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Short Communication

Molecular identification of cytoplasmic male sterility based hybrid GTH 1 and its parents in pigeonpea

Kishan A. Patel*, S. Acharya, Nishant Prajapati and J. B. Patel

Centre of Excellence for Research on Pulses, S. D. Agricultural University, Sardarkrushinagar 385 506 Gujarat

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Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is an important food legume of India that has all pervasive consumers' preference across the country. It is a rich source of protein, minerals and vitamins. Relative success in enhancing productivity of pigeonpea has by and large remained futile and the productivity of pigeonpea has remained static around lesser than one ton per hectare. Recently hybrid technology has given a new ray of hope for breaking yield plateau [1, 2]. Restricted availability of genetically pure seed of the released varieties is the major yield limiting factor. Conventionally genetic purity of seed is ascertained by conducting grow-out-test (GOT) that relies on morphological attributes. Measurement of such attributes is not only time and resources intensive but is enormously sensitive to environmental conditions too.

The first ever CGMS based pigeonpea hybrid GTH 1 (GT 288A x GTR 11) was developed using *Cajanus scarabaeoides* based CGMS system [2]. DNA fingerprinting of the hybrid and its parents can be used for the precise and early identification of the genetic purity of parents and hybrid with least influence of environment. Microsatellites or Simple Sequence Repeats (SSRs) being co-dominant markers that occur at high frequency and appear to be distributed throughout the genome as tandem repeats, seem better choice for genotype identification, seed purity evaluation, variety protection and paternity analysis [7]. Therefore, this study was undertaken to ascertain discrete SSR markers for precise identification of hybrid GTH 1 and its parents.

The world's first ever CGMS based male sterile line GT 288A was developed by transferring the sterile A_2 cytoplasm from *Cajanus scarabaeoides* [3]. The line was maintained through plant to plant mating with its fertile counterpart under insect proof net house. The seed of pigeonpea hybrid GTH 1 was developed by crossing with stable restorer line GTR 11 as male with CGMS based sterile line GT 288 A as female. The fertility restoration was confirmed by growing F_1 crossed seed (CMS GT 288A x GTR 11) by scoring for male fertile plants by visual observation of the pollen as well as the pollen fertility study by KI staining method [4]. This pollen fertility restoration is also confirmed finally by autogamous seed setting under insect proof net house conditions.

The genetically pure ten seeds of each of GT 288 A, GTR 11 and their hybrid GTH 1 were grown in individual pots at the Center of Excellence for Research on Pulses, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India under favourable condition of germination during *kharif* 2010. The juvenile leaf samples were collected from each genotype after 8 to10 days of germination for extraction of genomic DNA.

Using the Cetyl Tri-methyl Ammonium Bromide (CTAB) method with modification [5, 6]. The quality and concentration of extracted DNA was ascertained using Nanodrop spectrophotometer. DNA was diluted in TE buffer for PCR analysis.

[1]Corresponding author's e-mail: kishan.btech@gmail.com
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Forty SSR primers received from International Crop Research Institute for the Semi Arid Tropics (ICRISAT), Hyderabad were used for the study. The volume of the reaction mixture was 20 µl that comprised 1 µl (20 ng) DNA sample, 2 µl of diluted primers (2 pmols/ µl) vial containing both forward and reverse primers, 2 µl 10X *Taq* Buffer A (Tris with 15mM $Mgcl_2$), 2 µl dNTP mix (10 mM), 1.5 µl $Mgcl_2$ (25 mM), 1.5 µl Red *Taq* DNA Polymerase (1u/µl) and 10µl Protease and Nuclease free water.

The temperature profile used for PCR amplification comprised 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 55°C for 45 sec, 72°C for 1.30 min and ending up with 1 min at 72°C for the final extension. PCR products (7.0–7.5 µl) were used for electrophoresis on 2% agarose gels stained with Ethidium Bromide at 100–120 v/cm for nearly 2.5-3.0 hrs and UV trans-illuminated gels were photographed with gel documentation system.

Out of the 40 microsatellite primers, three primers *viz*., CcM0021, CcM0030 and CCB9 were selected for further analysis on account of their palpable polymorphism between the parents (CMS GT 288 A and GTR 11) and hybrid (GTH 1) (Table 1). These markers amplified DNA fragments in the size ranging from 30bp to 300bp with 33.3 to 50 per cent polymorphism. These three primers *viz*., CcM0021, CcM0030 and CCB9 produced three unique bands in parents and hybrid (Fig. 1a to 1c). Out of this one band of size 190bp produced

Table 1. Polymorphisms as revealed by microsatellite markers in parents (GTR 11 & GT 288A) and their hybrid (GTH1) in pigeonpea

S.No.	Primer	Range of amplification (bp)	Percent polymorphism (%)	Size of informative band (bp)	Status of presence of informative band in		
					CMS GT 288 A	GTR 11	GTH 1
1	CcM0021	30-150	50	150	Yes	No	Yes
2	CcM0030	30-190	50	190	No	Yes	Yes
3	CCB9	100-300	33	300	Yes	No	Yes

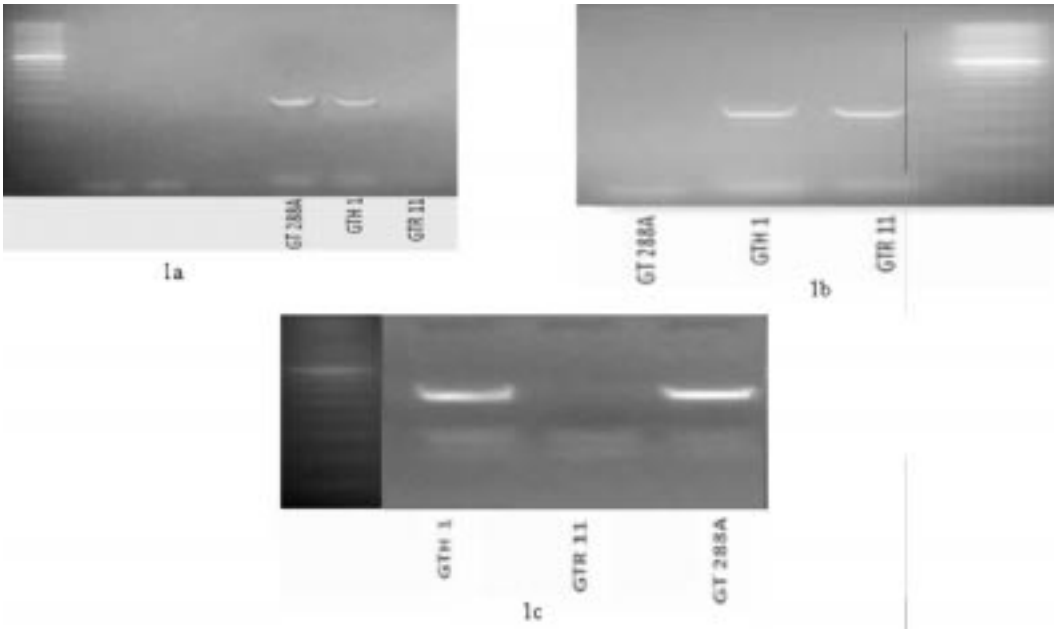

Fig. 1. Polymorphisms as revealed by three microsatellite markers *viz*., CcM0021 (1a), *CcM0030 (1b) and CCB9 (1c) in CGMS based hybrid GTH1 and its parents in pigeonpea*

by CcM0030 was conspicuous from hybrid seed identification point of view (Fig. 1b). This band was not present in female parent GT 288A but was present in male parent GTR 11. The band was conspicuously present in hybrid GTH 1 too that could facilitate in identifying the veracity of the hybrid seed produced on GT 288A with GTR 11. The other two bands of size 150bp and 300bp produced by CcM0021 and CCB9, respectively, though could distinguish male GTR 11 and female GT 288A, yet they were of little importance for identification of hybrid seed of GTH 1 as the band was present both in female GT 288A and hybrid GTH 1 (Fig. 1a & 1c). However, these two bands can be used to distinguish male GTR 11 and female GT 288A parental lines.

Thus, the hybrid seed harvested from female in seed production plot can be distinguished on the basis of 190bp male specific band attributable to CcM0030 microsatellite marker. The high discriminating power of SSR markers and inexpensive setup may be used as an added asset for affordable field based fingerprinting of parents (GT 288 A & GTR 11) and their hybrid GTH 1 for assessing genetic purity. The information of molecular fingerprinting of the CGMS line, restorer line and the hybrid assumes significance both for protecting Plant Breeders' Rights as also ensuring genetic purity of seed in each component line. Therefore, it is concluded that microsatellite marker technology can be a useful tool for ascertaining genetic purity of the pigeonpea hybrid GTH 1 at juvenile stage.

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