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Effects of cytoplasm from *Cajanus cajanifolius* on the performance of pigeonpea hybrids

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Abstract

This research communication reports results of the first study on the influence of cytoplasm on the performance of CMS-based pigeonpea hybrids. In this study two iso-nuclear lines with diverse cytoplasm were compared in hybrid combinations involving seven inbred fertility restorers. Pusa Ageti-(F) had cultivated pigeonpea cytoplasm, while Pusa Ageti-(A_4CMS) carried the cytoplasm of a wild species *Cajanus cajanifolius*. The trials conducted over two seasons, showed small cytoplasmic effects on the traits studied. For seed yield the hybrids with cultivated cytoplasm were better in performance; however the extent of the superiority of these hybrids varied. The greatest yield penalty of 19.1% due to the cytoplasm of the wild species was recorded in cross involving restorer line R-2364. For other traits no definite trend was observed in favour of any specific cytoplasm. The cytoplasmic effects on the hybrids also appeared to be influenced by genetic variation in the pollinator lines.

Key words: Cytoplasm, iso-nuclear lines, pigeonpea, wild species, yield components

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a crop of significance in various agricultural scenarios of tropical and sub-tropical regions and it is known for its significant role in subsistence agriculture. In order to overcome the decades old bottleneck of low productivity of the crop, a hybrid breeding technology, that was based on partial natural out-crossing and cytoplasmic nuclear male sterility (CMS) systems, was developed; and recently the world's first commercial pigeonpea hybrid was released (Saxena et al. 2013) for cultivation. Since this technology uses cytoplasm of a wild species, it was found necessary to generate information regarding effects of the alien cytoplasm on seed yield and other related traits in pigeonpea hybrids. This information may help in designing strategies for breeding elite hybrid parents. This paper, therefore, compares the performance of hybrids developed by using seven inbred restorer lines and two iso-nuclear CMS lines, one carrying the cytoplasm of cultivated pigeonpea (*Cajanus cajan*) and the other from *C. cajanifolius*, a wild relative of pigeonpea.

Materials and methods

To develop iso-nuclear lines with two different cytoplasms, a short duration cultivar Pusa Ageti (carrying cytoplasm of *Cajanus cajan*) and a CMS line ICPA 2039 with cytoplasm of *C. cajanifolius* were selected. A cross was made using ICPA 2039 as female and Pusa Ageti as male parent. The F_1 was found to be complete male sterile; and it meant that Pusa Ageti worked as a maintainer (B-) line with cytoplasmic background of *C. cajanifolius*, and it made the conversion job easy. The male sterile F_1 plants were backcrossed with Pusa Ageti and, subsequently, ten backcrosses were made in the same manner to ensure maximum substitution of the nuclear genome of ICPA 2039 with that of Pusa Ageti. Thus it became a new (converted) CMS with nuclear genome of Pusa Ageti and cytoplasm from *Cajanus cajanifolius*. In the literature on CMS systems, the cytoplasm from *Cajanus cajanifolius* was designated as A_4. Hence, hereafter it will be called as Pusa Ageti-(A_4CMS) while the cultivated type, carrying cultivated cytoplasm of *Cajanus cajan*, will be called Pusa Ageti-(F). The backcross generations were advanced in both open field (in rainy season) as well as glasshouse (in off-season). To develop hybrid cross combinations for

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this study, in 2009 rainy season both Pusa Ageti-(A_4CMS) and Pusa Ageti-(F) were crossed with seven known early maturing fertility restorers by hand pollinating the male-sterile flowers of Pusa Ageti-(A_4CMS) directly, while the flowers of Pusa Ageti-(F) were emasculated before pollinations. The 14 hybrids were evaluated in 2010 and 2011 rainy seasons in three replications using a RCB design in Alfisols at ICRISAT, Patancheru. The recommended package of practices was followed to raise a healthy crop. Each plot consisted of 4 rows of 4m length, with inter- and intra-row spacing of 75 cm and 25 cm, respectively. The trials in both the years were conducted under irrigation with appropriate insect management. Data on yield (kg/ha), days to flower and maturity were recorded on plot basis; while data on 100-seed weight (g), number of seeds/pod, and plant height (cm) were recorded on five competitive plants selected randomly within each plot. Data obtained from each trial were analyzed using 'procglm' procedure of SAS software version 9.3 for Windows with lines as fixed effects. To obtain their best linear unbiased estimates, least significant differences (LSD) were estimated to examine the genotypes which vary in the origin of their cytoplasm.

Results and discussion

The analysis of the data (ANOVA not reported here) showed that the differences among genotypes were significant in both the years for days to flower and maturity, plant height, seed size, seeds/pod and seed yield (Table 1). The results further showed that in both the seasons, days to flower, seeds/pod and plant height exhibited significant differences among the crosses involving the two cytoplasm sources. Seed size, however, exhibited non-significant variation in 2010, while in 2011 such differences were significant. On the contrary, days to maturity and seed yield exhibited the reverse trend with respect to these two seasons.

For seed yield the overall differences among the hybrids were significant in 2010 and non-significant in 2011. The mean data recorded in 2010 showed that the hybrids with cultivated cytoplasm (1511 kg/ha), as a group, were marginally superior (but statistically non-significant) than those with *C. cajanifolius* cytoplasm (1362 kg/ha). This means the advantage of 149 kg/ha was not enough to draw useful conclusions about the effect of cytoplasm on productivity. However, a trend in favour of cultivated cytoplasm although non-significant at some places was evident in all the seven crosses. The observed mean differences in the sets of crosses were from 6 to 241 kg/ha. Both the hybrids with restorer line R-2438 were the top performers in both the cytoplasmic backgrounds and it was followed by R-2363 hybrids. The performance of the hybrids with restorer R-3310 was almost similar with both the cytoplasms. These observations emphasized the significant role played by nuclear genome present in the fertility restorers in the manifestation specific combining ability in terms of hybrid vigour for yield. Some nuclear genomes may interact with the wild species cytoplasm to affect the performance adversely, while the other genome may not show any negative effect. In 2011, the mean 100-seed weight of the hybrids based on *C. cajan* cytoplasm (8.93 g/100 seeds) was similar to those based on *C. cajanifolius* cytoplasm (8.65 g/100 seeds). A similar trend was recorded for seeds/pod over the two years. In contrast to the above, the traits such as days to flower, days to maturity and plant height showed a reverse behavior where hybrids with *C. cajanifolius* cytoplasm showed an edge over the *C. cajan* hybrids (Table 1) but, their superiority was not large enough to affect the decision making in hybrid breeding programmes.

Cytoplasm is known to play an important role in the manifestation of male sterility. The hybrid breeding programme that is supported by a single cytoplasm may be at risk to potentially dangerous stresses like pest and diseases to cause yield losses. Hence, for a sustainable hybrid breeding programme the importance of cytoplasmic diversity has been advocated, particularly after the outbreak of corn leaf blight in the USA that was associated with Texas cytoplasm (Tatum 1971). Since then the role of cytoplasm in hybrid breeding has been a subject of investigation. Literature on different cytoplasmic sources does not exhibit any strong cytoplasmic influence on yield and other traits in some cases. For example in Brassica the effect of cytoplasm was expressed only in the floral traits (Chang et al. 2007). Moran and Rooney (2003) observed statistically significant but little cytoplasmic effect on sorghum yield. Subsequently, Hoffman and Rooney (2013) reported no effect of different cytoplasms on yield and any yield contributing traits in sorghum. Reddy et al. (2006) reported that the hybrids carrying different cytoplasms did not differ with respect to their combining ability and yield in sorghum. On the contrary, Virk and Brar (1993) reported positive and significant effect of male sterility-inducing cytoplasm on yield in pearl millet hybrids. They attributed the yield enhancement caused by CMS-inducing

Table 1. Comparative performance of crosses involving cytoplasm of cultivated (Pusa Ageti F) and wild (A_4CMS) wild species

Cross	Year	Yield (kg/ha)	100-seed wt (g)	Seeds/ pod	Days to flower	Days to mature	Plant ht (cm)
Pusa Ageti-(A_4CMS) x R-2364	2010	1249^{d}	9.5^{bc}	3.9^{cd}	70^{g}	117^{c}	155^{g}
	2011	1135^{de}	9.6^{a}	3.7^{bcd}	68^{h}	115^{f}	165^{f}
Pusa Ageti-(F) x R-2364	2010	1488^{cde}	9.8^{a}	4.0^{bcd}	66^{h}	111^{c}	163^{fg}
	2011	1150^{cde}	9.2^{ab}	4.0^{b}	68^{gh}	113^{g}	165^{f}
Pusa Ageti-(A_4CMS) x R-2363	2010	1566^{abc}	9.4^{bc}	3.9^{d}	70^{d}	114^{d}	195^{b}
	2011	1359^{bcd}	9.0^{bcd}	3.7^{bcd}	78^{bcd}	120^{bcd}	185^{bc}
Pusa Ageti-(F) x R-2363	2010	1807^{ab}	9.1^{cd}	4.0^{bc}	79^{d}	117^{a}	185^{bcd}
	2011	1502^{ab}	8.6^{de}	4.0^{b}	78^{bc}	119^{cde}	192^{ab}
Pusa Ageti-(A_4CMS) x R-2431	2010	1207^{f}	8.4^{ef}	4.2^{f}	82^{c}	122^{b}	207^{a}
	2011	1460^{ab}	7.7^{fgh}	3.7^{bcd}	79^{b}	120^{bcd}	192^{ab}
Pusa Ageti-(F) x R-2431	2010	1331^{def}	8.1	3.9^{cd}	82^{c}	124	187^{bc}
	2011	1566^{ab}	7.5^{h}	3.6^{cd}	84^{a}	122^{ab}	193^{a}
Pusa Aget-(A_4CMS) x R-2433	2010	1397^{cdef}	8.6^{def}	3.7^{bcd}	88^{a}	125^{a}	210^{a}
	2011	1429^{abc}	7.7^{gh}	3.8^{bc}	84^{a}	122^{abc}	185^{bc}
Pusa Ageti-(F) x R-2433	2010	1521^{cde}	8.6^{def}	3.5^{f}	66^{b}	125^{a}	215^{a}
	2011	1665^{e}	7.7^{fgh}	3.7^{bcd}	84^{a}	123^{a}	190^{ab}
Pusa Ageti-(A_4CMS) x R-2438	2010	1662^{abc}	8.7^{def}	4.0^{ab}	75^{e}	115^{ed}	178^{cde}
	2011	1522^{ab}	8.1^{fg}	3.8^{bc}	76^{d}	120^{bcd}	180^{cd}
Pusa Ageti-(F) x R-2438	2010	1831^{a}	8.9^{cd}	4.0^{bcd}	74^{ef}	115^{cd}	175^{de}
	2011	1445^{ab}	8.2^{ef}	3.9^{bc}	73^{e}	118^{de}	182^{c}
Pusa Ageti-(A_4CMS) x R-3310	2010	1269^{ef}	10.1	4.1^{ab}	67^{h}	110^{e}	183^{cd}
	2011	1144^{ade}	9.2^{ab}	4.0^{b}	69^{gh}	112^{g}	168^{ef}
Pusa Ageti-(F) x R-3310	2010	1275^{ef}	9.5^{b}	4.0^{bcd}	67^{h}	115^{cd}	177^{cd}
	2011	1065^{e}	9.1^{abc}	4.0^{b}	69^{fg}	117^{a}	173^{de}
Pusa Ageti-(A_4CMS) x R-2447	2010	1212^{f}	10.3^{a}	4.1^{ab}	73^{f}	113^{d}	182^{c}
	2011	1582^{ab}	9.3^{ab}	3.6^{cd}	77^{cd}	119^{cde}	192^{c}
Pusa Ageti-(F) x R-2447	2010	1321^{def}	9.8^{S}	4.1^{ab}	70^{g}	124^{cd}	168^{ef}
	2011	1330^{cd}	8.7^{cd}	4.4^{a}	75^{f}	115^{f}	178^{cd}
LSD	2010	282.5	0.65	0.1	1.8	2.46	10.2
	2011	289.5	0.40	0.12	1.5	2.40	7.7
CV%	2010	11.7	4.2	2.11	1.4	1.3	3.3
	2011	12.5	3.05	0.40	1.2	1.2	2.6
TMSS	2010	S	S	S	S	S	S
	2011	S	S	S	S	S	S
Pusa Ageti(-A_4CMS) vs	2010	S	NS	S	S	S	S
Pusa Ageti-(F) crosses MSS	2011	NS	S	S	S	NS	S

cytoplasm to pleiotropic effects of some extra-nuclear genes or heterogeneity at male sterility/fertility loci and /or at linked loci with over-dominance effect. Kong et al. (2014) reported significant effect of cytoplasm on pollen fertility/sterility of temperature sensitive genetic male sterility system in rice. Tao et al. (2011) recorded significant cytoplasmic effects on grain weight and filled grain ratio in rice. The scientific understanding about the interactions between mitochondrial and nuclear genomes which give rise to male sterility/ fertility at present is inadequate and it needs to be pursued further.

The hybrid technology in pigeonpea is of recent origin (Saxena et al. 2013) and it is based on the male sterility that has cytoplasm of a wild species *C. cajanifolius* (Saxena et al. 2005). This wild species belongs to the secondary gene pool of genus *Cajanus*. Based on various considerations, van der Maesen (1980) and De (1974) concluded that *C. cajanifolius* is the most closely related wild species to the cultivated type and it is a putative progenitor of the cultivated

type. However, based on the observations recorded in the present study, these two species exhibited genomic diversity with respect to their cytoplasm. Sinha et al. (2015) demonstrated that the male sterility in ICPA 2039 was due to deletion in the cytoplasmic DNA, and perhaps this information partially describes the cause of cytoplasmic diversity.

In the present study based on nuclear genome of Pusa Ageti-(F), indicated that cytoplasm of the wild species *per se* does not adversely affects yield and its components.; and there were no definite trends favouring any cytoplasm in the expression of traits studied.

The successful breeding of cytoplasmic-nuclear male sterility in pigeonpea allowed hybrid development programmes to break the yield plateau persisting over half century. The information on role of cytoplasm in the manifestation of high yields in the hybrid is not available in pigeonpea and the present investigation deals with a very small sample and hence, any generalization should be avoided. Even in this limited data the estimated mean yield losses over crosses due to the cytoplasm of wild species alone accounted for 10.5% which are non-significant, the maximum being 19.5%. Large scale efforts based on greater genetic variability in breeding hybrids that is available among fertility restorers (Saxena et al. 2014) be studied and the restorers such as R-3310 be identified so that the harmful interaction with the cytoplasm of the wild species are avoided. The use of CMS lines carrying the cytoplasm of cultivated type (Mallikarjuna and Saxena, 2005) and/or environment-sensitive male sterility system (Saxena 2014) in hybrid programme may be the alternative pathways in exploiting hybrid vigour in pigeonpea. These, however, may throw new limitations which are to be resolved amicably.

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