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GENETIC ANALYSIS OF MALE STERILITY GENES IN DIFFERENT A AND B SORGHUM LINES

Z.M. LAWAN, M.Y. YEYE[1], M.F. ISHIYAKU[1], S.M. BUGAJE[1], H.O. AHMED[1] and A.S. SHAIBU

Department of Agronomy, Faculty of Agriculture, Bayero University, Kano, Nigeria
[1]Department of Plant Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria
Corresponding author: asshuaibu.agr@buk.edu.ng

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ABSTRACT

Hybrid seed production requires use of cytoplasmic male sterility (CMS). Without this system, hybrid seed production would not be economically feasible. There is, therefore, need for developing A and B sorghum lines, as an essential step for development of hybrid sorghum industry. A genetic study of male sterility in different A and B sorghum (*Sorghum bicolor* (L.) Moench) lines was conducted at the research farm Institute for Agriculture Research in Samaru and Kadawa. Chi-square test revealed goodness-of-fit to single gene of observed proportion of sterile and fertile plants to the expected ratio in all the backcross generations, thereby upholding the assumption of single gene inheritance for the traits studied. Stability of male sterile genes across generations of backcrosses indicated that sterility was inherited 68 to 95% among the different genotypes. Based on high number of sterile plants, crosses 159 x 160 and 421 x 422 are the best in terms of breeding potential for male sterility.

Key Words: Backcrosses, Chisquare, *Sorghum bicolor*

RÉSUMÉ

La production des semences hybrides nécessite l'utilisation de la stérilité mâle cytoplasmique (CMS). Sans ce système, la production des semences hybrides ne serait pas économiquement faisable. Il y a donc, un besoin pour développer des lignées A et B du sorgho, comme une étape essentielle dans le développement d'industrie de sorgho hybride. Une étude génétique de la stérilité mâle dans différentes lignées A et B du sorgho (*Sorghum bicolor* (L.) Moench) a été conduite à la ferme d'expérimentation de l'Institut des Recherches Agricoles de Samaru et Kadawa. Le test de Chi-carré a révélé la qualité d'association d'un seul gène de la proportion observée de plants stériles et fertiles au ratio observé dans les générations de rétrocroisement, ainsi soutenant l'hypothèse de l'héritage d'un gène pour les traits étudiés. La stabilité de gènes de la stérilité mâle à travers les générations du rétrocroisement a indiqué que la stérilité était héritée entre 68 à 95% entre les différents génotypes. Sur la base du nombre élevé de plantes stériles, les croisements 159 x 160 et 421 x 422 sont les meilleurs en termes du potentiel d'amélioration génétique pour la stérilité mâle.

Mots Clés: Rétrocroisement, Chi-carré, *Sorghum bicolor*

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop in the world after rice, wheat, maize and barley (FAO, 2015). It constitutes the main grain food for over 750 million people, who live in the semi-arid tropics of Africa, Asia and Latin America (FAO, 2015). Sorghum is indigenous to semi-arid tropics of Africa (Dewar, 2003). Being a C4 species with higher photosynthetic ability, and greater nitrogen and water-use efficiency, sorghum is genetically suited to hot and dry agro-ecologies where it is difficult to grow other food crops, especially due to frequent droughts.

The demands for cereals, including grain sorghum is progressively increasing due to population growth, yet total production is not sufficient to cover the internal demands (Ali *et al.,* 2011). Furthermore, the demand for higher productivity over a unit area, increasing population growth and decrease in arable land are reasons for necessity for hybrid production. Due to these reasons, it will be important to increase the yield of sorghum. One of the best ways of increasing sorghum productivity is by the use of hybrids. Moreover, it is common knowledge that open pollinated varieties (OPVs) are generally lower yielding than hybrids.

For successful sorghum hybrid production, the first step in hybrid development is the development of the A and B lines. For the production of hybrid seed, mechanism for exclusion of selfing is essential. This can be achieved through removal of anthers. Manual removing of anthers is a tedious and time consuming process in some crops except in Maize and Castor which are monoecious.

The discovery of cytoplasmic male-sterility in sorghum by Stephens and Holland (1954) and Dogget (1969) facilitated cross pollination, and thus the commercial utilisation of hybrid vigor. Male sterility is characterised by nonfunctional pollen grains; while female gametes function normally. It occurs in nature sporadically, perhaps due to mutation. In sorghum, the most common type is cytoplasmic male sterility (CMS) system, which is based on male-sterility-inducing cytoplasm that is complemented by allele in the nuclear genome which either restore fertility or maintain sterility. In the CMS system, lines that have (A) cytoplasm have a dominant allele present in the nuclear genome to restore male fertility. If the line lacks the dominant allele for fertility restoration, the plant will be male-sterile (Murty *et al*., 1994). B lines, commonly referred to as maintainers, possess an N cytoplasm (N- normal), and also lack a dominant Rf allele. The A-and B-lines are genetically identical, except that the A-line has a sterility inducing cytoplasm while the B-line has normal fertile cytoplasm. Thus, A-line plants that are male-sterile can be pollinated with pollen from B-line plants to regenerate seeds of the A-line (Murty *et al*., 1994).

Hybrid cultivars make use of male sterility to enhance the combining ability of the parent lines, resulting in heterosis and significant increases in phenotypic traits such as plant height and days to flowering (Reddy *et al*., 2006). Also hybrid seed production requires the use of cytoplasmic male sterility (CMS); without this system, hybrid seed production would not be economically feasible. There is, therefore, the need for developing A and B sorghum lines as an essential step for development of hybrid sorghum industry. There is little information on the behavior of this sterility gene under different environments. Therefore, the objective of the present study was to determine (i) number of genes controlling sterility in A and B sorghum lines, (ii) relative stability of sterility across the generations of backcrosses, (iii) relationship between sterility and grain yield in sorghum lines, and (iv) identify combination of A and B sorghum lines adapted to savanna agro-ecology of Nigeria for A/B development.

MATERIALS AND METHODS

The study was conducted at the Institute for Agriculture Research (I.A.R.) Ahmadu Bello University, Samaru, Zaria (11^0 11' N, 7^0 38' E, 600 m above sea level) in the Northern Guinea savannah, and I.A.R. Irrigation Research Station Kadawa (11^0 39' N, 08^0 2' E, and 469 m above sea level) in the Sudan savanna.

The genetic materials for this study comprised of six A/B pairs (Table 1), which were selected based on their potential as good source of A and B lines obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Mali. A lines, commonly referred to as females, possess an A cytoplasm (A), lacking a dominant RF (restoration of fertility) allele in the nuclear genome; resulting in a male sterile plant. The B lines, commonly referred to as maintainers, possess an N cytoplasm (N- normal), and also lack a dominant *Rf* allele (Murty *et al*., 1994). The sole purpose of the B line was to perpetuate or maintain the A line. The A and the B lines are isocytoplasmic, meaning that they are genetically identical; except that the A line plants that are male sterile can be pollinated with pollen from B line plants to produce the next generation of A lines or male sterile plants.

The F_1s' were developed at ICRISAT in Mali. The backcross one (BC_1) population were developed during the 2013 wet season at the I.A.R. farm. The A and corresponding B line were grown each separately each in a single row, side by side in a plot. At anthesis, 100% sterile plants were identified among the A-lines, together with their corresponding B lines, and were bagged separately. Pollen was taken from a single B line plant to pollinate a single A line plant, which gave rise to BC_1F_1 plants.

BC_2F_1. The BC_1F_1s' were then grown and backcrossed to the B line, to produce the BC_2F_1 population. Planting activities were carried out during 2014 dry season. The A- line ($BC_1F_{1)}$ is the non- recurrent parent, while the B- line is the recurrent parent.

BC_3F_1. BC_3F_1 was obtained by crossing A-line (BC_2F_1) the non- recurrent parent with B-line, during 2014 wet season.

Entries consisting of four generations (F_1, BC_1F_1, BC_2F_1, BC_3F_1) of each of the 6 crosses involving the six different A- and B- lines combinations, were evaluated during 2015 dry season.

Treatments were laid out in a randomised complete block design (RCBD), with two replications at Samaru and Kadawa. Each plot consisted of a single 5 m long row spaced 75 cm and 30 cm inter- and intra-row spacing, respectively. The plots were sown with 5 seeds per hill, and later thinned to one seedling per stand, at two weeks after sowing. The missing hills were compensated for by transplanting. Split application of NPK fertiliser (20:10:10) at the rate of 80 kg ha^{-1} was done at three and six weeks after sowing (WAS). Weeds were controlled manually by hoeing as and when found necessary. Birds were scared off the plants, from flowering to harvest time.

TABLE 1. Pedigree of the lines used in the study of male sterility at Kadawa and Zaria in Nigeria

Entry	A lines (Sterile)	Entry	B lines (Fertile)
67	ISX-09001-11-2-2-BC-6	68	ISX-09001-11-2-2-6
85	ISX-09002-4-1-2-BC-7	86	ISX-09002-4-1-2-7
157	ISX-09003-8-5-1-BC-10	158	ISX-09003-8-5-1-10
159	ISX-09003-8-5-2-BC-12	160	ISX-09003-8-5-2-12
421	ISX-09005-11-7-1-BC-6	422	ISX-09005-11-7-1-6
477	ISX-09001-7-1-1-BC-13	478	ISX-09001-7-1-1-13

Data were collected for number of sterile plants, whereby the number of plants that did not produce pollen in A-line plants, in each plot, were observed and counted relative to the total number of plants in a plot. Pollen viability test was carried out using staining method to determine pollen sterility or fertility. Flower buds with matured and undehisced anthers from each plot were collected and preserved in 70% alcohol. Slides were prepared by dusting the pollen on the middle of the slide; then 1 to 2 drops of 1% acetocarmine solution was added and the slides were covered with cover glasses. The slides were examined under compound microscope, pollen grains filled with cytoplasm and distinct nuclei stained red, and have a regular shape and size were considered as fertile pollens, while colorless, empty pollen grains (without cytoplasm and nuclei) having irregular shape and size were considered as sterile types.

The data collected from each location were subjected to combined analysis of variance (ANOVA) using the Generalised Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 2015). The means, coefficient of variability (CV) and standard error (SE±) for each trait were estimated.

In order to determine the number of genes controlling sterility in different A and B sorghum lines, the expected values corresponding to observed values for each character were calculated on the theoretical ratios expected under each backcross generation. The expected ratio for single gene for BC_1, BC_2, and BC_3 are 3:1, 7:1, and 15:1 respectively. The deviations of observed from expected were subjected to chi-square test by using the formula given by Little and Hills (1978) as follows;

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Where:

χ^2= chi- square, O = Observed value, and E = Expected value

To estimate the stability of genes across the generation of backcrossing from F_1 to BC_3, linear regression analysis was used following the formula of Hoshmand (2006):

$$y = \alpha + \beta x$$

Where: y = Proportion of sterility
α = Intercept of the regression line
β = Slope of the regression line
x = Generation

Similarly, linear regression analysis was used to determine the nature of relationship between sterility and grain yield as follows:

$$y = \alpha + \beta x$$

Where: y = Proportion of yield
α = Intercept of the regression line
β = Slope of the regression line
x = Sterility

RESULTS

Table 2 indicates that 50% heading, days to maturity, number of sterile plants and number of fertile plants were highly significant ($P<0.01$). Panicle appreciation showed a significant difference ($P< 0.05$); while the rest of the characters studied. The result also showed highly significant ($P<0.01$) interactions between genotype and environment for 50% heading and days to maturity. However, the interaction between genotype and location was not significant for number of sterile and fertile plants.

The performance of the genotypes across locations for all the traits measured are presented in Table 3. The number of sterile plants ranged from 1 to 20, with genotype 85 x $86F_1$ having the lowest and 421 x 422 BC_3 the highest number of sterile plants, respectively. The number of fertile plants ranged from 1 to 20, genotype 85 x $86F_1$ had

TABLE 2. Mean square for sterility and agronomic traits different generation of backcross

Source of variation	Df	Grain weight (g)	Number of sterile plants	Number of fertile plants	Grain yield (kg ha^{-1})
Loc	1	6537.30	11.34*	12.04*	29281.60
Block (Loc)	2	14120.82	0.76	1.04	63249.37
genotype	23	16099.11	155.36**	154.33**	72110.44
Loc*genotype	23	12664.22	2.04	2.00	56725.06
Error	46	13937.80	2.13	2.11	62429.59

*, and ** Significant at 0.05 and 0.01 probability levels, respectively. Loc = Location

TABLE 3. Performance of sorghum in different generation of backcross across locations

Genotype	Grain weight (g)	1000 grain weight (g)	Grain size	Number of sterile plant	Number of fertile plant	Grain yield (kg ha^{-1})
67 x 68F_1	151.3	28.0	1.3	2.0	19.0	320.2
85 x 86F_1	299.0	27.3	1.3	1.0	20.0	632.8
157 x 158F_1	244.5	28.0	1.2	2.0	19.0	517.4
159 x 160F_1	242.6	26.3	1.2	3.0	18.0	513.4
421 x 422F_1	242.9	26.5	1.2	2.0	19.0	514.1
477 x 478F_1	168.1	27.5	1.3	4.0	17.0	355.8
67 x 68BC_1	301.6	28.5	1.2	15.0	6.0	638.3
85 x 86BC_1	162.5	27.8	1.2	15.0	6.0	344.0
157 x 158BC_1	196.1	28.8	1.3	14.0	7.0	414.9
159 x 160BC_1	204.3	26.8	1.2	16.0	5.0	432.4
421 x 422BC_1	232.2	26.3	1.2	15.0	6.0	491.5
477 x 478BC_1	99.3	27.5	1.2	15.0	6.0	210.1
67 x 68BC_2	237.7	27.0	1.1	14.0	7.0	503.1
85 x 86BC_2	190.5	27.0	1.2	14.0	7.0	403.1
157 x 158BC_2	93.1	26.3	1.2	15.0	6.0	197.0
159 x 160BC_2	131.1	27.8	1.2	16.0	5.0	277.5
421 x 422BC_2	95.6	27.3	1.3	17.0	4.0	202.4
477 x 478BC_2	220.7	27.8	1.3	16.0	5.0	467.1
67 x 68BC_3	95.7	26.5	1.2	15.0	6.0	202.4
85 x 86BC_3	192.2	26.0	1.3	19.0	2.0	406.8
157 x 158BC_3	210.0	28.3	1.2	17.0	4.0	444.5
159 x 160BC_3	242.7	27.5	1.2	19.0	2.0	513.5
421 x 422BC_3	133.5	26.5	1.1	20.0	1.0	282.4
477 x 478BC_3	108.6	28.5	1.2	18.0	3.0	229.8
Means	187.30	27.30	1.20	13.00	8.00	396.40
CV	63.03	6.87	8.90	11.51	17.47	63.03
SE(±)	10.87	1.37	0.33	1.21	1.20	15.81

TABLE 4. Test of goodness of fit to theoretical genetic ratios for different backcross generations

Generations	Ratios	Crosses					
		67 x 68	85 x 86	157 x 158	159 x 160	157 x 158	159 x 160
		χ^2	χ^2	χ^2	χ^2	χ^2	χ^2
F_1	50:50 (1:1)	13.76**	17.19**	13.76**	10.71**	13.77**	10.71**
BC_1F_1	75:25 (3:1)	0.14	0.14	0.78	0.14	0.78	0.14
BC_2F_1	87.5:12.5 (7:1)	8.33**	8.33**	4.96*	2.46	4.96*	2.46
BC_3F_1	93.75:6.25 (15:1)	17.86**	0.38	5.87*	0.38	5.86*	0.53

χ^2 = chi-square

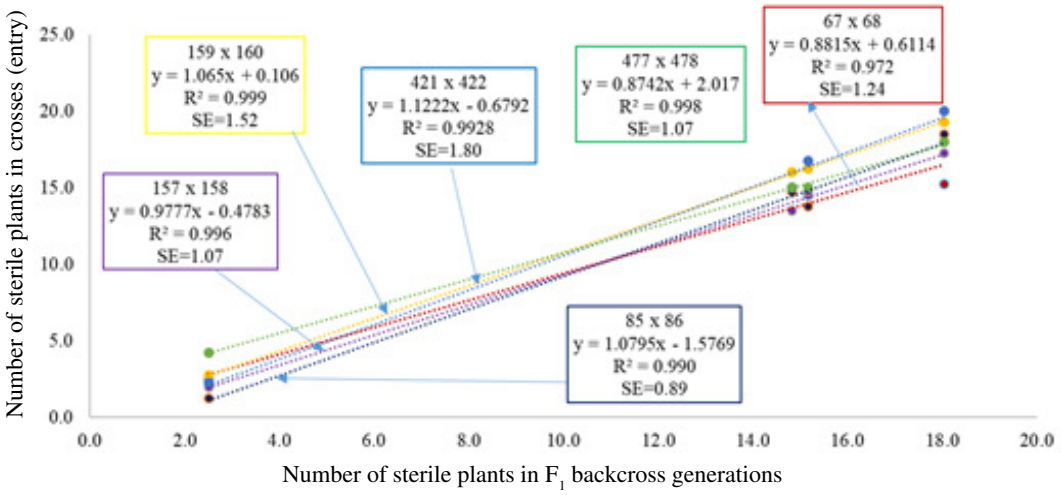

Figure 1. Stability of sterility across generation of backcrosses derived from crosses involving different pairs of A and B lines evaluated in Samaru and Kadawa during 2014/2015 dry season.

the highest number of fertile plant (20.0); while genotype 421 x 422 BC_3 had the lowest number (1.0).

Data for the goodness of fit to the genetic ratios expected for different generations of backcrossing are presented in Table 4. The chi-square test was not significant (P>0.05) for BC_1F_1 in all the crosses. Non-significant chi-square tests were also observed for cross 159 x 160, and 421 x 422 in BC_2F_1 and BC_3F_1. Also, non-significant chi-square test were observed for cross 85 x 86 and 477 x 478 in BC_3F_1. On the other hand, significant chi-square test in BC_2F_1 and BC_3F_1 were observed for cross 67 x 68 and 157 x 158. The chi-square tests for the F_1s' in all the crosses were significant.

Results for regression analysis for stability of male sterility genes across the generation of back crosses are presented in Figure 1. The results show that slope and sterility increasing across the generations of backcrossing. The crosses, 67 x 68, 85 x 86, 157 x 158, 421 x 43 and 477 x 478 had high r^2 values.

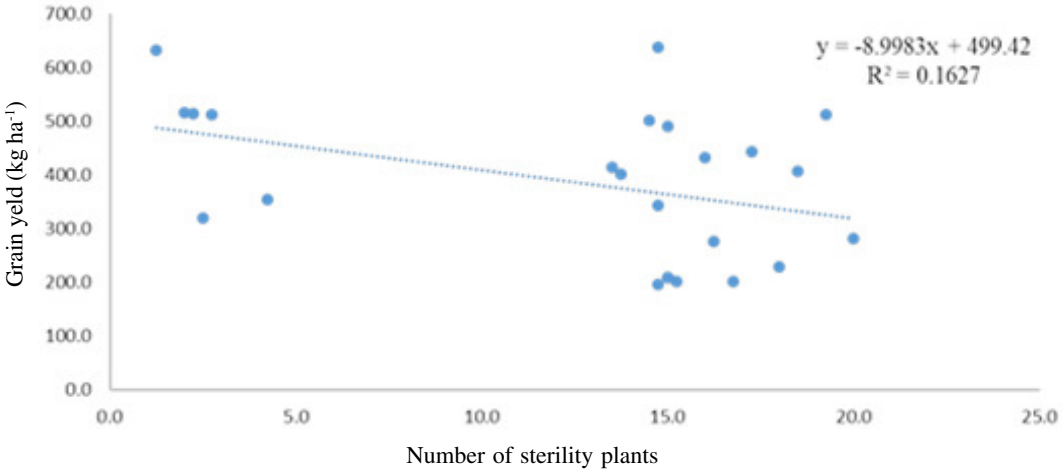

Figure 2. Relationship between yield and sterility in different backcross generations evaluated in Samaru and Kadawa during 2014/2015 dry season.

The regression analysis for the relationship between yield and sterility showed a negative relationship with R^2 of 0.1627 (Fig. 2).

DISCUSSION

The significant differences observed for number of sterile and fertile plants (Table 2) indicate significant genetic variability within the mentioned traits in each of the location, thus genetic improvement can be achieved for the characters studied. The amount of genetic improvement that can be achieved among a given set of genotypes depends on the amount of genetic diversity that is present within the population (Falconer and Mackey, 1996). The non-significant effects of genotype x location for number of sterile plants and number of fertile plants, indicate that the performance of the genotypes in terms of number of sterile and fertile plants at both locations was similar, which means that the expression of sterility in sorghum is not controlled or influenced by environment, rather by genotype. Thus, breeding for sterility can be done in any location.

The observed variation among the genotypes for each generation across locations for the traits studied (Table 3) indicate significant differences among the genotypes. This, provides a clear indication of the superiority of some of the genotypes over others. Cross 85 x 86 recorded the highest yield. The high number of sterile plants observed in cross 159 x 160 and 421 x 422, suggests the possibility of using the genotype for breeding sterility.

For each generation of a backcross, the expectation that homozygosity is increased; while heterozygosity is reduced by 50% were met (Table 4). From the results obtained in this study, F_1 in all crosses were fertile, indicating dominance of fertility over sterility. In all crosses and for the F_1 hybrid, chi-square values at expected segregation ratio 50:50 (fertile: sterile) were significant in all the crosses. Therefore, chance alone was not responsible for the observed differences; so we reject the null hypothesis, indicating poor-fit of the observed ratios. Reddy *et al.* (2010) also reported a goodness of fit between restorer parents and A-lines.

The expected segregation ratio 75:25 (fertile: sterile) was not significant ($P>0.05$) in BC_1F_1 in all crosses, indicating a good-fit of the observed ratio; also that whatever deviation observed from the expected, was due to chance and the null hypothesis holds true in this case. The good-fit of 75:25 segregation ratio in the above crosses, indicates the

preponderance of sterile genes over fertile ones in crosses; and that the assumption that sterility is governed by single recessive gene, holds in the crosses made. Similarly, the expectation that homozygosity is increased; while heterozygosity is reduced by 50% was observed. Similar findings was also reported by Reddy *et al*. (2010). The significant chi-square tests for BC_2F_1 and BC_3F_1 in crosses 67 x 68 and 157 x 158, indicated lack of fitness of the ratios. The ratio 93.75:6.25 was not significant in BC_3F_1 for cross 85 x 86 and 477 x 478, indicating that sterility is governed by single recessive gene. Similar results were obtained by Elkonin *et al.* (2009) and Nikolova *et al*. (2012). These differences in the type of gene interaction could also presumably be due to the influence of female parent and/or a probable variable expression of the weaker gene in different genetic backgrounds (Reddy *et al*., 2010). Certain modifier genes have been reported to be responsible for changing the segregation ratio in different generations of study as reported in rice (Govinda and Virmani, 1988) and wheat (Mann, 1985).

The number of sterile plants increased at each successive backcross generation, as indicated from the straight line obtained from the regression analysis (Fig. 1), which increased positively. For almost all genotypes, the co-efficient of determination (R^2) indicates that about 99% of the sterility can be accounted for, and only 0.1% of the variance may be due to error. The stability of genes across the generation of backcrosses for cross 421 x 422 and 159 x 160 from their standard error, indicated that the sterility genes are 95% were stable. For crosses 67 x 68, 85 x 86, 157 x 158 and 477 x 478, their standard error indicated that the sterility genes were 68% stable across the generations of back crosses. This corroborates with the work of Hoshmand (2006), who reported that if the scatter about the regression line is normally distributed in a large sample, approximately 68% of the points in the scatter diagram will fall within 1SE above and below the regression line; 95% of the points will fall within 2SE above and below the regression line.

From the regression analysis, a negative relationship existed between yield and sterility (Fig. 2). This implies that as sterility increases, yield decreases. This physiologically indicates that existence of more sterile flowers leads to low yield. The coefficient of determination of 0.1627, indicate that the variation in yield was explained only 16%. Similar results were obtain by Farzaneh (2013), who reported a negative relationship between yield and sterility.

CONCLUSION

The results show that sterility is governed by a single recessive gene. Stability of male sterility genes in the crosses remained between 68 or 95% across the generation of backcrosses. A negative relationship exist between grain yield and sterility. Cross 159 x 160 and 421 x 422 are found to have good breeding potential for sterility compared to other crosses.

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