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Mutation breeding in six rowed barley: Screening for stem rust resistance in certain induced mutants

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ABSTRACT

During the induced mutagenesis in six rowed barley, 18 mutant lines were isolated from the M_2 and M_3 generations. Thirteen lines were induced by gamma rays, four by EMS and one by HA. These lines were screened for resistance to stem rust and loss assessment. Mutants were grown in field as well as in earthen pots. Artificial inoculums were provided in condition suitable for rust occurrence. Rust reaction was recorded according to modified Cabb's scale and the rust intensity was classified as 0,5, 10, 25, 40 and 65 percent. Lines K50-38, K50-97, K50-102 and K40-73 were completely free from infection (0 %) and thought to be immune lines(I). K50-47, KE-4-47 and G50-47 exhibited traces of symptoms and placed under 5% intensity and were very resistant(R). Line KE2-63 exhibited 10% intensity and classified as moderately resistant(MR). Line K50-44; K40-11, K40-67, K30-91, KE3-75, G50-35 and GH3-32 showed 25% intensity of pustules and were put into moderately susceptible(MS) class. One line K30-82 showed 40% intensity and classified as susceptible(S), while line KE4-87 exihibited 65% intensity and was classified as heavily susceptible. Class 100 percent was not observed. Immune, very resistant and moderately resistant were identified as resistant lines and selected for further study, while moderately susceptible and heavily susceptible lines were identified as susceptible lines and discarded. Reduction in yield contributing traits *i.e.* number of grains/spike, test weight, seed yield and biological yield was also observed which was positively correlated with disease intensity.

Figure : 00 References : 20 Tables : 03
KEY WORDS : Evaluation, Immune, Inoculums, Pustules intensity, Resistant, Susceptible

Introduction

Barley is one of the most important cereal crops of the world which has got special significance, being cultivated in almost all parts of the world. The grains supply carbohydrates and proteins in feed and food. Barley malt products are superior than wheat and rye. The diseases reduce total biomass production and the grain yield. In case of severe infection the total crop my be lost. Rust, powdery mildew, stripe disease, covered smut, bacterial streak and yellow dwarf are the major diseases of barley. Among these diseases the rust disease is a potential threat to barley production. The stem rust caused by *Puccinia graminis tritici* may reduce the yield upto 58 percent[5]. Use of resistant varieties is only effective method to combat this disease because in recent years there has been an increased focus on the utilization of host resistance for diseases control in cereals to avoid the hazardous effects of fungicides. The present paper reports the results of some barley mutant lines evaluated for resistance against stem rust fungi with hope that these lines would provide effective stem rust resistance in barley.

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)[15] (Table-1).

Collection and multiplication of inoculum

The uredospores of *Puccinia granninis tritici* 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[11] LBC-23 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[10,11]. After 15 days of germination plants

TABLE-1 : Mutants and mother cultivars under study

S.No.	Mutant	Mother variety	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	"	EMS	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)	Gamma rays	50Kr
17	G50-47	"	Gamma rays	"
18	GH3-32	"	HA	0.3%

were inoculated with uredospores of rust. Both the 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.

Pot Screening

Earthen pots 10" diameter containing sterile soil and 30 percent compost were used for sowing mutants. Ten seeds of each mutant were sown separately in pots containing optimum moisture for good germination. After germination these pots were kept in alkathene house and were irrigated at regular interval to maintain the proper moisture in pots and humidity in alkathene houses.

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.

Recording rust reaction and pustules intensity

The reaction in form of rust intensity was recorded according to modified Cobb's scale which is widely used for recording rust intensity on cereal throughout the world. The pustules appeared on leaf and stem were carefully counted from 5 randomly selected plants in each treatment and data were recorded. On the basis of intensity of pustules the mutants were classified.

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 was used here to represent immune class. Different classes were (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 (Table 2).

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.

Immune, very resistant and moderately resistant lines were identified as resistant lines and were selected for further study (Table 3).

Evaluation of mutant lines on basis of loss caused

The loss caused was estimated as the reduction of value in traits *i.e.* number of grains/spike, 100 seed weight, seed yield/plant and biological yield. The reduction in these traits as compared to respective disease free mutants was estimated in percentage.

Reduction in number of grains ranged from 0.96% to 21.7% depending upon the intensity of disease. When there was 5% intensity in K5047, the loss was the minimum 0.96% and when there was 65% intensity, the maximum loss (27.76%) was recorded in KE4-87. The spikes become short and number of spikelets reduced resulting into the low number of grains (Table-2)

The seed became shriveled resulting the low test weight. The range was 0.75% to 28.9% **.**Due to low number and shriveled seeds the seed yield/plant reduced which ranged from 2.99% to 32.36%**.** The reduction in total biomass ranged from 2.75 to 37.44%. The plant height, number of tillers, leaf size and spike length reduced resulting in the loss of biological yield. The maximum loss occurred in mutant K40-12(36.44%) and the minimum 2.75% in KE4-47.

TABLE-2 : Rust intensity appeared on different lines and reduction in traits due to disease

Mutant	Rust intensity	Reduction (%)			
		Number of grains/spike	100 seed wt. (g)	Seed yield/ plant (g)	Biological yield/ plant(g)
K50-38	-	-	-	-	0
K50-44	25	7.20	10.23	18.54	10.95
K50-47	5	0.96	0.75	3.42	4.11
K50-97	-	-	-	-	0
K50-102	-	-	-	-	0
K40-11	25	7.61	9.79	13.66	13.62
K40-12	65	19.95	28.97	32.36	36.44
K40-67	25	9.37	11.92	13.06	12.15
K40-73	-	-	-	-	0
K30-82	40	12.92	25.15	27.74	25.37
K30-91	25	8.27	12.54	15.85	16.53
KE2-63	10	3.03	2-01	5.38	7.49
KE3-75	25	7.64	0.59	9.47	10.42
KE4-47	5	1.35	1.55	2.99	2.75
KE4-87	65	21.76	27.08	30.84	33.17
G50-35	25	6.53	12.70	11.23	17.50
G50-47	5	2.15	1.95	2.45	2.32
KH3-32	25	5.47	13.50	15.43	15.26

Discussion

Mutation breeding is the use of mutants either directly as new cultivars or indirectly *via* crosses with other genotypes to develop improved germplasm sources and cultivars[1]. A number of induced barley mutants have been utilized as cultivars and subsequently given rise to other cultivars through cross breeding. The semi dwarf mutant 'Jotum' (Norway) has given size to M21 and M22 in Minnesota[16]. In USA mutant "Luther" induced by dES has given rise to the newer winter barley cultivars 'Boyer', 'Hesk' and 'Mal'[20]. The other induced mutant cultivars

TABLE-3 : Classes of mutants and rust intensity.

Resistance/rust intensity	Mutant line
Immune (0 %)	K50-38, K50-97, K50-102, K40-73
Very resistant (5%)	K50-47, KE4-47, G50-47
Moderately resistance (10%)	KE2-63
Moderately susceptible(25%)	K50-44, K40-11, K40-67, K30-91, KE2-75, G50-35, GH3-32
Susceptible(40%)	K30-82
Heavily susceptible(65%)	KE4-87

include 'Mari' (Sweden), 'Amagi Nijo 1' (Japan) and 'Markeli' (Bulgaria).

Any new variety or mutant requires its evaluation in different agro-climatic conditions for yield, adaptability and resistance to biotic and abiotic stress. Keeping the above point in view the present mutants were screened for resistance against stem rust.

The stem rust caused by *Puccinia graminis tritici* infect barley plant and produce symptom in form of pustules. The colour arrangement and part affected differ according to the rusts. On this ground the rusts are identified externally in the field. Malic hydrazide in small concentration increases the susceptibility for multiplication of inoculums[7,10,11,17].

The rust reaction in form of pustule intensity are recorded on the basis of methods recommended by several workers. Infection types of *Puccinia graminis tritici* described[18] are generally used for other cereal rusts also with due modifications necessary to suit the individual characteristics of rust[11].

The rust intensity of cereal crops are measured with the help of diagrammatic scales proposed from time to time. The first diagrammatic scales was proposed which represent five degree of resistance[4]. In 1917 the Office of the Cereal Investigation of Plant Industry, USDA adopted a modification of the Cobb's scale and it was published[13]. It is usually referred as modified Cobb's scale and is widely used for recording rust intensity on cereals throughout the world. In this scale rusted plants have been classified in six classes.

The reaction of stem rust varied from 0-65%. The 5% intensity was observed in three, 10% in one, 25% in seven, 40% in one, 65% in two mutants. The 100% rust intensity was not observed in any mutant. The four mutants were identified as immune, three resistant, one moderately resistant, seven moderately susceptible, one susceptible and two heavily susceptible. These results are in agreement with the earlier[19].

Some workers[3.5.9] observed some resistant or moderately resistant and some moderately susceptible and others[8] classed barley varieties as resistant(R), moderately resistant(MR), moderately susceptible(MS) and susceptible(S). Similarly workers[12] evaluated barley varieties against stem rust and observed different degree of uredospore production. These references also support the findings of present investigation.

Stem rust reduced the number of grains/spike which ranged from 0.96% to 21.7%, 100 seed weight which ranged from 0.75% to 28.9%, seed yield which ranged from 2.99% to 32.36% and biological yield ranging from 2.75 to 37.44%. There was reported 58% grain yield loss in susceptible variety 'Galleon' and 12% loss in moderate resistant variety 'Grimmett'[5]. The reduction was associated with reduced grain size and weight. The loss 12% in resistant, 21-26% in moderate resistant, 30-37% in moderate susceptible and 53% in susceptible variety were there[8]. There were highly significant on test weight which ranged from 7-43%. There was no significant effect of rust on plant height and days to heading.

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