International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 9, Issue 9, 2017, pp.-3968-3971.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

STUDY ON GENE ACTION AND FERTILITY RESTORATION IN F_1 HYBRIDS INVOLVING CMS LINES AND RESTORER LINES IN PIGEONPEA [*Cajanus cajan* L. Millsp]

SONI NEETU*[1], PATEL P.T.[2], SURESH K.[3] AND TAK VIBHA[4]

[1]Department of Genetics and Plant Breeding, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, 385506, Gujarat, India
[2]Seed Spices Research Station, Sardarkrushinagar, Jagudan, 382710, Gujarat, India
[3]Department of Crop Physiology, International Crops Research Institute for the Semi-Arid Tropics, ICRISAT, Hyderabad, 502319, Telangana, India
[4]Department of Agricultural Meteorology, Anand Agricultural University, Anand, 388110, Gujarat, India
*Corresponding Author: Email-neetusoni639@gmail.com

Received: January 19, 2017; Revised: February 11, 2017; Accepted: February 12, 2017; Published: February 24, 2017

Abstract- Line x tester analysis was carried out involving six newly converted cytoplasmic male sterile lines and five fertility restorer lines for gene action and fertility restoration in pigeonpea [*Cajanus cajan* L. Millsp.]. Synthesized hybrids were evaluated with check Gujarat Tur Hybrid 1, Gujarat Tur 101 and Vaishali at Sardarkrushinagar. Combining ability analysis revealed presence of both additive and non additive gene effects. The values of average degree of dominance were more than unity (>1) and predictability ratio was less than unity (<1) for all the traits except days to flowering, days to maturity, plant height and biological yield, which indicated preponderance of non-additive gene action. The CMS lines, restorer lines, standard checks and hybrids were tested for fertility restoration by observing the pollen fertility status. The male sterile lines (P_1) exhibited 100 per cent pollen sterility. The pollen fertility in fertility restorer lines (P_2) varied from 98 to 100 per cent. The F_1 generation of all the crosses exhibited consummate fertility restoration with pollen fertility varying from 92 to 100 per cent. Thus it is evident that there were striking differences between the male sterile and male fertile lines for pollen fertility and that of resulting hybrids. Thus the success in development of pigeonpea hybrids largely depends on availability of effective pollen fertility restorer lines for evaluating fertile hybrids.

Keywords- Cytoplasmic male sterile lines, Fertility Restorers, Hybrids, Pigeonpea

Citation: Soni Neetu, et al., (2017) Study on Gene Action and Fertility Restoration in F_1 Hybrids Involving CMS Lines and Restorer Lines in Pigeonpea [*Cajanus cajan* L. Millsp.]. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 9, Issue 9, pp.-3968-3971.

Academic Editor / Reviewer: Kaur Harjeevan, Sinha Sweta

Introduction

Pigeon pea [*Cajanus cajan* L. Millsp.] is second most important pulse crop of India after chickpea commonly known as Red gram, Tur or Arhar. It is consumed on a large scale mainly in south Asia and is a major source of protein for the population of the subcontinent .The chromosome number of all *Cajanus* species is n=11. It's a perennial legume from the family Fabaceae [1]. However, the progress in the genetic improvement of yield potential has been limited and the improved cultivars failed 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, fruits, and vegetable crops was attempted in pigeonpea to enhance the yield [2].

The development of hybrid technology in pigeonpea was initiated with the discovery of two sources of genetic male sterility (GMS) sources from germplasm [3]. However, the technology suffers from a major technical bottleneck when it comes to large scale seed production. The need for rouging out of 50% of the fertile plants from the female parent by the use of genetic markers was a costly and skill oriented operation which escalated seed cost. To overcome the seed production problems associated with GMS, new CMS (Cytoplasmic Male Sterile) systems were developed using various wild relatives of pigeonpea.

Fertility restoration is a vital component of CMS-based hybrid technology. Cytoplasmic Nuclear-Male Sterility (CMS) is maternally inherited and associated with specific (mitochondrial) genes without otherwise affecting the plant [4]. The fertility restorer (***Rf*** or ***Fr***) genes in the nucleus suppress the male-sterile phenotype and allows commercial exploitation of the CMS system for the production of hybrid seeds. The system viable commercially to produce high yielding and heterotic hybrids as it eliminates need for labour and time intensive tedious hand emasculation [5].

Materials and Methods

Experimental Site and Agro-Climatic Details

The study was conducted at the experimental field of the Main Pulses Research Station, Sardar krushinagar Dantiwada Agricultural University, Sardar Krushinagar, Gujarat. The experiment conducted during *kharif* 2015. The latitude and longitude were 24^0 12' N and 72^0 12' E. The altitude and soil type were 154.5 m and loamy sand, for these location.

Experimental material

The experimental materials comprised of six cytoplasmic male sterile line used as fertile counterpart, five pollen fertility restorer line as male parents, thirty synthesized hybrids and standard check *viz*. GTH 1, GT 101 and VAISHALI were obtained from the Main Pulses Research Station, Sardar Krushinagar, Gujarat.

Land Preparation and Plot Layout

The field was ploughed, harrowed twice and ridged with a farm tractor. The experiment was laid out in a randomized block design (RBD) with three replications. Each genotype was represented by a single row plot of 4.0 m length.

The inter and intra row distances were 60 and 20 cm, respectively which accommodated 20 plants per plot. All the agronomical practices and plant protection measures were followed for raising the good crop.

Measurement of growth Characteristics

The observations were recorded both as visual assessment on plot basis and measurement on individual plants. The individual plant observations were recorded on five randomly selected competitive plants. The border plants were excluded for such observations.

Pollen fertility (%)

Single flower from each selected plant of genotype were used for microscopic pollen fertility, the test for fertility and sterility of pollen grains was done as per 2 % aceto-carmine stain method proposed by [6].

$$\text{Pollen fertility (\%)} = \frac{\text{Number of stained pollens}}{\text{Total number of pollengrains examined}} \times 100$$

Statistical Analysis

The replication wise mean values of each genotype for different characters were calculated and data obtained was subjected to analysis of variance (ANOVA) used for statistical analysis as per the procedure of Randomized Block Design as suggested by [7]. The computer facility available at Department of Agricultural Statistics, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar were utilized for statistical analysis. The data were analyzed for combining ability following method suggested by [8].

Results and Discussions

The magnitude of estimate of variance [Table-1 and 2] of lines was observed greater for days to flowering, days to maturity, plant height, seed yield per plant, biological yield and leaf area, which showed contribution of female lines to these traits more than male parents. The interaction variances due to lines x testers were highly significant for all the characters except plant height, exhibited that the specific combining ability variances were important for the inheritance of significant traits.

Table-1 *Analysis of variance for combining ability for different characters in pigeonpea*

Parents	d.f	Days to flowering	Days to maturity	Plant height (cm)	Number Of branches per plant	Number of pods per plant	Number Of seeds per pod	Pod length (cm)
Replications	2	2.02	0.84	320.48	0.17	185.06	0.03	0.07
Females (Lines)	5	1552.55**	1301.51**	2973.53**	2.38	6186.35	0.31	0.39
Males (Testers)	4	3.00	75.62	604.50**	11.32**	7522.87*	0.35	0.35
Females x Males	20	22.59**	33.92*	190.60	2.54**	3373.54**	0.36**	0.56**
Error	58	6.84	16.07	135.88	0.45	286.87	0.05	0.21
Components of Variance								
σ^2 Females		101.99**	84.51**	185.51**	-0.01	187.52	0.10	0.06
σ^2 Males		0.52	2.32	22.99**	0.49**	230.52*	0.10	0.03
σ^2_{gca}		46.64**	39.67**	96.86**	0.26*	210.97	0.0016	0.026
σ^2_{sca}		5.25**	5.96*	18.25	0.69**	1028.89**	0.09**	0.028**
σ^2_{gca} / σ^2_{sca}		8.80	6.65	5.30	0.37	0.20	0.0177	0.93

*, ** indicate P< 0.05, P< 0.01 respectively

Table-2 *Analysis of variance for combining ability for different characters in pigeonpea*

Parents	d.f	100 seed weight (g)	Seed yield per plant (g)	Harvest index (%)	protein content (%)	Biological Yield	Leaf area (cm²)
Replications	2	0.25	62.91	2.32	0.13	729.42	5961.95
Females (Lines)	5	0.05	3120.50**	21.60**	1.41	58428.80**	1047870**
Males (Testers)	4	0.24	2013.78**	14.58**	1.29	29897.33**	577783.10**
Females x Males	20	0.33**	931.44**	38.24**	0.97**	6912.26**	154289.10**
Error	58	0.14	34.46	4.19	0.20	485.62	14484.99
Components of Variance							
σ^2 Females		-0.02	145.94**	-1.11**	0.03	3434.44**	59572.08**
σ^2 Males		-0.01	60.13**	-1.31**	0.02	1276.95**	23527.44**
σ^2_{gca}		-0.0115	99.13*	-1.22	0.02	2257.62**	39911.37**
σ^2_{sca}		0.06**	298.99**	11.12**	0.25**	2142.22**	86512.73**
σ^2_{gca} / σ^2_{sca}		0.17	0.33	-0.10	0.09	1.05	0.46

*, ** indicate P < 0.05, P < 0.01 respectively

The ratio of variance due to specific vs. general combining ability [σ^2_{gca} / σ^2_{sca}] being more than unity was found for days to flowering, days to maturity, plant height and biological yield, which suggested greater role of additive genetic variance in the inheritance of these traits. These traits can be improved further as a source of favourable genes for earliness and yield through selection of desired transgressive segregants from segregating generation. The above results were in accordance with the findings of [9]. For plant height and biological yield additive gene action reported by [10,11]. For remaining traits non-additive type of gene action was predominant. and its components have also been reported by [12]. Preponderance of non-additive genetic variance suggested the relevance of heterosis breeding in pigeonpea.

Cytoplasmic Genetic Male Sterility (CGMS) system of hybrid technology, which is based on cytoplasmic-nuclear male-sterility, is expected to increase the productivity of pigeonpea seed yield. Fertility restoration of CMS-based hybrids is an integral part of breeding hybrids and the development of new hybrid parents with desirable agronomic traits. The availability of diverse male sterile lines and their fertility restoration had played an important role in exploiting hybrid vigor at commercial scale including pigeonpea also. Pigeonpea being an often cross pollinated crop, even the minor genetic purity can onset cyclic genetic upheavals that may jeopardize and bluster the whole heterosis breeding programme. Many genes are involved for fertility restoration and the knowledge of genetic control of fertility restorations is equally seminal for precise handling of the genes during transfer of fertility restoration gene(s) for diversification of restorer lines that forms another important aspect of heterosis breeding [13].

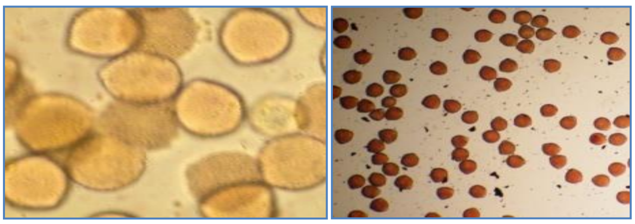
Pollen fertility test carried out for confirmation of fertility in F_1 hybrids is one of the important factor for commercialization of CGMS system based hybrids at door step of farming community. Thirty crosses were developed using six cytoplasmic male sterile lines as female parents and five pollen fertility restorer lines as male parents. All plants of parents (‘A’ lines and male parents) were checked for pollen shedding.

The differences observed due to pollen fertility as tested pollen grains (with 2% acetocarmine solution) of parents ('A' lines and male parents), hybrids and standard checks are given in [Table-3]. The male sterile lines (P_1) exhibited (100 per cent) pollen sterility. The pollen fertility in fertility restorer lines (P_2) varied from 98 to 100 per cent. The F_1 generations of all the crosses exhibited pollen fertility restoration varying from 92 to 100 percent. Standard checks GTH 1, GTH 101 and VAISHALI exhibited 97, 98 and 97 per cent pollen fertility respectively. The pollen fertility was observed under light microscope and on the basis of its staining properties, pollen grains were categorized as fertile (deep to light red color) and sterile (shrivelled, hyaline, transparent) of different parents (P_1 and P_2), all the F_1 crosses and standard checks given in [Fig-1]. The anthers of male sterile lines, restorer lines, F_1 hybrids and standard checks were observed on visual basis for pollen fertility status, indicated male sterile lines had translucent type anthers where as fertile lines (male parents, hybrids and checks) had normal pollen shedding in anthers. The results of present findings were in agreement to findings of [14].

Table-3 *Pollen fertility status recorded for female, male parent, F_1 hybrids & standard checks in pigeonpea*

Sr. No.	Genotype	R I*		R II*		R III*		Total		Total pollen observed	Plant fertility	Pollen Fertility (%)
		Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	sterile	Fertile			
P1	GT 301A	69.40	-	74.40	-	58.20	-	202.00	-	202.00	S	0.00
P2	GT 302A	85.20	-	76.40	-	62.00	-	223.60	-	223.60	S	0.00
P3	GT 33A	81.60	-	92.00	-	73.20	-	246.80	-	246.80	S	0.00
P4	GT 288A	52.00	-	63.20	-	86.40	-	201.60	-	201.60	S	0.00
P5	GT 601A	76.20	-	58.80	-	67.60	-	202.60	-	202.60	S	0.00
P6	GT 603A	91.20	-	75.20	-	83.80	-	250.20	-	250.20	S	0.00
P7	GTR 8	-	104.60	-	86.20	-	112.00	-	302.80	302.80	F	100.00
P8	GTR 18	-	96.20	-	110.20	-	78.40	-	284.80	284.80	F	100.00
P9	GTH 23	-	131.20	-	108.60	-	120.00	-	359.80	359.80	F	100.00
P10	GTH 52	4.00	87.80	-	111.40	2.00	124.40	6.0	323.60	329.60	F	98.18
P11	GTH 95	1.20	140.00	3.20	122.00	1.40	107.20	5.80	369.20	375.00	F	98.45
12	CMS GT 301 A X GTR 8	-	165.60	-	98.20	-	130.00	-	393.80	393.80	F	100.00
13	CMS GT 301 A X GTR 18	3.00	156.20	2.00	87.00	5.40	122.60	10.40	365.80	376.20	F	97.24
14	CMS GT 301 A X GTR 23	-	145.20	-	101.40	-	79.20	-	325.80	325.80	F	100.00
15	CMS GT 301 A X GTR52	10.20	136.40	8.60	87.20	3.20	142.40	22.00	366.00	388.00	F	94.33
16	CMS GT 301A X GTR 95	2.40	134.00	1.40	93.20	3.00	117.20	6.80	344.40	351.20	F	98.06
17	CMS GT 302 A X GTR 8	-	122.00	-	146.40	-	109.40	-	377.80	377.80	F	100.00
18	CMS GT 302 A X GTR 18	4.40	162.20	6.20	132.40	5.00	118.20	15.60	412.80	428.40	F	96.36
19	CMS GT 302 A X GTR 23	-	96.80	-	89.00	-	132.00	-	317.80	317.80	F	100.00
20	CMS GT 302 A X GTR 52	6.20	158.60	9.40	142.60	12.00	126.40	27.60	427.60	455.20	F	93.94
21	CMS GT 302 A X GTR 95	11.40	123.20	14.00	146.80	7.20	116.00	32.60	386.00	418.60	F	92.21
22	CMS GT 33 A X GTR 8	-	98.20	-	133.20	-	153.20	-	384.60	384.60	F	100.00
23	CMS GT 33 A X GTR 18	-	148.40	-	88.20	-	128.40	-	365.00	365.00	F	100.00
24	CMS GT 33 A X GTR 23	3.60	137.00	1.40	130.00	3.40	143.00	8.40	410.00	418.40	F	97.99
25	CMS GT 33 A X GTR 52	1.20	117.20	4.00	144.60	2.00	96.40	7.20	358.20	365.40	F	98.03
26	CMS GT 33 A X GTR 95	-	98.20	-	123.40	-	156.00	-	377.60	377.60	F	100.00
27	CMS GT 288 A X GTR 8	-	167.60	-	128.20	-	107.20	-	403.00	403.00	F	100.00
28	CMS GT 288 A X GTR 18	2.00	146.40	1.60	118.20	3.20	113.40	6.80	378.00	384.80	F	98.23
29	CMS GT 288 A X GTR 23	-	127.00	-	138.60	-	106.20	-	371.80	371.80	F	100.00
30	CMS GT 288 A X GTR 52	6.20	132.40	3.20	105.40	9.20	128.40	18.60	366.20	384.80	F	95.17
31	CMS GT 288 A X GTR 95	-	112.20	-	153.00	-	147.80	-	413.00	413.00	F	100.00
32	CMS GT 601 A X GTR 8	3.40	144.60	1.20	123.20	2.40	74.60	7.00	342.40	349.40	F	98.00
33	CMS GT 601 A X GTR 18	-	163.00	-	116.40	-	135.00	-	414.40	414.40	F	100.00
34	CMS GT 601 A X GTR 23	4.20	78.40	3.00	148.80	2.00	124.20	9.20	351.40	360.60	F	97.45
35	CMS GT 601 A X GTR 52	5.00	94.40	8.40	126.40	4.20	137.40	17.60	358.20	375.80	F	95.32
36	CMS GT 601 A X GTR 95	-	138.20	-	115.00	-	130.00	-	383.20	383.20	F	100.00
37	CMS GT 603 A X GTR 8	-	156.60	-	121.20	-	103.60	-	381.40	381.40	F	100.00
38	CMS GT 603 A X GTR 18	2.40	149.60	1.20	137.20	5.60	125.00	9.20	411.80	421.00	F	97.81
39	CMS GT 603 A X GTR 23	3.20	89.20	2.00	148.20	1.40	127.00	6.60	364.40	371.00	F	98.22
40	CMS GT 603 A X GTR 52	-	107.60	-	105.00	-	135.20	-	347.80	347.80	F	100.00
41	CMS GT 603 A X GTR 95	11.20	136.00	7.40	162.40	5.00	101.20	23.60	399.60	423.20	F	94.42
42	GTH 1	6.40	96.40	1.00	103.80	2.20	115.60	9.60	315.80	325.40	F	97.05
43	GT 101	3.00	121.40	2.60	89.60	1.80	106.40	7.40	317.40	324.80	F	97.72
44	VAISHALI	1.00	78.20	4.00	135.00	2.20	109.20	7.20	322.40	329.60	F	97.82

*** =** Number of fertile/sterile pollen grains, **S** = Sterile, **F** = Fertile, **CMS =** Cytoplasmic Male Sterile, **GTR** Gujarat Tur Restorer



Sterile pollen **Fertile pollen**

Fig-1 Microscopic images for pollen fertility

Conclusion

The concept of gene action is a major landmark in understanding the inheritance and genetic architecture of populations. The ratio of σ^2_{gca} / σ^2_{sca} indicate gene action for the various traits which can be used in further improvement as a source of favourable genes for yield improvement through selection of desired transgressive segregants from segregating generation.

Based on the fertility restoration studies, it concluded that CMS sources used were diverse as in different agronomic background and offers scope to incorporate in heterosis breeding programmes. Thus the success in development of pigeonpea hybrids largely depends on availability of effective pollen fertility restorer lines.

Acknowledgement

The present work was supported and facilities are provided by the Main Pulses Research Station and Department of Genetics and Plant Breeding, Sardar krushinagar Dantiwada Agricultural University, Sardar krushinagar, Gujarat.

Author Contributions: The work is a product of the intellectual environment of the whole team; and that all members have contributed in various degrees to the analytical methods used, to the research concept, and to the experiment design.

Abbreviations: CMS- Cytoplasmic Male sterile System, GTH1-Gujarat Tur Hybrid1.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: None declared

References

[1] Van der Maesen L.J.G. (1980) In: Arends, J.C.; Boelema, G.; de Groot, C.T.; Leeuwenberg, A.J.M. (eds) Liber gratulatorius in honorem H. C.D. de Wit land bou who geschool Miscellaneous paper no 19. Veenman H, Zonen BV, Wageningen, Netherlands. 257–262.

[2] Singh N., Singh P. and Singh P.K. (1996) *International Chickpea and Pigeonpea Newsletter*, 3, 60-62.

[3] Saxena K.B., Byth D.E., Wallis E.S. and Dundas I.S. (1983) In: International Pigeonpea Newsletter, 2, 20 - 21.

[4] Budar F. and Pelletier G. (2001) *Life Sciences*, 324,543-550.

[5] Kyu L.K. and Saxena K.B. (2011) *Journal of Food Legumes*, 24(4), 273-276.

[6] Alexander M.P. (1969) *Stain technolog*, 44, 117-22.

[7] Panse V. G. and Sukhatme P. V. (1967) Statistical methods for agricultural workers.ICAR New Delhi., *2nd Edn.* pp.381.

[8] Kempthrone O. (1957) An introduction to genetic statistics. John Wiley and Sons Publishing.

[9] Sreelakshmi C. H., Sameer Kumar C.V. and Shivani D. (2011) *Electronic Journal of Plant Breeding*, 2, 413-416.

[10] Patel P.T. and Tikka S.B.S. (2014) *Indian Journal of Genet*ics, 74(2), 257-260.

[11] Bhadru D. (2008) *Research on Crops*, 9(3), 661-662.

[12] Pandey N. (2004) *Legume Research*, 27(2), 79-87.

[13] Nadrajan N., Ganesh S.R. and Indira K.P. (2008) *Madras Agricultural Journal*, 95 (7-12), 320 – 327.

[14] Chaudhari S., Tikle A., Uttamchand N., Saxena K.B. and Rathore A. (2015) *Journal of Crop Improvement*, 29(3), 269-280.