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Modified CTAB Method for High Quality Genomic DNA Extraction from Medicinal Plants

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From centuries plants are helping the human population by protecting them and their animals from a number of diseases. Plants are enriched with some defending compounds (phytochemicals) e.g., flavonoids, alkaloids, phenols, saponins, glycosides, tannins etc., which have nutritive value and can inhibit disease causing agents (Hassan *et al*., 2007; Sohail *et al*., 2011; Karim *et al*., 2011). These compounds are the metabolic products of plant cells and DNA being the regulatory code plays an important role in their production (Fraser *et al*., 2009). Thus to get huge benefit from these plants, the genetic based phytochemical study is the basic need, which is usually performed through PCR analysis (Park *et al*., 2011). For this the extraction and isolation of plant DNA is first and important step, which can be made through the grinding and cell lyses of plant material (Ahmed *et al*., 2009). After which some buffers, ethers and alcohols are added to maintain the genetic stability and to remove non-DNA compounds. But low quality and quantity of extracted DNA limits the validity of extraction methods. As DNA extraction kits are used to extract large quantities of DNA relatively in short time (3 h), thus more samples can be processed (Ehli *et al*., 2008). But contamination of extracted DNA with kit extraction reagents can induce error in PCR analysis; hence reduce the efficiency of procedure (Mohammadi *et al*., 2005). For DNA extraction other practices DNeasy Plant Mini Kit, Wizard extraction, CTAB (Cetyl Trimethyl Ammonium Bromide) etc. are also used, these methods can provide DNA applicable to PCR studies (Cankar *et al*., 2006). These methods differ in their efficiencies of removing non-DNA substances, where reagents like DNA extraction solution used in these techniques can bring large scale faults in PCR results. Hence to magnify the benefits of medicinal plants, modification in DNA extraction techniques are required. As the efficiencies of these procedures are limited by low quality PCR results derived from extracted DNA.

According to Demeke *et al*. (2009), CTAB extraction method is better than Wizdar extraction and DNeasy Plant Mini Kit, as it produces large quantity of DNA. Moreover CTAB extracted DNA has less ratios of Abs (Absorbance) 260/280 and Abs260/230 indicating the purity of DNA, but this method has need of more modifications. The polyvinylpyrrolidone modified CTAB technique is able to give more DNA yield than miniprep and non-modified CTAB, regardless the age and plant's growth condition (Nazhad and Solouki, 2008). Thus modifications in CTAB may enable it to fulfill the quality and quantity requirements of PRC reactions. Recently Tiwari *et al*. (2012) modified CTAB technique by adding more concentrations of NaCl, Ethylene Diamine Tetra Acetic acid (EDTA) and mercaptoethanol. These modifications enhanced the CTAB extraction and purification activity. As additional quantities of NaCl and mercaptoethanol enhanced the DNA extraction and proteins degradation respectively, while increased concentration of EDTA protected DNA. They also increased the water bathing time and temperature for effective extraction. The researchers inspected the CTAB efficiency in extracted DNA of five medicinally important plant. These plants were Catharanthus roseus, Tridax procumbens, Tinospora cordifolia, Aloe barbadensis and Cissus quadrangularis, belonged to different genera. For all plants CTAB extraction technique was effective and the DNA concentration derived from this technique ranged between 179-833 μg mL^{-1}. High DNA concentration was obtained from nodal stem and meristematic region of *T. cordifolia*, which was 833 and 603.37 μg mL^{-1}, respectively. Whereas minimum concentration (179 μg mL^{-1}) was obtained from *T. procumbens* leaves and *A. barbadensis* nodal stems. Among other extractions of leaves, nodal stems and meristems, significant DNA concentrations were obtained from meristematic regions. This was due to high growth rate (more DNA) and low secondary metabolites of meristems than leaves and stems. Metabolic products lowered the affectivity of extracting agents possibly via reacting with them. Due to this meristematic derived DNAs were also more purified (having less values of Abs260/280) than leaves and nodal stems extractions. However extreme values of DNA purifications were obtained from nodal stems as highly purified DNA (1.93) was from *A. barbadensis,* while least purified (1.26) was from *C. quadrangularis* nodal stem. Thus this modified CTAB protocol was efficient in extracting high quality and quantity of DNA from plants of different genera. The difference in extracts values might be the result of different physiological reactions but this modified CTAB was better than previously done modifications in CTAB

technique. As Dehestani and Kazemi Tabar (2007) modified CTAB by adding polyvinylpyrrolidone and more concentrations of EDTA and mercaptoethanol, this extracted only 100-250 μg DNA per gram of plant tissue. Thus modification in CTAB buffer system and water bathing was more effective in extracting high quality DNA.

Medicinal plants are important part of biotechnology due to their curative phytochemicals. But biotechnological activities are often limited by the poor extraction of plant DNA; it is an important step for genetic studies. Tiwari *et al*. (2012) through his research proved that modified CTAB protocol produced considerable quality and quantity of DNA from plant of distinct genera. They brought some changes in composition of CTAB, which gave 833 μg mL^{-1} DNA with maximum purification of 1.93. Thus this technique is effective in gaining high yield of DNA from number of plants and can help in upgrading the present status of plant biotechnology.

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