RESEARCH PAPER International Journal of Agricultural Sciences, Vol. 7 Issue 2 (June, 2011) : 421-423

# Study of parental polymorphism in castor (*Ricinus communis* L.) using SSR and EST SSR markers

**P. VENKATA RAMANA RAO, V. GOURI SHANKAR*, V. RAJESH[1], J.V.P. PAVANI AND A. VISHNUVARDHAN REDDY**

Oilseeds Scheme, Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Palem, MAHABUBNAGAR (A.P.) INDIA

## ABSTRACT

**The present investigation has been undertaken to study the polymorphism between the four diverse castor genotypes (DPC 9, RG-72, Haritha and Kranthi) selected for making three crosses (DPC 9 x RG-72 and DPC 9 x Haritha for tolerance to drought; Kranthi x Haritha for resistance to wilt). The variation among these parents was characterized using 22 SSR and 143 EST SSR markers. Four each of SSR and EST SSR markers showed polymorphism with all the four parental lines. Five microsatellite markers each were polymorphic between RG 72 / DPC 9 and DPC 9 / Haritha (22.72%), and seven markers between Kranthi and Haritha (31.81%). Twenty four EST SSR markers were polymorphic between RG 72 / DPC 9 and Kranthi / Haritha (16.78%). Fourteen markers were polymorphic between DPC 9 and Haritha (9.79%). The results indicating that there is high conservation of coding sequences among the genotypes within the species.**

Rao, P. Venkata Ramana, Shankar, V. Gouri, Rajesh, V. , Pavani, J.V.P. and Reddy, A. Vishnuvardhan (2011). Study of parental polymorphism in castor (*Ricinus communis* L.) using SSR and EST SSR markers. *Internat. J. agric. Sci.*, **7**(2): 421-423.

**Key words :** Castor, Parental polymorphism, SSR, EST SSR markers

## INTRODUCTION

Oilseeds occupy a pride place in Indian economy next to food grains. Castor (*Ricinus communis* L.) plays an important role in the country's vegetable oil economy. India accounts for 59% of global castor area and 81% of world castor production and ranks first in area and production in the world. India meets more than 80% of world's requirement of castor oil and its derivatives and earns foreign exchange of about Rs. 2253 crores annually through its exports. Castor is the third most important oilseed crop of Andhra Pradesh in terms of acreage and economy after Groundnut and Sunflower. In Andhra Pradesh castor is cultivated in an area of 1.57 lakh ha, with a production of 0.80 lakh tones and productivity of 511 kg/ha (2008-09). The major reasons for the dismal state of production in the state are erratic rainfall and poor management practices. Apart from which, biotic and abiotic stresses constitute the major yield destabilizing factors which do not as well realize the full potential of the currently available varieties. Among the biotic constraints, Fusarium wilt and Botrytis grey rot contribute significantly to yield losses. Though wilt resistant varieties were developed and are under cultivation, breakdown of resistance has become a serious concern. Further, clear indications were observed for existence of more than one race, which is common for soil borne pathogens. Among abiotic stresses, drought is very important multidimensional stress affecting factor that acts on the growth and development of plant at various levels of their organization. It is the largest single factor for reduction of yield globally. Castor is cultivated mostly in shallow and less fertile soils during *Kharif* season under rainfed conditions in South India in which intermittent dry spells occur affecting the crop. Even though Castor is a drought hardy crop, evolving crop genotypes which have enhanced drought tolerance is the most successful and the cheapest strategy to cope up with drought. So breeding for drought tolerance is a major objective to maximize yield levels.

Molecular marker technology, among its variety of applications, enables precision in selection/screening at genotype level and in unfolding the hitherto hidden variability of breeding value. However, recent advances in molecular biology have equipped scientists with a wide choice of marker assisted techniques to identify both quantitative and qualitative traits. Among them the marker associated quantitative trait loci (QTLs) relating to quantitatively inherited traits like yield and drought tolerant

*** Author for correspondence.**

[1]Department of Oilseeds, Seed Research and Technology Centre, Acharya N.G. Ranga Agricultural University, Rajendranagar, HYDERABAD (A.P.) INDIA

related traits has become handy to plant breeders to identify germplasm / segregants with positive yield genes. Of the several PCR based markers employed in genetic mapping studies, microsatellite markers, also known as simple sequence repeats (SSR), are genetically more informative, crop and trait specific, co-dominant and robust. An EST is a unique stretch of DNA within a coding region of a gene that is useful for identifying full-length genes and serves as a landmark for mapping and is derived from cDNA. In castor alone, 66,000 ESTs are available in NCBI database. These ESTs can be easily downloaded and EST SSRs can be developed. EST SSRs are highly effective in assessing the genetic diversity existing in the genotypes as well as in genetic mapping studies.

## MATERIALS AND METHODS

Three accessions *viz*., DPC-9 (drought susceptible, pistillate line, green stem, zero bloom and spiny capsules), RG-72 (drought tolerant, germplasm line, red stem, double bloom and spiny capsules) and Haritha (drought tolerant, high yielding variety, green stem, double bloom and spiny capsules) with diverse reaction to drought tolerance were identified based on drought tolerance to make two crosses *viz*., DPC-9 x RG-72 and DPC-9 x Haritha and further to develop mapping population.

Two high yielding varieties *viz*., Kranthi (susceptible to Fusarium wilt, high yielding variety, red stem, double bloom, spiny capsules) and Haritha (resistant to Fusarium wilt, high yielding variety, green stem, double bloom and spiny capsules) released from Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Palem with diverse reaction to wilt were selected to generate mapping population. These genotypes are being used as national checks for wilt susceptibility (Kranthi) and resistance (Haritha). The selected four parents (RG-72, DPC-9, Kranthi, Haritha) were studied for polymorphism using SSR and EST SSR Markers.

Genomic DNA was extracted from young tender leaves from a random sample of five plants from each parent following the standard cTAB method with minor modifications (Doyle and Doyle 1987). The DNA quantification was done by using a Nanodrop spectrophotometer (Thermo Scientific) as well as using known amount Lambda DNA (Bangalore Genei) as standards. PCR optimization for SSR and EST SSR markers was done by varying concentration of template DNA, Taq polymerase, dNTPs, primers and $MgCl_2$.

The amplification reaction with SSR and EST SSR primers was carried out in a final volume of 10 µl in DNA Thermo cycler (Eppendorf Mastercycler Pro S). Each reaction mixture contained 1.0 µl 10 X reaction buffer containing 2.0 mM $MgCl_2$, 1.0 U of Taq DNA polymerase (Bangalore Genei), 0.1 mM dNTP (Bangalore Genei), 10.0 picomoles each of forward and reverse primer (synthesized by Eurofins) and approximately 50 ng/µl of template DNA.

The PCR amplification conditions are as follows: initial denaturation at 94 $^0$C for 4 min followed by 35 cycles of denaturation at 94 $^0$C for 45 sec, primer annealing at 54 $^0$C for 45 sec and elongation at 72 $^0$C for 1 min, followed by final elongation at 72 $^0$C for 5 min. 10 ìl of the amplified PCR product from each reaction was separated on 3.0% agarose gel (Lonza) containing ethidium bromide in 1 x TAE buffer at 130 V, finally visualized and photographed using gel documentation (Syngene Ingenious Bioimaging). In the present investigation, 22 SSR markers and 143 EST SSR markers were used.

## RESULTS AND DISCUSSION

Polymorphism among four diverse parental lines of Castor (RG-72, DPC-9, Haritha and Kranthi) was studied using SSR and EST SSR markers. Out of 22 SSR markers used for screening, four were polymorphic with all the four parents studied (Fig. 1). Each polymorphic primer was tested at least twice to determine if both the polymorphism and banding pattern were reproducible. Five microsatellite markers each were polymorphic between RG 72 / DPC 9 and DPC 9 / Haritha (22.72%) and seven markers between Kranthi and Haritha

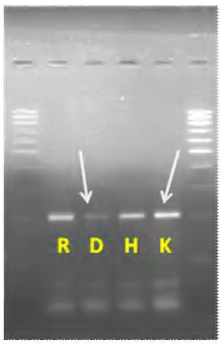


**Fig. 1 : Gel picture showing polymorphism between parents (RG 72 and DPC 9) and (Haritha and Kranthi) with RC SSR 11**

(31.81%).

One hundred and forty three EST SSR markers were used for screening of parental lines. Four markers were polymorphic with all the parents (2.79%) (Fig. 2). Twenty four EST SSR markers were polymorphic between RG 72 and DPC 9 (16.78%) and 14 markers were polymorphic between DPC 9 and Haritha (9.79%). Twenty four markers were polymorphic between parents Kranthi and Haritha (16.78%)

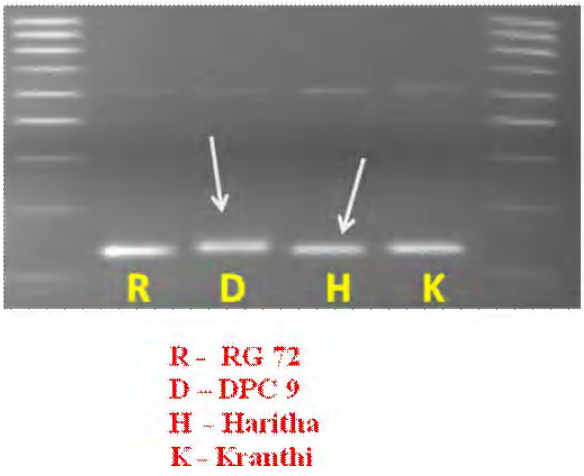


**Fig. 2 : Gel picture showing polymorphism between parents (RG 72 and DPC 9) and (Haritha and Kranthi) with RCE SSR 11**

The level of polymorphism studied using EST SSRs was low which is perhaps due to higher conservation of the coding regions among genotypes with in a species (Eujayl *et al.,* 2002). DNA based markers such as AFLP (Allan *et al.,* 2008) and SSRs (Allan *et al.,* 2008; Bajay 2009) have been reported for the genetic diversity studies in castor. The above study indicates that SSR and EST SSR markers can be used to study parental polymorphism and can also be utilized for genetic diversity analysis, association mapping and mapping studies in Castor.

**Acknowledgement :**

The authors would like to thank Rashtriya Krishi Vikas Yojana (RKVY), Govt. of India, New Delhi for funding under the project entitled "Development of drought, wilt and botrytis resistant castor (Biotechnology approach)".

## REFERENCES

**Allan, G., Williams, A., Rabinowicz, P.D., Chan, A.P., Ravel, J. and Keim, P. (2008).** Worldwide genotyping of castor bean germplasm (*Ricinus communis* L.) using AFLP and SSRs. *Genetic Resource & Crop Evolution,* **55**: 365-378.

**Bajay, M.M., Pinheiro, J.D., Batista, C.E.A., Nobrega, M.B.M. and Zucchi, M.I. (2009).** Development and characterization of microsatellite markers of castor (*Ricinus comminis* L.), an important oleaginous species for biodiesel production. *Conservation Genetics Resources,* **1**(1): 237-239.

**Doyle, J.J. and Doyle, J.L. (1990).** Isolation of plant DNA from fresh tissue. *Focus,* **12**: 13-15.

**Eujayl, I., Sorrells, M. E., Baum, M., Wolters, P. and Powell, W. (2002).** Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. *Theor. Appl. Genet.,* **104**: 339-407.

*Received : March, 2011; Accepted : May, 2011*