



Development of CGMS Systems in Pigeonpea with Special Reference to A₂ Source of Cytoplasm

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10.18805/LR-5032

ABSTRACT

Background: Exploitation of hybrid vigour is quite possible in cross-pollinated crops. However, pigeonpea is a grain legume crop with a moderate level of cross-pollination (20-70%), with diploid ($2n = 2x$) chromosome number of 22 and genome size of $1C = 858$ Mbp. Outcrossing mainly aided by insect pollinators. Commercialization of CMS based hybrid is constrained because of the labor intensiveness of seed production and concerns about seed purity. In pigeonpea, two dominant genes (Rf1 and Rf2) have been identified and reported by Saxena *et al.* (2011), which impart fertility restoration to the hybrid plants. The fertility restorer (Rf or Fr) genes in the nucleus suppress the male-sterile phenotype and allow the production of high yielding CGMS-based hybrids. Cytoplasmic male-sterile would effectively circumvent these constraints and revolutionize the hybrid seed industry. CGMS based hybrid breeding can be found more reliable to give productive hybrids in pigeonpea but need some expansion.

Methods: In the present study, the objective was to develop cytoplasmic-genetic male-sterile (CGMS) lines in pigeonpea through wide hybridization involving conventional backcrossing. Five CGMS lines viz. BDN 2004-1A, BDN 2004-2A, BDN 2004-3A, BDN 2004-4A and BSMR 736A were developed from *Cajanus scarabaeoides* cytoplasmic background. This paper discusses development of A and B lines by using A₂ CMS systems available for pigeonpea.

Result: Our investigations on Development of CGMS systems in Pigeonpea with special reference to A₂ source of cytoplasm have allowed us to diverse the male sterile lines based on *Cajanus scarabaeoides* (A₂) cytoplasm in pigeonpea which can be used as marker for easy identification, however characterization of these mentioned five CGMS lines will also help in the predicting the performance of progenies in the different breeding programmes.

Key words: CGMS system, Maintainer lines, Pigeonpea, Sterile lines.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] $2n = 2x = 22$, is an important drought tolerant pulse crop of India and one of the important grain legumes for semi-arid tropics, also called as poor man's meat due to its high protein content. It is an often cross pollinated (20-70%) crop (Saxena *et al.*, 1990). Among all the pulses, pigeonpea is the fourth important pulse crop in the world and second important pulse crop in India after chickpea. Pigeonpea seeds are the primary source of protein for the vegetarian population (Saxena, 2006). Although, out crossing is an essential trait in hybrid breeding technology, it is not desirable in maintaining the genetic purity of cultivars in pigeonpea breeding as it creates variation, which may lead to reduced yields and elimination of disease resistance genes. To overcome this problem, cytoplasmic male sterility (CMS) system was developed (Saxena *et al.*, 2010). As a first step, hybrids based on genetic male sterility (GMS) were developed in pigeonpea (Saxena *et al.*, 2010) but the disadvantages of genetic male sterility (GMS) based hybrids are labour-intensive seed production and seed purity. As rouging of 50 per cent fertile plants from the female rows resulted in loss of population and low hybrid seed yield, hence commercially not successful. The availability of male sterility system, exhibiting large variation in natural out crossing with precise selection of pollen fertility restorers recognized as an important tool for genetic improvement of

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How to cite this article: Patil, D.K., Gite, V.K., Girnare, V.B. and Abubakkar, A.J.S. (2025). Development of CGMS Systems in Pigeonpea with Special Reference to A₂ Source of Cytoplasm. *Legume Research*. **48(11)**: 1829-1834. doi: 10.18805/LR-5032.

Submitted: 30-08-2022 **Accepted:** 07-02-2024 **Online:** 27-09-2024

yield and may serve as a major fruitful technique to break existing yield barriers through heterosis breeding (Saxena and Sharma, 1990). Moreover, the CMS system is indispensable for pigeonpea, as its productivity was stagnant at 700 kg/ha for decades in spite of more than 50 varietal releases (Malikarjuna *et al.*, 2012). Tikka *et al.* (1997) developed, the first cytoplasmic genetic male sterile (CGMS) line, GT-288A using *Cajanus scarabaeoides* (A₂) cytoplasm. CGMS-based hybrids SKNPH-10 (GTH-1) and ICPH-2671 (Pushkal) were released for cultivation in Gujarat and Madhya Pradesh, respectively (Saxena *et al.*, 2013). In order

to meet the ever-growing demand for pigeonpea, So far, eight CMS systems have been reported in pigeonpea (Mallikarjuna *et al.*, 2012; Saxena, 2013). The first report on the development of CMS from *C. scarabaeoides* was by Ariyanayagam *et al.* (1993) however, Tikka *et al.* (1997) and Saxena and Kumar (2003) reported the development of CMS lines by combining the cytoplasm of *Cajanus scarabaeoides* with the genome of a cultivated type. In an F₂ population, male sterile segregants were recovered by Tikka *et al.* (1997) and subsequently, a perfect male sterility maintainer line, ICPL 288, was also identified. This led to the demise of the hybrid breeding program based on A₂ cytoplasm. In the present investigation, report has been made for the development of a CGMS system derived from basic CGMS line GT 288A as a source of diversification with *Cajanus scarabaeoides* cytoplasmic background.

MATERIALS AND METHODS

Location of experiment

The present research work was carried out at research field of Agricultural Research Station, Badnapur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra State (India). It has a black soil with considerable variation in texture and depth with average annual rainfall ranges from 650 to 750 mm. with latitude 19.86°N, longitude 75.70°E and latitude 586.65 m.

Observation recorded

The observations were recorded for nineteen (qualitative and quantitative) characters *viz.* Sterility %, fertility restoration (%), anther colour, flower colour, petal streak, keel structure, pod streak, seed color, growth habit, branching pattern, days to 50% flowering, days to maturity, plant height (cm), no. of primary branches, number of secondary branches, number of pods per plant, seed per pod, 100 seed weight (g) and yield per plant (g). Five randomly selected plants were used for recording the observations.

Diversification programme

In the diversification process, CMS line GT 288A having source of A₂ cytoplasm was crossed with five well adapted and agronomically superior lines, *viz.* BDN 2004-1, BDN 2004-2, BDN 2004-3, BDN 2004-4 and BSMR 736 during kharif season, 2012-13. Sterile plants were identified in F₁ generations of each cross during 2012-13 based on anther color, pollen fertility and anther dehiscence. Sterile plants with translucent anthers were selected for further backcrossing. For testing male sterility, anthers were taken randomly from 3 to 5 flowers from each plant at 50% flowering and crushed on slide with 1% acetocarmine stain and examined under simple microscope. Completely stained (deep blue colored) pollen grains were considered as fertile while, unstained pollen grains and anthers devoid of pollen grains were considered as sterile. In BC₁F₁,

generation sterile plants similar to recurrent parents were selected and sib mated with recurrent parents during backcrossing. In the similar way sterile plants were identified and backcrossed with recurrent parents upto the six generation (BC₆) and remaining plants were discarded. The maintenance breeding was carried out along with the observations recorded up to sixth generation for development of perfect male sterile lines with their respective maintainer. Different marker character wise plants were selected at the advent of flowering in each line during backcrossing. These five lines and their resulting maintainer are sown at Agricultural Research Station, Badnapur in pair row fashion. Schematic diagram of backcrossing programme for diversification of CGMS lines used in the research plan is given in Fig 1.

Characterization of CMS lines

Characterizations of these new CMS lines was done from BC₃F₁ generation after getting complete sterility and homogeneity between A and B-line of respective lines. Observations were recorded on sterility, seed color, plant growth habitat, flower color, days to 50% flowering, plant height and number of primary branches. Evaluation of yield and yield contributing traits was undertaken in the kharif season 2015-16. Observations were recorded in B-lines of respective CGMS lines on days to maturity, pods per plant, grains per pod, 100 seed weight (g) and grain yield per plant (g).

RESULTS AND DISCUSSION

Diversification of CMS

Cytoplasmic nuclear-male sterility (CGMS) was maternally inherited and was known to be associated with specific (mitochondrial) genes without otherwise affecting the plant (Budar and Pelletier, 2001). The fertility restorer (Rf or Fr) genes in the nucleus suppress the male-sterile phenotype and allows commercial exploitation of the CGMS system for the production of hybrid seeds. In the present study, source of cytoplasmic male sterile was used from GT 288A CMS line which was developed from A₂ cytoplasmic (*C. scarabaeoides*) source. Line BDN 2004-1A had a white flower mutant and white seeded derived from GT 288A × (White flower mutant of BSMR 853), BDN 2004-2A had red flower, white bold seed, long podded variety from crossing GT 288A × (BSMR 853 × ICP 9145), BDN 2004-3A obtained from GT 288A × (Selection from BPG 111) showed traits like early maturity, white seed and yellow flower however, the line BDN 2004-4A produced from GT 288A × (Open flower mutant of BDN 2003-2) had open flower and obcordate leaf mutant, the best line BSMR 736A had high yielding and Well adapted, red seed, yellow flower, green pods variety which was obtained from the cross GT 288A × BSMR 736, All this sterile lines were produced by Backcross method. The pedigree and special features of CGMS lines are depicted in Table 1.

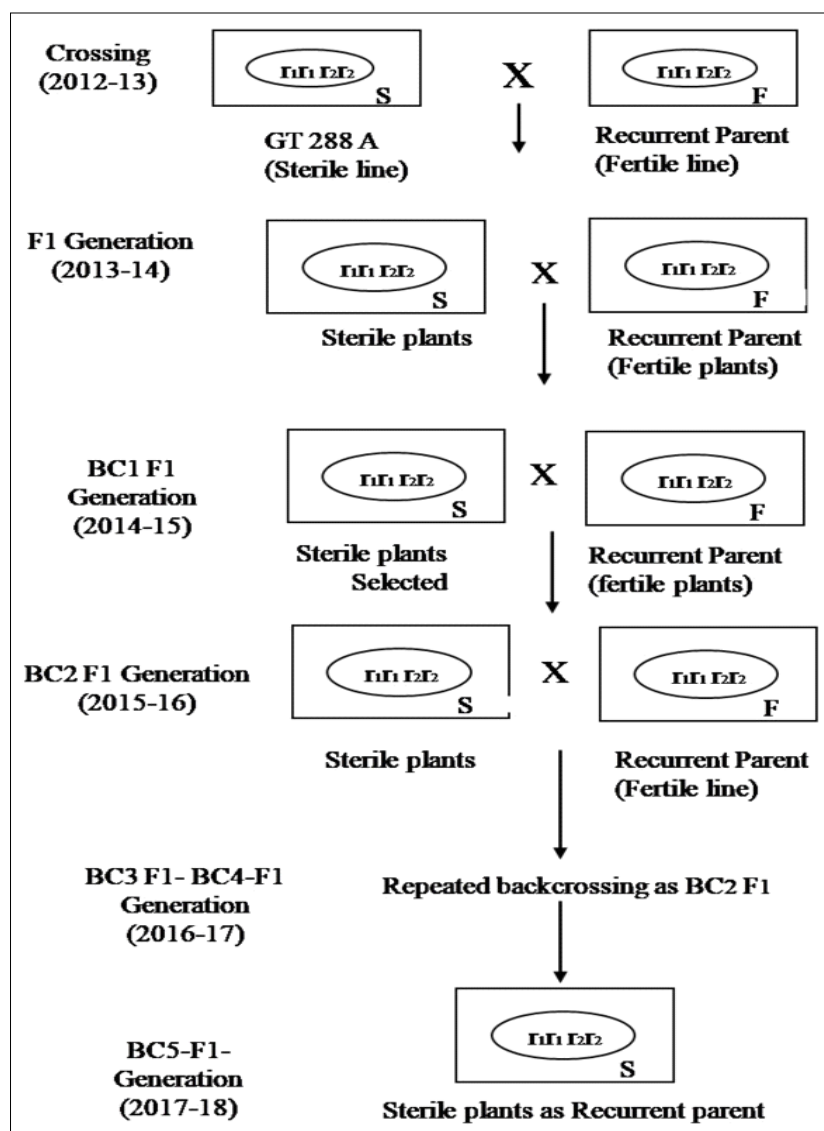


Fig 1: Schematic of crossing programme used for diversification of CMS lines.

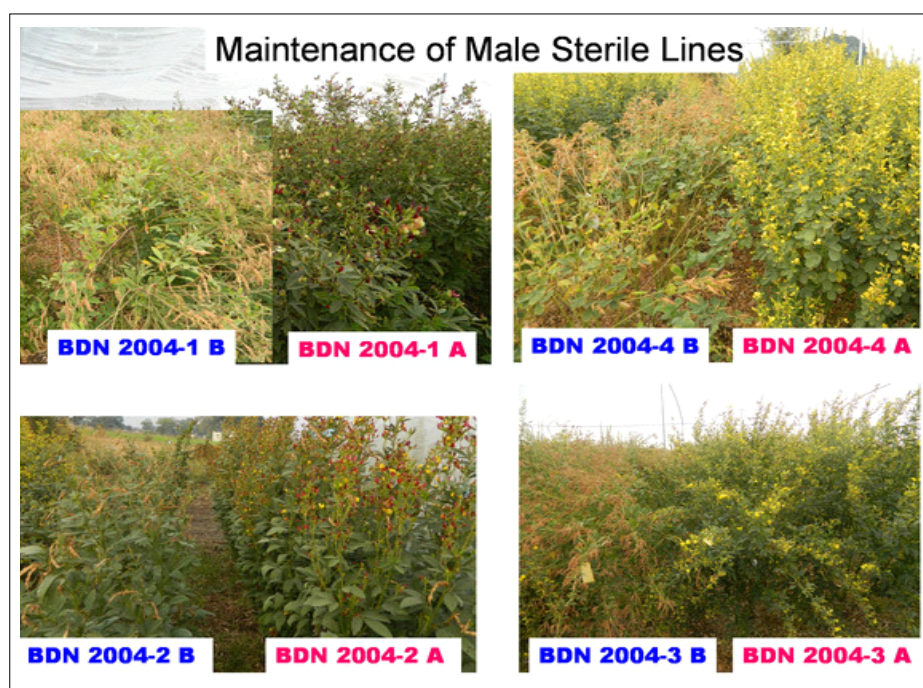
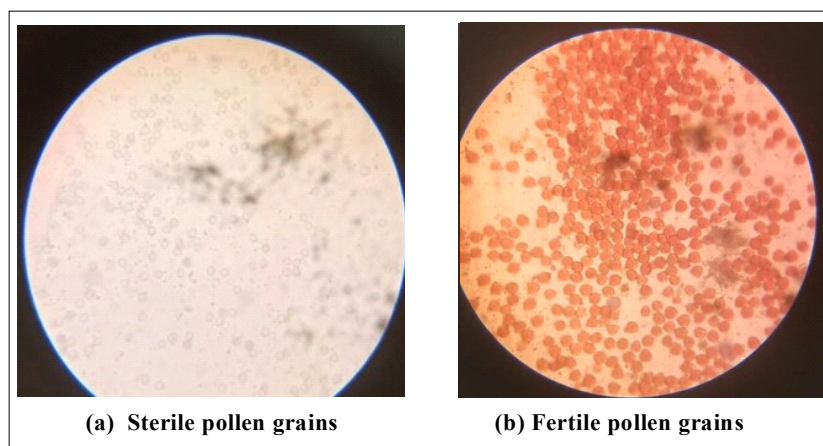
Descriptions of morphological traits

Characterization of newly developed breeding lines is important that will help breeders to select the desirable parents and to predict the performance of the progenies in the different breeding programmes. In the present study, new CGMS lines observed for various morphological characters and descriptions are given in the Table 2 and Fig 2. There is a difference in the anther color of five CGMS lines and their respective maintainer lines. All CGMS lines exhibited white translucent anthers which are characterized by absence of pollen grains. Such traits were closely associated with complete male sterility against shriveled anthers. Similar results had been reported by Gadekar *et al.* (2017), however, B-lines revealed yellow anthers. Pollen grains were absent in the translucent anther (Fig 3a-b)

and present in ample amount in yellow anthers (Fig 3c-d). None of the line exhibited pigmented streak on the standard petals of flower whereas, different flower colour were exhibited by all the lines. The line BDN 2004-1A showed white color flower, outer side of standard is red and inner side of standard is white colour while red color flower with outer side of standard is red and inner side of standard is yellow was reported in BDN 2004-2A and three lines *viz.* BDN 2004-3A, BDN 2004-4A and BSMR736 A indicated yellow flower colour. In Pigeonpea keel petals of the flowers are mostly closed in the present cultivars, such closed structure also found in all CGMS lines that enhance the self pollination except the line BDN 2004-4A having open keel structure with obcordifoliate leaf. Open keels or reduced keel of flower may enhance the cross pollination

Table 1: Features of CMS line.

Cytoplasm source	CGMS line	Selection/Pedigree	Special features/Morphological traits
<i>Cajanus scarabaeoides</i> (A ₂)	GT 288A	<i>C. scarabaeoides</i> × GT 288	Basic CGMS line used for diversification
<i>Cajanus scarabaeoides</i> (A ₂)	BDN 2004-1A	GT 288A × (White flower mutant of BSMR 853) Backcross method	White flower mutant, white seed
<i>Cajanus scarabaeoides</i> (A ₂)	BDN 2004-2A	GT 288A × (BSMR 853 × ICP 9145) Backcross Method	Red flower, white bold seed, long pod
<i>Cajanus scarabaeoides</i> (A ₂)	BDN 2004-3A	GT 288A × (Selection from BPG 111) Backcross method	Early maturing variety (BDN 711), white seed, yellow flower
<i>Cajanus scarabaeoides</i> (A ₂)	BDN 2004-4A	GT 288A × (Open flower mutant of BDN 2003-2) Backcross method	Open flower and obcordate leaf mutant
<i>Cajanus scarabaeoides</i> (A ₂)	BSMR 736A	GT 288A × (BSMR 736) Backcross method	High yielding and well adapted variety, red seed, yellow flower, green pods

**Fig 2:** Diverse male sterile lines based on *Cajanus scarabaeoides* (A₂) cytoplasm in pigeonpea.**Fig 3 (a and b):** Differences of pollen grain in 1% per cent acetocarmine staining.

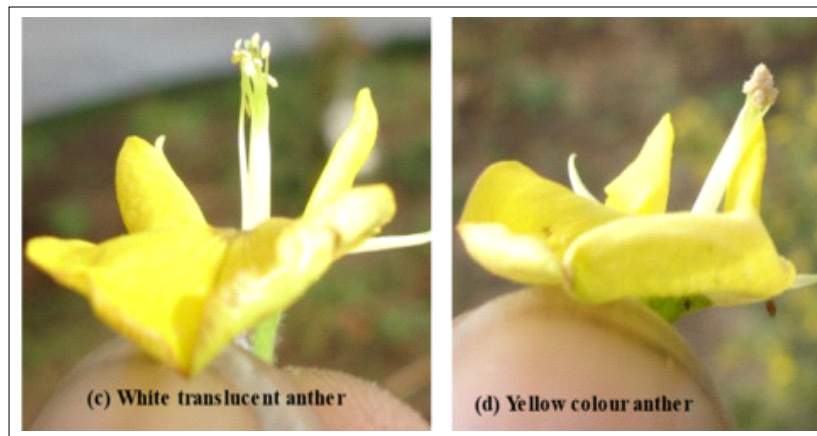


Fig 3 (c and d): Differences among colour of anthers in flower.

Table 2: Morphological characters and description of different CMS lines.

Characters	BDN 2004-1		BDN 2004-2		BDN 2004-3		BDN 2004-4		BSMR 736	
	A line	B line	A line	B line	A line	B line	A line	B line	A line	B line
Sterility %	100	0	100	0	100	0	100	0	100	0
Fertility restoration (%)	0	100	0	100	0	100	0	100	0	100
Anther colour	White translucent	Yellow	White translucent	Yellow	White translucent	Yellow	White translucent	Yellow	White translucent	Yellow
Flower colour	White	White	Red	Red	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Petal streak	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Keel structure	Closed	Closed	Closed	Closed	Closed	Closed	Open	Open	Closed	Closed
Pod streak	Present	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent
Seed color	White	White	White	White	White	White	White	White	Red	Red
Growth habit	IDT	IDT	IDT	IDT	IDT	IDT	IDT	IDT	IDT	IDT
Branching pattern	Semi spreading	Semi spreading	Semi erect	Semi erect	Semi spreading	Semi spreading	Semi spreading	Semi spreading	Spreading	Spreading
Days to 50% flowering	-	115	-	112	-	109	-	117	-	119
Days to maturity	-	169	-	164	-	156	-	175	-	179
Plant height (cm)	-	183.6	-	219.4	-	209.3	-	177.8	-	226.5
No. of primary branches	-	11.6	-	7.8	-	12.6	-	10.2	-	14.2
No. of secondary branches	-	22.4	-	12.4	-	20.8	-	17.8	-	25.4
No. of pods/plant	-	208.6	-	219.2	-	218.00	-	217.1	-	259.4
Seed/pod	-	4.0	-	5.1	-	4.1	-	4.2	-	4.4
100 seed wt.(g)	-	10.4	-	14.9	-	11.0	-	10.4	-	10.1
Yield/plant (g)	-	50.00	-	45.00	-	60-65	-	35-40	-	65-70

for maximum seed setting under open pollination. The male sterile line showed pigmentation on pod i.e black streaks pigmentation on BDN 2004-1A and BDN 2004-2A and brown streaks pigmentation on BDN 2004-3A while green pigmentation on pod was reported in CGMS line BDN 2004-4A and BSMR 736A. In the characterization, lines were distinct from each other majorly for height and for many other traits. Characterization of new lines will help in the predicting the performance of progenies in the different breeding programme. Pigeonpea shows continuous variation for maturity, which ranged from <90 to >250 days. The

performance for days to 50% flowering was ranged from minimum 109 days to maximum 119 days. Vales *et al.* (2012) reported pigeonpea genotypes mature in <90 days at ICRISAT. Among all five CGMS line BDN 2004-3A was promising one. The BDN 2004-3A, male sterile line is maturing in 150-155 days and high yielding which can be used for developing stable, early and high yielding hybrids in pigeonpea. The super early types are useful in diversifying pigeonpea cultivation in the areas characterized by a short growing season. Days to maturity observed for earliness in 155 days to late 179 days. Each line had

characteristic height, minimum 177.8 cm (BDN 2004-4) with semi spreading branching pattern and indeterminate growth habit to maximum 226.5 cm (BSMR 736). The bold seed size (seed weight 14.9 g) was reported in line BDN 2004-2B while BSMR 736B had small seed size (seed weight 10.1 g) among the male sterile lines evaluated, for the trait seed yield per plant, the performance was ranged from BDN 2004-4 (35-40 g) to BSMR 736 (65-70 g). However, while observing the yield parameters, highest pod bearing (259.4) and yield per plant (65-70 g) was exhibited by CGMS BSMR 736A. While line BDN 2004-3A had average yield per plant ranged from 60-65 gm/plant. This line will be best suited in high density planting with comparable yield. Further, it was suggested to verify the performance of these male sterile lines over the season and over the location for the development of more heterotic, widely adaptable and stable high yielding hybrids for commercial exploitation in pigeonpea. Data is presented in Table 2.

CONCLUSION

Diverse male sterile lines based on *Cajanus scarabaeoides* (A₂) cytoplasm in Pigeonpea can be used as marker for identification of CGMS lines, however characterization of new evaluated CGMS lines will also help in the predicting the performance of progenies in the different breeding programmes. The female parent BDN 2004-3A was earlier to flower and maturity and can be utilized for breeding early duration pigeonpea hybrids.

Conflict of interest

All authors declare that they have no conflicts of interest.

REFERENCES

- Ariyanayagam, R.P., Rao, A.N. and Zaveri, P.P. (1993). Gene-cytoplasmic male-sterility in pigeonpea. *Int. Pigeonpea News*. **18**: 7-11.
- Budar, F. and Georges, P. (2001). Male sterility in plants: Occurrence, determination, significance and use. *Sciences de la vie*. **324(6)**: 543-550.
- Gadekar, M., Meshram, M., Patil, A. and Nandanwar, R. (2017). Exploitation of the leaf variants in CGMS system derived from A₂ cytoplasm in pigeonpea [*Cajanus cajan* (L.) Millsp.]. *International Journal of Current Microbiology and Applied Science*. **6(11)**: 483-493.
- Mallikarjuna, N., Jadhav, D.R., Saxena, K.B., Srivastava, R.K. (2012). Cytoplasmic male sterile systems in pigeonpea with special reference to A7 CMS. *Electron J. Plant Breed*. **3**: 983-986.
- Saxena, K.B. (2006) Seed production systems in pigeonpea. International Crops Research Institute for the Semi-Arid Tropics, Patancheru. p 76. ISBN 92-9066-490-8.
- Saxena, K.B. (2013). A novel source of CMS in pigeonpea derived from *Cajanus reticulatus*. *Indian J. Genet. Plant Breed*. **73**: 259-263.
- Saxena, K.B. and Kumar, R.V. (2003). Development of cytoplasmic nuclear male sterility system in pigeonpea using *C. scarabaeoides* (L.) Thouars. *Indian Journal of Genetics and Plant Breeding*. **63**: 225-229.
- Saxena, K.B. and Sharma, D. (1990). Pigeonpea: Genetics. In: The Pigeonpea. [Nene, Y.L., Hall, S.D. and Sheila, K. (Ed.)], CAB International Wallingford, Oxon, UK. (pp. 137-158).
- Saxena, K.B., Singh, L., Gupta, M.D. (1990). Variation for natural out-crossing in pigeonpea. *Euphytica*. **46**: 143-148.
- Saxena, K.B., Sultana, R., Mallikarjuna, N., Saxena, R.K., Kumar, R.V., Sawargaonkar, S.L., Varshney, R.K. (2010). Male-sterility systems in pigeonpea and their role in enhancing yield. *Plant Breed*. **129**: 125-134.
- Saxena, K.B., Sultana, R., Saxena, R.K., Kumar, R.V., Sandhu, J.S., Rathore, A., Kishor, P.B.K. and Varshney, R.K. (2011). Genetics of fertility restoration in A4 based, diverse maturing hybrids of pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Crop Sci*. **51**: 1-5.
- Sultana R. and Saxena, K.B. (2017). Identification of new fertility restorers for development of early maturing pigeonpea hybrids. *Legume Research*. **40(4)**: 639-642. doi: 10.18805/ijar.v0iOF.7647.
- Tikka, S.B.S., Parmar L.D. and Chauhan, R.M. (1997). First record of cytoplasmic-genic male-sterility system in pigeonpea [*Cajanus cajan* (L.) Millsp.] through wide hybridization. *Gujrat Agricultural University Research Journal*. **22**: 160-162.
- Vales, M.I., Srivastava, R.K., Sultana, R., Singh, S., Singh, I., Singh, G., Patil, S.B. and Saxena, K.B. (2012). Breeding for earliness in pigeonpea: Development of new determinate and non determinate lines. *Crop Science*. **52**: 2507-2516.