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Identification and categorizationof restorers and maintainers for CMS lines of rice (*Oryza sativa* L.) focusing on hybrid development

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Abstract

Cytoplasmic male sterility plays an important role in hybrid seed production. Three CMS lines (IR5805A, IR79156A and IR68897A) were crossed to 20 rice parental lines following line × tester mating design during *kharif*, 2022to identify potential restorers and maintainers followed by evaluation of F_1 and their respective parents during *kharif*, 2023 at Rice and Wheat Research Centre, Malan (Himachal Pradesh). The performance varied across CMS lines where in two genotype HPR2866 and Varundhan exhibited full restoration potential based on pollen and spikelet fertility for CMS line IR-5805A while genotypes HPU2216, HPR2143 and HPR2880 showed the same for CMS line IR79156A. There waslack of effective maintainers in the material though there were 17, 14 and 15 partial restorers for IR5805A, IR79156A and IR68897A, respectively. Overall, CMS line IR68897A demonstrated the highest pollen fertility (92.13%), while IR79156A had the highest spikelet fertility (64.29%).

Keywords: Fertility, maintainers, restorers, rice

Foodgrain is a core strategic issue for a country's social and economic stability in a changing geopolitical crisis and era of war, and it is also an important cornerstone for ensuring national security. Rice (*Oryza sativa* L.) is widely consumed staple food crop on this planet (Rezvi *et al.* 2022). It is also popular as staple food crop known for its extravagant quality viz., high protein, carbohydrates, vitamins, minerals and low-fat levels (Ullah Zaid *et al.* 2019; El-Mowafi *et al.* 2021; Abd El-Aty *et al.* 2022). With the global population growing, there is an increasing demand for rice. However, the area dedicated to rice cultivation is decreasing. Increasing rice yield per unit area is the key to alleviating the food problem. To encounter this condition, which is a rising challenge for human being hybrid rice technology, a sustainable, long-lasting and tested method for raising rice productivity and production plays an important role, as it typically yields 15% to 30% more than modern inbred varieties (Virmani 1994, 1996; Annonymous 1997; Barclay 2010; Suresh *et al.* 2012). Hybrid rice based on the CMS framework has shown over a 20% increase in grain production compared to conventional rice varieties (Toriyama *et al.* 2019).

The primary obstacle to developing hybrid seeds for commercial use in rice, a self-pollinating crop. In self-pollinating crops like rice, hybrid seeds are produced by crossing CMS female lines with appropriate restorers. The CMS system proves valuable for leveraging hybrid technology commercially and cultivating high-yielding hybrid rice (Liao *et al.* 2021). Due to its high seed cost and limited ability for hybrid seed production, hybrid rice is not widely cultivated. There are more than 200 plant species, which revealed cytoplasmic male sterility (CMS), an inherited phenomenon marked by the absence of viable pollen (Chen and Liu 2014). The degree of heterosis in hybrids is mostly determined by diversity among the parents (Sharma *et al.* 2007) or the genetic distance between the restorer and the seed parent; the greater the genetic distance, the more heterosis and vice versa. Due to its key role in heterosis in three-line hybrids, the restorer gene(s) responsible for fertility restoration in CMS should have good combining and great fertility restoration ability. A consistent male sterile system could evade the labour-intensive steps of emasculation and pollination in F_1 seed production, reducing to a cost of hybrid seed production (Lata *et al*. 2023; Lata and Sharma 2024). Identifying restorers and maintainers in rice is crucial for developing hybrid varieties, as it allows breeders to harness heterosis (hybrid vigor) by ensuring that only desired male sterile lines, maintained by the maintainer line, can regain fertility through crossing with a restorer line. This step is vital for utilizing cytoplasmic male sterility (CMS) in hybrid rice production. To maximize heterosis in the final hybrids, breeders employ a CMS system that guarantees the fertilization of female gametes by the restorer line. To prevent unintended fertility restoration and maintain the genetic integrity of the male sterile line, a maintainer line is essential for preserving the male sterile cytoplasm in the CMS line. By accurately identifying restorer lines, breeders can effectively select the best genetic material for high-yield hybrid rice. Molecular markers can also help to identify restorer genes, streamlining and enhancing the selection of desirable genotypes. In order to evaluate fertility restoration and determine the optimum restorer cross combinations for commercial use, the current study was conducted and restorers and maintainers were identified using spikelet and pollen fertility percent.

Materials and Methods

Three CMS lines, IR 5805A, IR 68897A and IR 79156A were utilised, along with 20 improved or traditional varieties suitable for Himachal Pradesh's agro-climatic conditions, used as testers, and crossed using a line x tester mating design. The details of these elite genotypes are listed in Table 1. The 60 experimental hybrids were developed in *kharif*, 2022, by crossing three CMS lines IR 5805A, IR 79156A and IR 68897A with twenty genotypes in a line-x tester mating design. The trial was conducted in kharif, 2023, at the Rice and Wheat Research Centre (RWRC), Malan, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176062 (HP), India. In randomised block design (RBD) with three replications, the parents, including three CMS lines

Table 1. Details of three CMS lines and twenty rice varieties used in the study

S.N.	Parent	Symbol	Parentage/Source
1.	IR 5805 A	L1	IIRR Hyderabad
2.	IR 79156 A	L2	IIRR Hyderabad
3.	IR 68897 A	L3	IIRR Hyderabad
4.	Kasturi	T1	Basmati 370/CRR 88-17- 1-5
5.	HPR 2866	T2	HPR 2143/AC 19146//VL 30424
6.	HPR 2749	T3	Hasansarai/T 23//IR 66295-36-2
7.	HPU2216	T4	IR 8/IR 2033-521-1-1//IR 36
8.	China 988	T5	Secondary selection from China 988
9.	HPR 1156	T6	IR 32429-122-3-1-2/IR 31868-64-2-3-3-3
10.	Himalaya a!	T7	Sabarmati/Ratana
11.	HPR 2143	T8	HPR 9020-2-2-2-1-1-1Phul Patas/HUP 741
12.	Nagardhan	T9	Ching Shi 15(Acc.36852)
13.	HPR 2612	T10	Hassan serai/T 23//IR-66295-36-2
14.	Varundhan	T11	Kunjen 4 (HPR K 2001) IET 16020
15.	VL 221	T12	IR 2053-521-1-1-1/China 1039
16.	HPR 1068	T13	IR 42015-83-3-2-2/IR9758-K2
17.	HPU 741	T14	CR 126-42-5/IR 2061-213
18.	Himalaya ‘!	T15	IR 8/Tadukan
19.	HPR 2880	T16	HPU 2216/Tetep
20.	HPR 2703	T17	RP 2421/VL 221
21.	HPR 2720	T18	Selection from Begmi
22.	HPR 2795	T19	Selection from IC 3131180 germplasm
23.	HPR 2840	T20	Kalizini/HPR2143// HPR2143

(IR 5805A, IR 79156A and IR 68897A) with their maintainer lines (IR 5805B, IR 79156B and IR 68897A) and 60 F_1s were planted in a single row of 2 meters in length with a spacing of 20 cm x 15 cm between rows and plants, respectively.

During the flowering stage, 5-8 spikelets from each panicle of each replication were collected in vials containing 70% ethanol for microscopic examination. The pollen fertility percentage was determined by averaging data from all three replications for each cross using the below-given method. For spikelet fertility, five randomly selected plants from each line and replication, along with one panicle from each, were collected in panicle-size cover paper and sun-dried. After sun drying, spikelets from the panicles were collected in trays and both filled and unfilled spikelets were counted. Spikelet fertility was calculated using a specified formula, followed by averaging the fertility from the randomly selected plants and then the averages from all three replications were computed for each cross and parent.

Fertility scoring and analysis

Estimation of Pollen fertility

To study pollen fertility, in a vial with 70% ethanol, 5–10 spikelets from the just-emerged panicles of randomly chosen plants were gathered. Using forceps, all six spikelet anthers were removed, and the pollen grains were dyed with a 1% I_2–KI solution after being placed on a glass slide. Under an optical microscope, each plant's dark-blue (stainable), transparent (unstainable), and typical abortive pollen grains were counted. The percentage of stainable pollen grains was referred to as the pollen fertility.

$$\text{Pollen fertility} = \frac{\text{Number of fertile pollen}}{\text{Total number of pollen}} \times 100$$

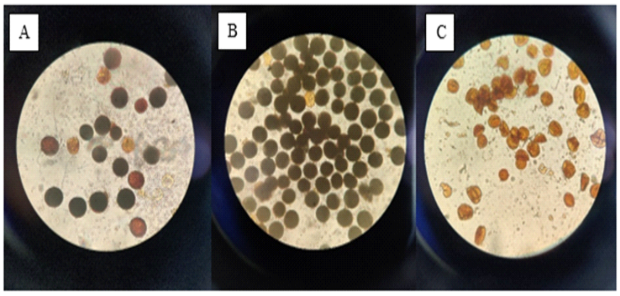

Fig. 1 A= anther with fertile and sterile pollens, B= Anther with fertile pollens, C= Anther with sterile pollens

Estimation of Spikelet fertility

Twenty days after flowering, one panicle is selected from a single plant. The number of filled and unfilled grains was counted to calculate the average seed-setting rate, also known as spikelet fertility.

$$\text{Spikelet fertility} = \frac{\text{Number of filled spikelet}}{\text{Total number of spikelet}} \times 100$$

Classification for pollen fertility restoration

The percentage of stainable pollen grains was the primary criterion used in the fertility estimation to distinguish between sterile and fertile plants. Plants with less than 5% stainable pollen grains were considered sterile (Table 2), while those with more than 90% stainable pollen were deemed fertile. Restorers had over 80%, partial restorers had 50.1–80%, partial maintainers had 1.1–50%, and maintainers had 0-1%.

Table 2. Classification of genotypes into restorers and maintainers (Virmani *et al.* 1997)

Category	Pollen fertility range (%)	Spikelet fertility range
Maintainers	0-1	0
Partial maintainers	1.1-50	0.1-50
Partial restorers	50.1-80	50.1-75
Restorers	>80	>75

Results and Discussion

The use of high-yielding, disease-resistant hybrids instead of conventional open-pollinated varieties is the crucial factor for increasing productivity (Lata *et al.* 2023). Kepping that in view, the highest pollen fertility range for CMS IR5805A was observed in hybrid IR5805A/HPR 2749 (96.24%) and the minimum in hybrid IR 5805 A/Nagardhan (23.71%), while for CMS IR79156A, maximum pollen fertility was seen in hybrid IR 79156 A/HPR 2720 (96.42%) and the lowest in hybrid IR 79156 A/Varundhan (10.14%). For CMS IR 68897 A, the highest pollen fertility was depicted by hy brid IR68897 A/Varundhan (96.62%) and the lowest in hybrid IR 68897 A/HPR 2143 (80.12%) (Table 3).

In CMS line IR 5805A, two genotypes HPR 2866(84.49%) and Varundhan (78.64%) showed complete restoration potential in terms of spikelet fertility. For CMS line IR 79156 A, genotypes HPU 2216 (91.97 %), HPR 2143 (91.03 %) and HPR 2880 (75.2 %) demonstrated full restoration potential. None of the genotypes exhibited full restoration potential for

Table 3. Percent pollen and spikelet fertility of 20 rice genotypes involving three cytoplasmic male sterile lines.

Testers	Lines								
	IR 5805 A			IR 79156 A			IR 68897 A		
	PF (%)	SF (%)	FC	PF (%)	SF (%)	FC	PF (%)	SF (%)	FC
Kasturi	92.93	61.75	PR	77.21	67.92	PR	95.73	46.44	PM
HPR 2866	89.41	84.49	R	91.55	66.94	PR	93.14	55.31	PR
HPR 2749	96.24	65.95	PR	21.39	43.81	PM	90.45	53.38	PR
HPU 2216	84.44	59.38	PR	91.97	81.28	R	81.16	73.54	PR
China 988	94.25	59.65	PR	52.87	69.29	PR	92.30	44.53	PM
HPR 1156	91.1	69.83	PR	68.88	64.00	PR	96.42	42.68	PM
Himalaya II	89.42	54.51	PR	90.64	71.89	PR	93.96	52.26	PR
HPR 2143	91.41	56.35	PR	91.03	76.84	R	80.12	61.68	PR
Nagardhan	23.71	41.02	PM	7.84	70.32	PR	95.31	58.36	PR
HPR 2612	82.88	63.58	PR	48.72	51.71	PR	96.59	51.12	PR
Varundhan	15.58	78.64	R	10.14	37.88	PM	96.62	36.95	PM
VL221	53.10	52.91	PR	11.64	46.73	PM	95.41	56.43	PR
HPR 1068	54.69	63.25	PR	88.68	59.66	PR	81.56	64.74	PR
HPU 741	91.77	55.62	PR	92.8	67.55	PR	93.69	52.55	PR
Himalaya II	87.71	73.22	PR	45.22	60.08	PR	87.56	54.81	PR
HPR 2880	81.42	61.33	PR	78.79	75.17	R	92.92	50.86	PR
HPR 2703	95.11	66.27	PR	53.63	70.38	PR	96.45	50.60	PR
HPR 2720	89.42	61.37	PR	96.42	66.75	PR	96.06	72.90	PR
HPR 2795	52.27	52.40	PR	93.51	70.90	PR	95.97	46.50	PM
HPR 2840	95.61	74.68	PR	95.39	62.89	PR	91.2	50.92	PR

PF=Pollen Fertility; SF=Spikelet Fertility; FC= Fertility Classification

CMS line IR 68897 A. The study also revealed the absence of an effective maintainer within the material under scrutiny, with a notably higher frequency of partial restorers in sample forty-seven. Only nine partial maintainers were identified. Genotypes Kasturi, HPR 2749, HPU 2216, China 988, HPR 1156, Himalaya aI, HPR 2143, HPR 2612, VL 221, HPR 1068, HPU 741, Himalaya II, HPR 2880, HPR 2703, HPR 2720, HPR 2795 and HPR 2880 were identified as partial restorers for CMS line IR 5805. In the case of CMS line IR 79156, genotypes Kasturi, HPR 2866, China 988, HPR 1156, Himalaya II, Nagardhan, HPR 2612, HPR 1068, HPU 741, Himalaya II, HPR 2703, HPR 2720, HPR 2795, and HPR 2880 were recognised as partial restorers. The partial restorers found for CMS line IR 68897 A acknowledged genotypes HPR 2866, HPR 2749, HPU 2216, Himalaya aI, HPR 2143, Nagardhan, HPR 2612, VL221, HPR 1068, HPU 741, Himalaya II, HPR 2880, HPR 2703, HPR 2720, and HPR 2880 (Table 3).

For CMS line IR 5805 A, Nagardhan was identified as a partial maintainer. HPR 2749, Varundhan and VL221 were highlighted as partial maintainers for CMS line IR 79156. For CMS line IR 68897 A, genotypes Kasturi, China 988, HPR 1156, Varundhan and HPR 2795 were marked as partial maintainers. It's noteworthy that no single genotype was found to be a restorer for CMS line IR 68897 A. Genotypes Himalaya AII, HPR 2612, HPR 1068, HPU 741, Himalaya II, HPR 2703 and HPR 2720 were identified as partial restorers for all three CMS lines. The finding revealed that no effective maintainer was found for all three studied CMS lines (Table 3).

For CMS line IR 5805A, the percentage of partial restorer was 85%, restorer 10%, and partial maintainer 5% (Figure 2A). CMS line IR 79156 A showed 70% partial restorer, 15% restorer, and 15% partial maintainer (Figure 2B). CMS line 68897A exhibited 75% partial restorer and 25% partial maintainer (Figure 2C). Fifteen genotypes depicted more than 85 per cent pollen fertility for CMS line IR 5805 A and twelve for CMS line IR 79156 A. For CMS line IR 68897 A all tester depicted more than 80 per cent pollen fertility that showed high degree of fertility restoration. The average pollen and spikelet fertility percentages for

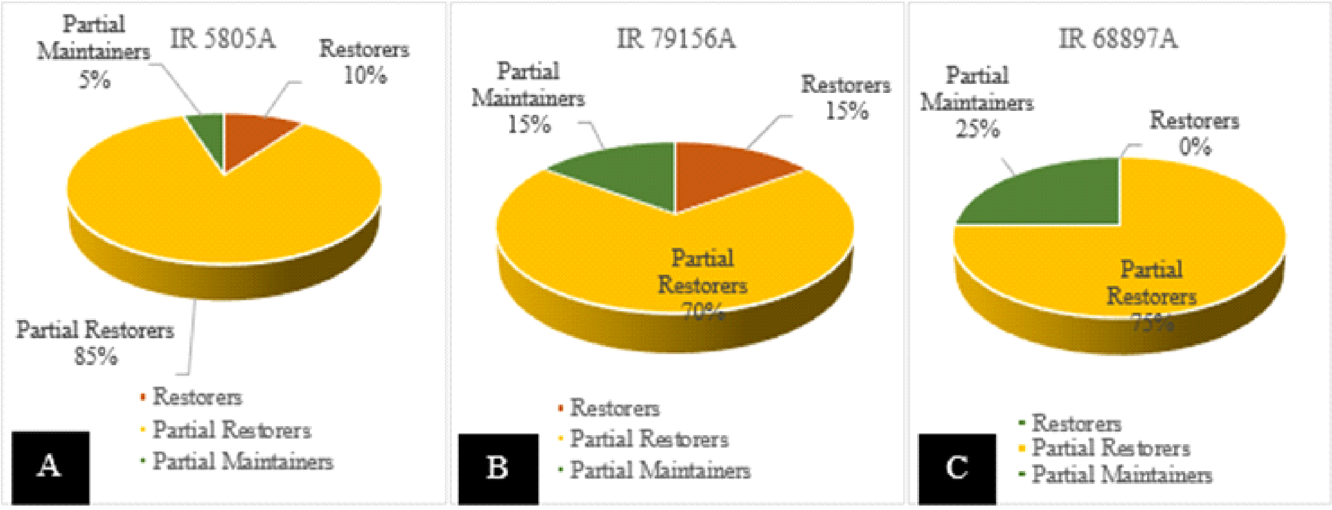

Fig. 2 Percent Restorers, Partial restorers, Maintainers and Partial maintainers for CMS line IR 5805A (A), IR 79156A (B) and IR 68897A (C)

CMS line IR 5805A were 77.64% and 62.71%, respectively. For CMS line IR79156A, the values are 67.47% and 64.20%, respectively and for CMS line IR 68897 A, the values are 92.13% and 54.32%, respectively (Figure 3).

In CMS line 'IR 68897 A, the pollen fertility is high in comparison to spikelet fertility. This phenomenon is occurred due to heat stress during the flowering stage affects various processes in rice, including anther dehiscence, stigma deposition, germination, pollen grain dispersal, pollen tube elongation (Wu *et al.* 2019) and embryo sac fertilization (Shi *et al.* 2018), ultimately reducing spikelet fertility. The main factor in this reduction is the inadequate deposition of pollen

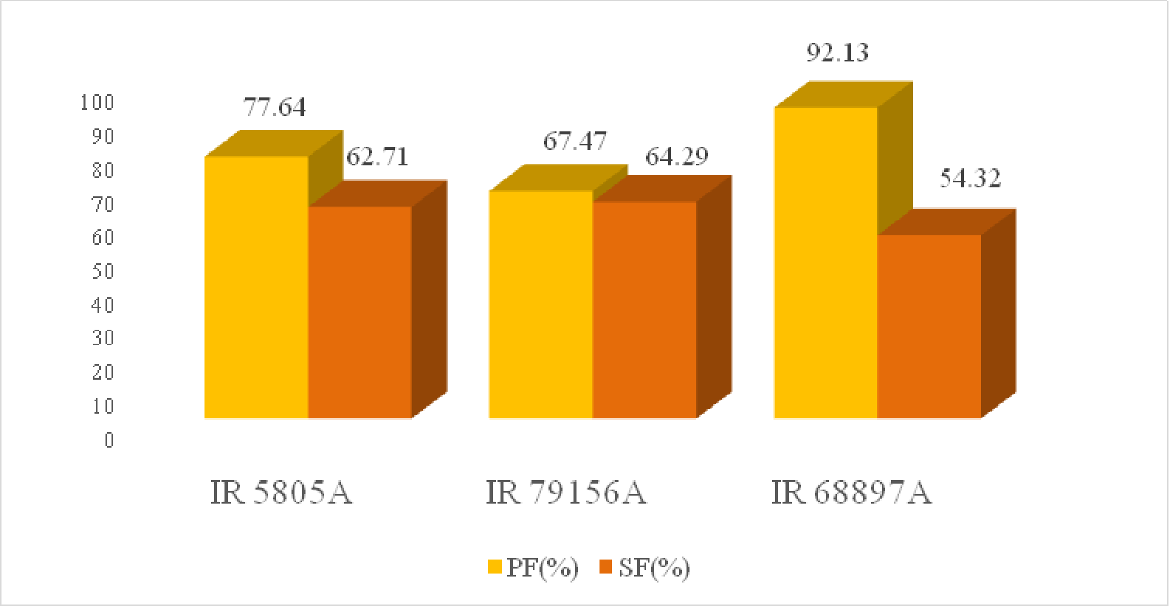

Fig. 3 Average pollen percent fertility and spikelet per cent fertility of 3 CMS line for 20 testers.

grains on the stigma due to disrupted pollen shedding (Matsui *et al*. 1997). For successful pollination and fertilization, more than 10 germinated pollen grains are needed on a stigma, which requires over 20 pollen grains to be deposited. However, heat diminishes pollen germination, necessitating an even higher pollen count on the stigma to achieve the required number of germinated grains (Sawada 1974; Matsui 2005). Therefore, increasing the deposition of pollen grains on stigmas is crucial to mitigate the impact of heat stress on spikelet fertility during flowering.

Conclusion

Variations in fertility restoration among genotypes may arise from different nuclear-cytoplasmic interactions between pollen (tester) and female (CMS line) parents, influenced by distinct fertility restoration genes or variations in their penetrance and expressivity. Additionally, modifier genes in the male parent (Ganesan and Rangaswamy 1998) and the genetic background of the female parent or CMS line (Hossain *et al*. 2010) may also play a role. Promising complete restorers can be further utilized in heterosis breeding by crossing them with male sterile lines to develop high-yielding hybrids.

Conflict of interest: Authors declare no competing interest.

Reference

Abd El-Aty MS, Abo-Youssef MI, Galal AA, Salama AM, Salama AA, El-Shehawi AM and El-Tahan AM 2022. Genetic behavior of earliness and yield traits of some rice (*Oryza sativa* L.) genotypes. Saudi Journal of Biological Sciences **29 (4):** 2691-2697.

Annonymous 1997. Hybrid Rice Breeding Manual 192. Laguna, Philippines: International Rice Research Institute Los Baños.

Barclay A 2010. Hybridizing the world. Rice Today **9 (4):** 32-5.

Chen L and Liu YG 2014. Male sterility and fertility restoration in crops. Annual review of plant biology **65 (1):** 579-606.

El-Mowafi HF, Al Kahtani MD, Abdallah RM, Reda AM, Attia KA, El-Hity MA and El-Esawi MA 2021. Combining ability and gene action for yield characteristics in novel aromatic cytoplasmic male sterile hybrid rice under water-stress conditions. Agriculture **11 (3):** 226.

Ganesan KN and Rangaswamy M 1998. Combining ability studies in rice hybrids involving wild abortive (WA) and Oryza perennis sources of CMS lines. *Oryza* **35:** 113-116.

Hossain MS, Singh AK and Fasih-uz-Zaman FUZ 2010. Genetics of fertility restoration of 'WA'-based cytoplasmic male sterility system in rice (*Oryza sativa*) using indica/japonica derivative restorers. Science Asia **36:** 94-99.

Lata H and Sharma A 2024. Exploitation of heterosis in chilli using genetic male sterile lines for red fruit yield with special reference to high rainfall conditions. Scientific Reports 14: 23061. https://doi.org/10.1038/s41598-024-73728-7

Lata H, Sharma A, Thakur H, Thakur A, Rana RS and Kaur M 2023. Heterosis and combining ability vis-à-vis association for green fruit yield and component traits involving male sterile lines in chilli (*Capsicum annuum*) under wet temperate zone of North Western Himalayas. Plant Breeding **142:** 547–562.

Liao C, Yan W, Chen Z, Xie G, Deng XW and Tang X 2021. Innovation and development of the third-generation hybrid rice technology. The Crop Journal **9 (3):** 693-701.

Matsui T 2005. Function of long basal dehiscence of the theca in rice (*Oryza sativa* L.) pollination under hot and humid condition. Phyton (Horn) **45 (4):** 401-407.

Matsui T, Omasa K and Horie T 1997. High temperature-induced spikelet sterility of japonica rice at flowering in relation to air temperature, humidity and wind velocity conditions. Japanese journal of crop science **66 (3):** 449-455.

Rezvi HUA, Tahjib-Ul-Arif M, Azim MA, Tumpa TA, Tipu MMH, Najnine F, Dawood MF, Skalicky M and Brestic M 2022. Rice and food security: Climate change implications and the future prospects for nutritional security. Food and Energy Security**12:** e430.

Sawada S 1974. Minimal number of dehiscent anthers and pollen grains on the stigma required for fertilization in rice plants. Obihiro University of Agriculture and Veterinary Medicine Academic Research Report. Part **I 9 (1):** 165-171.

Sharma A, Singh G, Sharma S and Sood S 2007. Combining ability and heterosis for pod yield and related horticultural traits in garden pea (*Pisum sativum* L.) under mid hill sub-temperate and high –hill dry temperate conditions of Himachal Pradesh.Indian Journal of Genetics **67 (1):** 47-49.

Shi W, Li X, Schmidt RC, Struik PC, Yin X, and Jagadish SK 2018. Pollen germination and in vivo fertilization in response to high temperature during flowering in hybrid and inbred rice. Plant, Cell & Environment **41 (6):** 1287-1297.

Suresh PB, Srikanth B, Kishore VH, Rao IS, Vemireddy LR, Dharika N, Rao KR, Viraktamath BC and Neeraja CN 2012. Fine mapping of Rf3 and Rf4 fertility restorer loci of WA-CMS of rice (*Oryza sativa* L.) and validation of the developed marker system for identification of restorer lines. Euphytica **187 (3):** 421-435.

Toriyama K, Kazama T, Sato T, Fukuta Y and Oka M 2019. Development of cytoplasmic male sterile lines and restorer lines of various elite Indica Group rice cultivars using CW-CMS/Rf17 system. Rice**12:** 1-4.

Ullah Zaid I, Tang W, He J, Ullah Khan S and Hong D 2019. Association analysis uncovers the genetic basis of general combining ability of 11 yield-related traits in parents of hybrid rice. Annals of Botany Plants **11 (1):** ply077.

Virmani SS 1994. Heterosis and hybrid rice breeding. Monograph on theoretical & applied general 22. International Rice Research Institute.

Virmani SS 1996. Hybrid rice. Advances in agronomy **57:** 377-462.

Virmani SS, Viraktamath BC, Casal CL, Toledo RS, Lopez MT and Monaldo JO 1997. Hybrid rice breeding manual, International Rice Research Institute, Philippines.

Wu C, Cui K, H, Q, Wang W, Nie L, Huang J, and Peng S 2019. Enclosed stigma contributes to higher spikelet fertility for rice (*Oryza sativa* L.) subjected to heat stress. The Crop Journal 7 (3): 335-349.