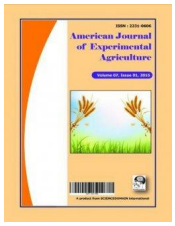

American Journal of Experimental Agriculture
8(1): 40-45, 2015, Article no.AJEA.2015.144
ISSN: 2231-0606

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Screening for Stem Rust (*Puccinia graminis* f. sp Tritici, Eriks. & e. Henn.) Resistance in Mutant Barley (*Hordeum vulgare* l.) Lines

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Authors' contributions

This work was carried out in collaboration between all authors. Author IJO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author FMM reviewed the experimental design and all drafts of the manuscript. Authors MGK and OKK managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2015/16332
Editor(s):
(1) T. Muthukumar, Root and Soil Biology Laboratory Department of Botany, Bharathiar University, India.
Reviewers:
(1) Klára Kosová, Division of Plant Genetics, Breeding and Crop Quality, Crop Research Institute, Prague, Czech Republic.
(2) Gyula Oros, PPI HAS, Budapest, Hungary.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1075&id=2&aid=8604

Original Research Article

Received 26th January 2015
Accepted 19th February 2015
Published 28th March 2015

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- M_O) were sent to Vienna in Austria for irradiation at the International Atomic Energy Agency at a dosage of 250 gray. The M_1 seeds were multiplied in University of Eldoret experimental field. Thousand plants were randomly selected from the M_1 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 M_2. 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

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183 lines were selected from the two sites. These lines were again replanted in university of Eldoret as M_3 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.

1. INTRODUCTION

Barley 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 [1]. This is the area that has the highest probability of being the geographical area within which wild barley was domesticated about 8000 B.C. [2]. 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 [3]. 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 [4]. 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 [5]. Stem rust of barley and wheat, (caused by *Puccinia graminis* f. sp *tritici* Eriks. & E. Henn.) is historically one of the most important plant diseases. Devastating stem rust epidemics often result in major grain losses [6]. Stem rust "Ug99"-infected plants may suffer up to 100% loss [7] 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 [8]. The induced mutations aid in development of many agronomic important traits in major crops such as wheat, barley rice and peanuts [9]. 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 [10]. 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 [11]. Barley production in Kenya was estimated to be 75,000MT in 2007 [12]. The aim of this study was to screen for stem rust resistance (Ug99) in mutant barley lines at M_2 and M_3.

1.1 Sites Description

The study was conducted at the University of Eldoret, The geographical coordinates are 0^0 30′ 0″ North, 35^0 15′ 0″ East. The site is located 10 Km of Eldoret town, in Uasin Gishu county of enya. It is located at an altitude of 2180 m above sea level; it consists primarily of an agro-ecological zone LH3 [13]. 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 16.6ºC. The soils are shallow, ferralsol, well drained, non humic cambisols with low nutrient availability and moisture [13].

The study was also conducted at Kenya Agricultural Research institute, Njoro in Nakuru county, (0º20'S 35º56'E), located in the lower highlands (LH3), at an altitude of 2166 meters above sea level. The temperature ranged between 18-28ºC during the period of study, while the average annual rainfall was about 1,000 mm. The soils are deep, well drained, fertile *Vitric Mollic Andosols* [13].

1.2 Irradiation

Six hundred grams of M_0 seeds (non mutated seeds) of the barley variety Nguzo, obtained from East African Maltings in Molo, Kenya were sent to International Atomic Energy Agency in Vienna and subjected to gamma radiation at an iradiation dose of 250 gy (gray) to obtain M_1 (mutated seed that gives rise to the first generation of mutants).

1.3 Seed Multiplication and Selection

The land to be planted was disc ploughed and harrowed to fine tilth suitable for barley planting. The irradiated M_1 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 M_1 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.

1.4 Planting and Field Management

The M_2 seed from each entry were sown in 1M rows. The experimental units were separated by 0.3 m and 0.5 m wide alleyways within and between the blocks, respectively. Sowing was done at an equivalent seeding rate of 125 kg/ Ha. At planting time, Di-ammonium phosphate fertilizer was applied at an equivalent rate of 125 kg/ha. Weeds growth was restricted by applying both pre - and post - emergent herbicides. Stomp® 500 EC (pendimethalin) a broad spectrum, pre-emergent herbicide was applied at an equivalent rate of 2.5 l/ha. At tillering stage (Zadoks' Growth stage 20-29) [13] the plots were sprayed with Buctril MC (bromoxynil + MCPA) at an equivalent rate of 1.5l/ha to control broad-leaved weeds. The trial was top dressed with Calcium Ammonium Nitrate (CAN) at stem elongation stage (Zadoks' GS 30).

1.5 Preliminary Data Collection and Selection

Rust development was closely monitored on the test plants and response to rust infection at the adult plant stage was termed "infection response". According to the size of the pustules and associated necrosis or chlorosis infection responses were classified into four categories; R = resistant, MR = moderately resistant, MS = moderately susceptible and S = susceptible [14]. Stem rust severity was assessed using the modified Cobb scale [15]. Entries were evaluated for response to infection and stem rust severity between heading and plant maturity and resistant lines selected and harvested to be advanced to M_3. 74 lines were selected from Njoro whereas in University of Eldoret 109 lines were selected. These showed acceptable levels of resistance based on infection type and severity. Each harvested line was harvested and kept in a separate bag to avoid mechanical mixture. The rest of the materials were bulked together.

1.6 Experimental Procedure

A field experiment was established on land previously under wheat and was disc ploughed and harrowed to fine tilth suitable for barley planting. Each entry was sown in double rows measuring 0.2 × 0.75 m. The entries were randomly assigned within a block. The experiment was layed out in RCBD replicated three times. The experiment was managed as described above for agronomic management and inoculation done as described for preliminary evaluation.

1.7 Seedling Screening of M_3 Plants for Resistance to Stem Rust in the Greenhouse

Greenhouse experiment was conducted at the University of Eldoret greenhouse. It was put in a Completely Randomized Design (CRD) with three replicates and repeated three times. The selected 183 lines were evaluated for seedling resistance to Ug99 plus their parent as a check. They were planted in Plastic cups measuring 4 cm diameter by 6 cm height filled with about 200 g of a mixture of soil and sand in a ratio of 3:1. The plastic cups were placed in a greenhouse that was maintained at 23ºC and 60% relative humidity (RH). In each pot, 3-4 seeds were planted at approximately 2 cm deep and the cups were then placed in large non-draining trays measuring 60 cm × 240 cm and watered to field capacity. The seedlings were inoculated with urediniospores after 14 days using a hand sprayer when the first leaf was fully developed and placed in dark moist chamber maintained at 100% relative humidity, temperature at 13ºC for 18 hours. Thereafter, the seedlings were then transferred to a growth chamber that was maintained at about 22ºC day and 20 - 21ºC night temperature. After the disease had developed, scoring was done according to Stakman et al. [16].

2. RESULTS AND 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 [12]. 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 [17]. Mutation has been used to produce many cultivars with improved economic value and study of genetics and plant developmental phenomena [18,19]. 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.

Table 1. Summary of seedling resistance infection type and adult plant resistance of the mutant barley lines at M_3

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

KEY: S-Susceptible, MS-Moderate Susceptible, MR-Moderate Resistant, R-Resistant; 1.Resistant 2.Resistant to moderately resistant 3.Moderately resistant/moderately susceptible 4 Susceptible

3. CONCLUSION

Mutation by irradiation was successfully applied and generated the much needed variability that inferred resistance to the mutant barley lines at M_2 and M_3 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).

4. 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.

ACKNOWLEDGEMENTS

University of Eldoret-EABL Project for funding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1075&id=2&aid=8604