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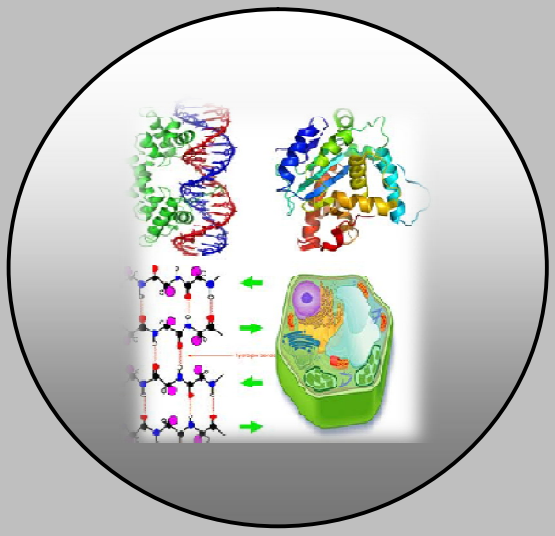

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RESEARCH PAPER

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Mutagen Induced CLS (*Cercospora* Leaf Spot) Resistance in Mungbean

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ABSTRACT

In the present investigation, attempts have been made to induce genetic variability in three CLS susceptible cultivars (TARM-1, TARM-2 and TARM- 18) of Mungbean [Vigna radiata (L.) Wilczek] developed by BARC Mumbai. The main objective of the study is to select CLS (Cercospora leaf spot) resistant/ tolerant plant types. In our attempt, we have raised EMS (Ethyl methanesulphonate) and gamma ray treated populations and the M_2 population was subjected to selection. The screening of M_2 population let us to select eight resistant plant types. The resistance status of these mutants was first decided by correlating their CLS percentage infection with the susceptible parent and resistant check and then confirmed by analyzing antimicrobial compound (phytoalexin) Genestein in their leaves, biochemically. All the selected mutants were observed with negligible infection and high phytoalexin content. Performance of these mutants was investigated up to M_4 generation for the stability of character. The progeny showed segregation, but improvement in the form of resistance to CLS.

Key Words: Vigna radiata, Cercospora, Genestein, Resistant and Susceptible.

INTRODUCTION

In India, *Vigna radiata* (Mungbean) occupies the important place in pulse crops. However, this crop is susceptible to several pathogens. The most damaging diseases are mildew, leaf spots and root rot caused by *Erysiphe polygoni*, *Cercospora canescens* and *Rhizoctonia* respectively, which results into huge losses in yield. Therefore, development of high yielding cultivars with multiple disease resistance is essential in mungbean. The cultivars resistant to mildew *(Erysiphe polygoni)* (Reddy *et al.* 1987, Reddy and Pawar, 1997) and MYMV (Mungbean yellow mosaic virus) (Raje and Rao, 2002) have already been developed.

However, there are no reports on the development of resistant cultivars, for *Cercospora*. Therefore, in the present investigation attempts have been made to induce the genetic variability for the traits related to CLS resistance, using gamma rays and EMS. This paper deals with the induction of mutations and selection of plant types showing resistance to *Cercospora* leaf spots, and their evaluation up to M_4 for stabilization of characters.

MATERIAL AND METHODS

In the present investigation, the seeds of TARM-1, TARM-2 and TARM-18 were procured from BARC, Mumbai. 100 seeds each of TARM-1, TARM-2 and TARM-18 were treated with 40, 60, 80 kR of gamma rays and 0.2, 0.3, 0.4% aqueous solution of EMS for 18h. For each treatment separate control was maintained. Soon after both the treatments, seeds were sown in the experimental field to raise the M_1 populations. The seeds of M_1 plants were collected and used to raise the M_2 population, in next growing season. Gamma rays and EMS treated M_2 populations of all three cultivars were screened thoroughly for plant types showing the resistance to *Cercospora* leaf spots. Initially the selections were made on the basis of percentage infection of *Cercospora canescens* on the leaves of plant. Then the resistant status was confirmed by analyzing phytoalexin content in their leaves. The phytoalexin Genestein was analyzed using HPLC (Shimadzu). The HPLC settings were: column- ODS (250 x 4 mm), oven temperature - 35^o C, solvent- 50% aqueous Acetonitrile, flow rate- 1.5 ml/min., ·$_{max}$- 265 nm, injection volume- 20·l.

RESULTS AND DISCUSSION

In the present study, EMS and gamma rays were found effective in inducing wide spectrum of genetic changes affecting characters related to defense genes in *Vigna radiata*. During the investigation, eight plant types showing resistance to *Cercospora canascens* have been isolated in M_2 populations of TARM-1, TARM-2 and TARM-18. In field grown, M_2 populations of TARM-1, two plants in EMS (0.3%) treatments and two with gamma ray treatment showed negligible amount of leaf spots (0.0 to 2.2%) caused by *Cercospora*. However, in these plants the spots mostly appeared on the aging leaves but the severity is negligible, while, the parental check showed heavy CLS infection (28.2-31.5%). The selected plant types also revealed high Genestein content in their leaves (68.2 to 212.5 ·g/g fresh tissue weight).

In another cultivar, TARM-2, three plants showing tolerance to *Cercospora* was observed. The disease incidence on these plants was very low (0.0 to 3.2%) and level of phytoalexin Genestein, was appreciably high (65- 216.5··g/g fresh tissue weight) indicating the possibility of mutation for biochemical trait (Table 1). From the mutagenized populations of TARM-18 cultivar, only one plant with negligible disease incidence (4.6%) and appreciably high Genestein content was selected (Table1).

Table 1. Data on the resistant plant types isolated in M_2 generation and their performance in M_3 and M_4 generation.

Cultivar	Treatment	M2 plant		M3 plant		M4 Plant	
		% inf	PA	% inf	PA	% inf	PA
TARM-1	Control	30.5%	7.8	28.6	8.5	31.5	6.8
	Gamma Rays (60kR)	30/C/3-1		30/C/3/1-1		30/C/3/1/1-1	
		0.0	212.5	0.0	190.5	0.0	145.9
						30/C/3/1/1-2	
						0.0	222.2
						30/C/3/1/1-20	
						0.0	150.9
				30/C/3/1/1-2		30/C/3/1/2-2	
				0.0	168.3	0.0	174.5
						30/C/3/1/2-12	
						0.0	193.7
						30/C/3/1/2-18	
						0.0	162.7
						30/C/3/1/2-25	
						0.0	208.3
	Gamma Rays (60kR)	30/D/18-1		30/D/18/1-1		30/D/18/1/1-1	
		1.8	68.2	1.5	60.3	2.6	52.8
						30/D/18/1/1-5	
						1.8	62.8
				30/D/18/1-7		30/D/18/1/7-4	
				1.9	62.5	2.3	55.7
						30/D/18/1/7-9	
						2.9	49.9
						30/D/18/1/7-15	
						2.8	51.5
	EMS 0.3%	25/D/25-1		25/D/25/1-4		25/D/25/1/4-1	
		0.0	155.3	0.0	160.8	0.0	228.4
						25/D/25/1/4-2	
						0.0	160.3
						25/D/25/1/4-9	
						0.0	186.3
						25/D/25/1/4-24	
						0.0	119.3
				25/D/25/1-9		25/D/25/1/9-1	
				0.0	120.8	0.0	109.4
						25/D/25/1/9-5	
						0.0	160.3
						25/D/25/1/9-15	
						0.0	240.2
						25/D/25/1/9-24	
						0.0	221.5
						25/D/25/1/9-27	
						0.0	159.32
		24/C/1-2		24/C/1/2-1		24/C/1/2/1-1	
		2.2	79.5	2.0	89.6	2.3	77.6
						24/C/1/2/1-3	
						0.0	83.8
						24/C/1/2/1-5	
						0.0	268.9
						24/C/1/2/1-6	
						0.0	155.5
						24/C/1/2/1-7	
						3.7	62.6
						24/C/1/2/1-8	
						0.0	189.4

						24/C/1/2/1-15	
						2.8	55.4
TARM-2	Control	28.5	7.5	31.8	5.9	29.6	6.5
	Gamma Rays (60kR)	29/C/18-2		29/C/18/2-1		29/C/18/2/1-5	
		0.0	126.4	0.0	115.6	0.0	110.8
						29/C/18/2/1-16	
						0.0	107.9
						29/C/18/2/1-17	
						0.0	118.8
				29/C/18/2-4		29/C/18/2/4-2	
				0.0	125.9	0.0	157.3
						29/C/18/2/4-21	
						0.0	137.6
	Gamma Rays (80kR)	29/D/22-4		29/D/22/4-9		29/D/22/4/9-5	
		0.0	216.5	0.0	188.6	0.0	199.1
						29/D/22/4/9-9	
						0.0	185.8
						29/D/22/4/9-13	
						0.0	203.6
						29/D/22/4/9-14	
						0.0	206.3
TARM-18	Control	43.8	5.9	46.5	5.3	45.2	4.6
	Gamma rays (60kR)	14/B/18-34		14/B/18/34-5		14/B/18/34/5-4	
		4.6	57.6	4.9	59.2	2.8	102.8
						14/B/18/34/5-6	
						2.5	88.8
						14/B/18/34/5-7	
						4.6	67.2
						14/B/18/34/5-12	
						2.6	95.7
Pant M-3	Resistant check	0.0	92.5	0.6	82.5	0.0	88.2

% inf= percent infection of CLS, Phy= phytoalexin Genestein content□□□g/g fresh tissue wt).

The M_3 population was found to be heterogeneous and segregating for above character. Therefore, only the desirable (with low CLS infection and high genestein content) progeny plants of M_2 selections were screened in M_3 and carry them for M_4 generation. All mutants showed low disease incidence and appreciably high phytoalexin content (Table 1). M_4 population shows few degree of stabilization in the character. The CLS percentage infection in all selected mutants was very low (0.0% to 4.6%) and all of them revealed higher amount of Genestein in their leaves (Table 1). Mungbean is a major pulse crop in India but most of the varieties grown here are susceptible to the *Cercospora* leaf spot (CLS) disease and root rot caused by *Cercospora canescens* and *Rhizoctonia* sp. respectively, which leads to the huge losses in the yield. Hence, producing variety with multiple disease resistance is top priority in Mungbean improvement. Owing to inherent problems in conventional breeding, a selection method based on plant defense has been employed in this investigation. The mutagen treated population was screened for high level of defense compound phytoalexin. In crop plants, several workers have induced the desirable traits using variable chemical and physical mutagens (Reddy *et al.*1994, Kothekar *et al.* 1996, Sarin and Koul, 1999). Physical and chemicals mutagens were successfully used to induce multiple disease resistance (Reddy and Pawar, 1997, Gautam *et al.,* 1998, Raje and Rao 2002). Sharma *et al.* (2003) assessed the mutagen induced potential resistance in Maize against *Bipolaris maydis*. Yadav *et al.* (2003) and Sheeba *et al.* (2003) made the similar reports in pearl millet and *Sesamum indicum* L. respectively.

Kale (1998) correlated the production of phytoalexin content in *Arachis hypogaea* mutants and their resistance against pathogens. The mutagen treatment may cause the micromutations in the nuclear genes that are involved in the synthesis of antimicrobial compounds like phytoalexins and defense related proteins. The high level of these compounds confers the resistance to the plants against pathogens (Hain *et al.*, 1993, Reddy and Pawar, 1997, Raje and Rao, 2002) our results are in conformation with these reports. Siemans *et al.*, (2009) and Majid *et al.* (2011) in there review articles indicated that, the secondary metabolites play vital role in disease resistance status of crop plants.

In Mungbean, it seems that the genes involved in the biosynthesis of Genestein have been mutated by mutagen treatment and these mutant alleles are hyperactive which ultimately led to Genestein synthesis at high level. This probably, resulted in expression of resistance to CLS disease, in these mutants.

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REFERENCES

Gautam, R. K., Sheti, G. S., Rana, M. K. and Sharma, S. K. 1998. Induction, inheritance pattern and agronomic performance of awned mutants in a multiple disease resistant bread wheat. *Indian Journal of genetics and Plant Breeding* 58 (4): 417-422.

Han, R., Reif, H. J., Krause, E., Lengbartels, R., Kindl, H., Vorman, B., Weise, W., Schmelzer, E., Schoreier, P. H. and Stocker, R. H. 1993. Disease resistance results from foreign phytoalexin. *Nature* 14**:**153-154.

Kale, M. C. and Choudhary A. D. 2001. Induction of phenylalanine ammonia- lyase in groundnut cutltivars in response to biotic and abiotic stress. *Indian Phytopathology* 54(3): 288-292.

Kothekar, V. S., Khndelwal, A. R. and Harshulkar, A. M. 1996. Low trypsin and chymotrypsin inhibitor mutants in winged bean (*Psophocarpus tetragonolobus* (L.) DC. *Dr. Babasaheb Ambedkar Marathwada University Journal* 27: 71-80.

Majid, M., Khan, T. A. and Mohammad, F. 2011. Role of secondary metabolites in defense mechanism of plants. *Biology and Medicine* 3(2): 232-249.

Raje, R. S. and Rao, S. K. 2002. Screening of mungbean (*Vigna radiata* L. Wilczek) germplasm for yellow mosaic virus and *Cercospora* leaf spot. *Legume Research* 25(2): 99.

Reddy, K. S. and Pawar, S. E. 1997. Powdery mildew (*Erysiphe polygoni* DC) resistance in mungbean In Proceeding of International conference Genetics and Biotechnology in crop improvement Meerut, pp 4-5

Reddy, K. S., Pawar, S. E., Wanjari, K. B. and Bhatia, C. R. 1987. Screening of powdery mildew (*Erysiphe polygoni* DC) resistance in mungbean (*Vigna radiata* (L) Wilczek) using excised leaves. In *Proceeding of Indian Academy of Sciences* (*Plant Sci*) pp-365.

Reddy, K. S., Pawar, S. E., Wanjari, K. B. and Bhatia, C. R. 1994. Development of powdery mildews resistant and high yielding varieties of mungbean. In International Symposium On Pulses Research New Delhi. pp 146-147.

Siemans, D.H., Haugen, R., Matzner, S. and Vanasma, N. 2009. Plant chemical defence allocation constrains evolution of local range. *Molecular Ecology*, 18(23): 4974-4983.

Sarin, S. and Koul, P. 1999. Mutation breeding in improvement of *Plantago ovata* Forsk. *Indian Journal of genetics and Plant Breeding* 59 (3): 337.

Sharma, R. C., Rai, S. N., Mukherjee, B. K. and Gupta, N. P. 2003. Assessing potential of resistance source for enhancement of resistance to Maydis leaf blight (*Bipolaris maydis*) in Maize (*Zea mays* L.). *Indian Journal of genetics and Plant Breeding* 63 (1**):** 33-35.

Sheeba, A., Ibrahim, S. M., Yogaminakshi, P. and Balu, S. 2003. Effect of mutagens on quantitative traits of M2 generation of Sesame (*Sesamum indicum* L). *Indian Journal of genetics and Plant Breeding* 63 (2): 173-175.

Yadav, O. P., Weltzein- Ruttunde, W. and Bidinger, F. R. 2003. Genetic variation in draught responses in landraces of pearl millet (*Pennisetum glaucum*). *Indian Journal of genetics and Plant Breeding* 63 (1): 37-41.

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