Electronic Journal of Plant Breeding, 2(1): 100-106 (Mar 2011)
ISSN 0975-928X

Research Article

Cytological causes of blond psyllium for male sterility

Ashish G. Vala*, Fougat. R.S. ,Roshni.S. and Vinay Kumar

Department of Agricultural Botany, Anand Agricultural University, Anand-388 110, Gujarat
*Email: ashishbiotechnology@gmail.com

(Received:07 Dec 2010; Accepted:09 Dec 2010)

Abstract:
Cytological male sterility is a useful trait in plant breeding, especially in medicinal plants such as *Cassia angustifolia, Commiphora wightii, Asparagus racemosus* .Abnormalities appeared to be the cause of male sterility in plants, we carried out this research in an attempt to make clear the characteristics and inheritance of this male sterility. In *Plantago ovata* germplasm cytoplasmic abnormality was found to be the cause of abnormality. cytomixis varied from 2% to 48 % in plants. Maximum numbers of PMCs carrying B-chromosomes were recorded in male sterile plants of line JI-214(62%). No B-chromosomes were observed in fertile, sterile and partial male sterile line of JI-107 and maximum abnormal chromosomal segregation was recorded in JI-206. These cytological abnormality parameters can be used for hetrosis breeding and hybrid seed production to improve quality of psyllium.

Key words*: P. ovate*, cytoplasmic male sterility, pollen mother cell, sex

Introduction

Cytoplasmic male sterility (CMS) is a common phenomenon among plants and has received much attention due to its potential use in heterosis breeding and hybrid seed production. Male sterility is characterized by the failure of the plant to produce viable or functional pollen. *Plantago ovata* is gynodioecious in nature. In gynodioecious populations, the male sterile plants vary from 2 to 50 % (Lewis, 1942). Paliwal and Hyde (1959) reported that male sterility in *Plantago cornopus* was due to the presence of B-chromosomes. Atal (1958) reported that male sterility in *P. ovata* was of cytoplasmic type. He further reported that sterile plants could be readily distinguished from the normal plants by the shriveled appearance of their anthers as compared to the membraneous, well developed anther of normal plants. However, Atal could not obtain any evidence of fertility restorer gene, and hence cytoplasmic nature of male sterility was not established.

In plants, cytological events of gametogenesis are controlled by a large number of genes that act from pre-meiotic to post-mitosis. Mutations in these genes cause abnormalities that may impair fertility and many abnormalities affecting plant fertility or causing total male sterility have been detected in some species (Maria Suely, 2000). The genetic determinants for cytoplasmic male sterility reside in the mitochondrial genome. Several nuclear genes are known to control expression of cytoplasmic male sterility. Different cytoplasmic male sterility types are distinguished by their specific nuclear genes (rfs) which restore pollen fertility. The occurrence of cytoplasmic variation within population has now been shown in several gynodioecious species e.g., *Plantago lanceolata* (Van Damme and Van Delden, 1982); *P. cornopus* (Koelewijn and Van Damme, 1995).

The chromosome complement of male sterile *P. ovata* plants was identical to that of male fertile plants. The pairs of chromosomes undergo regular meiosis as described by Hyde (1953), but the microspores degenerate soon after separation and no viable pollen grains are formed. This indicated that the probable cause of male sterility may be molecular at gene level rather than cytological. The male sterile plants, when pollinated with pollens from male fertile ones set seed profusely and the progeny was entirely male sterile. Several generations of controlled crossing by different workers indicated that the sterility in *P. ovata* is probably cytoplasmic. So, in order to decipher the causes of male sterility whether cytological or molecular, PMC's were screened for presence of abnormalities such as cytomixis, irregular segregation, presence of univalent, multivalents, translocations, variation in chromosome number, presence of B-chromosome etc., which are known to be the causes of male sterility in many crop plants.

Material and methods

The experimental material for the present investigation comprised of fifteen germplasm lines of *P. ovata*. All of them were found to have all three sex morphotypes (Fig. No. 01). Total of 45 treatments (15X3 sex types i.e. 15 sets) were raised in RBD design with two replications. Different floral biological observations were recorded to characterize male sterility. Genotypes used in the present investigation were Gujarat Isabgol-2, Jagudan Isabgol – 189, 216, 227, 192, 206, 214, 107, 127, 129,130, 131, 132, 137, and 150.

Meiotic studies were carried out on all three sex morphotypes from all lines: For the purpose young spikes were collected during early morning hours between 5:30 to 6:30AM. Collected spikes were fixed in 3 parts absolute ethyl alcohol and 1 part glacialacetic acid (carnoy's fluid), added with pinch of ferric chloride for 24 hours. After 24 hours, the spikes were stored in 70% ethyl alcohol at 4ºC in a refrigerator till further use. Pollen mother cell meiosis was studied by crushing anthers of appropriate size in a drop of acetocarmine stain over micro slide. Slides were prepared following standard procedure. Observations were recorded on 50 dividing PMC's to decipher the cytological cause of male sterility if any. PMC's were screened for presence of abnormalities such as cytomixis, irregular segregation, univalent, multivalent, translocation, variation in chromosome number, presence of B-chromosome etc., which are known to be the cause of male sterility in many crop plants.

Pollen viability (%): Viability of pollen grains was measured by acetocarmine stainability test. The pollen grains from freshly dehisced anthers were collected in watch glass with the help of fine brush over few drops of acetocarmine stain. The stained pollen grains were mounted on micro slide and observed under microscope. Deeply stained pollen grains were counted as viable while unstained, yellowish or shriveled ones as non-viable.

Result and discussion

Meiosis is an event of high evolutionary stability which culminates in reduction of chromosome number to half. In plants cytological events of gametogenesis are controlled by a large number of genes that act from premeiotic to postmitosis. Mutations in these genes cause abnormalities that may impair fertility and many abnormalities affecting plant fertility or causing total male sterility have been detected in some species (Maria Suely, 2000).

Variation in chromosome number: A total of 50 PMC's were screened among all three sexmorphotype of each line to look for variation in chromosome number if any. Variation was observed only in JI-107 of pollen mother cells (PMC's) showing variation in chromosome number. Presence of abnormality was recorded in male fertile and partial male sterile line of JI-107 with 1(2%) followed by male sterile line with 21(42%) [Table 1, Plate. No.1.]

Cytomixis***:*** Cytomixis represents the migration of chromatin material from one cell to another and was observed in pollen mother cells (PMC's) of *Plantago ovata* where a total 50 cells were studied from each sex morphotype in each line. The percentage of PMC's showing cytomixis ranged from 2.0 to 48.00%. Maximum cytomixis was recorded in sterile morphotype of line JI-189 (48%) and minimum in PMC's of fertile morphotype of line JI-192 and few others (2%) [Table No. 1]. Cytomixis was observed at all stages of meiosis, but became less frequent in the later stages. No cytomixis observed in PMC's of JI-214,JI-150, JI-129, JI-227. Direct nuclear fusion or cytoplasmic strands between cells were noted, so that sometimes a series of PMC's were connected together (Plate. No.2). Cytomixis was often accompanied by other meiotic aberrations, such as univalent, laggards etc. Level of pollen stainability was less in plants showing high percentage of cytomixis. Pagliarini (2000) reported in *Paspalum subciliatum* that the most frequent abnormalities included irregular chromosome segregation, cytomixis, chromosome stickiness, mixoploidy, chromosome fragmentation, syncyte formation, abnormal spindles and failure of cytokinesis which caused total male sterility in this species. Nirmala, and Kaul. (1994) studied male sterility in mutant plants of pea where cytological examination showed Cytomixis and meiotic arrest in one-third of the PMC's. The main abnormalities being desynapsis, incipient metaphase I plate formation, irregular anaphase I and II disjunctions and production of dyads, tetrads and polyads of unequal size. All the microspores degenerated and the mutant was completely male sterile. Cytological abnormalities named above were reported to be the cause of male sterility.

B-chromosome***:*** Male sterile plants in some lines carried a single extra chromosome which was largely hetrochromatic, shorter and did not pair with any of the other chromosomes (Plate. No.3). In microscopic examination total 50 PMC's cells were studied from each sex morphotype of each line. Percentage of

pmc's carrying B- chromosomes ranged from 2 to 62 %. Maximum chromosome carrying PMC's were recorded in sterile line of JI-214 with 31(62%), [Table No.1.] No B-chromosomes were observed in fertile, sterile and partial male sterile line of JI-107. Meiosis was regular in pmc's carrying B-chromosome. The accessory chromosome usually did not divide and moved to one pole during first division of meiosis which probably lead to non functional pollens in male sterile plants. Generally B-chromosome was absent in fertile plants but was regularly observed in partial male sterile and male sterile plants. Palliwal and Hyde (1959) also reported that the male sterility in *P. ovata* was probably cytoplasmic but at the same time also reported that the male sterility in *P. cornopus* was associated with the B-chromosomes. In the present study also presence of single B-chromosome appears to be the likely cause of male sterility in *P. ovata*. Roman (1948) reported that maize sperm with two B-chromosomes preferentially fertilize the egg in competition with sperm containing no B chromosome. Thus, these chromosomes may be considered as useful in the pollen grain, through they may not necessarily be useful or beneficial to every fertilized egg that receives them. However in the present study reverse has been noticed. Randolph (1941) reported for the first time that in maize, when too many accessory chromosomes are present in the same nucleus, there was some reduction in the vigor and fertility. Muntzing (1949), Ostergren (1947), and Bosemark (1957) and Ehrendorfer (1957) all have reported that accessory chromosomes, when accumulated beyond an optimal number, result in reduction of vigor and fertility. The optimal dosage of these accessory chromosomes is governed by various factors including the genotype of the individual whereas in Swedish rye two accessory chromosomes produced a significant effect, on fertility. The condition in *Platnago coronopus* was however unique. Plants containing a single B-chromosome were marked by complete male sterility. However, in *P. ovata* this does not hold true, through fertility was reduced.

Translocations ***:*** A total of 50 PMC's were screened among all three sexmorphotype of each line to look for presence of translocations if any. Maximum PMC's showing translocation were recorded in male sterile line of JI-132 with 15(30%) followed by partial male sterile line with 11(22%) [Table No.1, Plate. No.5] Minimum translocations were served in partial male sterile line of JI-189 with only 2(4%) pmc's carrying translocation. No translocations were recorded in JI-127, JI-129, JI-130, JI-192 and JI-216. Sharma *et al*., (1984) studied translocation hetrozygote which was detected in the *P.lanceolata* L. population. Sixty to sixty four per cent pmc's showed translocations. At diplotene, chromosomes were paired as bivalents, trivalents, quadrivalents, and hexavalents.

Chromosome segregation***:*** The observations were recorded on chromosome segregation at anaphase-I among all the three sexmorphotype of each line. Majority of the pmc's followed regular and normal segregation with 4:4 chromosome segregating to two poles. However, number of lines depicted pmcs undergoing unequal segregation [Plate.No.6]. Results pertaining to chromosomal segregation and chromosomal abnormalities are presented in Table 1. For this purpose total of 50 pmcs were studied from each sex type of each line. Maximum pmcs with abnormal chromosomal segregations were recorded in male sterile line of JI-206 *i.e.*, 29 (58%) followed by male sterile line of JI-150 i.e., 28 (56%). No abnormal chromosomal segregation was recorded in any of the sex morphotype of JI-189. In Gujarat Isabgol-2, no irregular chromosomal segregation was recorded in fertile line but partial male sterile line had 3(6%) and male sterile line recorded 23(46%) per cent pmcs showing irregular segregation respectively. Koul and Sharma (1986) also reported certain segregation abnormalities which were deviations from the normal chromosome segregation. Such abnormalities affected the pollen fertility and lead to male sterility. They studied the inherent property of non-disjunction of some bivalents in *P. ovata* and four of its wild allies, namely *P. drummondii*, *P. lagopus*, *P. lanceolata and P.major*. Non-disjunction and delayed disjunction upset chromosome segregation. In at least two of these species, genetically imbalanced gametes were observed, which on getting involved in fertilization lead to the formation of trisomics. The cause for non-disjunction was not quite clear. According to them among the other factors which control disjunction, the site and size of heterochromatin were also significant. Chromosomes of *P. ovata* and its allies are highly heterochromatic; the heterochromatin flanks the centromere in most of the cases. Similar observations were recorded during the present study also.

Abnormal Pairing**:** A total of 50 PMC's were screened among all three sexmorphotype of each line to look for abnormal pairing if any. Maximum PMC's showing abnormal pairing were recorded in male sterile line of JI-130 and JI-132 followed by JI-216 with 38(76%).

Pollen viability**:** Pollen viability was determined by acetocarmine stainability test. The pollen grains which stained deep red and looked normal in shape under microscope were counted as viable, while shriveled and unstained pollen grains were considered as non-viable. Within the group, pollen viability varied from 81.46% to 96.41% (Table No.1) in fertile plants whereas partial male sterile and male sterile ranged 21.36to 32.18, 0 to 0.6 %. Maximum viability was recorded in fertile line of JI-132 with 96.41 %. Completely non viable pollen observed in male sterile line of GI-2, JI-214, JI-206, JI-107, JI-129 and JI-130. Lower the pollen viability, higher the chromosomal abnormality seems to be the reason in the present study also which caused male sterility and non stain pollen grains. Similar type of results were found by Koul and Sharma (1986) and Zadoo and Farooqi (1977) who reported 95.0% pollen viability in *P. ovata.* Plants are classified into different fertility – sterility group as was done by Chaudhary *et al*. (1981) where in those with more than 60% fertile pollen grouped as fully fertile, 1-30% fertile pollen as partial sterile and 0% complete sterile. Hyde (1953) reported that the chromosome complement of male sterile *P. ovata* is identical to that of male fertile plants. Though 4 pairs of chromosomes undergo regular meiosis, the microspores degenerate soon after separation and no viable pollen grains are formed.

References

Atal, C. K. (1958). Cytoplamic male sterility in *plantago ovata* Forsk. Curr Sci. 27: 268.

Bosemark, N. O. (1957). On accessory chromosomes in *Festuca pratensis.* V. Influence of accessory chromosomes on fertility and vegetative development. *Hereditas*. **43**: 211-235.

Chaudhary, R. C.; Virmam. S.S. and Khash. G. S. (1981). Pattern of pollen abortion in some cytoplasmic – genetic male sterile line of rice. *Hereditas*.**18**: 140-142.

Ehrendorfer, F. (1957). Akzessorische chromosomen, Kreuzungs fertilitat und polyploidie beim *Achillea millefolium Komplex* (*Compositeae*). *Naturwiss.* **44:** 505-506.

Hyde B B (1953). Differentiated chromosomes in *Plantago ovata. American Journal of Botany.* **40:** 809-815.

Koul A K and Sharma P K (1984). Cytogenetic studies of *Plantago ovata* Forsk. and its wild allies. Reprinted from Genetics and Crop Improvement, Ed. P. K.Gupta and J. R. Bahl , Rastogi and Company, Meerut. Pp 359-366.

Koelewijn, H. P. and Van Damme, J. M. M. (1995). Genetics of male-sterility in gynodioecious *Plantago coronopus* cytoplasmic variation. *Genetics.* **139:** 1749-1758.

Maria Suely, P.(2000). Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. *Genet and Mol Biol.* **23**(4): 997-1002.

Muntzing A (1949). Cytological studies of extra fragment chromosomes in rye. II transmission and multiplication of standard fragments and isogragments. *Hereditas.* **31**: 457-477.

Nirmala C and Kaul (1994). Male sterility in pea VIII: deterministic degenerative male meiosis. *Journal of Phytological Research*. **9**(1): 81-88.

Lewis, D. (1942). The evolution of sex in flowering plants. *Biol Rev.* **17:** 46-47.

Ostergen G (1947). Heterochromatic B-chromosomes in *Anthoxanthum. Hereditas.* **33:** 261-269.

Pagliarini M S (2000). Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. *Genetics and Molecular Biology*. **23**(4): 997-1002.

Paliwal R L and Hyde B B (1959). The association of a single B- chromosome with male sterility in *Plantago coronopus*. *Amer. J. Bot.* **46**: 460-66.

Pakozdi K, Taller J, Alfoldi Z and Hirata Y (2002). Pepper *(Capsicum annuum* L.) cytoplasmic male sterility. *Journal of Central European Agriculture. **3***(2): 149-157.

Randolph, L. R. (1941). Genetic characteristics of the B-chromosome in maize. *Genetics.* **26**: 608-631.

Roman, H. (1948). Directed fertilization in maize. *Proc. National Acad. Sci.* **34** : 36-42.

Sharma, P. K.; Langer, A. and Koul, A. K. (1984). Genetic diversity among *Plantagos* VI. Satellite associations in *Planatgo ovata* Forsk. *Curr. Sci*. **58** (6): 321-323.

Van Damme, J. M. M. and Van Delden, W. (1982). Gynodioecy in *Plantago lanceolata* L. I polymorphism for plasmon type. *Heredity.* **49**: 303-318.

Zadoo, S. N. and Farooqi, M. I. H. (1977). Performance of autotetraploid blond psyllium. *Indian Journal of Horticulture*. **34**: 294-300.

.

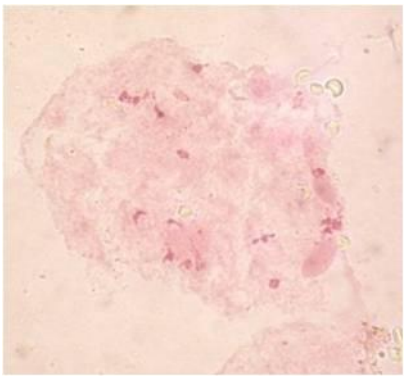

Plate. 1 Meiosis in male sterile plant of Plantago ovata; showing varying chromosome number.

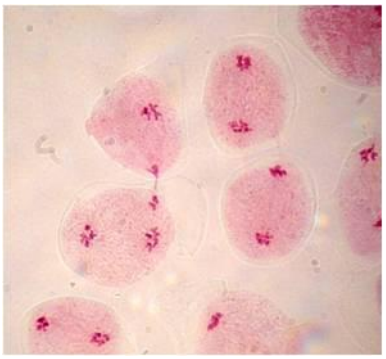

Plate 2. Cytomixis in male sterile plant

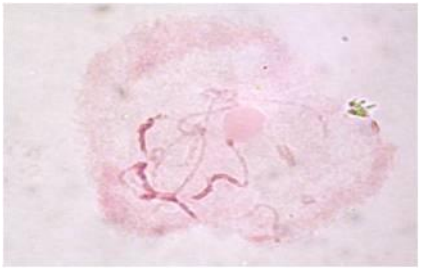

Plate 3. A pmcs showing one B- chromosome in *P. ovata*

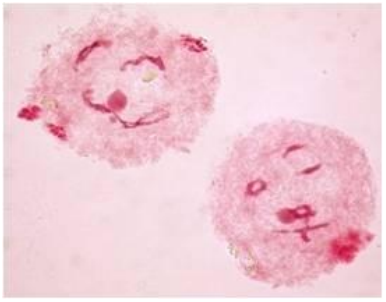

Plate 4. A pmcs showing translocations in *P. ovata*.

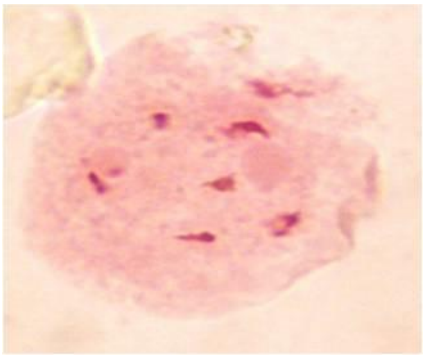

Plate 5. Meiosis in male sterile plant showing chromosomal irregular segregation.

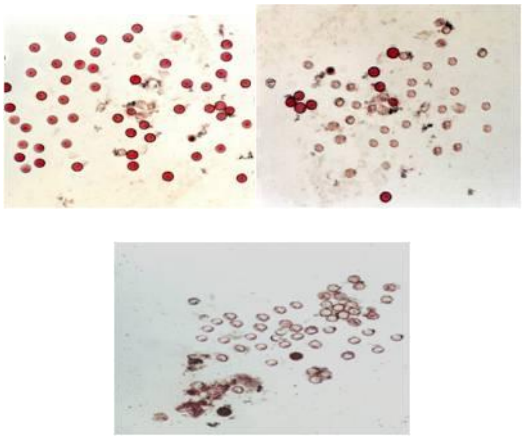

Plate 6. Pollengrains of different fertile, partial male sterile and male sterile morphotypes of *P. ovate*

Table 1. . Cytological analysis of different germplasm lines for cytological causes of male sterility

Germplasm 1	Total cell's studied 2	Normal PMC (%) 3	Number and percent of pmcs showing abnormality						Pollen Viability (%) 10
			Varying chromosome number 4	Cytomixis 5	B-chromosome 6	Translocation 7	chromosomal segregation 8	Abnormal pairing 9	
GI-2									
Fertile	50	49(98%)	-	1(2%)	-	-	-	-	93.33
Partial male sterile	50	28(56%)	-	-	6(12%)	9(18%)	3(6%)	4(8%)	29.46
Male sterile	50	6(12%)	-	9(18%)	12(24%)	-	23(46%)	-	0
JI-216									
Fertile	50	47(94%)	-	-	-	-	3(6%)	-	86.39
Partial male sterile	50	31(62%)	-	6(12%)	5(10%)	-	8(16%)	-	28.35
Male sterile	50	4(8%)	-	4(8%)	4(8%)	-	-	38(76%)-	0.5
JI-214									
Fertile	50	47(94%)	-	-	1(2%)	-	2(4%)	-	87.38
Partial male sterile	50	35(70%)	-	-	7(14%)	-	4(8%)	4(8%)	29.41
Male sterile	50	3(6%)	-	-	31(62%)	8(16%)	3(6%)	5(10%)	0
JI-150									
Fertile	50	49(98%)	-	-	-	-	1(2%)	-	85.96
Partial male sterile	50	31(62%)	-	-	5(10%)	4(8%)	10(20%)	-	31.10
Male sterile	50	2(4%)		-	17(34%)		28(56%)	3(6%)	0.4
JI-192									
Fertile	50	48(96%)	-	1(2%)	-	-	-	1(2%)	91.36
Partial male sterile	50	35(70%)	-	5(10%)	1(2%)	-	4(8%)	5(10%)	29.34
Male sterile	50	2(4%)	-	18(36%)	13(26%)	-	7(14%)	10(20%)	0.6
JI-206									
Fertile	50	48(96%)	-	-	1(2%)	-	1(2%)	-	94.38
Partial male sterile	50	33(66%)	-	-	5(10%)	3(6%)	9(18%)	-	21.37
Male sterile	50	2(4%)	-	3(6%)	-	-	29(58%)	16(32%)	0
JI-107									
Fertile	50	46(92%)	1((2%)	-		-	-	3(6%)	93.49
Partial male sterile	50	27(54%)	1(2%)	3(6%)	-	7(14%)	12(24%)	-	26.47
Male sterile	50	2(4%)	21(42%)	-	-	-	22(44%)	5(10%)-	0
JI-132									
Fertile	50	49(98%)	-	-	1(2%)	-	-	-	96.48
Partial male sterile	50	29(58%)	-	1(2%)	6(12%)	11(22%)	-	3(6%)-	28.54
Male sterile	50	3(6%)	-		2(4%)	15(30%)	13(26%)	17(34%)	0.2
JI-189									
Fertile	50	46(92%)	-	3(6%)	-	-	-	1(2%)	81.46
Partial male sterile	50	34(68%)	-	5(10%)	-	2(4%)	-	9(18%)	29.14
Male sterile	50	1(2%)	-	24(48%)	17(34%)	-	-	8(16%)	0.2
JI-129									
Fertile	50	43(96%)	-	-	1(2%)	-	1(2%)	-	93.21

Table 1 . contd..

Germplasm	Total cell's studied	Normal PMC (%)	Number and percent of pmcs showing abnormality						Pollen Viability (%)
			Varying chromosome number	Cytomixis	B-chromosome	Translocation	chromosomal segregation	Abnormal pairing	
1	2	3	4	5	6	7	8	9	10
Partial male sterile	50	31(62%)	-	-	4(8%)	-	7(14%)	8(16%)	25.54
Male sterile	50	1(2%)	-	-	16(32%)	-	21(42%)	12(24%)	0
JI-130									
Fertile	50	49(98%)	-	1(2%)	-	-	-	-	82.43
Partial male sterile	50	37(74%)	-	3(6%)	7(14%)	-	2(4%)	1(2%)	23.16
Male sterile	50	2(4%)	-	9(18%)	12(24%)	-	9(18%)	18(36%)	0
JI-227									
Fertile	50	48(96%)	-	-	-	-	2(4%)	-	91.23
Partial male sterile	50	37(74%)	-		3(6%)	-	9(18%)	1(2%)	27.59
Male sterile	50	3(6%)	-	-	12(24%)	7(14%)	17(34%)	11(22%)	0.2
JI-127									
Fertile	50	47(94%)	-	-	1(2%)	-	2(4%)	-	91.26
Partial male sterile	50	31(62%)	-	4(8%)	7(14%)	-	5(10%)	3(6%)	30.18
Male sterile	50	2(4%)	-	17(34%)	11(22%)	-	5(10%)	15(30%)	0.5
JI-137									
Fertile	50	48(96%)	-	-	2(4%)	-	-	-	91.21
Partial male sterile	50	28(56%)	-	1(2%)	7(14%)	5(10%)	9(18%)		21.36
Male sterile	50	5(10%)	-	10(20%)	14(28%)	9(18%)	-	12(24%)-	0.3
JI-131									
Fertile	50	46(92%)	-	3(6%)	-	-	-	1(2%)-	90.45
Partial male sterile	50	33(66%)	-	7(14%)	5(10%)	3(6%)	-	2(4%)-	32.18
Male sterile	50	4(8%)	-	13(26%)	9(18%)	-	9(18%)	15(30%)	