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Studies on stripe rust resistance in Induced Barley (*Hordeum Vulgare*) mutants. Isolation and evaluation of resistant mutants.

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Abstract:

Mutation was caused in mother verities of barley i.e. Gitanjali and K555 with the help of gamma rays and EMS. Eighteen mutant vatities were identified. Total twenty varients that includes mother varities and mutant varities were grown in randomized block design. Infection was caused by stripe rust fungi *Puccinia striiformis* f. sp. *Hordei*. The intensity of pustule development was calculated on modified cobb's scale. All the mutants were evaluated for Number of grains/ spike, 100 seed weight (g), seed yield/ plant(g) and biological yield/plant(g).Minimum loss occurred in mutant line K30-82(3.22%) followed by KE4-47(3.65%), KE2-63(5.69%), KH3-32(6.64%) and K40-12 for number of grains per spike, (K40-12), 5.20% (KE4-47) , 7.49%(KE2-63) and 8.20% (KH3-32) for 100 seed weight, K 40-12(6.03%) followed by KE2-63(7.49%), KH3-32(8.93%) and K30-91(12.83%) for seed yield per plant, KE3-47(5.06%) followed by K30-82(6.7%), KE2-63(8.98%), K40-12(9.27%) and KE232(9.39%) for Biological yield.

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Introduction

Stripe rust is caused by the fungus *Puccinia striiformis* f. sp. *hordei*, damages barley crops, sometimes completely wiping out crops. The fungus produces stripes of rust pustules between the veins of leaves, and sometimes on barley heads. The pustules may be more yellow than orange, so the disease is sometimes referred to as yellow rust. Barley stripe rust can build up rapidly if conditions are cool and wet, ruining crops. Rust spores are small, light and can survive away from plants for several days so that once introduced, the disease can be easily spread over large distances by wind and attached to clothing, machinery and tools.

The rust disease reduce total biomass production by the crop in different ways (1) killing whole plant or its branches (2) General stunting (3)leaf damage leading to reduced photosynthetic area(4) Damage of reproductive organs including fruits and seeds. Barley stripe rust poses a significant threat to India's barley crops since around 80 per cent of the varieties that we grow are susceptible. The host varieties are classified as susceptible or resistance according to their response to the pathogen. The different reactions of the host may be grouped into- (i) susceptible, (ii) immune (iii) resistant and (iv) tolerant.

In case of **susceptible** reaction the disease development is profuse and is not checked by the genotypes of host. When host does not show any symptom of disease it is known as **immune** reaction. The **resistance**

denotes less disease development than the susceptible variety and is a relative attribute. Infection and establishment do take place but the growth of the pathogen in the host tissues is restricted. **Tolerance** implies that the host is attacked by pathogen but there is no loss in biomass production or yield.

The rust disease can be controlled either by use of resistant varieties or use of fungicides, insecticides, weedicides which cause toxic effect on our ecosystem. Hence the use of chemical against disease should be minimized. Use of resistant varieties is only effective method to combat rust disease in barley.

The present research work aim to develop rust resistant varieties with the following objectives - 1. To study the reaction of stripe rust fungi to different mutants in field and in alkathene house. 2. Classification of reaction on the basis of symptoms produced in different environments (field condition and green house condition). 3. Evaluation of mutants on basis of loss caused by rust fungi and biomass produced. 4. Selection of resistant and superior mutants. 5. To study the performance of resistant mutants.

MATERIALS AND METHODS

The materials of the present research work are the induced mutant lines isolated from the M_2 and M_3 generations as the result of physical and chemical mutagens treatment of seeds of cultivar K555 (Hulled) and Gitanjali (Hull-less) (Table-1) (PRAJAPATI, 2005).

S.No.	Mutants	Mother varieties	Mutagen	Dose/conc.
1	K50-38	K555(hulled)	Gamma rays	50Kr
2	K50-44	"	"	"
3	K50-47	"	"	"
4	K50-97	"	"	"
5	K50-102	"	"	"
6	K40-11	"	"	40Kr
7	K40-12	"	"	"
8	K40-67	"	"	"
9	K40-73	"	"	"
10	K30-82	"	"	30Kr
11	K30-91	"	"	30Kr
12	KE2-63	"	"	0.2%
13	KE3-75	"	EMS	0.3%
14	KE4-47	"	EMS	0.4%
15	KE4-87	"	EMS	0.4%

16	G50-35	Geetanjali(hull-less)	Gama rays	50Kr
17	G50-47	"	Gama rays	"
18	GH3-32	"	HA	0.3%
19	K555	Mother variety	Control	-
20	Geetanjali	Mother variety	"	-

COLLECTION AND MULTIPLICATION OF INOCULUM

The uredospores of stripe rust fungi *Puccinia striiformis* f. sp. *Hordei*.were collected from Simla (India) growing on susceptible varieties. Samples containing uredospores which included well infected leaves and stems were kept in rough paper envelops specially meant for this purpose. The samples were dried for 12 hours in shade in order to remove the excess surface moisture and kept in refrigerator.

Identified susceptible variety LBC-23 (JOSHI *et al*., 1988) was used to multiply the inoculums. This variety was sown in pots 20 days before the experimental materials were sown. After 5 days of germination the pots containing 10-15 seedlings were irrigated with 0.12 percent solution of maleic hydrazide to increase the susceptibility as recommended by JOSHI (1965) and JOSHI *et al.*, (1988). After 15 days of germination plants were inoculated with uredospores of rust. Both surfaces of leaves of seedlings were sprayed with uredospore suspension in water using bottle sprayer for two days two times. The inoculated plants were kept in cloth tent chamber to create the favorable atmosphere for infection. After few days the uredospores appeared in plenty amounts.

FIELD SCREENING

Mutants were sown in Randomized Block Design with three replications in three rows of 3 m length in plots. An additional disease free treatment was also maintained to compare the loss caused by rust. All the optimum cultural practices were provided to obtain a good commercial crop. The experiment was conducted for two consecutive years in winter.

PREPARATION OF UREDOSPORE SUSPENSION AND INOCULATION.

For inoculation the uredospore suspension in water was prepared using infected parts of susceptible variety containing uredospores. For this purpose the uredospore containing parts were clipped off and put in spraying bottle containing distilled water. Proper shaking was done for homogenous suspension. Thorough spraying of uredospore suspension was done on whole plant in field and in pots, two times morning and evening for two days. Inoculated plants rows in field were then covered with cloth tents specially meant for this purpose which were kept wet to ensure good humidity essential for infection. The pots were kept in alkathene chamber. The susceptible mutant lines showed symptoms 7-10 days after inoculation.

JUDGING THE LOSS CAUSED BY RUST

The data were also collected from 5 randomly selected plants from rust free and diseased plots on number of grains/spike, 100 seed weight, seed yield /plant and biological yield to judge the loss caused by disease. The value of characters of diseased plants were subtracted from the value of rust free sown mutant plants.

RESULTS

STUDY AND CLASSIFICATION OF RUST REACTION ON THE BASIS OF PUSTULE INTENSITY:

The rust reaction was scored as the pustule intensity on mutant plants. Pustule intensity was classified according to modified Cobb's scale in six classes from 5-100%. Class zero is not in Cobb's modified scale but it is used here to represent immune class. Different classes are (1) 0%, (2) 5%, (3) 10%, (4)25%, (5) 40%, (6) 65% and (7) 100%.

The pustules were dark reddish which appeared on stems, leaf sheaths, both sides of leaves and spikes. In beginning they were scattered but coalesced later.

The mutant lines were placed in only six classes, because 100 percent intensity was not observed. **Lines K50-38, K50-97, K50-102 and K40-73** were completely free from infection. They did not show any symptom of rust. These mutant lines were thought to be *immune.*

Lines K50-47, KE4-47 and G50-47 exhibited traces of symptoms and placed under 5% intensity.There appeared no major loss. These lines were classified as *very resistant*. The uredia were extremely minute and surrounded by necrotic areas.

Line KE2-63 exhibited 10% intensity and was classified as *moderately resistant*. The uredia, were small to medium in size along with hyper sensitive area surrounding them.

Seven mutant lines K50-44, K40-11, K40-67, K30-91, KE3-75, G50-35 and GH3-32 showed 25% pustule intensity along with a considerable amount of loss and were put into *moderately susceptible* class. The uredia were medium in size without necrotic area.

The only line K30-82 exhibited 40% pustule intensity and classified as *susceptible* to stem rust. The pustules occupied a large portion of stems, leaf sheaths, leaves, glumes and awns.

Line KE4-87 showed 65% intensity of pustules on stems, leaf sheaths, leaves, glumes and awns. The pustules were large and closer. Hence it was classified as *heavily susceptible*.

S.No.	Mutants	Rust Intensity (%) Stripe rust
1	K50-38	5
2	K50-44	65
3	K50-47	65
4	K50-97	0
5	K50-102	5
6	K40-11	65
7	K40-12	25

8	K40-67	65
9	K40-73	0
10	K30-82	5
11	K30-91	40
12	KE2-63	25
13	KE3-75	40
14	KE4-47	10
15	KE4-87	65
16	G50-35	40
17	G50-47	0
18	GH3-32	25

Immune, very resistant and moderately resistant lines were identified as resistant lines and were selected for further study.

Loss caused by stem rust Number of grains/spike The reduction in number of grains ranged from 0.96% to 21.76

Loss caused by stripe rust

The stripe rust caused decrease in the several yield contributing characters.

S.No.	Mutants	Reduction in percentage			
		Number of grains per spike	100 seed weight	seed yield	Biological yield
1	K50-44	14.75	20.08	21.24	23.43
2	K50-47	15.97	17.27	19.16	20.89
3	K40-11	12.67	14.16	14.75	12.21
4	K40-12	7.48	5	6.03	9.27
5	K40-67	18.29	15.11	16.49	13.65
6	K30-82	3.22	2.55	5.47	6.7
7	K30-91	14.14	15.8	12.83	13.63
8	KE2-63	5.69	7.49	6.41	8.98
9	KE3-75	12.36	16.41	32.93	27.79
10	KE4-47	3.65	5.2	4.43	5.06

11	KE4-87	16.9	22.12	24.01	22.61
12	G50-35	11.58	12.63	15.88	18.03
13	KH3-32	6.64	8.2	8.93	9.39

Number of grains / spike The decrease in number of grains/spike was observed in different mutants which varied from 3.22% to 18.29%. The spikes emerged short having lower number of spikelets. The maximum loss was exhibited by mutant K40- 67(18.29%) followed by KE4-87(16.90%), K50-47(15.97%), K50-44(14.75%), K30-91(14.14%), K40-11(12.67%), KE3-75(12.36%) and G50-35(11.58%). The minimum loss occurred in mutant K30-82(3.22%) followed by KE4-47(3.65%), KE2-63(5.69%), KH3-32(6.64%) and K40-12(7.48).

100 seed weight The loss in 100 seed weight ranged from 2.55% (K30-82) to 22.12%(KE4-87). The loss in other mutants was 22.08% (K50-44), 17.27%(K50- 47), 16.41%(KE3-75), 15.80% (K30-91), 15.11%(K40-67) and 12.63% (G50-35). The low loss observed was 5.0% (K40-12), 5.20% (KE4-47) , 7.49%(KE2-63) and 8.20% (KH3-32).

Seed yield per plant The decrease in seed yield ranged from 4.43 (KE-47) to 32.93%(KE3- 75). The next highest loss occurred in mutant KE4-87(24.01%) followed by K50- 81 44(21.24), K50-47(19.16), K40-67(16.49), G50-35(15.88%) and K40-11(14.75%). The loss was less observed in K40-12(6.03%) followed by KE2-63(7.49%), KH3-32(8.93%) and K30-91(12.83%)

Biological yield The biological yield of mutant was also affected by rust infection. The maximum loss was observed in KE3-75 where the loss was 27.79% and rust intensity was 40% followed by K50-44(23.43%), KE4-87 (22.61%), K50- 47(20.89%) and G50-35(18.03%). The minimum loss was recorded in mutant KE3-47(5.06%) followed by K30-82(6.7%), KE2-63(8.98%), K40-12(9.27%) and KE232(9.39%).

Performance of resistant mutants comparing mother cultivars A total of five mutant were identified as stripe rust resistant mutants. Mutant K50-38 is significantly superior to all the rest of mutants and mother varieties. Mutant K40-73 is superior to K50-102, K50-102 is superior to 88 K50-97, K50-97 is superior to G50-47, G50-47 is superior to mother variety K555 and mother variety K555 is superior to Gitanjali.

K50-38>K40-73>K50-102>K50-97>G50-47> K555>Gitanjali

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