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Optimization of DNA Extraction for Molecular Marker Studies in Rose (Rosa) an Ornamental Plant

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ABSTRACT

Studies were undertaken for optimization of DNA extraction from 7 different Rose cultivars with different colours (red, yellow, orange, white, yellow and pink, light pink and dark pink).DNA extraction from the rose is a bit difficult because of presence of large amount of polysaccharides, polypenols and other compounds. Presences of these compounds create hindrance in extraction of DNA. So, a simple but efficient protocol is designed to extract high amount of DNA, this is the modified method of CTAB procedure which involves high concentration of NaCl in clean up buffer and CTAB (0.5%), β-mercaptoethanol (0.2%) and EDTA (0.5M) in extraction buffer to remove polypenols and polysaccharides and then use of potassium acetate and cholorform to remove protein and polysaccharides. After that DNA is precipitated in isopropanol and finally DNA is purified by giving RNase treatment and quality of DNA was determined by agarose gel electrophoresis.

Keywords : DNA extraction, Polyphenols, Rose, Polysaccharides.

Introduction

The Rose (Rosa) is the most popular and important ornamental plant in the country. Rose is a diploid plant with chromosome number 2n=14. It belongs to the family Rosaceae. According to the system of Rehder (1940), it is divided into four subgenera: Hulthemia, Platyrhodon, Hesperhodos and Eurosa, in which the first 3 subgenera include only few species. The subgenus Eurosa comprises 10 sections, out of which, Caninae and Cinnamomeae, are the largest and comprise about 50 and 80 species, respectively (Wissemann, 2003). Uses of rose are multifaceted and speard across different aspects like gardening, medicines, cosmetics etc. The germplasm is an important component for efficient and effective management of plant genetic resources Mohapatra et al. (2005). A number of methods for DNA isolation from plants containing high levels of polyphenols and polysaccharides have been developed Aljanabi and Martinez, (1997); Dixit, (1998). DNA isolation from rose species is particularly difficult because of their large amounts of polysaccharides and polyphenols and other compounds. These substances not only decrease the yield but the quality of DNA is almost unusable Jabbarzadeh et al. (2009). High contents of polyphenolics and polysaccharides in plant leaves are problematic during the isolation of high-quality/-quantity intact genomic nucleic acids Karaca et al. (2005); Angeles et al. (2005). Roses contain high levels of polysaccharides, polyphenols and other secondary metabolites Xu et al. (2004). So, a effort was carried out with an approach to design a simple and efficient protocol for DNA extraction from rose.

Materials and Methods

Plant Material: Seven cultivars of rose were used in the present study for analysis of genetic diversity. The different cultivars were obtained from Kurukshetra University, Kurukshetra. Fresh leaf samples from grown plants were taken for DNA extraction.

Table 1: List of rose cultivars that were investigated during the study.

S. No	Rose Cultivar	Petal colour	Flower diameter (cm)	Blooming period
1.	Red rose	Red	3	Feb-early june; Early sept-early dec
2.	Yellow rose	Yellow	2.5	Feb-early june; Early sept-early dec
3.	Orange rose	Orange	2.7	Feb-early june; Early sept-early dec
4.	Yellow and pink rose	Yellow-white Pink-white	3	Feb-early june; Early sept-early dec
5.	White rose	White	3	Feb-early june; Early sept-early dec

6.	Light pink rose	Light pink	2.3	Feb-early june; Early sept-early dec
7.	Dark pink rose	Dark pink	2.7	Feb-early june; Early sept-early dec

DNA Extraction:

DNA of all the seven cultivars of rosa was extracted from young leaf samples using CTAB extraction method of Murray and Thompson (1980), modified by Saghai-Maroof et al. (1984) and Xu et al. (1994). Fresh leaves from plant were taken for the DNA extraction. 5 grams of leaves were taken and powdered with liquid nitrogen using pre-sterilized mortar and pestle. The powder obtained was thoroughly mixed with 10 ml of pre warmed 0.5% CTAB extraction buffer in sterilized centrifuge tubes. The samples were thoroughly mixed with the extraction buffer by inverting the tubes several times gently so as to avoid shearing of DNA and were incubated in water bath at 65°C for 3 hour, for the purpose of standardization of the DNA extraction protocol. Gentle mixing was given in between the incubation time. After incubation in water bath, samples were allowed to cool at room temperature for few minutes. The above mixture was then centrifuged at 8000 rpm for about 10 minutes. Supernatant was taken in another autoclaved centrifuge tube and the debris obtained was discarded. Then equal volume of chloroform: isoamylalcohol mixture (24:1) was added into the supernatant. Mixture was mixed thoroughly by gently shaking the centrifuge tubes for at least 15 minutes and then this mixture was centrifuged at 5000 rpm for about 5 minutes at 4°C. Upper layered mixture was separated and again chloroform: isoamylalcohol mixture (24:1) was added in equal volume. Gentle shaking was given to thoroughly mix the contents and then centrifuged at 5000 rpm for 5 minutes. This washing was done to obtain clear upper phase. The clear upper phase was taken and then equal volume of chilled isopropanol for precipitation of DNA was added and then incubated at -20°C for overnight. On the next day, mixture was centrifuged at 2000 rpm for about 3 minutes. Supernatant was discarded and then the pellet obtained was sterilized by adding 200 µl of 70% ethanol. This solution was centrifuged at 2000 rpm for 2 minutes. The pellet was air dried for at least 2 hour so that complete drying of the pellet takes place, minimum amount of TE buffer was added to preserve the DNA solution at -20°C for further use.

Purification of DNA:

0.1 µl of RNAse (10 mg/ml) was added to 100 µl of DNA extracted. This mixture was gently mixed and then incubated at 37°C for 10 minutes. Then the vials were kept at room temperature for few minutes and stored the DNA at 4°C for further use.

Quality estimation of DNA:

Quality of DNA was determined by agarose gel electrophoresis. It was checked by running DNA samples along with gel loading buffer in 0.8% agarose gel.

Results and Discussion

DNA extraction was improved by modifying some of the steps in the original CTAB DNA isolation protocol (Murray and Thompson 1980). This modified method was found effective as compared to phenol– chloroform method. Present procedure resulted in extracting, high quality, low polysaccharide genomic DNA from all the seven Rose cultivars. DNA isolated from modified method was clear while the latter was partially soluble and formed a gelatinous pellet. DNA was smeared, when agarose gel visualized under U.V light which indicated high levels of protein and polysaccharide impurities in samples isolated from phenol – chloroform method as compared to modified CTAB method. Azeem et al. (2012) extracted DNA from rose leaves at 65°C for 30 min. using 1.5 ml of CTAB to characterized the rose germplasm by random amplified polymorphic DNA (RAPD) markers. The presence of polyphenols which are powerful oxidizing agents present in medicinal plants can reduce the yield and purity by binding covalently with the extracted DNA making it useless for most research applications. Certain polysaccharides are known to inhibit RAPD reactions they distort the results in many analytical applications and therefore lead to wrong interpretations (Kotchoni et al., 2003; Padmalatha 2006). Several modifications were introduced to CTAB method for removal of impurities, combination of high concentration of CTAB (3%w/v) and sodium chloride (3M) in the extraction buffer along with two successive washes with Phenol- chloroform and final precipitation with salt proved very effective. Addition of PVP along with CTAB may bind to polyphenolic compounds by forming a complex with hydrogen bonds and may help in removal of impurities. Several methods on removal of polysaccharides from DNA have been extensively reviewed (Maryam Sarwat et al., 2006) of which salt precipitation has been recommended to be most effective . Salts when in precipitation increase the solubility of polysaccharides in ethanol thus preventing its coprecipitation with DNA. In the present study reprecipitation of dissolved DNA with salt at end of process ensured complete removal of residual polysaccharide in the sample (Fig 1).We found these modified steps necessary to standardize and increase the quality and quantity of genomic DNA. The present study on the development of protocol for isolation of high purity DNA may serve as an efficient tool for further molecular studies.

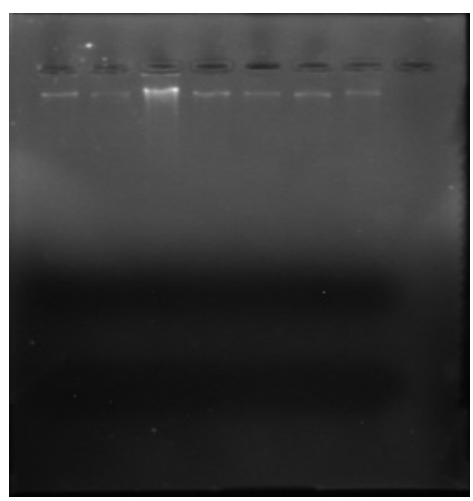

Figure 1: Electrophoretic pattren of DNA samples showing sharp, distinct & clear bands in samples of seven Rose cultivars

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