Himachal Journal of Agricultural Research 50(2): 182-189 (2024)

Genetic assessment of blast resistance in Red × Basmati and Red × White rice crosses under North Hill Zone of India

Varadharajan Nareshkumar[1], Vakul Sood[2], Gaurav Sharma[1] and Neelam Bhardwaj[1*]
Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya,
Palampur-176062

*Corresponding author e-mail: neenabhardwaj@gmail.com

Manuscript received: 29.5.2024; Accepted: 16.8.2024

Abstract

A total of 11 parent lines and 24 hybrids were assessed for blast resistance at RWRC, Malan during *Kharif* 2022 and 2023. The experiments were conducted under both field conditions and controlled conditions. Among parents, eight red rice lines were found susceptible for both leaf and neck blast and all the hybrids exhibited resistant reaction due to the presence of dominant resistance genes in three testers utilized in crossing program during *Kharif* 2022 whereas, during *Kharif* 2023 the disease incidence was very low in parents as well as in hybrids due to less pathogen load built up as a result of various environmental factors. F_2 progenies resulting from ten cross combinations were systematically screened under controlled conditions at Palampur (H.P.) to investigate the genetic inheritance of resistance against blast isolate RML-29. The analysis revealed that genotypes Pusa Basmati 1637 and DHR 9 carried a single blast resistance gene against blast isolate RML-29, while RB 13 possessed two independently segregating blast resistance genes against the same isolate.

Keywords: Blast resistance, chi square test, duplicate gene interaction, red rice

Rice (*Oryza sativa* L.) is the second most important cereal crop in the world after wheat and feeding over half of the world population. Rice can be grown in diverse ecological conditions like; rainfed low land, rain fed upland and flood prone /deep water environment due to its wide range of adaptability and hardiness for different agro-climatic zone (Khush 2005) and its production scenario is rapidly changing in different agroclimatic regions with the change in farming situations in India (Sharma *et al*. 2015). The diseases of rice are estimated to cause annually about 10 per cent losses in its production. Paddy blast appears in an epidemic proportion in many areas year after year and is the main yield limiting factor, especially in mid hill region, where rainfall is very high during rice growing season (Prasad *et al*. 2015). Blast resistance; however, has tended to be unreliable as cultivars initially released as resistant usually succumb to the disease withinfew years of their release. Blast resistance breakdown has been attributed to the existence of extreme virulence present in the pathogen population (Zeigler *et al*. 1995). Considerable success has been achieved in developing blast resistant varieties but due to variability in pathogenicity of the fungus, varieties with long lasting resistance have never been developed. Host resistance represents a low-cost, environmentally-safe and sustainable mean of controlling the disease (Rana *et al*. 2023). Dynamic changes in the race composition of pathogen have often caused breakdown of resistance in most of the improved resistant varieties and the cultivars containing a single major resistance gene become susceptible within few years. Stacking of more than one major resistance gene has proven as one of the effective methods to deliver durable resistance against rice blast (Joshi & Nayak 2010; Koide *et al*. 2010). It is both prohibitive and hazardous to the environment to rely on chemicals and so, the optimum strategy to manage the disease is to use host resistance (Sharma *et al*. 2013). Blast resistance, especially regulated by major genes, may fail in the field conditions (Bonman

---

[1] Department of Genetics and Plant Breeding, [2] Department of Plant Pathology

and Mackill 1988) therefore, effective blast control strategy involves the discovery and deployment of novel sources of resistance, particularly those imparting partial resistance. Therefore, there is an urgent need to identify rice genotypes with broader resistance spectrum for the rice breeding programmes. For breeding durable rice blast resistance and stacking a number of genes into a single cultivar, the knowledge of inheritance pattern of blast disease is prerequisite. Keeping all these facts in view, the present investigation was carried out to assess the blast resistance in field conditions and to study inheritance of seedling blast resistance in broad spectrum resistant germplasm.

Materials and Methods

Field screening of hybrids for leaf and neck blast during Kharif 2022 and 2023

Eight red rice land races of Himachal origin, one basmati line and two white rice lines (Table 1) were taken for the current study. A total of 24 F_1 hybrids were produced using Line × Tester mating design. The hybrids along with parents were evaluated for leaf and neck blast during *Kharif* 2022 and 2023 under field conditions at Rice and Wheat Research Centre (RWRC), Malan. Disease scale of 0-9 was used for the assessment of disease reaction under field conditions seedling and tailoring stage for leaf blast (Table 2).The

Table 1. List of rice genotypes and their parentage/source used in the study

	Accessions	Parentage/Source
	Lines	
1.	*Bongal Dhan*	Landrace collected from Kangra, Himachal Pradesh.
2.	*Totu*	Landrace collected from Kangra, Himachal Pradesh.
3.	*Shimla Collection*	Landrace collected from Shimla, Himachal Pradesh.
4.	*Kalizhini*	Landrace collected from Kangra, Himachal Pradesh.
5.	*Gosha*	Landrace collected from Kangra, Himachal Pradesh.
6.	*Lal Nakanda*	Landrace collected from Kangra, Himachal Pradesh.
7.	*Jattoo*	Landrace collected from Kullu, Himachal Pradesh.
8.	*Sukara Red*	Landrace collected from Chamba, Himachal Pradesh.
	Testers	
1.	*Pusa Basmati 1637*	MAS selection from Pusa basmati 1.
2.	*DHR-9*	Collected from Dept. of Agricultural Biotechnology, CSK HPKV, Palampur.
3.	*RB-13*	HPR 2143$*^4$/Pusa Basmati 1637//HPR 2143$*^4$/DHMAS 164

Table 2. Disease scale used for field screening of parents and hybrids against leaf blast (SES, 2013)

Scale	Description/Symptoms
0	No lesions observed
1	Small brown specks of pinhead size without sporulating centre.
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm indiameter, with a distinct brown margin
3	Lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves
4	Typical susceptible blast lesions 3 mm or longer, infecting less than 4 per cent of the leaf area
5	Typical blast lesions infecting 4-10 per cent of the leaf area
6	Typical blast lesions infection 11-25 per cent of the leaf area
7	Blast lesions infecting 26-50 per cent leaf area
8	Typical blast lesions infection 51-75 per cent of leaf area and leaves are dead
9	More than 75 per cent leaf area affected

genotypes were evaluated for neck blast reaction using Standard Evaluation System (SES) of IRRI for rice on a 0-9 scale during maturity stage (20-25 days after heading) with score 0 as No incidence, 1 (<5%), 3 (5-10%), 5 (11-25%), 7 (26-50%) and 9 (50%).

Evaluation of F_2 seedlings under controlled conditions

For inoculation and disease assessment, stored cultures of the pathogen isolate RML-29 were revived by inoculating onto oat meal agar slants. The resultant mycelia from 10-days-old slants were homogenized in 5 ml of distilled water and plated onto Mathur's medium to induce sporulation. Subsequently, a spore suspension was prepared and Tween 20 was added. Parental genotypes were screened for leaf blast susceptibility using RML-29 isolate (Figure1).

Furthermore, crosses involving susceptible parents were subjected to screening under controlled conditions to decipher the genetic mechanism responsible for resistance to seedling leaf blast. The F_2 progenies resulting from 10 crosses, along with their parental lines, were raised in plastic trays and maintained at a temperature of 25±1°C. Following the application of spore suspension by spraying, data were collected after 7 days of inoculation using a 0-5 scale (Figure 2) as described by Mackill and Bonman (1992) that describe 0 (No evidence of infection), 1 (Brown specks smaller than 0.5 mm in diameter), 2 (brown specks about 0.5-1.0 mm in diameter), 3 (roundish to elliptical lesions about 1-3 mm in diameter with gray centres and brown margins), 4 (typical spindle-shaped blast lesions) and 5 (same as 4 but half of one or more leaves killed by coalescence of lesions). Plants with scores ranging from 3 to 5 were classified as susceptible, while those with scores ranging from 0 to 2 were categorized as resistant.

Estimation of chi square test for inheritance of blast resistance

A powerful test for testing the significance of the discrepancy between theory and experiment was given by Plackett (1973) and is known as "Chi-square test for goodness of fit". It helps us to find if the theory is just by chance or is it due to inadequacy of the theory to fit the observed data (Sharma *et al*. 2024).

If O_i, (I = 1,2,3,…., n) is a set of observed (experimental frequencies) and E_i (i = 1,2,3,…., n) is the corresponding set of expected (theoretical or hypothetical) frequencies, then,

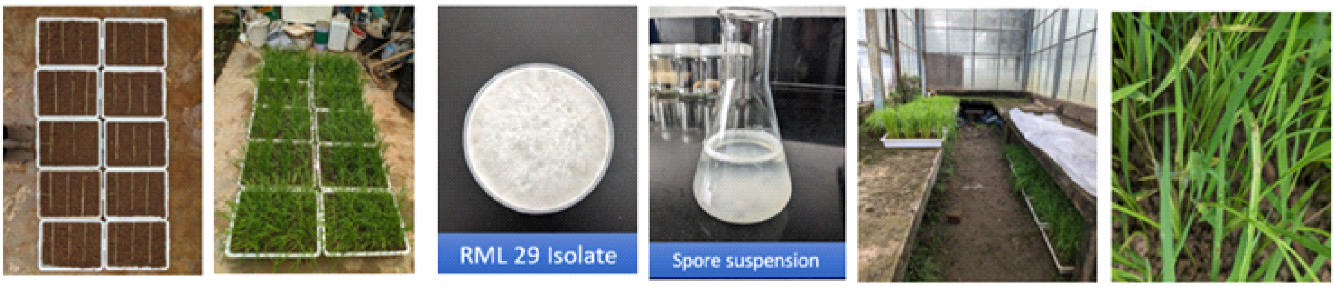

Fig 1: Genetic inheritance of blast resistance in F_2 Seedlings

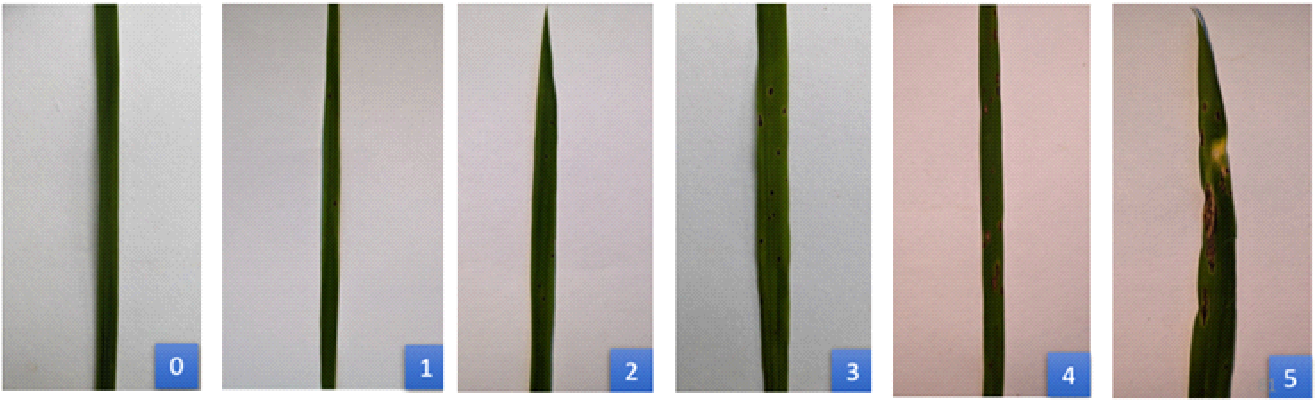

Fig 2: Disease scale 0-5 (Mackill and Bonman, 1992)

$$X2 = \sum_{i=1}^{n} \frac{Oi - Ei}{Ei} \sim X2(n-1)\ \text{df}$$

Where,

N = Total number of classes

O_i = Observed frequencies

E_i = Expected frequencies

Results and Discussion

Leaf blast represents a significant threat to rice crops, often causing substantial damage. Infections typically occur during the seedling or tillering stages, with severe cases leading to a reduction in leaf area available for grain filling, thereby decreasing overall grain yield. Leaf blast can be lethal to rice plants at the seedling stage and can result in substantial yield losses under severe infection conditions. Neck blast stands out as one of the most detrimental diseases affecting rice cultivation, primarily because it directly targets the panicle through neck and node region, resulting in significant yield losses. Results obtained from field screening of leaf and neck blast were presented in Table 3.

Table 3. Reaction of 11 parents and 24 cross combinations to leaf and neck blast under field conditions *kharif* 2022

Parents/ cross combinations	Leaf blast score	Disease reaction	Neck blast score	Disease reaction
Bongal Dhan	6	S	5	MS
Totu	6	S	5	MS
Shimla Collection	7	S	5	MS
Kalizhini	6	S	7	S
Gosha	7	S	7	S
Lal Nakanda	7	S	7	S
Jattoo	6	S	5	MS
Sukara Red	7	S	5	MS
Pusa Basmati 1637	0	HR	0	HR
DHR-9	0	HR	1	R
RB-13	0	HR	0	HR
HPR 2880	2	R	1	R
HPR 3106	4	R	3	MR
HPR 2143	4	MR	3	MR
Bongal Dhan × Pusa Basmati 1637	2	R	1	R
Bongal Dhan × DHR-9	4	MR	5	MS
Bongal Dhan × RB-13	1	R	1	R
Totu × Pusa Basmati 1637	2	R	1	R
Totu × DHR-9	1	R	1	R
Totu × RB-13	3	R	1	R
Shimla Collection × Pusa Basmati 1637	1	R	1	R
Shimla Collection × DHR-9	3	MR	5	MS
Shimla Collection × RB-13	1	R	1	R
Kalizhini × Pusa Basmati 1637	3	R	1	R
Kalizhini× DHR-9	4	MR	3	MR
Kalizhini × RB-13	1	R	3	MR
Gosha × Pusa Basmati 1637	4	MR	3	MR
Gosha × DHR-9	4	MR	3	MR
Gosha × RB-13	1	R	1	R
Lal Nakanda× Pusa Basmati 1637	1	R	1	R
Lal Nakanda × DHR-9	3	R	5	MS
Lal Nakanda × RB-13	1	R	1	R
Jattoo× Pusa Basmati 1637	2	R	1	R
Jattoo× DHR-9	1	R	1	R
Jattoo× RB-13	1	R	1	R
Sukara Red × Pusa Basmati 1637	2	R	1	R
Sukara Red × DHR-9	3	R	3	MR
Sukara Red × RB-13	1	R	1	R

All the F_1 hybrids exhibited high to moderate resistance reactions (SES score = 0-4) during *kharif*, 2022, while all eight red rice landraces displayed susceptible reactions (SES score = 6-7)for leaf blast both at seedling and tillering stage (Figure 3). During *kharif* 2022, all eight red rice land races exhibited moderate to susceptible reactions to neck blast (SES score = 5-7). Similarly, among the crosses examined, *Bongal Dhan* × DHR-9, *Shimla Collection* × DHR-9 and *Lal Nakanda* × DHR-9 were found to have moderate susceptibility (SES score = 5) (Figure 4). Rest of the hybrids were found to be highly resistant to moderately resistant reaction during *kharif*, 2022 for neck blast. Similar results of resistance and susceptibility under field conditions were also reported by Saikiran *et al*. (2019), Sidhu *et al*. (2021) and Kapoor *et al*. (2022) in parents and hybrids. However, during *kharif* 2023, none of the parental lines or cross combinations under the study were found susceptible to leaf blast or neck blast (Table 3).

During *Kharif* 2023, the disease incidence was found to be very low likely due to insufficient pathogen load resulting from variable environmental factors (Low relative humidity & rainfall), application of fungicides in nearby fields as well as cultivation of disease resistant varieties and hybrids at RWRC, Malan and surrounding region. These factors should be considered for field screening studies in non-hotspot locations/environments for efficient screening. On the other hand, developing resistance for major disease

Fig.3 Leaf blast in field condition

Fig.4. Neck blast in field condition

may increase the susceptibility of varieties and hybrids towards minor diseases.

Genetic inheritance of blast resistance in F_2 Seedlings

Out of eleven parents screened for blast resistance, genotypes Pusa Basmati 1637, DHR 9 and *Totu* exhibited highly resistant reaction (0-1) against blast isolate RML 29 whereas lines *viz*., *Bongal Dhan, Totu, Shimla Collection* and *Kalizhini* were recorded to have susceptible (SES score - 5) reaction.

In F_2 seedlings, a total of 352 (*Bongal Dhan*×Pusa Basmati 1637), 211 (*Totu*×Pusa Basmati 1637), 354 (*Kalizhini*×Pusa Basmati 1637), 185 (*Lal Nakanda*×Pusa Basmati 1637), 238 (*Totu*× DHR 9), 320 (*Kalizhini*× DHR 9), 246 (*Lal Nakanda*× DHR 9), 370 (*Bongal Dhan*× RB 13), 392 (*Totu*× RB 13) and 377 (*Kalizhini*× RB 13) seedlings were found to be resistant with a disease score of 0-3 (Table 4). Conversely, 106 (*Bongal Dhan*×Pusa Basmati 1637), 91 (*Totu*×Pusa Basmati 1637), 108 (*Kalizhini*×Pusa Basmati 1637), 47 (*Lal Nakanda*×Pusa Basmati 1637), 65 (*Totu*× DHR 9), 136 (*Kalizhini*× DHR 9), 104 (*Lal Nakanda*× DHR 9), 44 (*Bongal Dhan*× RB 13), 42 (*Totu*× RB 13) and 46 (*Kalizhini*× RB 13) seedlings exhibited susceptible reaction with a disease score of 3-5 (Table 4).

The F_2 seedlings of seven cross combinations which included Pusa Basmati 1637 and DHR 9 as one of the parents displayed a good fit to 3:1 ratio (Table 4). These results were in line with earlier reports that showed the presence of single dominant gene *Pi9* and *Pi-42* in Pusa Basmati 1637 and DHR 9, respectively (Khanna *et al*. 2015). In Pusa Basmati 1637, *Pi9* gene was inherited from Pusa Basmati 1637 through marker assisted selection. Whereas, cross combinations that included RB 13 as one of the parents displayed 15:1 phenotypic ratio against RML 29 blast pathogen (Table 4). This indicated the presence of two independently segregating dominant genes in RB 13 (*Pi9* and *Pi-ta*). In RB 13, the resistant gene *Pi9* was inherited from

Table 4: Estimation of chi square value for goodness of fit in F_2 seedlings for blast inheritance

Crosses	F_1	Disease score in F_2 seedlings						Observed ratio	Expected ratio	Chi square value (calculated)	P value At 5%
		Resistant			Susceptible						
		0	1	2	3	4	5				
Bongal Dhan × Pusa Basmati 1637 (*Pi9*)	R	135	116	101	23	39	44	352:106	3:1	0.841	7.815
Totu× Pusa Basmati 1637 (*Pi9*)	R	77	56	78	21	41	29	211:91	3:1	4.242	7.815
Kalizhini × Pusa Basmati 1637 (*Pi9*)	R	135	91	128	18	54	36	354:108	3:1	0.649	7.815
Lal Nakanda × Pusa Basmati 1637 (*Pi9*)	R	81	44	60	8	16	23	185:47:00	3:1	2.781	7.815
Totu × DHR-9 (*Pi-42*)	R	101	96	31	22	11	32	238:65	3:1	2.034	7.815
Kalizhini × DHR-9 (*Pi-42*)	R	125	122	73	33	45	58	320:136	3:1	5.660	7.815
Lal Nakanda × DHR-9(*Pi-42*)	R	124	61	61	24	41	39	246:104	3:1	4.148	7.815
Bongal Dhan × RB-13 (*Pi9* & *Pi-ta*)	R	115	158	107	12	16	16	370:44	15:1	13.542	24.996
Totu × RB-13 (*Pi9* & *Pi-ta*)	R	138	140	114	9	15	18	392:42	15:1	8.701	24.996
Kalizhini ×RB-13 (*Pi9* & *Pi-ta*)	R	158	115	104	14	10	22	377:46	15:1	15.44	24.996

Pusa Basmati 1637 and Pita was inherited from DHMAS 164 line through three generations of back cross (Chauhan *et al*. 2021).

Previous studies have indicated that the hereditary traits governing disease response vary across different strains of the pathogen (Flores 1981). Nonetheless, resistance is frequently governed by one or two dominant genes (Yu *et al*. 1987). Rana *et al*. (2017) investigated the hereditary transmission of resistance to *P. grisea* races MSF-9 and DSN-37-1, revealing that a single locus in TDH251 and TDH 10 conferred resistance to race DSN-37-1, while two loci were responsible for resistance to race MSF-9. Our current investigation identified blast-resistant genotypes, namely Pusa Basmati 1637, DHR 9, and RB 13, as promising candidates for breeding resilience against blast disease in rice.

Conclusion

Rice blast, caused by *Magnaporthe oryzae*, is considered as the most damaging disease in rice due to its high pathogen plasticity and mutation rate. Rice blast can devastate entire rice crop within 15 to 20 days, leading to yield losses up to 100%. In our present study, almost all F_1's were found to be resistant to leaf and neck blast under field conditions during both the seasons clearly indicating the inheritance of blast resistant genes *Pi9* from Pusa Basmati 1637, *Pi-42* from DHR-9 and *Pi9*, *Pi-ta* from RB-13. These results were also supported with chi square analysis for "goodness of fit" which indicated monogenic ratio for crosses involving Pusa Basmati 1637 and DHR-9 and duplicate gene interaction (15R:1S) for crosses involving RB-13. Moreover, it is proposed that the pyramiding of multiple resistance (R) genes could provide substantial benefits in combating the diverse pathogenic races that arise under natural field conditions.

Acknowledgments: The authors would like to express their gratitude to the Heads, Department of Genetics and Plant Breeding, CSK HPKV, Palampur (H.P.) for providing financial support and research facilities.

Conflict of interest: Authors declare no competing interest.

References

Bonman JM and DJ Mackill 1988. Durable resistance to rice blast. Oryza **25:** 103-110.

Chauhan R, Kapila RK and Rathour R 2021. Introgression of rice blast and bacterial leaf blight resistance genes into HPR2143 rice variety through marker assisted selection and anther culture. International Journal of Chemical Studies **9 (1):** 991-997.

Flores JA 1981. Inheritance of resistance to leaf blast *Pyricularia oryzae* Cav. in some rice lines and varieties. Master's thesis, University of the Philippines, Los Baños.

Joshi RK and Nayak S 2010. Gene pyramiding-A broad-spectrum technique for developing durable stress resistance in crops. Biotechnology and Molecular Biology Reviews **5:** 51-60.

Kapoor P, Rana SK, Upmanyu S and Katoch S 2022. Screening and identification of resistant sources against sheath blight of rice. Himachal Journal of Agricultural Research **48 (01):** 142-144.

Khanna A, Sharma V, Ellur RK, Shikari AB, Gopala Krishnan S, Singh UD, Prakash G, Sharma TR, Rathour R, Variar M and Prashanthi SK 2015. Development and evaluation of near-isogenic lines for major blast resistance gene (s) in Basmati rice. Theoretical and Applied Genetics **128:** 1243-59.

Khush GS 2005. What it will take to feed 5.0 billion rice consumers in 2030. Plant Molecular Biology **59:** 1–6.

Koide Y, Kawasaki A, Telebanco YM, Hairmansis A, Nguyet N, Bigirimana J, Fujita D, Kobayashi N and Fukuta Y 2010. Development of pyramided lines with two resistance genes, Pish and Pib, for blast disease (*Magnaporthe oryzae* B. Couch) in rice (*Oryza sativa* L.). Plant Breeding **129:** 670-675.

Mackill DJ and Bonman JM 1992. Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology **82 (7):** 746-9.

Plackett RL 1973. Karl Pearson and the chi-squared test. International statistical review/revue internationale de statistique **1:** 59-72.

Prasad R, Sharma A and Sehgal S 2015. Influence of weather parameters on occurrence of rice blast in mid hills of Himachal Pradesh. Himachal Journal of Agricultural Research **41 (2):** 132-136.

Rana C, Sharma A, Rathour R, Bansuli, Banyal DK, Rana RS and Sharma P 2023. In vivo and in vitro validation of powdery mildew resistance in garden pea genotypes. Scientific Reports **13:** 2243.

Rana D, Paul YS and Rathore R 2017. Studies on inheritance of resistance to paddy blast. Plant Disease Research **32**

(1): 58-60.

Saikiran V, Krishna L, Jagadeeshwar R, Mohan YC and Shankar VG 2019. Identification of superior hybrids and parents for blast resistance in rice (*Oryza sativa* L.). Journal of Pharmacognosy and Phytochemistry **8 (3):** 3010-3013.

Sharma A, Dhole VJ, Lata H, Kumar N and Thakur A 2024. Isolation of mutants using gamma rays and studying genetics of afila and 3-flowers/pedicel attributes in garden pea (*Pisum sativum* L.). Bazilian Archives of Biology and Technology 67: e24230622. https://doi.org/10.1590/1678-4324-2024230622

Sharma A, Kapur P, Katoch V, Singh Y and Sharma JD 2013. Development of powdery mildew resistant genotypes in garden pea (*Pisum sativum* L.) through generation mean analysis approach. Indian Journal of Genetics **73 (4):** 371-377.

Sharma SK, Rana SS, Subehia SK and Negi SC 2015. Production potential of rice-based cropping sequences on farmers' fields in low hills of Kangra district of Himachal Pradesh. Himachal Journal of Agricultural Research **41 (1):** 20-24.

Sidhu PS, Sidhu N, Upmanyu S and Gill RS 2021. Screening of aromatic rice genotypes for resistance against bacterial blight and neck blast diseases. Journal of Pharmacognosy and Phytochemistry**10:** 307-311.

Yu ZH, Mackill DJ and Bonman JM 1987. Inheritance of resistance to blast in some traditional and improved rice cultivars. Phytopathology **77 (2):** 323-326.

Zeigler RS, Cuoc LX, Scott RP, Bernardo MA, Chen DH, Valent B and Nelson RJ 1995. The relationship between lineage and virulence in Pyricularia grisea in the Philippines. Phytopathology **85:** 443-451.