

Original Research Article

SCREENING FOR STEM RUST(*Puccinia graminis* f.sp *tritici*, Eriks. ~~and~~ & E. Henn.) RESISTANCE IN MUTANT BARLEY (*Hordeum vulgare* L.) LINES

ABSTRACT

Stem rust is a devastating disease in barley that is caused by a fungi (*Puccinia graminis* f.sp *tritici*, Eriks. and E. Henn.).The disease has been controlled for quite some time due to the presence of cultivars carrying the resistant gene *Rpg1* .It has been effective in controlling the various races of stem rust. This was so until the emergence of the race Ug99 from Uganda in the year 1998. This race did break all the resistant genes that were there hence the need to get new sources of resistance. In the current study, mutation breeding was used to create variation for stem rust resistance (Ug99). Seeds of barley (Nguzo variety- M₀) were sent to Vienna in Austria for irradiation at the International Atomic Energy Agency at a dosage of 250gray.The M₁ seeds were multiplied in University of Eldoret experimental field.Thousand plants were randomly selected from the M₁ population, two ears were harvested of each plant that were subsequently divided within two groups. One group was planted at University of Eldoret experimental field while the other group of a thousand ears were planted at KARI Njoro as M₂. Each ear formed a row/line. A susceptible line of wheat was planted as a spreader and inoculated with stem rust -Ug99 in both sites. A total of 183 lines were selected from the two sites. These lines were again replanted in university of Eldoret as M₃ in a RCBD design with three replicates in the field to determine adult plant resistance and in the green house in a CRD design to determine the seedling resistance. The non mutated parent,Nguzo was used as a check. The following lines did show resistance both at the seedling level and adult plant level (1,2,9,21,26,49,55,58,59,69,76 and 78). Mutational breeding is therefore recommended for continual screening of these lines as this race may mutate further.

Keywords: Stem rust (Ug99), Mutation.

Introduction

Barley (*Hordeum vulgare* L.), is one of the most important cereal crops, in Kenya. Currently about 30,000 ha of land is used for the crop but potential for expansion remains [1]. Rust fungi, responsible for diseases like stem rust, yellow rust and leaf rust is a major contributor to the sub optimal yields realized by farmers. Breeding for resistance has been used as the main method of protection against the fungi. The rust fungi can however overcome host resistance genes and spread new strains through wind dispersal of spores [2]. Stem rust of barley and wheat, (caused by *Puccinia graminis* f.sp *tritici* Eriks. ~~and~~ & E. Henn.) is historically one of the most important plant diseases. Devastating stem rust epidemics often result in major grain losses [3].In the current study mutation breeding has been utilised to create variation for resistance.The use of mutagens such as gamma rays for inducing variation is well established.Induced mutations have been used especially by the FAO/IAEA division of nuclear techniques in Agriculture.More than 1800 cultivars obtained either as direct mutants or derived from their crosses have been released worldwide in over 50 countries [4].The induced mutations aid in development of many agronomic important traits in major crops such as wheat ,barley rice and peanuts [5]. Barley (~~*Hordeum vulgare* L.~~) is one of the founder crops of Old World agriculture. Genetic markers point to the origin of barley being the Fertile Crescent especially the Israel-Jordan area in the southern part of the Fertile Crescent where there is the highest diversity of the species [5]. This is the area that has the highest probability of being the geographical area within which wild barley was domesticated about 8000 B.C. [6].The Agriculture

Sector, which is the backbone of Kenya's economy and its growth is dependent on increasing barley production amongst other crops and livestock [7]. The importance of barley as a major crop in Kenya cannot be overlooked because it is the fourth most important cereal in Kenya and the world after maize, wheat and rice [8]. Some of the Major barley producing areas in Kenya includes; Timau, Moiben, Nakuru, on the wetter escarpment of Samburu District near Maralal Town, Molo, and Mau Narok[9]. The total land area that is deemed suitable for barley production in Kenya is 85,000 hectares. Only about 20,000 hectares of this total land area suitable for barley production is under barley production, thus 65,000 hectares has not been utilized [1]. Barley production in Kenya was estimated to be 75,000MT in 2007 [10]. The aim of this study was to screen for stem rust resistance (Ug99) in mutant barley lines at M₂ and M₃.

Comment [R1]: Put these as the introductory sentences at the top.

Comment [R2]: This is redundant of lines 27-28

Comment [R3]: Include some information on the yield reduction in barley due to *Puccinia graminis* infestation in Kenya.

Comment [R4]: Was this screening carried out at two sites?

Site description

The study was conducted at the University of Eldoret, The geographical coordinates are 0° 30' 0" North, 35° 15' 0" East. The site is located 10 Km of Eldoret town, in Uasin Gishu county of Kenya. It is located at an altitude of 2180m above sea level; it consists primarily of an agro-ecological zone LH3 [11]. The site is among major wheat growing regions in Kenya. University of Eldoret receives a unimodal rainfall which begins in March. The average annual rainfall range is between 900 mm and 1100 mm and mean annual temperature of 16.6°C. The soils are shallow, ferralsol, well drained, non humic cambisols with low nutrient availability and moisture storage [11].

The study was also conducted at Kenya Agricultural Research institute, Njoro in Nakuru county, (0°20'S 35°56'E), located in the lower highlands (LH3), at an altitude of 2166 meters above sea level. The temperature ranged between 18-28°C during the period of study, while the average annual rainfall ~~is was~~ about 1,000 mm. The soils are deep, well drained, fertile *Vitric Mollic Andosols* [11]

Irradiation

Six hundred grams of M₀ seeds (non mutated seeds) of the barley variety Nguzo, obtained from East African Maltings in Molo, Kenya and were sent to International Atomic Energy Agency in Vienna and subjected to gamma radiation at an irradiation dose of 250 gy (gray) to obtain M₁ (mutated seed that gives rise to the first generation of mutants).

Seed Multiplication and Selection

The land to be planted was disc ploughed and harrowed to fine tilth suitable for barley planting. The irradiated M₁ seeds were planted in University of Eldoret for seed multiplication. The mutated seed were drilled on a plot measuring 125 m by 40 m. Drills were 5 cm apart. All the agronomic practices like insect pest control, diseases control and weed control were done up to harvest time to ensure good crop establishment. Thousand plants were randomly selected from the M₁ population and two ears harvested from each selected plant and divided into two corresponding groups. One group planted at University of Eldoret experimental field while the corresponding group of a thousand ears was planted at KARI Njoro. Each ear formed a line/row.

77 **Planting and field management**

78 The M₂ seed from each entry were sown in 1M rows. The experimental units were separated by 0.3m and 0.5m
79 wide alleyways within and between the blocks, respectively. Sowing was done at an equivalent seeding rate of
80 125 kg/ Ha. At planting time, Di-ammonium phosphate fertilizer was applied at an equivalent rate of 125kg/ha.
81 Weeds growth was restricted by applying both pre - and post - emergent herbicides. Stomp® 500 EC
82 (pendimethalin) a broad spectrum, pre-emergent herbicide was applied at an equivalent rate of 2.5 l/ha. At
83 tillering stage (Zadoks' Growth stage 20-29) [12] the plots were sprayed with Buctril MC (bromoxynil +
84 MCPA) at an equivalent rate of 1.5l/ha to control broad-leaved weeds. The trial was top dressed with Calcium
85 Ammonium Nitrate (CAN) at stem elongation stage (Zadoks' GS 30).

86 **Preliminary data collection and selection**

87 Rust development was closely monitored on the test plants and response to rust infection at the adult plant stage
88 was termed "infection response". According to the size of the pustules and associated necrosis or chlorosis
89 infection responses were classified into four categories; R = resistant, MR = moderately resistant, MS =
90 moderately susceptible and S = susceptible [13]. Stem rust severity was assessed using the modified Cobb scale
91 [14]. Entries were evaluated for response to infection and stem rust severity between heading and plant
92 maturity and resistant lines selected and harvested to be advanced to M₃. 74 lines were selected from Njoro
93 whereas in University of Eldoret 109 lines were selected. These showed acceptable levels of resistance based on
94 infection type and severity. Each harvested line was harvested and kept in a separate bag to avoid mechanical
95 mixture. The rest of the materials were bulked together.

96 **Experimental Procedure**

97 A field experiment was established on land previously under wheat and was disc ploughed and harrowed to fine
98 tilth suitable for barley planting. Each entry was sown in double rows measuring 0.2 × 0.75m. The experimental
99 units were separated by 0.3m and 0.5m wide alleyways within and between the blocks, respectively. Sowing
100 was done at an appropriate seeding rate. The entries were randomly assigned within a block. The experiment
101 was laid out in RCBD replicated three times. The experiment was managed as described above for agronomic
102 management and inoculation done as described for preliminary evaluation.

Comment [R5]: These have been mentioned already.

103 **Seedling screening of M₃ plants for resistance to stem rust in the greenhouse**

104 Greenhouse experiment was conducted at the University of Eldoret greenhouse. It was put in a Completely
105 Randomized Design (CRD) with three replicates and repeated three times. The selected 183 lines were
106 evaluated for seedling resistance to Ug99 plus their parent as a check. They were planted in Plastic cups
107 measuring 4 cm diameter by 6 cm height filled with about 200g of a mixture of soil and sand in a ratio of 3:1.
108 The plastic cups were placed in a greenhouse that was maintained at 23°C and 60% relative humidity (RH). In
109 each pot, 3-4 seeds were planted at approximately 2 cm deep and the cups were then placed in large non-
110 draining trays measuring 60cm × 240cm and watered to field capacity. The seedlings were inoculated with
111 urediniospores after 14 days using a hand sprayer when the first leaf was fully developed and placed in dark
112 moist chamber maintained at 100% relative humidity, temperature at 13°C for 18 hours. Thereafter, the

seedlings were then transferred to a growth chamber that was maintained at about 22⁰C day and 20 - 21⁰C night temperature. After the disease had developed, scoring was done according to Stakman et al. [15].

Comment [R6]: How old were the seedlings at the time of measurements?

Results and discussion.

Table 1-Summary of Seedling Resistance Infection type and Adult Plant Resistance of the mutant barley lines at M₃.

Mutant lines	Seedling resistance infection type(IT)	APR (Severity % and infection type)
1	1	5 MR
2	2	20 MR
5	4	30 S
7	4	20 MS
8	3	20 MS
9	2	15 MR
21	1	20 MR
23	2	20 MS
26	2	10 MR
27	3	40 MS
34	2	35 MS
36	2	15 MS
41	2	20 MS
44	3	10 MS
49	2	10 MR
54	2	20 MS
55	2	25 MR
58	2	20 MR
59	1	25 MR
62	1	5 MR
69	1	10 MR
76	1	5 MR
78	2	20 MR
90	3	45 MS
95	1	5 MR
124	3	20 MS
126	2	30 MS
130	3	25 MS
156	3	20 MS
161	3	15 MS
163	2	35 MS
165	2	20 MR
173	2	30 MS
184	3	40 S

Comment [R7]: What does the numbers 1-4 indicate?

KEY: S-Susceptible, MS-Moderate Susceptible, MR-Moderate Resistant, R-Resistant

Discussion

The germplasms (mutant barley lines) showed varying levels of resistance to stem rust (Ug99) table 1. At seedling stage, the infection levels ranged from 0 to 4, whereas at adult plant stage the severity ranged from MR to S. The resistance at seedling stage may probably be because the germplasm had resistance conferred by one

single major gene that was broken down at adult plant stage. The genotypes that showed some resistance at adult stage may contain a major single gene that remained resistant at seedling and at adult plant stage or they may have minor genes that are working together to reduce the disease [13]. Mutations are used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops [16]. Mutation has been used to produce many cultivars with improved economic value and study of genetics and plant developmental phenomena [17,18]. In the present study gamma rays were used to induce variation in barley lines especially for the gene governing stem rust (Ug99) resistance (*Rpg1*). The induced variations that were brought about by the gamma rays had some molecular basis, i.e., change in the base sequence of the DNA molecule coding for the protein. The change in the base sequence can be through base substitution, base addition or deletions. This could explain the induced variations in terms of Ug99 resistance in the barley lines. There some lines which had low infection type in their seedling screening and were showing resistance to Ug99 in the field at adult plant stage.

Conclusions

Mutation by irradiation was successfully applied and generated the much needed variability that inferred resistance to the mutant barley lines at M₂ and M₃ at the seedling level and adult plant level. The following lines did show resistance both at the seedling level and adult plant level (1,2,9,21,26,49,55,58,59,69,76 and 78).

Recommendations

This study recommends the following.

- Continual screening of these lines as this race may mutate further((1,2,9,21,26,49,55,58,59,69,76 and 78).
- Stabilization of these mutant lines through double haploid techniques and backcrossing to reduce the effects of mutation.
- Agronomic traits should be evaluated on the resistant lines to identify superior lines for release as new varieties for commercial purposes.

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Comment [R9]: ????

Comment [R10]: Cite journal names as per journal format.