RESEARCH ARTICLE | *International Journal of Plant Sciences,* (January to June, 2010) Vol. 5 Issue 1 : 233-235

Molecular characterization of transgenic castor and identification of polymorphism between sunflower ray and non-ray florets

K. TULASI RAMAKRISHNAN AND V. SIVAKUMARI

Accepted : November, 2009

SUMMARY

The present investigation was carried out with an objective of the conformation of the transgenic in T_1 progeny of the transgenic plants of castor and sunflower by using PCR technique. A total of 74 plants in the T_1 progeny were tested for transgenic conformation by using PCR technique for the hpt primers. In them 59 showed PCR positive and 15 PCR negative. The presence of polymorphism between these two rays and non-ray florets are of interesting task in the development of hybride varieties. Considering the importance of these issues the present work was conducted using molecular markers (RAPD) for assessment of diversity among the various collections of *Helianthus annuus*.

Key words : Sunflower, Castor, PCR technique and RAPD markers

Castor (*Ricinus communis*) is an important non-edible oilseed crop widely cultivated in tropical, sub-tropical and temperate countries for its high utilitarian value (Atsmon, 1989). Castor seed oil and its derivatives have become important commodities and an increasing number of uses are being found for them in the industrial world. The derivatives are used in a range of sectors including agriculture, the textile industry, plastics engineering, rubber and pharmaceuticals (Vignolo and Naughton, 1991).

Sunflower (*Helianthus annuus*) is an important oilseed crop worldwide. Successful cultivation of the crop is limited by the vulnerability of the crop to several biotic stresses (Morris *et al*., 1983; Sujatha *et al*., 1997). Genetic improvement of any species through genetic engineering techniques requires an efficient *in vitro* regeneration system, which is rapid, reproducible and applicable to a broad range of genotypes (Sujatha and Reddy, 1998). This study reveals that wild *Helianthus* species not only have valuable genes to the improvement of cultivated sunflower but also preserve large genetic variability in them that can be exploited for further improvement of this crop.

MATERIALS AND METHODS

Materials were collected from DOR field, Rajendar Nagar, Hyderabad. The plant material are been young leaves of *Helianthus annuus* and *Ricinus communis* were collected from in and around Thanjavur, Tamilnadu.

Correspondence to:
V. SIVAKUMARI, Department of Environmental and Herbal Sciences, Tamil University, THANJAVUR (T.N.)
Authors' affiliations:
K. TULASI RAMAKRISHAN, Department of Biotechnology, Marudupandiyar College, THANJAVUR (T.N.) INDIA

Total genomic DNA was isolated from young leaves of 60 plants of T_1 progeny derived from PCR positive. To plant and untransformed (control) plants using the CTAB method (Doyle and Doyle, 1987). 100-150mg of leaf sample was taken in an eppendorf and was kept in liquid nitrogen for 30 minutes. The samples were ground to fine powder-using micro pestles followed by addition of 400 µl of extraction buffer and kept at 65°C in water bath. Then equal volumes of chloroform: iso-amyl alcohol (24:1) was added and kept for centrifugation at 8000 rpm for 8 minutes. The supernatant was collected and CI treatment was repeated. To the resultant supernatant 2/3 volume of isopropanol was added and kept at -20°C for 30 minutes. DNA was pelleted at 10,000 rpm for 15 minutes and washed with 70% alcohol, air dried and dissolved in 40-50 µl of $T_{10}E_1$. Quality and size of DNA were checked through agarose gel electrophoresis.

PCR analysis for Ricinus communis:

Genomic DNA extracted from the samples was subjected to PCR analysis for confirmation of hpt gene. The PCR reaction mixture (20 µl) contained 0.48 U Taq DNA polymerase, 10 mM Tris-Hcl (pH 9.0), 50mM KCL, 1.5 mM $MgCl_2$, 0.01% gelatin, 150 µM of each dNTP, 1 µl of each forward and reverse primer at a final concentration of 0.25 µl and 100 ng template DNA. For the positive control, 50 Pg of the Pcambia 1304 DNA was used. DNA from untransformed control and reaction profile included 30 cycle of strand separation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 1 min. The program was extended for 5 min at 72°C. The amplification products were analyzed on 1.4% agarose ethidium bromide gels. Further, to overrule the possibility of nonspecific amplification in PCR-amplified