P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2020; 8(3): 2677-2680

© 2020 IJCS

Received: 01-03-2020

Accepted: 03-04-2020

BS Thorat

Department of Agriculture
Botany Genetics and Plant
Breeding, Dr. BSKKV, Dapoli,
Maharashtra, India

RL Kunkerkar

Department of Agriculture
Botany Genetics and Plant
Breeding, Dr. BSKKV, Dapoli,
Maharashtra, India

SR Kadam

Department of Agriculture
Botany Genetics and Plant
Breeding, Dr. BSKKV, Dapoli,
Maharashtra, India

SG Bhav

Department of Agriculture
Botany Genetics and Plant
Breeding, Dr. BSKKV, Dapoli,
Maharashtra, India

JP Devmore

Department of Agriculture
Botany Genetics and Plant
Breeding, Dr. BSKKV, Dapoli,
Maharashtra, India

Corresponding Author:**BS Thorat**

Department of Agriculture
Botany Genetics and Plant
Breeding, Dr. BSKKV, Dapoli,
Maharashtra, India

Study of anther dehiscence, pollen viability and stigma receptivity in rice (*Oryza sativa* L.)

BS Thorat, RL Kunkerkar, SR Kadam, SG Bhav and JP Devmore

DOI: <https://doi.org/10.22271/chemi.2020.v8.i3am.9616>

Abstract

A present study was carried on floral biology of rice that cover anther dehiscence, pollen viability and stigma receptivity was carried out with the aim to improve the efficiency of hybridisation in rice breeding work at the Experimental farm of Regional Agriculture Research Station, Karjat (Raigad) during *kharif* 2016. In this study, three CMS lines viz., IR58025A, RTN 12A and RTN17A and four testers i.e., Chedo Local, CR-2829-PLN-36, NPQ-49 and RP-5898-19-8-6-1-1-1 of rice were used. The results reported that the time required for dehiscence of anther ranged from 8 - 20 minutes in testers of rice. The results showed that time of anthesis in both the CMS lines and testers of rice since 08.00 AM. In the pollen viability study, results suggested that pollens are more viable from single minute to 11 minutes after anther dehiscence at fully blooming stage. The range of pollen viability percentage was observed from 90.79% to 94.92%. However, there are variations among the genotypes for the optimum viability of pollen at maximum blooming stage. In both CMS lines and testers of rice, maximum blooming stage of panicles was recorded 9.00 am to 1.00 pm while 8.00-11.00 days took for fully completion of anthesis. In CMS lines of rice, receptivity of stigma ranged from 36 to 42 hrs (two to three days). There was no difference in stigma receptivity stages among the three CMS lines of rice. Thus, the precise knowledge of floral biology, which includes structural and functional aspects of rice inflorescence, is essential for breeders to plan and execute breeding strategies.

Keywords: Rice, anthesis, anther dehiscence, pollen viability and stigma receptivity

Introduction

Rice (*Oryza sativa* L.) is basically a highly self-pollinated crop, with limited degree of out crossing (< 0.5%). The factors limiting the receptivity of rice flowers to out crossing include a short style and stigma, short anthers, limited pollen viability and brief period between opening of florets and release of pollen (Morishima, 1984; Oka, 1988) [14, 15]. Among many genetic approaches being explored to break the yield barrier in rice and increased productivity, hybrid rice technology appears to be the most feasible and readily adaptable one (Virmani *et al.*, 1997) [19]. Over 75% of the world supply is consumed by people in Asian countries and thus rice is of immense importance to food security of Asia. The demand for rice is expected to increase further in view of expected increase in the population (Viraktamath, B.C., 1997) [19]. Since the yield of high yielding varieties (HYVs) of rice is plateauing, it is rather difficult to achieve this target with the present day inbred varieties. Among the limited options, hybrid technology is the only proven technology currently available for stepping up rice production significantly. Hybridization ensures a wide variety exists among the cultivars of rice ensuring quality attributes and yield. Therefore, the development of hybrids and popularization of their production technology are feasible and readily adoptable to achieve targeted production (Ayyappan *et al.*, 2012). Among many, the precise knowledge of floral biology, which includes structural and functional aspects of rice inflorescence, is essential for breeders to plan and execute breeding strategies. The objectives of this study are to determine the time of anthesis, the nature of pollen viability and the stigma receptivity of rice, while in tandem; the aim is also to improve the efficiency of innovative approaches in rice breeding.

Materials and Methods

The present investigation was carried out at the Experimental farm of Regional Agriculture Research Station, Karjat (Raigad) during *kharif* 2016. Three CMS lines viz., IR58025A, RTN 12A and RTN17A and four testers Chedo Local, CR-2829-PLN-36, NPQ-49 and

RP-5898-19-8-6-1-1-1 were used for this study (table 1). The experiment was laid out in experimental laboratory at Regional Agriculture Research Station, Karjat (Raigad) during *khari*, 2016. The experimental material consisting three CMS lines and four restorers were sown on 21st June 2016. Then twenty-five days old seedlings were transplanted in the pot at 20 x 15 cm spacing with single seedling per hill. The recommended fertilizers @ 100 kg N, 50 kg P₂O₅ and 50 kg K₂O along with 7.5 tonnes of FYM per hectare were applied. All standard agronomic recommended practices and plant protection measures were adopted for raising healthy crop. The data for each trait was analyzed by using standard statistical procedure (Panse and Sukthame, 1978).

Table 1: List of CMS lines and testers of rice for the study

Sr. No.	CMS Lines	Testers
1	IR58025A	Chedo Local
2	RTN12A	CR-2829-PLN-36
3	RTN17A	NPQ-49
4		RP-5898-19-8-6-1-1-1

Anther Dehiscence

The anthers selected for the study were at the pre-dehiscence stage. Observation, with each minute interval each time, was carried out starting from early morning until anther dehiscence was noticed. The preliminary judgment on anther dehiscence was made on the basis that whitish powder-like pollens were seen by the naked eye. Then, the released pollens were confirmed through stereomicroscope observations.

Pollen Viability

Assessment of pollen viability by two methods which are given below

Iodine- potassium iodide Test (I₂KI): The technique described indicates viability and starch content of pollen grains. Iodine broke up in a watery arrangement of potassium iodide the tri-iodide-anion edifices with starch, creating blue-black colour.

Procedure: Dissolve 1g potassium iodide and 0.5g iodine in distilled water to make a final volume of 100 ml. Put 1 or 2 drops of the dye over pollen and mix thoroughly. Place a cover slip and after 5-10 min count the number of darkly stained (viable) pollen grains under the microscope.

Aceto-carmin test (2%): Carmine shows the presence of cytoplasm. The pollen nucleus is rich in chromatin material and viable pollen stains pink to deep red with aceto-carmin, while sterile (mostly shriveled) pollen does not take any stain and thus remains almost white and transparent (McKellar and Quesenberry, 1992; Marutani, *et al.*, 1993)^[13, 12].

Procedure: Weigh 2 g of carmine powder; dissolve it in 95ml of glacial acetic. Add distilled water to make a total of 100ml solution. Boil it, cool and filtered and stored in a refrigerator. Two to three drop of stain was placed on slide and pollen grains were dusted on it followed by covered with covers lip and pollen viability was recorded after 5-10 min. The dark red coloured grains are counted as viable pollens (Thorat, 2018)^[18]. The per cent pollen viability was calculated using formula

$$\text{Pollen viability (\%)} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains on slide}} \times 100$$

Stigma Receptivity

Freshly cut stigmas of rice inflorescences were collected from the CMS lines grown in the field. For each species we have taken a total 10 stigmas of different stage of flower development to evaluate the receptivity. Samples and experiments were repeated 3 times. In the present study, the peroxidase and alcohol dehydrogenase tests was performed to examine the stigmatic receptivity at different stages of flower blooming and thereby healthy seed set. Alcohol dehydrogenase based test known as Baker's procedure by Galen and Plowright (1987)^[4]. A 6% of hydrogen peroxide (H₂O₂) solution was placed on the stigma and the appearance of bubbles was observed (Knox, 1984)^[11].

Result and Discussion

The viable pollen percentage was ranged from 90.79% (RP 5898-19-6-1-1-1) to 94.92% (NPQ-49) (Table 2). The graphical representation of pollen viability of testers of rice in Figure 4. The maximum pollen viability (per cent) was observed in NPQ-49 (94.92%) followed by CR 2829 PLN-36 (93.47%). The viable and non viable pollen grains were seen in the microscopic slide (Fig. 1). Anthers were dehiscent within 8.00 minutes (NPQ-49) to 20.00 minutes (RP 5898-19-6-1-1-1) under artificial light. The less time required for dehiscence of the anther was reported in NPQ-49 (8 minutes) followed by CR 2829 PLN-36 (9 minutes). It is evident that, opening of inflorescence of all the rice genotypes since 8.00 AM under study. The fully blooming time of the inflorescence of testers was observed 09.00 AM to 01.00 PM, while CMS lines was fully opened in 09.00 AM to 12.00 PM. In the present investigation, it was observed that 8-11 days required for the complete the anthesis of all the genotypes under study (Table 3). The range of pollen viable from 6 minutes (RP 5898-19-6-1-1-1) - 11 minutes (NPQ-49) at fully blooming stage of the rice inflorescence. The pollens were viable for longer time was observed in NPQ-49 (11 minutes) followed by CR 2829 PLN-36 (10 minutes). The similar results also reported by Rathod *et al.* (2018)^[16], Dafni and Firmage (2000)^[2], Georgieva and Kureleva (1994)^[5], McKellar and Quesenberry (1992)^[13], Knox RB (1984)^[11] and Athwal and Kimber (1970)^[15].

The stigma receptivity was observed up to 7 days since flower opening; dry stigma type revealed greatest dehydrogenase activity on second and third day of blooming stage of flower. The graphical representation of stigmatic receptivity of CMS lines of rice in Figure 5. In the hydrogen peroxide test, the number of bubbles recorded on the stigma of flowers up to 7 days after the commencement of bloom stage, which indicates the degree of receptivity of stigma. The highest number of bubbles on second (IR 58025A) and third (RTN 12A and RTN 17A) day after floret opening. Hence, maximum stigmatic receptivity was observed in IR 58025A (42 hrs.) followed by RTN 12A (39 hrs.). The stigmatic receptivity was seen in the microscopic slide (Fig. 2). Seven (IR 58025A) and six (RTN 12A and RTN 17A) seeds per inflorescence were recorded on the fully blooming flowers, respectively (Table 4). Therefore, the 2 quick tests *viz.*, alcohol dehydrogenase and hydrogen peroxide are in conformity to suggest the potential role of receptivity of stigma in determining seed set. Owing to maximum receptivity in stigma, flowers showed dark violet stain and more number of bubbles as we have observed in CMS lines in rice crop. In the present investigation, stigma receptivity was maximum on second and third day after anthesis. The similar results also reported by Gupta *et al.* (2015), Marutani *et al.* (1993)^[12], Galen and Plowright (1987)^[4], Knox (1984 and King (1960)^[11, 10].

Table 2: Mean performance of viable pollens in the testers of rice at fully blooming stage

S. No.	Testers	Viable Pollens	Total pollens	Pollen Viability (%)
1.	Chedo Local	642	695	92.37
2.	CR-2829-PLN-36	716	766	93.47
3.	NPQ-49	729	768	94.92
4.	RP-5898-19-8-6-1-1-1	582	641	90.79
	SE	1.56	1.12	1.02
	CD @ 5 per cent	2.64	3.89	2.25

Table 3: Floral biology and pollen viability at different stages in testers of rice

S. No.	Testers	Time required for dehiscence of anthers (minutes)	Time of Anthesis	Time taken for completion of Anthesis (days)	Time of Maximum Blooming	Pollen Viability (Minutes)		
						One day before Anthesis	At the time of Anthesis	At highest Blooming Stage
1.	Chedo Local	15	08.00 AM	11	09.00 AM - 11.00 AM (2 hrs.)	1	4	7
2.	CR-2829-PLN-36	9	08.00 AM	9	10.00 AM - 12.00 PM (2 hrs.)	1	3	10
3.	NPQ-49	8	08.00 AM	8	10.00 AM - 01.00 PM (3 hrs.)	1	5	11
4.	RP-5898-19-8-6-1-1-1	20	08.00 AM	11	09.00 AM - 11.00 AM (2 hrs.)	1	4	6
	SE	1.12	-	1.03	-	0.05	0.41	0.35
	CD @ 5 percent	2.26	-	1.95	-	0.11	0.87	0.74

Table 4: Floral biology and stigma receptivity in CMS lines of rice

S. No.	CMS Lines	Time of Anthesis	Time taken for Anthesis (days)	Time of Maximum Blooming	Stigma Receptivity (Hours)	Stigma colour and type	Number of Bubbles on Stigma and seed set
1.	IR58025A	8.00 AM	8	09.00AM- 11.00 AM (2 hrs.)	42 (Third day)	Whitish yellow and dry	***** and seven seeds
2.	RTN12A	8.00 AM	9	10.00AM- 12.00 PM (2 hrs.)	39 (Second day)	Whitish yellow and dry	**** and six seeds
3.	RTN17A	8.00 AM	9	10.00AM- 12.00 PM (2 hrs.)	36 (Second day)	Whitish yellow and dry	**** and six seeds
	SE	-	0.86	-	0.57		
	CD @ 5 per cent	-	1.31	-	1.24

Fig 1: I₂KI Staining method for pollen viability **Fig 3:** Receptive stigma at second (i) and third (ii) day from anthesis **Fig 2:** Pollen viability by Acetocarmine solution **Fig 4:** Pollen viability at different stages in restorer lines of rice

Fig 5: Stigma Receptivity in CMS lines of rice

Conclusion

From above study, it is concluded that, testers viz., NPQ-49 and CR 2829 PLN-36 used as a male parent and CMS lines viz., IR 58025A and RTN 12A used as best female parents for hybridization programme for increase the grain yield. Thus, the precise knowledge of floral biology, which includes structural and functional aspects of rice inflorescence, is essential for breeders to plan and execute breeding strategies.

References

1. Athwal RS, Kimber G. Anther size and pollen longevity in wheat/rye addition lines, Wheat Infor. Ser. 1970; 30:30-32.
2. Dafni A, D Firmage. Pollen viability and longevity: Practical, ecological and evolutionary implications, Plant Systematic and Evolution. 2000; 222(1-4):113-132.
3. Firtz SE, Lukaszewski AJ. Pollen longevity in wheat, rye and triticale, Plant Breeding. 1989; 102:31-34.
4. Galen C, Plowright RC. Testing accuracy of using peroxidase activity to indicate stigma receptivity. *Can J Bot.* 1987; 65:107-11. <http://dx.doi.org/10.1139/b87-015>.
5. Georgieva LD, Kureleva MM. Cytochemical investigation of long term stored maize pollen, Euphytica. 1994; 72:87-94.
6. Grist DH. Rice Longmans, Green & Co. London U.K.
7. Johri, B.M. and Vasil, I.K. (1961). Physiology of pollen, Bot. Rev. 1953; 27:325-375.
8. Khatun S, Flower TJ. The estimation of pollen viability in rice, J. Exp. Bot. 1995; 46:151-154.
9. Khatun S, Flowers TJ. The estimation of pollen viability in rice. Journal of Experimental Botany. 1995; 46(282):151-154.
10. King JR. The peroxidase reaction as an indicator of pollen viability, Stain Tech. 1960; 36:225-227.
11. Knox RB. Pollen-pistil interaction In: Linskens HF, Heslop-Harrison J. (eds) Cellular interactions. Encyclopedia of Plant Physiology; 17 Springer, Berlin Heidelberg New York, 1984.
12. Marutani M, Sheffer RD, Kameto H. Cytological analysis of *Arthrium andraenum* (Araceae), its related taxa and their hybrids. Amer. J Bot. 1993; 80:93-103.
13. McKellar MA, Quesenberry KH. Chromosome pairing and pollen viability in *Desmodium ovalifolium* Wall x *Desmodium heterocarpon* (L.) DC hybrids. Aust. J Bot. 1992; 40:243-247.
14. Morishima H. Wild plants and domestication. Ch. 1, In: S Tsunoda, N Takahashi, eds. *Biology of rice*, 7, Elsevier, Amsterdam, 1984, 3-30.
15. Oka HI. Origin of cultivated rice, Elsevier, Amsterdam, 1988.
16. Rathod Vijayakumar, TK Behera, AD Munshi, Kumar Durgesh, GS Jat, Boopala Krishnan G *et al.* Pollen

viability and *in vitro* pollen germination studies in *Momordica* species and their intra and interspecific hybrids, Int. Jr. Che. Studies. 2018; 6(6):32-40.

17. Song ZP, Lu B, Zhu TG, Chen JK. Gene flow from cultivated rice to wild species (*Oryza rufipogon*) under experimental field condition, New phytologist. 2003; 157:657-665.
18. Thorat BS. Pollen viability, Practical manual of Breeding of Fruit and Plantation Crops, 2018, 33.
19. Virmani SS, Viraktamath BC, Casal CL, Toledo RS, Lopez MT, Manalo JO *et al.* Pollen sterility, Hybrid Rice Breeding Manual, 1997, 40-41.