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Development of new early maturing cytoplasmic genetic male sterile lines in pigeonpea (*Cajanus cajan* (L.) Millspaugh)

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ABSTRACT

Sufficient level of heterosis and out crossing (>70 %) in pigeonpea provides opportunity for the development of hybrid varieties. Earlier, GMS based hybrids were developed by different research institutes. However, due to inherent problem of maintenance of male sterility and high cost of hybrid seed production they could not be popularized. Thereafter work on development of CGMS lines was initiated by GAU, S.K. Nagar and ICRISAT, Hyderabad. The CGMS lines developed by these institutes were of medium to late maturity group and were not suitable for pigeonpea-wheat cropping system under north-western Indian conditions. Thus, a need was felt to develop early maturing CGMS lines to develop early maturing hybrids for this zone. In the first phase, early maturing and stable CGMS lines viz., AL100A, AL102A and AL103A in A_2 cytoplasmic background were developed, and utilized in hybrid breeding. In the second phase, fourteen new male sterile lines were developed in A_2 CMS background. These newly developed lines, were found stable for their cytoplasmic sterility across diverse agro-ecological locations across seasons. These lines can be used to develop early maturing high yielding hybrids suitable for pigeonpea-wheat rotation of north-western India.

Key words: *Cajanus cajan*, Hybrids, Male sterility, Pigeonpea

Pigeonpea is the second most important pulse crop of India after chickpea. It is grown on about 5 million ha globally in more than 50 countries. India accounts for about 80% (4 million ha) of global area (Wanjari and Rathod, 2012). There is considerable increase in area under this crop during recent years but average productivity remained around 7 quintals per ha (http:/faostat:fao.org/site/ 339/ default.aspx). Although major emphasis was given during last four decades on genetic improvement to increase area and productivity of pigeonpea and a large number of varieties have been developed through hybridization and selection (Singh, 2005), although progress has been limited. To have a quantum jump in the productivity, the research focus was shifted to the alternate approach of hybrid breeding. Pigeonpea is the only pulse crop showing very high degree of out crossing from 70% (Saxena, 2001) to 94.5% (Onim, 1981). This rate of out crossing is dependent on many factors like population of insect pollinators, barrier crops, wind velocity, *etc*. (Bhatia *et al*. 1981). A substantial level of natural out crossing provides an opportunity for exploitation of hybrid vigour but it could not be utilized in commercial hybrid development due to non availability of suitable male sterile source. Pigeonpea has substantial amount of non-additive genetic variation (Saxena *et al*. 1981) which can be utilized for heterosis breeding. Commercial utilization of heterosis in pigeonpea was made possible due to the discovery of stable genetic male sterility (Reddy *et al*. 1978) which lead to the development of first GMS based hybrid ICPH 8 in 1991 by ICRISAT. Thereafter, GMS based hybrid PPH 4 was developed and released in 1993 by PAU, Ludhiana (Verma and Sidhu, 1995). Afterwards the hybrids CoH 1 and CoH 2 by TNAU, Coimbatore and hybrids AKPH 4194 and AKPH 2022 by PDKV, Akola were also developed and released respectively in 1997 and 1998 (Wanjari *et al*. 1999). But hybrid seed production with genetic male sterility system invited problems of identification and removal of 50% male fertile plants in male sterile line lead to increased cost of hybrid seed production as it takes more time and labour. In this system there is always a risk of inefficiency of removal of pollen shedders which adversely affect quality of hybrid seed. To overcome this bottleneck, efforts were made to develop cytoplasmic genetic male sterile lines. Reddy and Faris (1981) were the first to develop CGMS lines by using the crossable wild relatives of pigeonpea. The first CGMS line GT 288A was developed using *C. scarabaeoides* at Gujarat Agricultural University (GAU), S.K. Nagar, India (Tikka *et al*. 1997) and consequently several CGMS lines were identified from interspecific crosses involving other wild relatives like *C. volubilis* (Wanjari *et al*. 2001), *C. acutifolius* (Rathnswamy *et al*. 1998; Malikarjuna and Saxena, 2002), *C. cajanifolius* (Saxena *et al*. 2005b) and from *C. cajan* itself (Mallikarjuna and Saxena 2005). At present there are seven CMS systems derived from different *Cajanus* species and designated as A_1 to A_7 (Saxena *et al*. 2010). Out of these, A_2 (*C. scarabaeoides*) and A_4 (*C. cajanifolius*) systems had shown promise because of their stability under various agroclimatic zones and availability of good maintainers and fertility restorers. Based on A_2 (*C. scarabaeoides*) system, Sardarkrushinagar Dantiwada Agricultural University (S.K. Nagar) developed first CGMS based hybrid GTH 1 in 2005 and later ICRISAT developed A_4 (*C. cajanifolius*) based

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hybrid ICPH 2671 which was released in Madhya Pradesh in 2010 (Saxena *et al.* 2010). This A_4 system has been reported to be highly stable under diverse environments (Dalvi *et al.* 2008a).

All of these CMS lines developed in A_2 CMS system were in late maturing genetic backgrounds and produced hybrids of about 150 days maturity which were suitable for central and peninsular India. However, under north-western Indian conditions, pigeonpea hybrids of <140 days maturity are required to fit in wheat-pigeonpea rotation. Hence there is a strong need to develop stable CMS lines in early maturing backgrounds to commercialize hybrids in this zone of India. Therefore, efforts were made for the development of early maturing (135-140 days) CGMS lines in pigeonpea at PAU, Ludhiana for their use to develop hybrids suitable for cultivation in north-western Indian conditions.

The seeds of male sterile line GT 288A (A_2) were procured from Sardarkrushinagar Dantiwada Agricultural University (S.K. Nagar) during 1999. Early maturing pigeonpea lines AL201 (released variety for Punjab state), AL1515 and AL1517 were involved in the crossing programme. The male sterile line GT 288A was crossed to develop F_1 crosses viz., GL288A x AL201, GT288A x AL1515 and GT288A x AL1517 during *Kharif* 1999 at PAU, Ludhiana. The F_1 hybrid plants were sown during rainy season of 2000 and matured anthers of the F_1 plants were observed under acetocarmine. All the F_1 plants were sterile in cross GT288A x AL201 and GT288A x AL1515. The F_1 plants of these two crosses were then backcrossed to respective male parents i.e. AL201 and AL1515 during *kharif season of 2000* and the resultant backcrossed plants in each advancing generation were crossed in subsequent *kharif* seasons to the respective recurrent parents for 6 generations. In each backcross generation the early flowering plants with more resemblance to the recurrent parents were chosen for next generation of crossing. In each backcross generation and the final male sterile BC_6F_1 plants were observed for sterility under microscope by using 2% acetocarmine to ensure male sterility. During 2006 these two lines were maintained in isolation for their seed multiplication by planting Line A and Line B in 2:1 ratio. The male sterile lines were again inspected to ensure 100% male sterility.

During *kharif* 2004, the male sterile line GT288A was also crossed with 10 new lines and F_1 hybrids grown during 2005 were examined for male sterility. The seven cross combinations with early maturing lines viz., PAU881 (AL1507), P2007, AL1518, AL1514, AL1571, IC245352 and IC 245522, showed 100% male sterile plants and were retained for conversion programme, while rest three crosses were discarded which showed segregation. These stable male sterile BC_6F_1 populations were bulked as male sterile lines. Later, during *kharif* 2006, a new male sterile line CGMS 67A was used as source for male sterility transfer to five early maturing lines viz., H2003-29, H04-23, AL1489, AL1520 and AL201 and after 6 backcrosse generations these new stable male sterile lines were designated as AL113A (H 2003-29), AL114A (H 04-23), AL115A (AL 1489), AL116A (AL1520) and AL117A (AL201). In addition, the already developed early maturing male sterile line AL100A (A_2 cytoplasm) was crossed with P 2002-2 and CORG 99041 whereas the male sterile line UPAS 120A (A_2) was crossed with CORG 99041 to develop new male sterile lines i.e. AL118A, AL119A and AL120A.

During *kharif* 1999 about 300 buds of GT 288A line were pollinated with AL201, AL1515 and AL1517 pollen. Pod set was very low and only 15 F_1 seeds were produced in cross I (GT 288A x AL201), 13 seeds in cross II (GT 288A x AL1515) and only 8 seeds were produced in cross III (GT 288A x AL 1517). All the plants in cross I and II were male sterile (Table 3) while in cross III, 5 plants were male fertile and 3 were male sterile, hence this cross was discarded and not carried forward for conversion programme. The plants of cross I and II were back crossed for 6 generations with respective pollen parents. All the BC_6F_1 plants resembled to the respective male parents and were stable for male sterility, hence designated as AL100A and AL102A, respectively. Similarly maintainers were designated as AL100B and AL102B.

During *kharif* 2004 about 100 buds of GT 288A were pollinated with 10 new lines and F_1 seeds produced from these crosses were grown in 2005. Out of 10, all the plants were found sterile in 7 cross combinations viz., GT 288A x PAU881, GT288A x P 2007, GT 288A x AL1518, GT288A x AL1514, GT288A x AL1571, GT288A x IC 245352 and GT 288A x IC 245522. In rest of the 3 cross combinations, about 30-40% plants were male fertile, hence they were discarded. The 7 F_1s were backcrossed to their respective pollen parents for 6 generations to produce BC_6F_1 populations which resembled their respective pollen parents. These stable male sterile lines, thus developed, were designated as AL103A, AL104A, AL105A, AL109A, AL110A, AL111A and AL112A during 2009. Similarly maintainers were designated as AL103B, AL104B, AL105B, AL109B, AL110B, AL111B and AL112B, respectively. During 2006 a new male sterile line CGMS 67A was used as source for male sterility to develop new male sterile lines in the background of new lines namely H2003-29, H04-23, AL1489, AL1520 and AL201. In addition the already developed male sterile line AL100A was used as source to develop new male sterile lines in the background of P 2002-2 and CORG 99041, while the male sterile line UPAS120A was used as source to develop new male sterile line in the background of CORG 99041. The plants of all these crosses were tested to confirm their male sterility. All the hybrid plants were found male sterile and were back crossed to respective pollen parents. After 6 back crosses the stable BC_6F_1 male sterile populations were designated as AL113A, AL114A, AL115A, AL116A, AL 117A, AL118A, AL119A and AL120A during 2012. Similarly their maintainer lines were designated as AL113B, AL114B,

AL115B, AL116B, AL117B, AL118B, AL119B and AL120B. Presently 3 male sterile lines viz., AL100A, AL102A and AL103A are being used extensively in hybrid breeding programme at PAU.

Stability of newly developed CMS lines

During 2006 the seeds of male sterile lines AL100A and AL102A and during 2010 seeds of AL103A were supplied to Regional Stations, Gurdaspur and Faridkot to evaluate them for stability for male sterility. These lines were able to maintain 100% male sterility at both the locations indicated their stability for male sterility across three diverse locations, Ludhiana, Gurdaspur and Faridkot. The three locations represent different agro-ecological zones of Punjab state viz. Ludhiana represents " central agro-ecological zone", Gurdaspur belongs to "sub mountainous." zone, Faridkot lies in "South western" zone of Punjab state (Table 1). At Ludhiana, these lines were evaluated for male sterility in the main season (June-October) as well as during off-season (November-March). These lines had stable expression of sterility at Gurdaspur and Faridkot during rainy season of 2010 (Table 4), indicating that these lines were stable for male sterility across the locations under different growing environments as well as under different temperatures and photoperiods. So, the seed production of these lines and any hybrid in future can be taken up easily at these locations and also in the off-season. Similar findings were reported earlier regarding stability of male sterile lines (A_2 cytoplasm) under different agroclimatic conditions in Tamilnadu (Kalaimagal *et al.* 2008).

Identification of maintainers and restorers for newly developed CMS lines

During *kharif* 2011, 53 F_1 hybrid combinations with cms line AL100A and 49 with cms line AL103A were developed and evaluated during *kharif* 2012 for their fertility/sterility reaction. More than 75% cross combinations were 100% sterile, while two combinations with both lines were found to be 100% fertile. Some cross combinations showed segregation for fertility. These results indicated the availability of large number of maintainers in early background which can be used to develop new male sterile lines, while on the other hand, identification of very less number of restorers warrants the involvement of diverse germplasm from different maturity groups to search some potential restorers.

Morphological description of newly developed CMS lines

Data on some morphological traits recorded during *kharif* 2010 are given in Table 5. The plants of all the three male sterile lines were erect and indeterminate. The flower colour of all the lines was yellow with faint red veins on standard petal. Stems were green in colour. The male sterile lines (A) and their maintainer lines (B) were identical in phenotypic appearance as no major difference was observed between the A and B lines. The anthers of male sterile lines were small, white and sticky with sterile pollen grains. The average plant height of male sterile lines AL100A, AL102A and AL103A was 255 cm, 225 cm and 215 cm, respectively. The seed colour of all the 3 lines was yellowish brown. The seeds per pod were 3.7, 3.6 and 3.7, respectively while 100-seed weight was 7.0g, 7.1g and 6.8g, respectively of AL100A, AL102A and AL103A. All these traits were comparable to their respective maintainer lines. All the 3 lines took 2-3 days more for flowering and maturity compared to their maintainer lines, however, all fell in to early maturity group. The detailed evaluation of the characteristic features of rest of the newly developed male sterile lines will be done over locations and over seasons in the coming seasons besides their stability for male sterility. The best and stable male sterile lines will be used in hybrid breeding programme.

The original CMS lines procured from S.K. Nagar in 1999 were very late and took 160-165 days to mature under Punjab conditions, hence could not be used as such for hybrid development. The newly developed male sterile lines are early in flowering and maturity and the hybrids, thus developed, using these lines will fit well in pigeonpea-wheat

Table 1. Information on location and weather parameters

Location	Latitude	Longitude	Altitude (m)	Rainfall (mm)	Soil type	Soil pH
Ludhiana	30^0 56' N	72^0 52' E	247	500-800	Loamy sand	8.0
Gurdaspur	32^0 02' N	75^0 24' E	254	900	Sandy loam	8.0
Faridkot	30^0 67' N	74^0 75' E	206	400-500	Sandy loam	8.0

Table 2. List of male sterile lines developed through back cross programme

Sr. No.	Name of CMS line	Female parent (cms source)	Male parent (recurrent parent)
1	AL100A	GT 288A	AL201
2	AL102A	GT 288A	AL1515
3	AL103A	GT 288A	AL1507
4	AL104A	GT 288A	P2007
5	AL105A	GT 288A	AL1518
6	AL109A	GT 288A	AL1514
7	AL110A	GT 288A	AL1571
8	AL111A	GT 288A	IC 245352
9	AL112A	GT 288A	IC 245522
10	AL113A	67A	H 2003-29
11	AL114A	67A	H 04-23
12	AL115A	67A	AL1489
13	AL116A	67A	AL1520
14	AL117A	67A	AL201
15	AL118A	AL100A	P2002-2
16	AL119A	AL100A	CORG 99041
17	AL120A	UPAS 120A	CORG 99041

rotation. These newly developed lines are being used extensively to identify good restorers for the development of hybrids with reasonable heterosis. Development and identification of good hybrid will help in increasing area and productivity of the crop in the state and ultimately will increase the income of the Punjab farmers.

Table 3. Fertility and sterility reaction of F_1 hybrid combinations at Ludhiana

Year	Hybrid combination	Number of plants		
		Total	Male Sterile	Male Fertile
2000	GT 288A x AL 201	15	0	15
	GT 288A x AL 1515	13	0	13
	GT 288 x AL 1517	8	5	3
2004	GT 288A x AL 1507	18	0	18
	GT 288A x P2007	14	0	14
	GT 288A x AL 1518	15	0	15
	GT 288A x AL 1514	19	0	19
	GT 288A x AL 1571	16	0	16
	GT 288A x IC 245352	11	0	11
	GT 288A x IC 245522	10	0	10
2006	67A x H 2003-29	13	0	13
	67A x H 04-23	18	0	18
	67A x AL 1489	12	0	12
	67A x AL 1520	14	0	14
	67A x AL 201	16	0	16
	AL 100A x P2002-2	25	0	25
	AL 100A x CORG 99041	20	0	20
	UPAS 120A x CORG 99041	11	0	11

Table 4. Stability of newly developed early maturing *CMS* lines AL100A, AL102A and AL103A over locations and seasons

	Over locations					
	Reg. Res. Station, Gurdaspur			Reg. Res. Station, Faridkot		
No. of plants	**AL100A** (2006)	**AL102A** (2006)	**AL103A** (2010)	**AL100A** (2006)	**AL102A** (2006)	**AL103A** (2010)
Total	46	42	48	44	46	47
Male sterile	46	42	48	44	46	47
Male sterility (%)	100	100	100	100	100	100
	Over seasons (PAU, Ludhiana-2010)					
	Main season (flowering in September)			Off season (flowering in February)		
	AL100A	**AL102A**	**AL103A**	**AL100A**	**AL102A**	**AL103A**
Total	60	62	58	58	60	57
Male sterile	60	62	58	58	60	57
Male sterility (%)	100	100	100	100	100	100

Table 5. Morphological description of pigeonpea male sterile lines and maintainer lines

Trait	AL100A	AL100B	AL102A	AL102B	AL103A	AL103B
Days to flowering	97	95	94	90	95	91
Days to maturity	143	144	140	138	137	134
Plant height (cm)	255	250	225	220	225	215
Plant type	Erect	Erect	Erect	Erect	Erect	Erect
Growth habit	In- determinate	In- determinate	In- determinate	In- determinate	In- determinate	In- determinate
Stem colour	Green	Green	Green	Green	Green	Green
Flower colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Seed colour	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown
Seeds per pod	3.7	3.5	3.6	3.6	3.7	3.5
100-seed weight	7.0	7.3	7.1	7.2	6.8	6.9

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