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Original Research Article

Genetics of Fertility Restoration in A_2 Cytoplasm based Hybrids of Pigeonpea [*Cajanus cajan* (L.) Millsp.]

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A B S T R A C T

In the present study, single crosses involving two CMS-lines and three known fertility restorers were studied to determine the genetics of fertility restoration in pigeonpea (*Cajanus cajan* L. Millsp.). The F_1 plants of all the four hybrids were selfed to produce F_2 seed and simultaneously crossed to their corresponding A-lines to produce BC_1F_1 seeds during 2015. The parents, F_1, F_2 and BC_1F_1 populations were planted in 2016 kharif season at Pulses Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.), India. The interaction of dominant nuclear genes of AKPR 303 and AKPR 359 with AKCMS 11A and AKPR 303 with AKCMS 10A produced 100% fertile F_1 plants and showed complete dominance for fertility restoration. The restorer AKPR 303 and AKPR 359 when crossed with AKCMS 11A similarly restorer AKPR 303 crossed with another female AKCMS 10A showed monogenic inheritance (3:1), while AKPR 324 when crossed with AKCMS 11A revealed digenic inheritance (15:1) of fertility restoration. In a cross between AKPR 324 and AKCMS 11A, the dominant gene of fertility restoration at either of two loci masked the expression of male-sterile recessive alleles at the two loci in such a way that it modified normal di-hybrid ratio in to 15:1 ratio and produced duplicate gene interaction.

Keywords

Pigeonpea, A_2 Cytoplasm, Genetics, Fertility restoration

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh], a short lived perennial member of family *Fabaceae*, is invariably cultivated as annual crop. It is an often cross-pollinated (20-70%) crop with 2n = 2x = 22 chromosomes. Globally, pigeonpea is grown on ~6.22 m ha land in more than 20 countries with an annual production of ~4.74 MT (FAOSTAT 2015). Pigeonpea is the second important pulse crop in India. It is grown in about 5.13 million hectares with a production of 4.23 million tonnes of grains (Anonymous 2016-17). Since 1976, pigeonpea has recorded globally a 56% increase in its area and production but the productivity has remained low at ~750kg/ha (FAOSTAT 2015). The per capita availability of protein in the India is 28 g/day, while WHO recommended it should be 80 g/day, consequently most serious problem of the malnutrition existing among the poor people, where most of the people have vegetarian diet and avoid the animal protein. Therefore it's become imperative to increase productivity of pulse crop for proper nutrition balance.

Singh *et al.*, (2005) reported that the progress through genetic improvement of yield potential is limited and the improved

cultivars developed through breeding has been inadequate in enhancing the productivity of the crop in the last five decades. Moreover, the genetic male sterility (GMS) based pigeonpea hybrids has not been commercialized because of high seed cost and difficulties in maintaining the genetic purity (Saxena *et al.,* 2006; Saxena and Nadarajan, 2010). Hence, the development of cytoplasmic nuclear male sterility (CMS) became imperative. Cytoplasmic nuclear male sterility (CMS) is a maternally inherited trait and do not follow Mendelian law of segregation; and this can originate from alternations in either nuclear or cytoplasmic genes. In this system the genetic determinants of male sterility generally inherit through the mitochondrial genome.

However, the nuclear genomes also play an important role in the expression of CMS phenotype (Newton, 1988). CMS has been reported in about 140 plant species belonging to 47 genera and 20 families (Kaul, 1988). In pigeonpea, seven CMS systems (A1, A2, A3, A4, A5, A6 and A7, A8) were developed by integrating the cytoplasm of wild species with the genome of cultivars through interspecific hybridization followed by selection and backcrossing (Saxena *et al.,* 2013).

Among these, A_2 and A_4 CMS system derived from a cross involving a wild relative of pigeonpea *C. scarabaeoides and C. cajanifolius* respectively and cultivated type (*C. cajan*) has shown great promise (Saxena *et al.,* 2005) because of its stable expression under various agro-climatic conditions, availability of reliable maintainers (B-lines), and stable fertility restoration. The presence of greater genetic diversity among fertility restorers enhances the probability of breeding widely adapted high yielding hybrids. The information regarding the number of genes controlling fertility restoration (*Rf* or *Fr* genes) and their eventual mapping in the pigeonpea genome will facilitate the development of new hybrids. Consequently, this will provide guidance in the introgression of fertility restoring genes in new genetic backgrounds. Keeping this in view, the present study was undertaken to study the genetics of fertility restoration system in pigeonpea using F_1, F_2, and BC_1F_1 generations in four pigeonpea hybrid combinations carrying A_2 cytoplasm.

Materials and Methods

The genetics of fertility restoration was studied in four hybrids along with their F_2 generation and their test crosses progenies. These hybrid combinations were selected on the basis of their genetic diversity of parental lines. During 2014-15 *kharif* season, the crossing programme involved by crossing two male-sterile lines (AKCMS 10A and AKCMS 11A) with three (AKPR 324, AKPR 359 and AKPR 303) restorer lines was planned and obtained hybrid crosses AKCMS11A x AKPR 324, AKCMS 11A x AKPR 359, AKCMS 11A x AKPR 303 and AKCMS 10A x AKPR 303. Crossing of these four hybrids with their respective CMS-lines to develop BC_1F_1 progenies was carried out during Kharif 2015-16 simultaneously, selfing of hybrid plants to produce F_2 seeds.

Evaluation of parents, F_1, F_2, and test crosses

Materials involving the parents (P_1 and P_2), F_1s, F_2s, and test crosses (A-line x F_1) listed above planted at Pulses Research Unit, Dr. PDKV, Akola during Kharif 2016-17. One row of parental lines, hybrids and test cross population and four rows of F_2 were grown with four meter row length, spaced at 90 cm between rows and 30 cm between plants.

Recording observations on male-fertility and male-sterility

Plant fertility/sterility observed in F_1, F_2, and test cross populations. The hybrids were tested for their fertility status at the initial flowering stage of each plant for each hybrid, their F_2 and back cross populations. The anthers of plants were critically visualized to identify sterility/ fertility of pollen grains on the basis of,

Anther colour

The visual observations for anther colour were taken on fresh flower after opening from fully grown buds.

The anther colour was either yellow or translucent white. The white translucent anthers were completely sterile.

Anther dehiscence

The observations on anther dehiscence were taken after opening the well-developed fresh buds. Based on release of pollen grains powder, the plant were classified as (a) good dehiscent having abundant pollen like cultivated variety as fertile or (b) poor dehiscent where the pollen powder was sparse or absence of pollen grain powder then it was classified as non- dehiscent type as sterile.

Statistical analysis

The goodness of fit in F_2 and test cross ratios was tested using a chi-square test (Panse and Sukhatme, 1985).

The confirmation of ratios obtained in F_2 segregating populations was done by the ratios obtained in test crosses. While applying χ^2 test correction were made as suggested by Yates (1934).

Results and Discussion

Cytoplasmic nuclear-male sterility (CGMS) is maternally inherited and is 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 addition the CGMS-*Rf* system provides an excellent model for the study of nuclear-mitochondrial interaction in multicellular organisms.

The gene action in F_1 generation and the nature of segregation in F_2 generation observed in this study are shown in the Table 1 to 4. A total of four F_1 hybrids were advanced to F_2 and backcross generations to study the segregation for fertility restoration. The F_1 plants were selfed under insect proof net and also backcrossed to the male-sterile parent. (P_1 and P_2), F_1s, F_2s, and test crosses (A-line x F_1) populations were grown in field during kharif 2016. Data on segregation for male-sterility and fertility were recorded in each plant of these populations. Chi-square ($\chi 2$) tests were applied for testing goodness of fit for each phenotypic ratio. In hybrid AKCMS 11A x AKPR 303 all the eleven F_1 plants were found male-fertile indicating the dominance of fertility restoring genes. As expected, the F_2 and BC_1F_1 population of this hybrid segregated for male-sterility and fertility. Out of 105 F_2 plants grown, 81 were fertile while 24 were male-sterile. This segregation fit well to the expected ratio of 3 fertile: 1 sterile ($\chi 2$ = 0.2677; P = 0.6932) ratio. In BC_1F_1 generation out of 20 plants, 12 were male-fertile and 8 had male-sterile anthers, which showed a good fit for 1 fertile: 1 sterile ($\chi 2$ = 0.450, P = 0.502) ratio (Table 1).

In AKCMS 11A x AKPR 359 hybrid, all the 13 F_1 plants were male-fertile. Among 61 F_2 plants, 48 were male-fertile and 13 were male-sterile. This segregation fit well to the expected ratio of 3 fertile: 1 sterile ($\chi 2$ = 0.267; P = 0.6048) ratio In BC_1F_1 generation out of 20 plants, 12 were male-fertile and 08 had male-sterile anthers, which showed a good fit for a 1 fertile: 1 sterile ($\chi 2$ = 0.450; P = 0.502) ratio (Table 2). Similar trend was also observed in a cross AKCMS 10A x AKPR 303 where all the 17 F_1 plants found fertile. In F_2, 147 plants segregate in 106 fertile and 41 male sterile. This segregation fit well to the expected ratio of 3 fertile: 1 sterile ($\chi 2$ = 0.5102; P = 0.4750) ratio. In test cross (BC_1F_1) generation out of 18 plants, 05 were male-fertile and 13 had male-sterile anthers, which showed a good fit for a 1 fertile: 1 sterile ($\chi 2$ = 2.7222; P = 0.0989) ratio (Table 3). All these crosses suggested that the fertility restoration was controlled by a single dominant gene.

In another hybrid AKCMS 11A x AKPR 324, all the 08 F_1 plants were also male-fertile but the observations in the F_2 generation revealed that out of 236 total plants, 225 were male fertile and 11 were male-sterile. This segregation fit well to a dihybrid ratio of 15 fertile: 1 sterile ($\chi 2$ = 0.7638, P = 0.3821) while, segregation in BC_1F_1 generation revealed that 11 plants were male-fertile and 07 were male-sterile, which showed a good fit for a 3 fertile: 1 sterile ($\chi 2$ = 1.1851, P = 0.2763) ratio (Table 4). It suggested that a digenic inheritance with duplicate gene action for fertility restoration.

Such phenomenon was also reported in pigeonpea by Dalvi *et al.*, (2008) in CMS line ICPA 2039 and its five fertility restorers and found that all the F_1 plants in 5 crosses were fully fertile indicating the dominance of fertility restoring genes. Among the 5 crosses studied, 3 (ICPA 2039 x ICPL 12320, ICPA 2039 x ICPL 11376, and ICPA 2039 x HPL 24-63) segregated in a ratio of 3 fertile: 1 sterile in F_2 generation and 1 fertile: 1 sterile in BC_1F_1 generation indicating the monogenic dominant nature of a single fertility restoring gene. The crosses ICPA 2039 x ICP 10650 segregated two dominant duplicated gene action with a ratio of 15 fertile: 1 fertile in F_2 and 3 fertile: 1 sterile in BC_1F_1, respectively. The rest cross ICPA 2039 x ICP 13991 had two complementary gene action of 9 fertile: 7 sterile in F_2 and 1 fertile: 3 sterile in BC_1F_1, respectively. Saxena *et al.*, (2010) also reported dominance of fertility restoring genes in hybrid ICPA 2067 x ICP 12320 and segregation of F2 fit well to a ratio of 13 fertile: 3 sterile (P = 0.01) while BC_1F_1 generation showed a good fit for a 3 fertile:1 sterile (P = 0.01) ratio.

These results suggested the presence of 2 dominant genes, with one basic and one inhibitory gene action for the determination of fertility restoration in ICPA 2067. Saxena *et al.*, (2011) reported the fertility restoration of extra-early-maturing hybrid (ICPA 2089 x PHR 31) was governed by mono gene with the segregation ratio of 3 fertile: 1 sterile in F2 and 1 fertile: 1 sterile in BC_1F_1 while early-maturing hybrids ICPA 2039 x ICPR 2438 and ICPA 2039 x ICPR 2447 were governed by digenic duplicate dominant ratio of 15 fertile: 1 sterile in F2 and 3 fertile: 1 sterile in BC_1F_1. Similarly, late-maturing hybrid ICPA 2043 x ICPR 2671 and ICPA 2043 x ICPR 3497 were also governed by two duplicate dominant genes. It was also observed that hybrids with two dominant genes produced a greater pollen load and expressed greater stability as compared with those carrying a single dominant gene. Sawargaonkar *et al.*, (2012) studied the four crosses and reported the similar results such as monogenic

inheritance (3:1) in two crosses while digenic inheritance (15:1) of fertility restoration in other two pigeonpea crosses while Sheikh *et. al.* (2016) observed the segregation of F2 for fertility restoration of A2 cytoplasm indicated 3F:1S segregation indicating dominant monogenic control. Saroj *et al.,* (2017) reported that out of two major genes governing the fertility restoration, one gene segregated in the ratio of 9:3:4 whereas the second gene in 12:3:1 due to the allelic differences.

Table.1 Inheritance of fertility restoration in a cross AKCMS 11A x AKPR 303

1.1 A Parents and F_1					
AKCMS 11A	AKPR 303	F_1			
Sterile	Fertile	Fertile			
1.1 B Population of F_2					
Class	Observed (O)	Expected E (3:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	81	78.50	2.25	0.06694	0.693287
Sterile	24	26.25	-2.25	0.20082	
Total	105	105	--------	0.26776	
1.1 C Population of test cross (BC1F1)					
Class	Observed (O)	Expected E (1:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	12	10	2	0.225	0.502335
Sterile	08	10	-2	0.225	
Total	20	20	--------	0.450	

Table.2 Inheritance of fertility restoration in a cross AKCMS 11A x AKPR 324

1.2.A Parents and F_1					
AKCMS 11A	AKPR 324	F_1			
Sterile	Fertile	Fertile			
1.2 B Population of F_2					
Class	Observed (O)	Expected E (15:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	225	221.5	3.75	0.04774	0.382129
Sterile	11	14.75	-3.75	0.716102	
Total	236	236	--------	0.763842	
1.2 C Population of test cross (BC1F1)					
Class	Observed (O)	Expected E (3:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	11	13.5	2.5	0.296296	0.276304
Sterile	07	4.5	-2.5	0.888889	
Total	18	18	--------	1.185185	

Table.3 Inheritance of fertility restoration in a cross AKCMS 11A x AKPR 359

1.3.A Parents and F_1					
AKCMS 11A	AKPR 359	F_1			
Sterile	Fertile	Fertile			
1.3.B Population of F_2					
Class	Observed (O)	Expected E (3:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	48	45.75	2.25	0.06694	0.604845
Sterile	13	15.25	-2.25	0.20082	
Total	61	61	--------	0.26776	
1.3.C Population of test cross (BC1F1)					
Class	Observed (O)	Expected E (1:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	12	10	2	0.225	0.502335
Sterile	08	10	-2	0.225	
Total	20	20	--------	0.450	

Table.4 Inheritance of fertility restoration in a cross AKCMS 10A x AKPR 303

1.4.A Parents and F_1					
AKCMS 10A	AKPR 303	F_1			
Sterile	Fertile	Fertile			
1.4 B Population of F_2					
Class	Observed (O)	Expected E (3:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	106	110.25	-4.25	0.127551	0.475052
Sterile	41	36.75	4.25	0.382653	
Total	147	147	--------	0.510204	
1.4 C Population of test cross (BC1F1)					
Class	Observed (O)	Expected E (1:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	05	9	4.00	1.361111	0.09896
Sterile	13	9	-4.00	1.361111	
Total	18	18	--------	2.72222	

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