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Cytological Study of Anthers for Pollen Sterility in the F_7 Generation of a Rice (*Oryza sativa* L.) Cross

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present research work was proficiently done at the experimental plot of Birsa Agricultural University, Kanke under the rainfed conditions of Jharkhand, India during kharif 2021. The F_7 generation seeds of the cross Pusa-1176 x BPT-5204 was sown as panicle to progeny rows in the seed bed nursery and thereafter transplanted into the field. In this investigation, the study has been oriented more towards the male sterility as it is more sensitive than female gametes based on the observation recorded in previous generation (F_6) regarding spikelet sterility percentage. Henceforth, the study has been focussed on the microscopic study of pollen grains to validate the reason behind the cause of spikelet sterility in the panicles. To understand this phenomenon, pollen viability test was done by staining the anthers with 1% Iodine- potassium iodide in order to know the extent of fertility or sterility present in the pollen grains of the rice segregants. Through microscopic study, segregation was observed in the pollen sterility % among the families studied which might be due to the change in the environmental conditions such as temperature, photoperiod and humidity etc. The expected genetic ratio obtained was 9:3:3:1(partially sterile+highly fertile/completely fertile/ highly sterile/completely sterile) revealing that it is controlled by 2 genes since the ratio fitted in digenic ratio.

Keywords: Sterility; chi-square; pollen viability; staining; fixatives.

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is a monocot plant that belongs to Poaceae family. Rice is one of the major staple food crops for more than half of the population who rely on this crop for almost 20% of their regular calorie intake and thereby considered as a lifeline for food and nutritional security [1]. It is basically cultivated under diverse ecosystems ranging from irrigated to rainfed upland to rainfed lowland to deep water. At the end of fiscal year 2020, India had approximately 44 million hectares of land area for cultivation of rice out of which the total area under rainfed lowland and upland rice is 14.4 and 6.3 million ha, respectively. This area under rice had been relatively consistent over during the past three years and it was the most produced food grain across the south Asian nation [2]. The yield of rice across India was estimated to be approximately 2.7 thousand kilograms per hectare. Although a consistent increase in the yield of rice was noted since fiscal year 1991 [2], the production and productivityis still thought to be enhanced in order to meet the demands of expected global population in future and reductions in available land. Therefore, the need of the hour is to increase the rice grain yield per unit area that is essentially crucial for ensuring food security, particularly in developing countries in Asia, such as in China and India.

The grain yield of the rice is determined by traits number of plants per unit area, number of panicles per plant, grain number per panicle (GNPP) and grain weight. Number of grains per panicle are dependent on the fertility of the spikelet. The fertility of the spikelet depends on many factors, among them male sterility is the crucial factor. Therefore, the present investigation is planned to know the genetic behaviour of the spikelet sterility. Thus, fertility is an important agronomic trait which is closely related to yield in rice. But a reduced amount of grain setting has been observed under the present investigation and therefore, the experiment would be conducted to identify the genetic reasons behind this phenomenon, which is also known as spikelet sterility. It may be caused due to the defective male or female reproductive parts of the plant. In recent years, a large scale of rice male organs and male-sterile mutants have been reported and successfully applied to heterosis breeding programme [3]. However, little effort has been devoted to female sterility [4] emphasizing more on sterile male reproductive organs. Male sterility is generally characterized by the impairment of the male reproductive development as a result of underlying genetic causes and leads to the malformation of male gametes and/or pollen. Hence, several genes responsible for the male sterility that leads to reduced amount of grain setting has been reviewed and studied such as vacuolation retardation 1 (vr1), tapetum desquamation (t), and many of the thermosensitive male sterility genes (tsgms) and photoperiod sensitive male sterility genes (psgms) contributing to environment sensitive genic male sterility. To date, 13 PTGMS genes have been identified in rice: pms1 [5], pms2 [6], pms3 [7], rpms1 [8], rpms2 [8], tms1 [9], tms2 [10], tms3 [11], tms4 [12], tms5 [13], tms6 [14], rtms1 [15] and Ms-h [16], which have been mapped to chromosomes 7, 3, 12, 8, 9, 8, 7, 6, 2, 2, 5, 10 and 9. Therefore to identify the defective male reproductive organs that results in futile grain setting, the objective of the present investigation was to test the pollen viability and it was accomplished by using iodine-potassium iodide staining method. Moreover, genetic segregation ratio was also computed by chi-square analysis. Therefore, present research programme will be focused on the genetic studies of spikelet sterility by observing the sterility in the pollen grains of the segregantsin the F_7 generation of the cross Pusa-1176 and BPT-5204.

2. MATERIALS AND METHODS

The present investigation was carried out at the experimental plot of Birsa Agricultural University, Kanke under the rainfed conditions of Jharkhand, India during kharif 2021. The F_7 generation seeds of the cross Pusa-1176 x BPT-5204 was sown on July 12th, 2021 as panicle to progeny rows in the seed bed nursery and thereafter transplanted into the field. Based on the observation in previous generation (F6) regarding spikelet sterility percentage, the study has been oriented more towards the sterility of male reproductive parts as it is more sensitivethan female gametes.Henceforth, the study has been focussed on the microscopic study of pollen grains to know the reason behind the cause of spikelet sterility in the panicles.Therefore, to understand this phenomenon, the spikelets were collected to store the anthers in the fixativeduring the flowering period in order to study the pollen cytologically through staining techniques.The fixative was prepared by blending acetic acid and ethanolin the ratio of 1:3 to preserve anthers for

longer duration. Then collections were done from the selected plants that showed sterility during grain setting and 2-3 spikelets were taken from the emerging panicles of the selected plants whose anthers were not yet exposed out. Later on, pollen viability test was done by staining the anthers with 1% Iodine- potassium iodide in order to know the extent of fertility or sterility present in the pollen grains of the rice segregants. The description of the staining procedure has been followed below.

2.1 Iodine-Potassium Iodide Test (I_2KI)

The procedure to prepare the 1% iodine-potassium iodide stain required1 g potassium iodide and 0.5 g iodine that was dissolved in distilled water to make up the volume of 100 ml. Then preserved spikelets were taken out from the fixative, anthers were separated and placed on the slide. The anthers were then ruptured by using needle for the exposure of pollen grains and stained by 1-2 drops of 1% iodine-potassium iodide. Immediately after 2-3 minutes, observations were recorded by scanning the field view of light microscope for sterility in the pollen grains. The pollen grains were classified into sterile and fertile on the basis of their shape, size and extent of staining. Thus, round, large and fully dark stained grains were considered as fertile whereas shrivelled, comparatively smaller and unstained pollen grains were the sterile ones. Based on the microscopic study of pollen sterility (Fig. 1), 5 classes have been designated for which chi-square analysis was done in order to know the number of genes governing the pollen sterilityphenomenon.

3. RESULTS

In total 228 segregants were collected from 85 different families to study the genetics of pollen sterility in the cross Pusa-1176 and BPT-5204. The observation on the pollen sterility was classified into 5 classes that has been described in the table given below (Table 1).

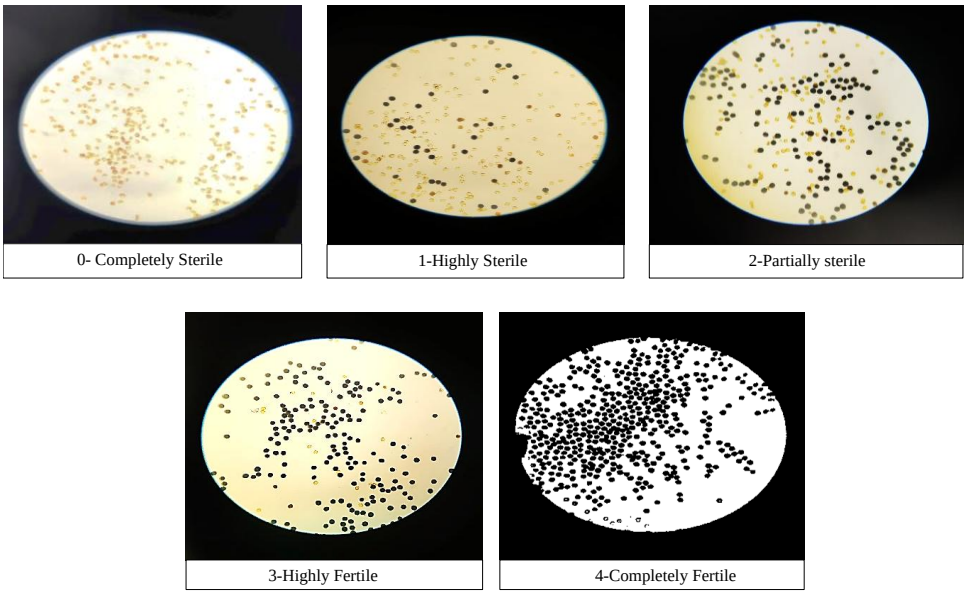

Fig. 1. Pictorial representation of the various classes obtained for pollen sterility through microscopic study. (Magnification: 50X)

Table 1. Classification of the pollen grains based on the pollen sterility percentage observed through microscopic study

S/No.	Class	Description on the basis of sterile pollens	Fertility percentage
1	0	Completely Sterile	0%
2	1	Highly Sterile	20-30%
3	2	Partially Sterile	50%
4	3	Highly Fertile	70-80%
5	4	Completely Fertile	100%

This classification was done based on the sterility % observed in the pollen grains under microscope and segregation was observed in the pollen sterility % among the families which might be due to the change in the environmental conditions such as temperature, photoperiod and humidity etc. Therefore, expected genetic segregation ratio was calculated for pollen sterility % among the families studied and tested for goodness of fit by using Chi-square test (Pearson, 1900) with the formula given below.

$$\chi^2 = \Sigma (O - E)^2 / E$$

Where, 'O' is the Observed frequency and

'E' is the Expected frequency

The expected genetic ratios obtained after testing the goodness of fit have been presented in the table given below (Table 2) which represents the number of genes governing the pollen sterility in rice and also revealed the scenario of genetic interactions among the genes.

Among the 5 classes, class 3 consisted of 67 segregants, class 2 pertained 56 segregants and both were considered as a single class for chi-square analysis but class 4 had 54 segregants, class 1 comprised of 33 segregantsand the class 0 consisted of 18 segregants which showed the genetic ratio as 9(5:4):3:3:1 respectively revealing that it is governed by 2 genes since the ratio fitted in digenic ratios. Moreover, the respective genotypes of each class has also been worked out and represented in the Table 2.

Moreover, number of families that showed segregation for the trait pollen sterility (Fig. 2) has been represented graphically according to their expected genetic ratios computed through chi-square test.

Table 2. Expected genetic ratios computed for pollen sterility in the F_7 generation families of the cross Pusa-1176 and BPT-5204

Class	Description of the class	Expected Genotype	Fitted genetic Ratio	Observed Value	Expected Value	Chi-Square test	P-value
0	Completely Sterile	abab	1	18	14.25	0.987	
1	Highly Sterile	aaB_	3	33	42.75	2.224	
2	Partially Sterile	AaBb	4	56	57	0.018	0.169
3	Highly Fertile	A_BB, AAB_, AABB	5	67	71.25	0.254	
4	Completely Fertile	A_bb	3	54	42.75	2.961	
		Total	**16**	**228**		**6.442**	

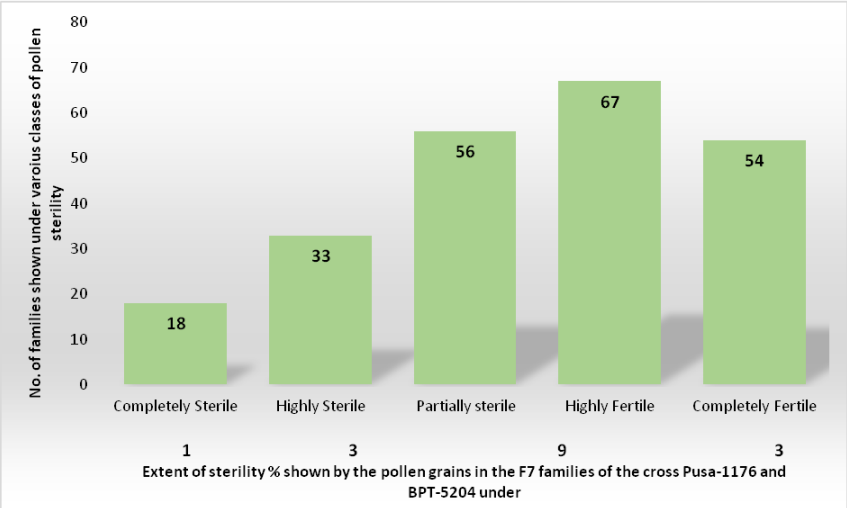

Fig. 2. Graphical representation of number of families segregating for the trait pollen sterility and the expected genetic ratios computed through chi-square test in F_7 families of the cross Pusa-1176 and BPT-5204

4. DISCUSSION

The principle behind the technique of testing the pollen grains with iodine-potassium iodide was the total starch content of pollen grains indicating its viability. In this method, Iodine broke up in a watery arrangement of potassium iodide, the tri-iodide-anion edifices with starch prevailing in the pollen grains that creates blue-black colour that is also reported by Durgesh et al. [17] in the completely stained pollen grains. Thereafter, expected genetic segregation ratio calculated for pollen sterility % among the families studied showed digenic ratio without any modification i.e. 9 (5+4):3:3:1 executing the Mendel's law of independent assortment that is governed by two genes. The digenic segregation pattern for pollen sterility phenomenon was also reported by Singh and Sinha [18]; Govinda Raj and Virmani [19]; Ramalingam et al. [20]; Singh et al. [21]; Kumari et al. [22]; Pradhan and Jachuck [23] and Sohu and Phul [24] but with epistatic interaction among the genes controlling the trait.

5. CONCLUSION

It was concluded from the present investigation that the trait pollen sterility is governed by two genes since it exhibited digenic segregation ratio but further analysis should be done through molecular markers for accuracy in the results. Moreover, the segregants that had completely sterile pollen grains but showed high grain setting may be utilized in two-line breeding system of hybrid rice seed production overcoming the difficulties of cytoplasmic-genic male sterility (three-line breeding system).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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