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RESEARCH ARTICLE

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High quality nucleic acid (DNA and RNA) extraction from Pea (*Pisum sativum* L.) seeds

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SUMMARY

Nucleic acid isolation is a prerequisite to the study of gene expression at the molecular level and has an increasingly important role in physiological diversity and genetic engineering based investigations in plants. High throughput DNA extraction from seed can significantly increase the efficiency of screening large mapping or mutation in a population. Many standard protocols are available for isolation of nucleic acid from plants but they do not work consistently well in plant tissues that are rich in polysaccharide and phenolics. With key modification in phenol chloroform extraction and precipitation reaction, a simplified, easy and reliable protocol for the isolation of high quality nucleic acids from pea (*Pisum sativum* L.) seeds has been developed. Standardized protocols offer a significant saving in time for the analysis of DNA and RNA.

Key words : *Pisum sativum*, Quality-DNA/RNA, Extraction protocol.

Pea is one of the most intensively studied plant in physiological, biochemical, genetic, molecular biological and breeding experiments. Pea has received attention as an experimental plant largely because it has large flowers, a self pollinating sexual mechanism and a wide array of easily observable seed, seedling and adult plant phenotypes. Several genetic markers have been located on each of its 7 chromosomes. It has been found that in addition to their morphological variation, great variations also exist in the protein content of the seeds in different types. These criteria makes pea a useful plant model for molecular genetic analysis of biosynthetic and developmental processes including organization and regulation of genes and for developing new material for breeding superior varieties of pea and other crop plants (Ramanathan, 1998).

Plant seeds vary dramatically in size and chemical composition with respect to the quantity and complexity of DNA, RNA, starches, lipids and proteins. Seed quality, traceability, hybridity and varietal purity are becoming increasingly important. DNA based methods can be a sensitive and rapid solution to meet these goals. The time required to grow seedlings to perform nucleic acid extractions from leaf tissues (3-7 days) is a significant delay in obtaining results. Standardized protocols for nucleic acid extraction directly from seeds may offer a significant saving in time for gene tests. Therefore, the objective of this research is to develop standardized nucleic acid extraction protocols for pea seeds that can be directly applied in high throughput extraction protocols for their efficiency in yielding high quality DNA/RNA from seeds. RNA isolation is a pre requisite to the study of gene expression at the molecular level and has an increasingly important role in physiological and genetic investigations of plant. However, RNA isolation is difficult in seeds that contain large amount of polysaccharides and polyphenol compounds. Therefore, because of the large amount of polysaccharides and polyphenol compounds, the common protocols for RNA isolation are tedious and usually result in poor yield, hence standardized RNA extraction protocol for pea seed is required. Besides, cells of seed contain high levels of food reserves such as carbohydrates, lipid and proteins, which interfere with extraction of nucleic acid, accounting for both poor yield and quality.

MATERIALS AND METHODS

Pea seeds (*Pisum sativum* L.) purchased from local market of Dehradun, were ground to a fine powder by grinder. Four methods tested for high quality DNA isolation were : CTAB (modified from Keb-Llanes, 2002), CTAB (Chakraborti *et al.*, 2006). SDS (Sadasivam and Manickam, 1996), Rapid method of DNA extraction (Sadasivam and Manickam, 1996). On the basis of results modified Keb-Llanes CTAB protocol was standardized for genomic DNA isolation from pea seeds. 0.3g of pea seeds powder was transfered to 1.5 ml eppendorf tube and then added 300µl EBA, 900µl EBB and 100µl SDS.

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