1990). Furthermore these methods avoid tedious grinding of each sample in liquid nitrogen. Zang and Stewart (2000) proposed a similar extraction protocol that was a drill machine for grinding.

## Conclusion

The experiments indicated that both DJ-Kim (unpublished) and Hosaka (2004) DNA extraction methods can work with cowpea. Repeated experiments with various combinations of treatments should be done to validate the protocols. They appeared to be faster than conventional methods of DNA extraction and can therefore be easily adapted in less advanced laboratories. The critical phase of marker assisted selection (MAS) is the DNA extraction, PCR, and gel electrophoresis stages. If these stages can be optimized and easily done in less advanced laboratories, marker assisted selection can successfully be applied in cowpea breeding programmes in National Agricultural Research Programmes (NARS). In all the experiments done, Hosaka (2004) method for DNA extraction seems to be more

promising than the other method for DNA extraction in cowpea because it gives better and more consistent DNA bands.

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