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Fertility restoration studies in short duration redgram (*Cajanus cajan* (L.) mill spp.) hybrids involving cgms system

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Abstract : Fertility restoration is a crucial requirement for successful hybrid synthesis using CGMS system in crops. This investigation was carried out with the objective to explore the extent of fertility restoration for various cytoplasmic sources across different usable male parent sources *viz.*, germplasm lines, advanced breeding lines and cultivars. One hundred and sixty eight CGMS based hybrids were synthesized by adopting L x T mating design with 12 CGMS lines and 14 testers. The hybrids were tested for fertility restoration by observing the pollen fertility status. The results indicated that 19 hybrids were restored out of 168 crosses evaluated accounting to 11.3 %. The extent of restoration varied from 9.5 to 14.3 % across the three cytoplasmic sources *viz.*, A_1, A_2 and A_4. Among the three sources of male parents selected, restoration was maximum in the germplasm inbreds as compared to advanced breeding lines and cultivars indicating need for intensive exploration across genetically and geographically diverse genetic resources. The implications of the results for augmenting hybrid breeding in pigeonpea and possible strategies for isolating new restoration sources through marker assisted selection are discussed.

Key words : Redgram, Cytoplasmic-Genic male sterility, fertility restoration.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Mill spp.) is an important pulse crop of India. It is grown in about 3.5 million hectares with a production of 2.4 million tonnes of grains. During last five decades, area under pigeonpea cultivation has remained static, and the productivity has been hovering around 600-700 kg/ha. On the otherhand, there is an ever growing demand for pigeonpea dhal. Thus, to meet the demand, concerted efforts are needed to boost the pigeonpea production through enhancement of its productivity. Pureline breeding in pigeonpea has not contributed much in recent times and the yield levels of pureline varieties had platued over past four decades. Hence, the productivity could be enhanced only through non-conventional breeding approaches especially the development of hybrid varieties offers enormous scope to achieve this breakthrough. The quantum jump in yield potential observed in some crops like rice, maize, cotton, *etc.*, in the past was primarily due to commercial exploitation of heterosis.

Heterosis, in pulses could not be exploited because of their limited or no outcrossing for hybrid seed production. But during the last decade in pigeonpea, a break through has been achieved in developing hybrid technology. The development of hybrid technology in pigeonpea was initiated with the discovery of two sources of genetic male sterility (GMS)sources from germplasm (Reddy *et al.*, 1978; Saxena *et*

Table1. Characters of CMS lines used

CMS lines	Cytoplasmic source	Plant type	Days to 50% flowering	Anther morphology
ICPA 2067	A_1	DT	70	Yellow, scaly
ICPA 2068	A_1	NDT	77	Yellow, scaly
ICPA 2052	A_2	NDT	73	Yellow ,scaly
GT 288A	A_2	NDT	79	White, translucent
GT 100A	A_2	DT	80	White, translucent
GT 33A	A_2	NDT	85	White, translucent
CRG 990047A	A_2	NDT	77	White, translucent
CRG 990052A	A_2	NDT	75	White, translucent
ICPA 2039	A_4	DT	76	Yellow
ICPA 2089-24	A_4	NDT	78	Pale yellow and scaly
ICPA 2155	A_4	NDT	81	Pale yellow and scaly
ICPA 2156	A_4	NDT	79	Pale yellow and scaly

NDT – Non-Determinate DT- Determinate

Table 2. Male parents used for crossing

Male parents	Source	Plant type	Days to 50% flowering	Flower Colour
CRG 9060	GMI	NDT	75	Yellow
CRG 9919	GMI	NDT	72	Yellow
CRG 9934	GMI	NDT	74	Yellow
CRG 9347	GMI	NDT	72	Red
CRG 9524	GMI	NDT	75	Yellow
CRG 9580	GMI	NDT	72	Yellow
CRG 06-12	ABL	NDT	75	Yellow
CRG 0711	ABL	NDT	75	Yellow
CRG 03-14	ABL	NDT	77	Yellow
CRG 9147	ABL	NDT	78	Yellow
CRG 9142	ABL	NDT	75	Yellow
Co(Rg) 7	CVS	NDT	71	Yellow
Co 5	CVS	NDT	72	Yellow
VBN 3	CVS	NDT	70	Yellow

GMI-Germplasm inbreds ABL- Advanced Breeding Lines CVS- Cultivars

Table 3. Experimental hybrids evaluated and their pollen fertility status.

Name of the cross	Mean pollen fertility (%)	Name of the cross	Mean pollen fertility (%)
ICPA 2067 x CRG 9060	48.6	ICPA 2052 x CRG 06-12	18.9
ICPA 2067 x CRG 9919	56.4	ICPA 2052 x CRG 0711**	91.8
ICPA 2067 x CRG 9934	49.6	ICPA 2052 x CRG 03-14	26.9
ICPA 2067 x CRG 9347	53.2	ICPA 2052 x CRG 9147	61.6
ICPA 2067 x CRG 9524	64.5	ICPA 2052 x CRG 9142	46.5
ICPA 2067 x CRG 9580	45.7	GT 288Ax CRG 9060	14.5
ICPA 2067 x Co(Rg) 7	76.5	GT 288Ax CRG 9919*	2.3
ICPA 2067 x Co 5	80.5	GT 288Ax CRG 9934*	1.5
ICPA 2067 x VBN 3**	93.4	GT 288Ax CRG 9347	15.6
ICPA 2067 x CRG 06-12	26.3	GT 288Ax CRG 9524	12.3
ICPA 2067 x CRG 0711	24.3	GT 288Ax CRG 9580	11.5
ICPA 2067 x CRG 03-14	26.8	GT 288Ax Co(Rg) 7	13.4
ICPA 2067 x CRG 9147	41.6	GT 288Ax Co 5	15.5
ICPA 2067 x CRG 9142	26.5	GT 288Ax VBN 3	16.3
ICPA 2068 x CRG 9060**	94.3	GT 288Ax CRG 06-12	12.5
ICPA 2068 x CRG 9919	25.6	GT 288Ax CRG 0711	16.9
ICPA 2068 x CRG 9934**	93.6	GT 288Ax CRG 03-14*	3.2
ICPA 2068 x CRG 9347	25.6	GT 288 A x CRG 9147	34.2
ICPA 2068 x CRG 9524*	0.0	GT 288 A x CRG 9142	24.8
ICPA 2068 x CRG 9580	24.6	GT 100A x CRG 9060	30.2
ICPA 2068 x Co(Rg) 7	12.3	GT 100A x CRG 9919	25.4
ICPA 2068 x Co 5	36.5	GT 100A x CRG 9934	16.3
ICPA 2068 x VBN 3*	0.0	GT 100A x CRG 9347	15.4
ICPA 2068 x CRG 06-12	26.3	GT 100A x CRG 9524	13.2
ICPA 2068 x CRG 0711**	95.3	GT 100A x CRG 9580**	91.2
ICPA 2068 x CRG 03-14	25.4	GT 100A x Co(Rg) 7	10.3
ICPA 2068 x CRG 9147	23.6	GT 100A x Co 5	15.4
ICPA 2068 x CRG 9142	52.8	GT 100A x VBN 3	16.3
ICPA 2052 x CRG 9060	45.5	GT 100A x CRG 06-12	10.3
ICPA 2052 x CRG 9919	16.4	GT 100A x CRG 0711	16.5
ICPA 2052 x CRG 9934*	3.6	GT 100A x CRG 03-14	16.3
ICPA 2052 x CRG 9347*	1.8	GT 100 A x CRG 9147	35.4
ICPA 2052 x CRG 9524	45.6	GT 100 A x CRG 9142	42.8
ICPA 2052 x CRG 9580**	92.5	GT 33Ax CRG 9060	17.6
ICPA 2052 x Co(Rg) 7	25.6	GT 33Ax CRG 9919	16.5
ICPA 2052 x Co 5	54.8	GT 33Ax CRG 9934	18.6
ICPA 2052 x VBN 3	75.6	GT 33Ax CRG 9347	14.6
GT 33Ax CRG 9524	16.3	ICPA 2039 x CRG 9347	52.4
GT 33Ax CRG 9580	19.5	ICPA 2039 x CRG 9524	25.1
GT 33Ax Co(Rg) 7	16.3	ICPA 2039 x CRG 9580*	2.3

Contd....

Table 3. Contd...

Name of the cross	Mean pollen fertility (%)	Name of the cross	Mean pollen fertility (%)
GT 33Ax Co 5	15.2	ICPA 2039 x Co(Rg) 7	25.6
GT 33Ax VBN 3	20.3	ICPA 2039 x Co 5	24.6
GT 33Ax CRG 06-12	16.8	ICPA 2039 x VBN 3	27.5
GT 33Ax CRG 0711	16.2	ICPA 2039 x CRG 06-12	64.5
GT 33Ax CRG 03-14*	3.2	ICPA 2039 x CRG 0711	17.6
GT 33 A x CRG 9147	35.4	ICPA 2039 x CRG 03-14	15.6
GT 33 A x CRG 9142	42.8	ICPA 2039x CRG 9147	11.7
CRG 990047Ax CRG 9060	16.2	ICPA 2039x CRG 9142	14.5
CRG 990047Ax CRG 9919	11.6	ICPA 2089-24 x CRG 9060	65.4
CRG 990047Ax CRG 9934	12.5	ICPA 2089-24 x CRG 9919	14.3
CRG 990047Ax CRG 9347	13.6	ICPA 2089-24 x CRG 9934	36.5
CRG 990047Ax CRG 9524	12.5	ICPA 2089-24 x CRG 9347	52.6
CRG 990047Ax CRG 9580**	93.6	ICPA 2089-24 x CRG 9524	26.5
CRG 990047Ax Co(Rg) 7	14.5	ICPA 2089-24 x CRG 9580	35.2
CRG 990047Ax Co 5	16.3	ICPA 2089-24 x Co(Rg) 7*	5.9
CRG 990047Ax VBN 3	8.6	ICPA 2089-24 x Co 5	11.2
CRG 990047Ax CRG 06-12	17.5	ICPA 2089-24 x VBN 3	14.5
CRG 990047 A x CRG 0711	24.6	ICPA 2089-24 x CRG 06-12	65.8
CRG 990047 A x CRG 03-14	62.0	ICPA 2089-24 x CRG 0711	16.5
CRG 990047 A x CRG 9147**	94.6	ICPA 2089-24 x CRG 03-14	24.6
CRG 990047 A x CRG 9142**	92.0	ICPA 2089-24 x CRG 9147	12.6
CRG 990052A x CRG 9060	19.5	ICPA 2089-24 x CRG 9142	16.5
CRG 990052A x CRG 9919	20.5	ICPA 2155 x CRG 9060	65.2
CRG 990052A x CRG 9934	12.3	ICPA 2155 x CRG 9919	53.6
CRG 990052A x CRG 9347	13.5	ICPA 2155 x CRG 9934	76.4
CRG 990052A x CRG 9524	16.7	ICPA 2155 x CRG 9347**	92.8
CRG 990052A x CRG 9580	15.4	ICPA 2155 x CRG 9524	65.3
CRG 990052A x Co(Rg) 7	17.2	ICPA 2155 x CRG 9580	42.1
CRG 990052A x Co 5	18.3	ICPA 2155 x Co(Rg) 7	12.4
CRG 990052A x VBN 3	19.5	ICPA 2155 x Co 5	15.8
CRG 990052A x CRG 06-12	14.2	ICPA 2155 x VBN 3**	94.5
CRG 990052 A x CRG 0711	44.8	ICPA 2155 x CRG 06-12	20.3
CRG 990052 A x CRG 03-14	82.0	ICPA 2155 x CRG 0711**	91.5
CRG 990052 A x CRG 9147**	91.7	ICPA 2155 x CRG 03-14*	0.0
CRG 990052 A x CRG 9142**	90.8	ICPA 2155 x CRG 9147	25.6
ICPA 2039 x CRG 9060	56.4	ICPA 2155 x CRG 9142	13.9
ICPA 2039 x CRG 9919**	93.5	ICPA 2156 x CRG 9060	56.4
ICPA 2039 x CRG 9934	37.3	ICPA 2156 x CRG 9919	45.8
ICPA 2156 x CRG 9934*	0.0	ICPA 2156 x VBN 3*	0.0
ICPA 2156 x CRG 9347	12.3	ICPA 2156 x CRG 06-12**	96.4
ICPA 2156 x CRG 9524	15.5	ICPA 2156 x CRG 0711	15.6
ICPA 2156 x CRG 9580	80.5	ICPA 2156 x CRG 03-14**	94.5
ICPA 2156 x Co(Rg) 7*	6.5	ICPA 2156 x CRG 9147	14.5
ICPA 2156 x Co 5**	95.3	ICPA 2156 x CRG 9142	18.8

* Maintained cross combinations, ** Restored cross combinations.

Table 4. Extent of restoration among the A lines investigated

Source	A lines	No. of restored hybrids	Restoration per cent
A_1	ICPA 2067	1	14.3
	ICPA 2068	3	
A_2	ICPA 2052	2	9.5
	GT 288A	Nil	
	GT 100A	1	
	GT 33A	Nil	
	CRG 990047A	3	
	CRG 990052A	2	
A_4	ICPA 2039	1	12.5
	ICPA 2089-24	Nil	
	ICPA 2155	3	
	ICPA 2156	3	

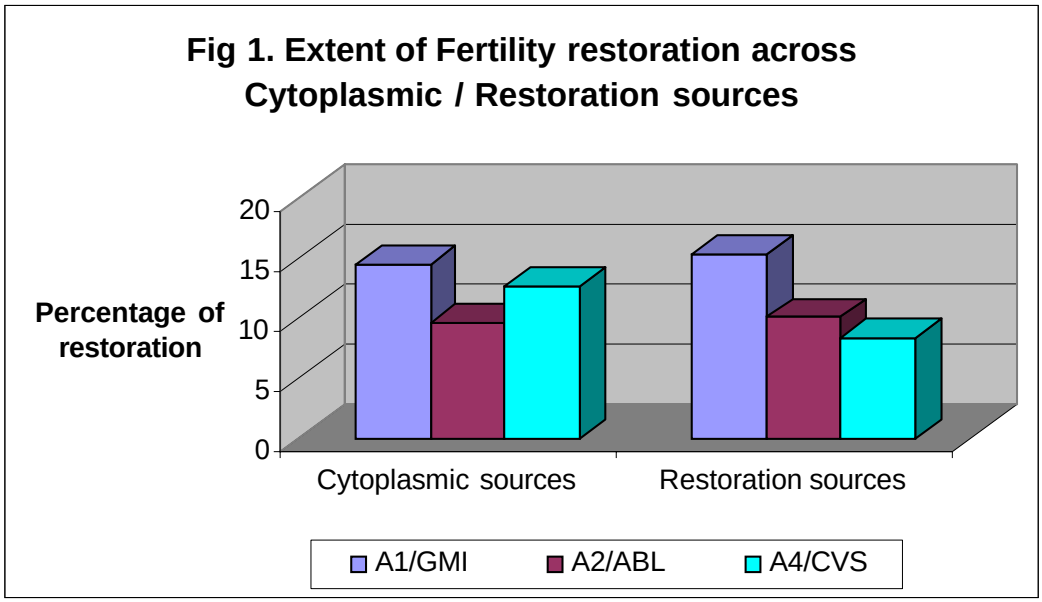
al., 1983). Six GMS based pigeonpea hybrids were released for commercial cultivation during the ninetees by ICRISAT and various SAU's including TNAU. However, the technology suffers from a major technical bottleneck when it comes to large scale seed production. The need for rouging out of 50% of the fertile plants from the female parent by the use of genetic markers was a costly and skill oriented operation which escalated seed cost.

To overcome the seed production problems associated with GMS, new CMS (Cytoplasmic Male Sterile) systems were developed using various wild relatives of pigeonpea. These include A_1 derived from *C. sericeus* (Ariyanayagam *et al*., 1995); A_2 from *C. scarabaeoides* (Saxena and Kumar.2003); A_3 from *C. volubilis* (Wanjari *et al*., 2001) and A_4 from *C. cajanifolius* (Saxena *et al.*, 2005). To augment hybrid breeding programme of TNAU, this investigation was conducted to explore the frequency of restoration available in the germplasm and advance breeding lines for three CGMS (Cytoplasmic- Genetic Male Sterile) sources *viz.*, A_1, A_2 and A_4.

Materials and Methods

The parental materials used in the study are detailed in Tables 1 & 2. The parental lines were chosen to represent all practically usable sources of 'A' lines and 'R' lines. The twelve 'A' lines chosen represented three sources *viz.*, A_1, A_2 and A_4 while the 'R' lines included inbreds from germplasm, advanced breeding lines and released varieties. Crosses were effected in a L x T mating design during *Kharif* 2007. All the 168 F_1 hybrids were raised in non replicated row plots with 20 plants per hybrid adopting a spacing of 60 x 20 cm in Summer 2008. Recommended agronomic practices were followed.

The hybrids were tested for pollen fertility status (Alexander, 1969) at the initial flowering phase on five randomly selected plants for each hybrid. To identify sterility/ fertility of pollen grains in F_1 hybrids, 1% I_2 – KI solution was used. Well developed flowers were collected from each plant at the time of anthesis (9-10 AM). Pollen grains were collected from the flower on a micro slide and mixed with



A1-A_1 cytoplasm GMI-Germplasm Inbreds

A2-A_2 cytoplasm ABL - Advanced Breeding Lines

A4-A_4 cytoplasm CVS- Cultivars

a drop of one per cent potassium iodide stain and examined under a light microscope. Three such microscopic fields were examined for each flower. The round and well stained pollen grains were counted as fertile and shriveled hyaline pollen grains were scored as sterile. The mean for all the microscopic fields were workedout and the proportion of fertile pollens was expressed in percentage on total for individual plants. Based on the number of stained and unstained pollen grains, the fertility status was computed as follows:

$$\text{Pollen fertility (\%)} = \frac{\text{Number of round and stained pollen}}{\text{Total number of pollen grains examined}} \times 100$$

Results and discussion

The cross combinations and their mean pollen fertility status are furnished in Table 3.The mean fertility varied from 0.0 to 96.4 per cent across the hybrids tested. Of the 168 hybrids investigated 19 were found to be completely restored (>90% fertility) accounting to 11.3%, 14 were found to be maintained (<10% fertility) and 135 were partially restored. The low restorability among the hybrids observed in the present investigation is parallel with the observations of Saxena, (2002). At ICRISAT, the observations made with 200 hybrids involving advanced breeding lines and germplasm of diverse origin indicated that the fertility restorers were available in both germplasm as well as advanced breeding lines but their frequency was low and many lines produced hybrids with partial fertility restoration (Saxena, 2004). Hence it

is imperative to make meticulous and continuous exploration to identify suitable restorers for different CMS systems for pigeonpea hybrid development.

Apart from identification of restorers, it is also equally important to diversify the parental lines, especially the male sterile lines, to avoid the problem of monoculture. Experience with T cytoplasm of Maize in the U.S. leading to serious outbreak of Corn Blight (Hooker, 1974 and Levings, 1990) stresses the importance of diversification of cytoplasmic sources. Hence in this study we had chosen to test verify three cytoplasmic sources *viz.,* A_1, A_2 and A_4. Fig .1 indicates the extent of restoration across the three cytoplasmic sources tested. In all the three sources, the frequency of partially restored hybrids was maximum as compared to the restored or maintained hybrids. Similar observations had also been recorded in the earlier studies by Chauhan *et al.* (2004) and Dalvi *et al.* (2008). The proportion of restoration for A_1 cytoplasm was higher (14.3 %) as compared to the other two sources. Such variable restoration among cytoplasmic sources with a same set of male parents had been reported by earlier workers (Saxena, 2003 and Saxena *et al.,* 2005). The frequency of restoration across the individual CMS lines is summarized in Table 4. Among the two CMS lines tested under A_1 source, the line ICPA 2068 registered higher frequency of restoration as compared to ICPA 2067. In the A_2 cytoplasmic source, the CMS line CRG 990047 A was best for restorability with three restored hybrids as compared to CRG 990052 A. The A lines ICPA 2155 and ICPA 2156 were superior for restorability in the A_4 source among the four CMS lines included. Such variable restoration among a common set of male parents within a single cytoplasmic source has also been reported by Dalvi *et al.* (2008) and Nithya (2008).

The R lines included in this study comprised germplasm lines, advanced breeding lines and cultivars. The frequency of restoration among the three groups of R lines is summarized in Fig.1. It is clear that the frequency of restoration is higher in the case of germplasm inbred lines compared to the advanced breeding lines and cultivars. This may be due to the fact that the germplasm lines represent a wider genetic and geographic diversity as against narrow genetic base of cultivars and advanced breeding lines. From this observation it may be concluded that intensive exploration of genetically diverse germplasm could be fruitful for identification of new restoration sources.

From the results of the present study and earlier reports it is obvious that the availability of restoration system in the germplasm and advanced breeding lines is very scarce to develop good heterotic CGMS based hybrids. Development of new restorer strains by conventional breeding requires repeated backcrossing of the restorer lines with the recurrent parent, followed by the selection of the fertile plants, which is extremely laborious and time consuming process. If molecular markers could be employed to tag the restorer (Rf) genes, it would reduce the time required to develop new restorer lines. Through this approach marker assisted development of new isogenic alloplasmic lines and fingerprinting of hybrids will also be possible, as also indicated by Souframanien *et al.* (2003).

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