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Study of Inheritance for Fertility Restoration in *Cajanus scarabaeoides* Cytoplasm based Pigeonpea [*Cajanus cajan* (L.) Millsp)] Hybrids

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A B S T R A C T

In the present investigation, the parents (P_1 and P_2), F_1's, F_2's and BC_1F_1's progenies of four selected hybrids involving four cytoplasmic genetic male sterile lines based on *Cajanus scarabaeoides* (A_2) cytoplasm and four restorers from diverse source were evaluated to determine the inheritance of fertility restoration in pigeonpea [*Cajanus cajan* (L.) Millsp)] hybrids. Among these restorers, three restorers *viz*. BDNHR 1, BDNHR 21-2 and BDNHR 22-1-2 were identified and developed from segregating materials of interspecific cross involving *C. scarabaeoides*, however one restorer *viz.* BDNHR 60-2 from interspecific cross involving *C. albicans* wild species. The results revealed that the hybrids *viz.* BDN 2004-1A x BDNHR 1, BDN 2004-4Ax BDNHR22-1-2 and BSMR 736A x BDNHR 21-2 showed the monogenic dominant gene action (3:1) with single fertility restoring gene. However, only one hybrid *viz.* BDN 2004-2A x BDNHR 60-2 showed digenic duplicate gene action with complete dominance for fertility restoration in Pigeonpea hybrids. The information generated on genetics of fertility restoration will help in knowing the selection of breeding methods and further transfer of fertility restorer genes in to elite backgrounds.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is an often cross pollinated crop (20–70%) with diploid (2n = 2x) chromosome number of 22 and genome size of 1C = 858 Mbp. It is a short-lived perennial shrub in which plants may grow for about five years and turn into small trees. It is the sixth most important legume crop, grown predominantly in the tropical and sub-tropical regions of Asia, Africa and Latin America. India is considered as the center of origin of pigeonpea because of its natural genetic variability available in the local germplasm and the presence of its wild relatives.

To promote the pigeonpea production, genetic improvement of pigeonpea was emphasized by researchers for more than five decades and a number

of cultivars were developed from hybridization programmes and selection of landraces (Singh *et al.*, 2005). However, the progress in the genetic improvement of production potential has been limited and the improved cultivars failed to enhance the productivity of the crop. Therefore, an alternative breeding approach such as hybrid technology, which has been profitably used in a number of cereals, vegetable and fruit crops was attempted in pigeonpea to enhance the yield. 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 yield and may serve as a major fruitful technique to break existing yield barriers through heterosis breeding (Saxena & Sharma, 1990).

Three lines system of hybrid technology, which is based on cytoplasmic-nuclear male-sterility, is expected to make a quantum increase in production and boost the productivity of Pigeonpea yield. Cytoplasmic male-sterility is under extra-nuclear genetic control (under the control of the mitochondrial or plastid genomes). They show non mendelian inheritance and are under the regulation of cytoplasmic factors. In this system, male sterility is inherited maternally and is never lost or diluted in the succeeding generations of reproduction. The success in development of pigeonpea hybrids largely depends on availability of effective restorers and precise basic knowledge on the genetics of fertility restoration of such lines. In pigeonpea, two dominant genes (Rf_1 and Rf_2) 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. In the exploitation of heterosis from potential crosses, the level of fertility restoration would likely be the key for added yield advantages. Therefore, a precise understanding of the inheritance of fertility restoration, pollen fertility (%) and amount of viable pollens produced by particular hybrid are important traits and had a basic need for the successful production of high yielding CGMS-based hybrids in pigeonpea.

Materials and Methods

The experimental materials comprising the parents (P_1 and P_2), F_1''s, F_2's and BC_1F_1's progenies of four selected hybrids involving four CGMS lines based on *Cajanus scarabaeoides* (A_2) cytoplasm and four restorers from diverse source were evaluated to determine the inheritance of fertility restoration in pigeonpea [*Cajanus cajan* (L.) Millsp)] hybrids. Among these restorers, three restorers *viz.* BDNHR 1, BDNHR 21-2 and BDNHR 22-1-2 were identified and developed from segregating materials from interspecific cross involving *C. scarabaeoide* wild species accession, however one restorer *viz.* BDNHR 60-2 was identified and developed from segregating materials of interspecific cross involving *C. albicans* wild species accession.

During *Kharif* 2018-19 season, the crossing programme involving four male-sterile lines (BDN 2004-1A, BDN 2004-2 A, BDN 2004-4 A and BSMR 736 A) with four restorers (BDNHR 1, BDNHR 60-2, BDNHR 22-1-2 and BDNHR 21-2) respectively was undertaken to obtain four hybrid crosses *viz.* BDN 2004-1A x BDNHR 1, BDN 2004-2A x BDNHR 60-2, BDN 2004-4A x BDNHR 22-1-2, BSMR 736A x BDNHR 21-2 and then crossing of these four hybrids with their respective CGMS-lines to develop BC_1F_1 progenies was carried out during *Kharif* 2019-20. Simultaneously, these four selected hybrid plants were selfed to produce F_2 seeds. The experimental materials involving the parents (P_1 and P_2), F_1's, F_2's and test cross (A-line x F_1) were sown at Agricultural Research Station, Badnapur during *Kharif* 2020-21. Two rows of parental lines and hybrids, four rows of test cross population and eight rows of F_2 will be grown with four meter row length, spaced at 90 cm between rows and 20 cm between plants. The observation on pollen fertility/sterility were observed in F_1, F_2, and test cross populations. The anthers of plants were critically visualized to identify sterility/ fertility of pollen grains at the initial flowering stage of each

plant for each hybrid, their F_2 and back cross populations.

The visual observations for anther colour were recorded on fresh flower after opening from fully grown buds. The anther colour is either yellow or translucent white. The white translucent anthers are completely sterile. However, the observations on anther dehiscence were taken after opening the well developed fresh buds. Based on release of pollen grains powder, the plants are classified as (a) good dehiscent having abundant pollen like cultivated variety or (b) poor dehiscent where the pollen powder was sparse or absence of pollen grain powder then it is classified as non- dehiscent type. Simultaneously, mature pollen grains were collected and stained with acetocarmin solution to distinguish sterile and fertile pollen grains under light microscope. Completely stained pollen grains were classified as fertile and partial or no stained pollen grains were classified as sterile. Pollen fertility counts were taken on individual plants of F_1's and their parents. Microscopic observations for pollen fertility were taken for all those plants of F_2 s and testcrosses, whose fertility cannot be judged manually/phenotypically. The slides were examined under the microscope at three microscopic fields to avoid all sources of error.

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) 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 present study, observed gene action in F_1 generation and the nature of segregation in BC_1 and F_2 generations are presented in the Table 1 to 4.

The hybrid BDN 2004-1A x BDNHR 1 was evaluated for inheritance of fertility restoration and revealed that all thirty two F_1 plants were found to be male fertile indicating the dominance of fertility restoring genes. The segregation for male sterility and male fertility was depicted in F_2 and BC_1F_1 population of the hybrid BDN 2004-1A x BDNHR 1. Among 302 F_2 populations grown, 237 plants were fertile while 65 plants showed male sterility indicating segregation for male fertility: male sterility was fit to the expected ratio of 3 fertile: 1 sterile ($\chi 2$ =2.947; P = 0.086) ratio. Whereas, out of 156 plants in BC_1F_1 generation, male fertility was noticed in 85 plants and male sterility was observed in 71 plants suggesting the segregation ratio was fit for 1 fertile: 1 sterile ratio ($\chi 2$ = 1.256, P = 0.2624). The segregation ratio of 3 fertile: 1 sterile in F_2 generation and 1 fertile : 1 sterile in BC_1F_1 generation of hybrid BDN 2004-1A x BDNHR 1 suggesting the monogenic dominant nature of a single fertility restoring gene.

The study of inheritance of fertility restoration in the hybrid BDN 2004-2A x BDNHR 60-2 revealed that all thirty five F_1 plants were found to be male fertile reflecting the dominance of fertility restoring genes. The segregation for male sterility and male fertility was recorded in F_2 and BC_1F_1 population of the hybrid BDN 2004-2A x BDNHR 60-2. Among 291 F_2 populations grown, male fertility was observed in 266 plants while male sterility was recorded in 25 plants indicating segregation for male fertility: male sterility was fit to the expected ratio of 15 fertile: 1 sterile ($\chi 2$ =2.722; P = 0.099) ratio.

However, out of 143 plants in BC_1F_1 generation, male fertility was noticed in 102 plants, while male sterility was observed in 41 plants suggesting the segregation ratio was fit for 3 fertile: 1 sterile ratio ($\chi 2$ = 1.028, P = 0.311).The segregation ratio of 15 fertile: 1 sterile in F_2 generation and 3 fertile: 1 sterile in BC_1F_1 generation of hybrid BDN 2004-2A

x BDNHR 60-2 indicating the two dominant genes with duplicate gene action.

The pollens of hybrid BDN 2004-4A x BDNHR 22-1-2 was examined for study of inheritance of fertility restoration and observed that all thirty eight F_1 plants were found to be male fertile depicting the dominance of fertility restoring genes. The segregation for male sterility and male fertility was noticeable in F_2 and BC_1F_1 population of the hybrid BDN 2004-4A x BDNHR 22-1-2. Among 275 F_2 populations grown, 213 plants were fertile while 62 plants were male sterile indicating segregation for male fertility: male sterility was fit to the expected ratio of 3 fertile: 1 sterile ($\chi 2$ =0.884; P = 0.347) ratio. Whereas, out of 133 plants in BC_1F_1 generation, male fertility was evinced in 76 plants while male sterility was registered in 57 plants suggesting the segregation ratio was fit for 1 fertile: 1 sterile ratio ($\chi 2$ = 2.714, P = 0.099). The segregation ratio of 3 fertile: 1 sterile in F_2 generation and 1 fertile: 1 sterile in BC_1F_1 generation of hybrid BDN 2004-4A x BDNHR 22-1-2 suggesting the monogenic dominant nature of a single fertility restoring gene governed fertility restiration in this cross.

When compared the thirty six F_1 plants for fertility restoration in the hybrid BSMR 736A x BDNHR 21-2, it was observed that all plants had male fertility which was governed by the dominance of fertility restoring genes. When this cross was advanced to F_2 and BC_1F_1 generations, segregation for male sterility and male fertility was noticed.

Out of 262 F_2 populations grown, 204 plants were fertile while 58 plants were male sterile indicating segregation for male fertility: male sterility was fit to the expected ratio of 3 fertile: 1 sterile ratio ($\chi 2$ =1.145; P = 0.2845). In BC_1F_1 generation, total 145 plants were examined for fertility restoration and evinced that 82 plants showed male fertility while male sterility was observed in 63 plants suggesting the segregation ratio was fit for 1 fertile: 1 sterile ratio ($\chi 2$ = 2.490, P = 0.1145). The segregation ratio of 3 fertile: 1 sterile in F_2 generation and 1 fertile: 1 sterile in BC_1F_1 generation of hybrid BSMR 736A x BDNHR 21-2 suggested the fertility restoration was predominantly due to monogenic dominant gene action with single fertility restoring gene in pigeonpea.

The four hybrids *viz.* BDN 2004-1A x BDNHR 1, BDN 2004-2A x BDNHR 60-2, BDN 2004-4A x BDNHR 22-1-2, BSMR 736A x BDNHR 21-2 were selected on the basis of diversity of parental lines for study of fertility restoration in pigeonpea hybrids. Among these hybrids, the segregation ratio of 3 fertile: 1 sterile in F_2 generation and 1 fertile: 1 sterile in BC_1F_1 generation were observed in three hybrids *viz.* BDN 2004-1A x BDNHR 1, BDN 2004-4A x BDNHR 22-1-2, BSMR 736A x BDNHR 21-2 indicating the fertility restoration was governed by monogenic dominant gene action with single fertility restoring gene in pigeonpea. While only one hybrid *viz.* BDN 2004-2A x BDNHR 60-2 involving restorer from diverse source evinced segregation ratio of 15 fertile: 1 sterile in F_2 generation and 3 fertile: 1 sterile in BC_1F_1 generation of hybrid BDN 2004-2A x BDNHR 60-2. In this hybrid, dominant gene of fertility restoration at either of two loci masked the expression of male-sterile recessive alleles at the two loci. These nuclear and cytoplasm gene interactions produced male-fertile and male-sterile progenies in F_2 generation in such a way that it modified normal di-hybrid ratio in to 15:1 ratio and produced duplicate gene interaction suggesting the two dominant genes with duplicate gene action in inheritance of fertility restoration in the hybrid BDN 2004-2A x BDNHR 60-2

In the present investigation, it was observed that fertility restoration in three *Cajanus scarabaeoides* cytoplasm based CGMS pigeonpea hybrids was governed by monogenic dominant single gene, however only one hybrid exhibited two dominant genes with duplicate gene action suggesting that inheritance of fertility restration in *Cajanus scarabaeoides* cytoplasm based CGMS pigeonpea was primarily depend on source and diversity of restorer lines utilized in pigeonpea hybrid development programme.

Table.1 Inheritance of fertility restoration in a cross BDN 2004-1 x BDNHR 1

1. Parents and F_1					
BDN 2004-1A	Sterile		BDNHR 1	Fertile	
Class	Total plants		Fertile	Sterile	
F_1	32		32	0	
2. Population of F_2					
Class	Observed (O)	Expected E (3:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	237	226.5	10.5	0.487	**0.086**
Sterile	65	75.5	-10.5	1.460	
Total	302	302	---	**2.947**	
3. Population of test cross (BC_1F_1)					
Class	Observed (O)	Expected E (1:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	85	78	7	0.628	**0.262**
Sterile	71	78	-7	0.628	
Total	156	156	---	**1.256**	

Table.2 Inheritance of fertility restoration in a cross BDN 2004-2 x BDNHR 60-2

1. Parents and F_1					
BDN 2004-2A	Sterile		BDNHR 60-2	Fertile	
Class	Total plants		Fertile	Sterile	
F_1	35		35	0	
2. Population of F_2					
Class	Observed (O)	Expected E (15:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	266	272.8125	-6.8125	0.170	**0.099**
Sterile	25	18.1875	6.8125	2.552	
Total	291	291		**2.722**	
3. Population of test cross (BC_1F_1)					
Class	Observed (O)	Expected E (3:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	102	107.25	-5.25	0.257	**0.311**
Sterile	41	35.75	5.25	0.771	
Total	143	143		**1.028**	

Table.3 Inheritance of fertility restoration in a cross BDN 2004-4A x BDNHR 22-1-2

1. Parents and F_1					
BDN 2004-4 A	Sterile		BDNHR 22-1-2	Fertile	
Class	Total plants		Fertile	Sterile	
F_1	38		38	0	
2. Population of F_2					
Class	Observed (O)	Expected E (3:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	213	206.25	6.75	0.221	**0.347**
Sterile	62	68.75	-6.75	0.663	
Total	275	275	---	**0.884**	
3. Population of test cross (BC_1F_1)					
Class	Observed (O)	Expected E (1:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	76	66.50	9.50	1.357	**0.099**
Sterile	57	66.50	-9.50	1.357	
Total	133	133	---	**2.714**	

Table.4 Inheritance of fertility restoration in a cross BSMR 736 A x BDNHR 21-2

1. Parents and F_1					
BSMR 736 A	Sterile		BDNHR 21-2	Fertile	
Class	Total plants		Fertile	Sterile	
F_1	36		36	0	
2. Population of F_2					
Class	Observed (O)	Expected E (3:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	204	196.50	7.50	0.286	**0.284**
Sterile	58	65.50	-7.50	0.859	
Total	262	262	---	**1.145**	
3. Population of test cross (BC_1F_1)					
Class	Observed (O)	Expected E (1:1)	Deviation (O - E)	X^2	P value for 1 degree of freedom
Fertile	82	72.5	9.5	1.245	**0.115**
Sterile	63	72.5	-9.5	1.245	
Total	145	145	---	**2.490**	

These findings were in consonance with earlier observations of Dalvi *et al.,* (2008) who reported that three hybrids *viz.* 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 while hybrid ICPA 2039 x ICP 10650 segregated two dominant duplicated gene action with a ratio of 15 fertile : 1 sterile in F_2 and 3 fertile : 1 sterile in BC1F1 and one 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 for inheritance of fertility restoration in pigeonpea. Further, Saxena *et al.,* (2011) evinced that extra-early-maturing hybrid (ICPA 2089 x PHR 31) was governed by mono gene with the segregation ratio of 3 fertile: 1 sterile in F_2 and 1 fertile : 1 sterile in BC_1F_1 while early-maturing hybrids ICPA 2039 x ICPR 2438 and ICPA 2039 x ICPR 2447 and late-maturing hybrid ICPA 2043 x ICPR 2671 and ICPA 2043 x ICPR 3497 were governed by digenic duplicate dominant ratio of 15 fertile: 1 sterile in F_2 and 3 fertile : 1 sterile in BC_1F_1. Similar findings were also obtained by Sawargaonkar *et al.,* (2012) and observed monogenic inheritance (3:1) in the hybrid ICPA 2092 x ICP 2766, however digenic inheritance (15:1) of fertility restoration in the hybrid ICPA 2043 x ICP 2766 in pigeonpea. Similar trends were also observed by Meshram and Patil (2018) indicating the three hybrids *viz.* AKCMS 10A x AKPR 303, AKCMS 11A x AKPR 303, AKCMS 11A x AKPR 359 exhibited a monogenic dominance of fertility restoring gene, while in only one hybrid AKCMS 11A x AKPR 324 exhibited two duplicate dominant genes for fertility restoration in pigeonpea.

Similarly, monogenic and digenic duplicate gene action with complete dominance for fertility restoration in F_1 hybrids derived from CMS lines having A4 cytoplasm was depicted by Choudhary and Singh (2015). However, Saroj *et al.,* (2015) revealed four different types of gene interaction i.e. inhibitory. supplementary, masking and complimentary gene actions showing 13: 3, 9 : 3 : 4, 12 : 3 : 1 and 9 : 7 and 3:1, 1 : 1 : 2, 2 : 1 : 1 and 1 : 3 in F_2 and BC_1F_1 populations, respectively for inheritance of fertility restoration in pigeonpea. The monogenic inheritance for fertility restoration were in conformity with those of Sheikh *et al.,* (2016). Sharma *et al.,* (2018) reported similar results on the basis of plant fertility and pollen viability, the genetic ratios of F_2, BC_1 F_1 and F_3 populations of hybrid AL 100A × AL 1599) revealed that fertility restoration was controlled by two duplicate dominant genes in pigeonpea.

The information generated on inheritance of fertility restoration will help in knowing the selection of breeding methods and further transfer of fertility restorer genes in to elite backgrounds. The number of genes identified will help to transfer in to other genotype by backcross methods. The data on fertility restoration of restorer lines may be used for diversification and for development of heterotic cross combinations.

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