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A novel source of CMS in pigeonpea derived from *Cajanus reticulatus*

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Abstract

The present study reports the development of a new CMSsource (A_8) with cytoplasm derived from *Cajanus reticulatus* (Dryander) F. V. Muell var. *grandifolius*, a wild relative of pigeonpea (*Cajanus cajan* (L.) Millsp.). The plants of this male-sterility system do not produce pollen grains and the androecium is underdeveloped. The paper also reports identification of six diverse male-sterility maintainers.

Key words: CMS, *Cajanus reticulatus,* pigeonpea, maintainer

Introduction

Wild relatives of cultivated species generally contain vast genetic variability and there are numerous examples [1], where valuable genes have been transferred from wild relatives to cultivated types through breeding. Pigeonpea (*Cajanus cajan* (L.) Millsp.) has 32 wild relatives with a large genetic variation and some of them have been used to enhance protein content [2], pod borer tolerance [3] and selection of unique traits such as cleistgamous flowers [4], and cytoplasmic-nuclear male-sterility [5].

One of the CMS sources (A_4) derived from *C. cajanifolius* cytoplasm was used to develop the world's first commercial pigeonpea hybrid ICPH 2671 [6]. This hybrid has demonstrated 46% yield advantage over the popular local cultivars in farmers' fields; and it is receiving acceptance from both commercial seed producers and farmers. Considering the vast potential of hybrid technology in breaking the decades old yield plateau and enhancing overall productivity of pigeonpea, it is essential to develop strong and broad-based hybrid parent breeding programmes. In this context, the genetic diversity of both male and female parents is important.

Pigeonpea breeders have so far identified seven different cytoplasm sources [5] which are capable of producing male-sterile genotype in specific combinations. However, at present, the commercial hybrid pigeonpea breeding programme is based on A_2 and A_4 cytoplasm sources. This paper describes the success in breeding a new CMS system using the cytoplasm of *Cajanus reticulatus*, another wild relative of pigeonpea.

Materials and methods

Description of Cajanus reticulatus

Cajanus reticulatus (Dryander) F.V. Muell var. *grandifolius* (= *Atylosiagrandifolia*), is a wild relative of pigeonpea and it occupies place in the secondary gene pool of genus *Cajanus*. It can be crossed manually with cultivated pigeonpea both as female or male parent. This wild species was first collected in Australia by Banks and Solander in 1770 around River Endeavor and subsequently, Mueller in 1883 included it in genus *Cajanus* (http://www.somemagneticislandplants.com.au). This species is endemic to northern Australia at the altitudes ranging up to 1000 m. It is resistant to pod borers [7] and bush fire [8]. *C. reticulatus*, a shrub that usually grows to about 150 cm, is characterized by long pod hairs, shattering of pods, small brown seeds with dark speckles and prominent strophiole. The stem and branches have conspicuous hairs. The flowers with yellow petals are attached to pedicels, about 5 mm in length. Both standard and keel petals are about one centimeter long. The pods are flat, 2-3 cm in length (Table 1), and have a prominent constriction between the two consecutive seeds.

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Selection of male-sterile genotypes

In 2008, open-pollinated seeds of *C. reticulatus* (accession ICPW 176) were acquired from ICRISAT's Gene Bank. Twenty seeds of this accession were scarified with a sharp blade and sown in a Vertisol field at the onset of rainy season in a two-row plot. Only nine seeds germinated. One of the plants in this lot appeared grossly different from the parental accession with large deviations observed in its morphology (Table 1). The author suspected it to have originated through natural out-crossing of *C. reticulatus* with an unknown pigeonpea plant. Like that of cultivated types [9], the wild relatives of pigeonpea are also prone to natural out-crossing [10]. In contrast to *C. reticulatus*, the natural hybrid had semi-spreading growth and its primary branches originated at an acute angle. The floral buds of this plant lacked normal pollen load. For studying pollen sterility, the floral buds were harvested randomly from different parts of the plant and their anthers were squashed on a glass slide and stained with 2% aceto-carmine solution. The observations on pollen viability were recorded under light microscope with 10X magnification. The pollen viability within the plant ranged between 30 and 40% as compared to > 95% in the *C. reticulatus*. The pods on the natural hybrid were longer and seeds bigger than that of ICPW 176. To look into the possibility of developing a new CMS system from this material, two branches of this plant were selfed using muslin cloth bags, and at the same time, it was crossed to three cultivated inbred lines ICP 28, ICPL 87119, and ICPL 20176 as male parent. These crosses (F_1), irrespective of their genotype, were considered as BC_1F_1 material, considering '*Cajanus cajan*' as a recurrent parent.

Table 1. Observations on some morphological traits of the natural F_1 hybrid plantand its parent ICPW 176 (*C. reticulatus)*

Trait	*C. reticulatus* (ICPW 176)	Out-crossed plant
Growth habit	Non-determinate	Non-determinate
Plant spread*	Spreading	Semi-spreading
Branch angle*	Wide	Narrow
Leaflets*	Elliptical	Semi-elliptical
Stipule	Persistent	Persistent
Petals	Deciduous	Deciduous
Pod length*	Small (< 2 cm)	Longer (3-4 cm)
Seeds/pod*	2-3	3-4
Pod beak*	Long & conspicuous	Small
Seed colour	Brown-grey	Brown-grey
Strophiole	Prominent	Prominent
Seed size (g/100)*	< 3g	4 -5 g
Pod hairs*	Long	Small

*Prominent distinguishing traits

The self-seeds harvested from the out-crossed plant were abnormally small and dark which failed to germinate. The pollinations on this plant with ICP 28 and ICPL 87119 as male parent did not produce any pod, while the cross with ICPL 20176 yielded only two seeds from a single pod and it was considered as BC_1F_1. In 2009, these seeds were sown in plastic pots in a glass house and the plants were examined for pollen sterility. The pollen grains of these plants exhibited 50-60% sterility. These partial male-sterile plants were again backcrossed (BC_2F_1) as female parent to a pigeonpea cultivar ICPL 85030 and from 14 pollinations only one pod was harvested that yielded two seeds.

In 2010, the two seeds were sown in a glass house and both the plants exhibited100% male-sterility. For further introgression of *C. cajan* genome, these plants were crossed (to develop BC_3F_1 seeds) with six pigeonpea genotypes of diverse origin (Table 2). In 2011, all the plants in each cross expressed 100% male-sterility. On these plants, a total of 1078 pollinations were made to complete another set of backcrosses (BC_4F_1) and the highest number of seeds (524) was obtained in the cross involving ICPL 85030. All the plants in BC_4F_1 generation were examined for pollen fertility in 2012.

Results and discussion

Pigeonpea is an important source of protein for rural masses of India, Nepal, Myanmar and Southern and Eastern Africa. Since the crop can grow well under a range of soils with low input, it has become an inseparable component of subsistence agriculture. In the last few decades the area under this crop has recorded significant increase, but in spite of numerous breeding efforts, its productivity has remained low at around 750 kg/ha [11]. In recent years, however, the development of hybrid breeding technology has given a hope for a breakthrough in the productivity of pigeonpea [6]. For sustainability of the hybrid technology and to avoid the potential dangers associated with a single cytoplasm, search for alternative male-sterility inducing cytoplasm is essential.

The development of a new CMS system originating naturally in the population of *C. reticulates* adds to the cytoplasmic diversity. The cytological studies conducted in a similar cross (*C. cajan* x *C. reticulatus*) by Reddy *et al*. [12] revealed the presence of some ring and rod shaped bivalents that lead to meotic disorders during meiosis. They further reported that the pollen fertility in the F_1 hybrid plants was low, and among different floral buds it ranged between 26 to 58% with a mean of 42% as compared to >90% in both the parents. In the present study also, almost similar results were obtained as far as pollen fertility of the natural F_1 hybrid plant was concerned.

The pod set in the first backcross generation (natural F_1 hybrid x ICPL 20176) was low (3.3%) and it increased marginally (7.14) in BC_2F_1, when crossed with ICPL85030 indicating continuation of cytological abnormalities in the plants. The third (45.3%) and fourth (42.7%) backcrosses recorded considerable improvement in pod set (Table 2). It was also observed that although the pod set in BC_3F_1 and BC_4F_1 was comparable, the ovule abortion was considerably high in the former with mean seed/pod of 0.28. In BC_4F_1 generation a ten-fold (2.08 seeds/pod) increase in the seed set was recorded. These observations suggested that backcrossing to the cultivated types significantly reduced the cytological disorders that permitted normal growth and development of the ovules. This situation is likely to improve further with additional backcrosses to the cultivated types. The new CMS genotype will be ready for use in breeding hybrids after a few more backcrosses.

Table 2. Pollination success and number of seeds harvested in various backcross generations at Patancheru, 2008-11

Year/Cross	Generation	Pollinations(no.)	Pods(no.)	Success (%)	Seeds (no.)
2008					
Natural hybrid (NH)	F_1	-	-	-	-
NH x ICP 28(C1)	BC_1F_1	30	0	0.0	0
NH x ICPL 87119(C2)	BC_1F_1	30	0	0.0	0
NHx ICPL 20176 (C3)	BC_1F_1	30	1	3.3	2
Total/mean		90	1	1.1	2 (2.0)
2009					
C_3 x ICPL 85030	BC_2F_1	14	1	7.14	2
Total/mean		14	1	7.14	2 (2.0)
2010					
BC_2 F_1 x ICPL 85030	BC_3F_1	43	21	48.8	40
BC_2 F_1 x ICPL 88039	BC_3F_1	80	45	56.3	9
BC_2 F_1 x Vaishali	BC_3F_1	130	63	48.5	15
BC_2 F_1 x ICPL 87119	BC_3F_1	50	28	56.0	3
BC_2 F_1 x ICP 14903	BC_3F_1	200	85	42.5	2
BC_2 F_1 x ICP 7035	BC_3F_1	60	13	21.7	2
Total/mean		563	255	45.3	71 (0.28)
2011					
BC_3 F_1 x ICPL 85030	BC_4F_1	251	214	85.3	524
BC_3 F_1 x ICPL 88039	BC_4F_1	309	55	17.8	90
BC_3 F_1 x Vaishali	BC_4F_1	284	119	42.3	189
BC_3 F_1 x ICPL 87119	BC_4F_1	90	21	23.3	43
BC_3 F_1 x ICP 14903	BC_4F_1	102	37	36.3	71
BC_3 F_1 x ICP 7035	BC_4F_1	42	14	33.3	38
Total/mean		1078	460	42.7	955 (2.08)

NH = Natural F_1hybrid; ( ) = Mean seeds/pod

A close examination of the floral buds of the male-sterile plants showed that unlike other CMS systems, the anthers and filaments of the new male-sterile genotype were not fully developed, while the stigma and style achieved their normal growth. This orientation results in the placement of stigma at much higher level than anthers (Fig. 1). This situation may be advantageous in breeding hybrids because of greater opportunities of insect-aided out-crossing and absolutely no chance of selfing even in the event of a breakdown of the male-sterility under any specific environment. The segregation data in the backcross generations of six crosses (Table 3) showed that the male-sterility trait is fast stabilizing. This male-sterility system has been designated as A_8. In the present study six diverse maintainers ICPL 85030, ICPL 88039, Vaishali, ICPL 87119, ICP 14903, and ICP 7035 were identified and these will provide necessary nuclear variability to the new CMS system. It is believed that the diversity both at the cytoplasmic as well as nuclear levels, generated from this research will strengthen the hybrid pigeonpea breeding programmes. To achieve this, a programme to select the restorers among germplasm has been launched. Besides this, a breeding programme to transfer fertility restorer gene from the cytoplasm donor (*C. reticulatus*) has begun. Soon the pigeonpea breeders would be able to use this new CMS source in hybrid breeding programmes.

Fig. 1. Androecium of normal pigeonpea cultivar (left) and new male-sterile plant (right) derived from *C. reticulatus*

Table 3. Segregation for male-sterility in different backcross generations of six crosses at Patancheru, 2012

Tester	Plants grown			Fertile plants			Sterile plants			Male-sterility (%)		
	BC_2F_1	BC_3F_1	BC_4F_1	BC_2F_1	BC_3F_1	BC_4F_1	BC_2F_1	BC_3F_1	BC_4F_1	BC_2F_1	BC_3F_1	BC_4F_1
ICPL 85030	2	35	173	0	0	0	2	35	173	100	100	100
ICPL 88039	-	9	36	-	0	0	-	9	36	-	100	100
Vaishali	-	13	70	-	0	0	-	13	70	-	100	100
ICPL 87119	-	3	35	-	0	0	-	3	35	-	100	100
ICP 14903	-	2	36	-	0	0	-	2	36	-	100	100
ICP 7035	-	2	36	-	0	0	-	2	36	-	100	100

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