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# A Simpleand reliable extraction of genomic DNA Formicrosatellite analysis in wheat: an easy approach

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**Abstract**

**For study of molecular biology applications such as microsatellite (SSR), rapid amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP),southern blotting and constructionof genomic and c-DNA libraries in crops are based on high efficiency of purified DNA samples.In this study a simple, rapid, low cost and efficient method for leaf DNA extractionwas optimized for microsatellite analysis in wheat with significant modification in Doyle and Doyle (1987) and Shahzadi and Shah (2010) DNA extraction method.The results showed that appropriate dilution of extracted DNA may be used directly as template DNA in PCR or PCR based techniques. Stored DNA from several months (5-6) has been successfully produced amplification with wheat microsatellite markers that show suitability of this extraction method after long-term storage of DNA.**

**Key word:** Microsatellite (SSR), RAPD, RFLP, AFLP, genemic and c-DNA, PCR

## Introduction

In recent years, molecular markers have been used for a variety of applications including examination of genetic relationships among individuals, mapping of useful genes, marker assisted selections and phylogenetic studies. Among the available molecular markers, microsatellites or SSRsare PCR based marker that are tandem repeats of 2-6bp long DNA motifs, have gained considerable importance in genetics and breeding of plant owing to many desirable genetic attributes including highly polymorphic, multiallelic nature, codominant inheritance, reprod-ucibility, highly abundant and random distribution, extensive genome coverage and chromosome specific location. The variability in SSR length may be demonstrated by polymerase chain reaction with primers designed from the conserved flanking region. In crop species,the successful amplification requiresgood quality and ample amount of isolated template DNA with reproducible result. Many DNA extraction methods are known for isolation ofgenomic DNA from various plant sources including Cetyl Tri-methyl Ammonium Bromide (CTAB) method(Doyle and Doyle**,** 1987) and its modifications (Ahmed *et al.,* 2009 and Huang *et al.,* 2000) were widely used in many laboratoriesbut also have associated limitations, such as these methods are time consuming.

Other conventional methods(Jobes*et al.,* 1995 and Cheng *et al.,* 2003) for DNA extraction requires large quantities of sample to be ground are not efficient because they require long time for plant growth. Some other protocol uses small quantities of sample but with certain limitation like specialized apparatus likethe matrix mill(Hill-Ambroz*et al.,* 2002).Extractions of high quenality DNA from plant materials are considered the most difficult because the key is properly prepare the sample tissue.Some method (Sharma *et al.,* 2002) involves the use of liquid nitrogen flash freezing followed by grinding of sample tissues with a mortar and pestle which is difficult to handle and is not considered to be safe in open laboratory environment such as classroom.Today, manycommercial DNA isolation kit are available, the advantage of DNA kit over crude method, is they are fast, minimal handling,that do not use hazardous reagents such as chloroform or phenol have these ideal properties; howeverwhich have high cost per sample ratio (Kang and Yang, 2004 and Ahmed *et al.,* 2009).We were uniformly unsuccessful in our attempts to amplify wheat genomicDNA by PCR using earlier reported methods (Dellaporta*et al.,* 1985; Doyle and Doyle, 1987; Wang *et al.,* 1993 and Davis *et al.,* 1995).

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Therefore,we found it necessary to devisean efficient plant DNA extraction protocol which use fresh sample is crucial when hundreds of samples need to be analysed rapidly, for many purposes in molecular biology and plant genetics analysis. In the present study, a method used for the simple,relatively quick and inexpensive extractionof genomic DNA from small amounts of plant leaf materialwas modified for the purpose of specially microsatellite analysis in wheat. This modifiedprotocol may be applicable to a variety of plant species, requiring small amount ofleaf sample with optimal amount of DNA and it is fast andcomplete DNA extraction can be achieved within 3-4 h.Samples (leaves) for microsatellite analysis were collected about 0.20g, from germinated 5-7 days old plant and putinto a sterile 1.5 ml microfuge tube.Macerate the leaf tissue for 20-30 s at room temperature, using a sterile tip as grinder, without anybuffer, and then add preheated freshly prepared 300µlof extraction buffer (1M Tris-HCl, 5 M NaCl, .5M EDTA and 20% CTAB) into the tube and mixed for 10 s. Put the tube in water bath at $65^0$C for 30-45 minwith gentle inversion 4-5 times. Subsequently, 250µlof chloroform: isoamyl alcohol (25:1,v/v) was added and centrifuge at 13000 rpm for 15 min.Transfer the supernatant (contains DNA only) in a new microfuge tube to which, an equal volume of absolute ice-cold isopropanol was added, mixed and centrifuge the tube at 13,000 rpm for 10 min. Discarded the supernatant and wash the pellet with 70% (v/v) ethanol.

Finally allow the DNA pellet to dry (approximately 15-20 min) at room temperature, avoid the DNA to over dry or it will be hard to re-dissolve and then dissolved in 50µl TE buffer. Add 2µl RNase A and incubate the tube at 37°C for 30 min.Visualize the DNA on 0.8% agarose gel and use double beam UV-VIS Spectrophotometer (UV 5704SS) to measure the absorbance of isolated DNA of eighteen wheat varieties at 260 nm and 280nm of light.

The purity of extracted DNA was conformed based on A260/A280 ratioaveraged >1.70for all organism, the yield was measured according to theformula (DNA (µg) = A260 x 50 x Dilution factor); prepare appropriate dilutions as per required concentration to be used as template in PCR or for other purposes.The values of absorbance were in the range of 1.83-1.94, indicating the good quality of the isolated DNA without any impurities.Some earlier workers (Lodhi*et al.,* 1994 and Sharma *et al.,* 2002) who noticed that long term storage of DNA does not give reproducible results; a major problem associated it because of freezing and thawing, which is expect to damage the DNA. But with our protocol, even more than 5-6 months old DNA dilution have been successfully amplified with microsatellite primer, showing suitability of this method for long term storage of DNA.Screening of quality of extracted DNA was done successfully through the use of different wheat simple sequence repeat marker like, xgwm, barc, xgdm, wmc and xpsp with this protocol.

This protocol possesses significant modifications of the methods suggested (Doyle and Doyle, 1987 and Shahzadi *et al.,* 2010), including times of centrifugation, amount of sample and concentration of DNA extraction buffer. Unlike all other protocols mentioned above, our protocol is relatively fast, consistent produced sparingly suitable DNA yields from extracted fresh leaf tissue for all wheat varieties assayed. All DNA was stable and may be amplified by PCR, requiring only 0.5 ng DNA in 20 µl reactions, for long term stored DNA. This technique has potential to be aneffective protocol for DNA extraction using both fresh and mature leaf tissue for wheat, rice and perhaps for other crop species in the family of*Gramineae*.By using this protocol, it was possible to isolate DNA and perform PCR-based techniqueon wide range of organism, for many samples efficiently in a short period at minimal cost and in laboratory lacking state-of-the-art equipment's and technology.

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