Determination of resistance to yellow rust in new breeding mutant lines of spring wheat at adult plant and seedlings stages

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Abstract. Stripe (yellow, YR) rust bringing about by fungal pathogen of *Puccinia striforms* Westend Westend. is a serious wheat disease that poses substantial threats to the global wheat yield. Currently, the appearance of virulent fungus races is overcomed restricted by the studed resistance genes of wheat. Seach and identification of new genetic sources with durable resistance genes can allow efficiently to incorporate these target genes into germplasm pools. To increase spring wheat genetic diversity, on the back phone of parental variety Kazakhstanskay-19 (WT) characterizing by rust resistance through physical mutagenesis bythe various gamma irradiation doses, namely 300-, 350-, and 400 Gy, new M_3 mutant lines were created. From these new mutant resources, the 75 genotypes having higher yield-associated parameters such as grains number and weight per main spike comparing the parent variety were selected by phenotyping as the adult plant resistant (APR) under rust infected field trial. To measure resistance of these breeding lines at seedling stage by microscopy through the number of haustorial mother cells formation induced by YR treatment with the isolate of *Puccinia striforms* "Warrior" as temporary responses was carried out. Almost all of spring wheat mutant lines (93.0%, 70 samples from 75 mutant lines) identified as APR to YR had a strong association with SR which is developed on longer time of infection. The most effective dose of irradiation to generate the genetic variation was 350 Gy with mean of 60.08 (p<0.005) according to Anova analysis.

1 Introduction

Wheat (*Triticum aestivum* L.) is the leading staple crop. For its global request is needed reach to 324 kg per year (per capita) by 2050 [1]. Fungi *Puccinia striforms* Westend. f. sp. tritici Erikss. (*Pst*) bringing about YR is the dangorus disease that decrease global wheat yield and grain quality [2, 3]. In the Kazakhstan, epiphytotic development of YR disease occurred in 2000 and 2002, its moderate development was in 2003, 2006 and 2009 [4]. The application of fungicides used for prevention of fungal spreading is known by the expenses and unfavorable environmental effects [5,7]. The growing wheat resistant cultivars allows to manage and control YR dissemination having high effectivity [2, 5]. In breeding practices, resistance to YR is characterized by two nain types and could be used [6]. The seedling resistance (SR) is valuable during growth and development, but not long-lasting and not effective against the novel virulent race of *Puccinia striforms*. The elevated level of resistance is inherent to the the stage of adult plan (APR). The APR is determined by temperature and is named as high-temperature APR (HTAP) [6] and it is charactrized by the prolonged impact and does not influence by by of new *Puccinia striforms* race [5, 7]. The ideal approach is the combination of APR with SR to YR with aim to reach the desirable level and permanence of resistance.

Currently, the YR resistance 85 genes and more than 300 described quantitative trait loci (QTL) are known [8, 9]. However, in wheat cultivars a number of genes to YR resistance widely used have been overcome by new races of Pst, and the minor QTL for APR has weak effectivity for practival breeding.

Wheat breeding objects of YR resistance to have had to rapidly adapt to the changing *Pst* races and that's why there is important request for constantly developing and searching new sources of genetic resistance to create the improved wheat varieties. Identification of new resistant germplasms to YR rust with the combined APR and SR is very relevant goal for effective suppression of illness. Induced mutagenesis especially the effective physical one, is a strong and widespread approach for generating novel genetic diversity in cereal crops that have its restriction. Induced mutagenesis has been greatly applied to wheat yield increase; however, has been not broadly used to reach desirable long-lasting rust resistance [10, 11]. It has been reported that new wheat mutants created by EMS-induced mutagenesis, MNR220, showed enhanced resistance to three types of rusts and powdery mildew [12]. Other wheat achievement is that fast-neutron-derived mutants showed resistance to multiple rust pathogens [13], and a space-induced wheat mutant line (R39) demonstrated the APR to stripe rust [14].

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Induced by mutagenesis multi-generation of new desirable alleles of genes that did not be in a germplasm pool is the great advantage. Due to that in a short breeding cycle, creation of the desirable mutations can be effectively directed for development of new mutant variety [11]. Mutant resources of crops are not considered to be the GMOs and therefore they are acceptable by society.

Our previous studies on creating new spring wheat mutant lines (M_3–M_7 generation) based on Kazakhstan varieties and expanding diversity revealed comparing the parental vvarieties their higher productivity and grain morphometry along with microelements bio-fortification ability [15-18]. In addition, grain Fe and Zn content genetically improved mutant lines demonstrated roots- and leaves-related gene expression patterns of genes set associated with Fe homeostasis regulation. This knowledge's provide new insights into regulation of Fe uptake, intracellular metals translocation, storage in wheat, and aid the prioritization of gene targets for increased grain Fe content and bioavailability [17]. We also showed a highly differential expression of Fe homeostasis-related genes in spring wheat mutant lines with increased grain metal content at Fe-deficiency response [18].

The goals of the current research were: 1) to expand the genetic diversity of spring bread wheat on the basis of the variety Kazakhstanskaya-19 characterizing by rust resistance via mutation breeding, and to develop new M_3 mutant lines through 300, 350, and 400 Gy gamma irradiation doses; 2) to carry out APR phenotyping of mutant lines in a rust-infection field trial; 3) to determine mutant lines identified as APR to YR for their SR by microscopic analysis.

2 Materials and methods

2.1 Plants material

To create M_3 mutant lines, spring wheat (*Triticum aestivum* L.) grains of variety Kazakhstanskaya-19 characterizing by rust resistance were irradiated with doses Gy irradiation (300, 350, and 400) from a ^{60}Co origin at the IAEA breeding and genetics laboratory. These Gy doses were emanated from test of radiosensitivity indicated that the LD_{50} was 330 Gy for the initial parent variety. The promoted single spikes were choiced to create the M_2 plants that was grown in the plots of Kazakh Research Institute of Agriculture and Plant Growing. Each M_3 mutant plant was produced only a single main spike. The grain weight per main spike of breeding genotypes comparing the initial variety value was s the screening test. Grain samples from each M_3 mutant population were evaluated together with variety Kazakhstanskaya-19 in the infectious field to evaluate the APR to YR.

2.2 Inoculation of wheat plants and seedlings with yellow rust

Inoculation with YR-uredospore in water suspension containing 0.001% Tween-20 was appleis two times wheat plants were at botting (10-12 days was an interval between fungal treatments). Plants were treated after preliminary moistening in the evening and conditions of high humidity were maintained. The infectious material was the spore inoculum of the fungus *P. striiformis* of the Kazakh rust population, provided by the Research institute of biological safety problems of RK. On the flag leaves type of infection was estimated (the end of May and June) when plants were reached the syages of boot and milk. The method for estimating YR symptoms were the following: I - immune; R -resistant; MR—moderately resistant; MS—moderately susceptible; and S—susceptible [19]. A total of 75 immune or resistant M_3 mutant lines were selected together with cv. Kazakhstanskaya-19 (wild type) for the SR evaluation.

The isolates of the fungal YR were initially propagated from single pustules on the susceptible genotype Akteur. The *P. striiformis* races are collectively known as Warrior (based on the virulence of one initial variant of this group to the wheat variety Warrior) and Kranich (based on the virulence of one variant to the wheat variety Kranich) [20, 21]. In this study, the races of Warrior(-), Warrior + YR27, Oakley,v7/Kranich and Triticale aggressive were used to check the SR of wheat mutant lines to YR. The races of *P. striiformis* determined through virulence analysis by K. Flath (JKI Kleinmachnow) and multiplied according to [22].

2.3 Analysis of the formation of fungal haustorial structures

Staining procedures using Calcofluor White M2R solution (0.2% in sterile water, w/v) [23] and microscopic analysis to calculate the formation of fungal haustorial structures (HMCs) as a temporary response, 5 and 10 days, after inoculation of YR isolate "Warrior" are in detail described in our paper [24]. To assess the number of HMCs, for each replication, 15 infection sites were used reaching the total counts of 30 infection sites (3 × 10). The fungal structures microscopy was performed using an Axioskop 50 and an Axiocam MRc connected with the software package Axiovision 4 (Carl Zeiss AG, Jena). The detailed description of using filter set for all microscopy procedures were presented in [11, 24].

3 Results and discussion

On the back phone the variety Kazakhstanskay-19 new M_3 mutant breeiding ines were developed by treatments of the different Gy irradiation (300, 350, and 400). These breeding objectives were generated to increase the studied YR resistant genetic resources of wheat that are able to combine APR and SR. From these three dosed mutant plants the 75 lines without any visible symptoms of disease expresstion according to [19] were selected as APR. During at development stage 42, 15, and 17 samples representing the 300-, 350-, and 400-Gy-treated breeding mutant lines of spring wheat,were identified, respectively, as APR. These new mutant lines along with the parent variety Kazakhstanskay-19, genotypes of Akteur and Tabasco, being the sensitive and resistant controls, respectively, were used for SR evaluation to YR in 14-days seedling as a time-dependent effect by 5 and 10 days of inoculation by the aggressive worldwide isolate of *P. striiformis* "Warrior" [22] in greenhouse experiments.

The SR evaluation of wheat genotypes is represented in **Fig. 1**. In 10 mutant lines (13%) including resistant variety Tabasco, the fungal haustorial mother cells (HMCs) were not observed or very little after 5 days YR treatment (**Fig. 1A)**. In other mutant lines, the HMCs number varied from 6.0 to 48.1 with a mean of 22.37±12.29 (n=975). Exsept variety Akteur, sensitive control, and 5 mutant lines (7.0%), the HMCs and sporulation were not produced after 10 days of YR infections (**Fig. 1B**). The obtained results allow to conclude that almost all of spring wheat mutant lines created (93.0%, 70 samples from 75 mutant lines) identified as APR to YR had a strong association with SR which is developed on longer time of infection. In our preveious paper, the leaf rust fungal isolate was applied to observe responses of incompatible and compatible resistance mechanisms in seedling sensitive and resistant Kazakhstanskay-19 MK/27 and MK/39 mutant lines, respectively, selected based on their significantly different HMCs counts after both inoculation periods [23]. According to these results, the development of an autofluorescence as an indicator of plant defense reactions counteracting the formation of HMCs was observed. Due to this phenomenon, no HMCs were formed in these SR genotypes at both rust infection periods.

Anova analysis used for comparing the different dose of irradiation to generate the genetic variation indicated that the 350 Gy was the most effective with mean of 60.08 (p<0.005), and more less the doses of 300 Gy (28.81, p< 0.005) and 400 Gy (18.96, p< 0.05) at time of YR infection 5 days.

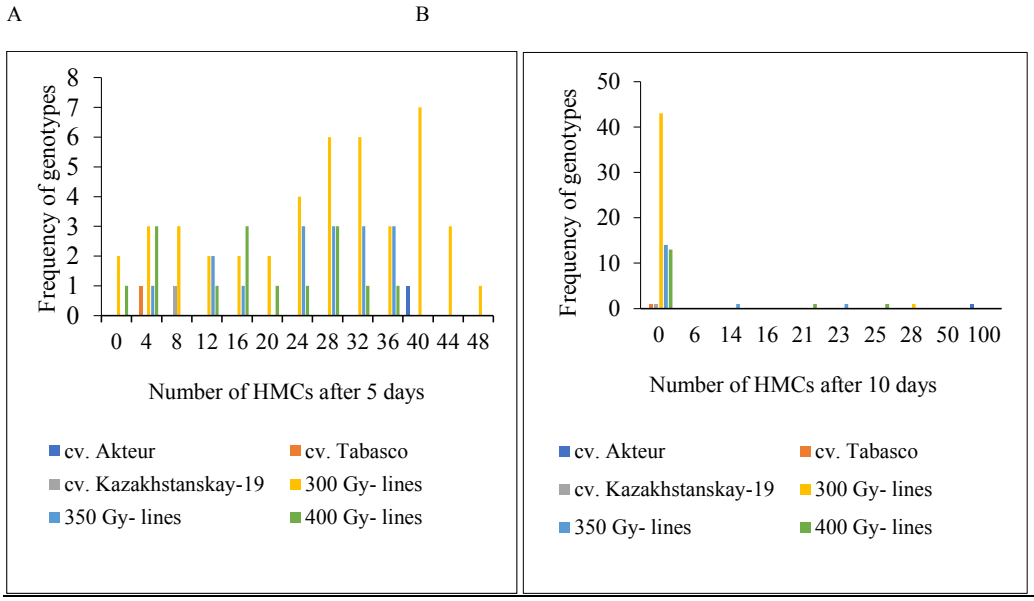

Fig. 1. Frequency distribution of genotypes in haustorial mother cells (HMCs) produced after 5 days, and B. − after 10 days inoculation by *P. striiformis* yellow rust fungal isolate of 14-days seedling in the cv. Kazakhstanskay-19 (WT), seedling sensitive and resistant varieties of Akteur and Tabasco, respectively, Kazakhstanskay-19 300-, 350- and 400-dosed spring wheat mutant lines. Values are presented as the mean of 15 leaf area infected sites ± standard deviation.

4 Conclusion

In this study, new spring wheat mutant (M_3 generation) lines created on variety Kazakhstanskaya-19 characterizing by rust resistance using gamma-induced mutagenesis through 300-, 350-, and 400-Gy doses were developed. In field trials after YR inoculation, during growth 75 mutant lines generated by treatment of different doses gamma radiation showed APR. During at development stage 42, 15, and 17 samples representing the 300-, 350-, and 400-Gy-treated breeding mutant lines of spring wheat,were identified, respectively, as APR. These new mutant lines of spring wheat along with the parent variety Kazakhstanskay-19, genotypes of Akteur and Tabasco, being the sensitive and resistant controls, respectively, were used for SR evaluation to YR in 14-days seedling as a time-dependent effect by 5 and 10 days of inoculation by the aggressive worldwide isolate of *P. striiformis* "Warrior" [22] in greenhouse experiments. The valuation of their SR by microscopic analysis based on number of in haustorial mother cells (HMCs) produced at yellow rust *P. striiformis* fungal isolate "Warrior"was carried out. Most of the created mutant lines (93.0%) were characterized as having both types of reistance to YR as APR and SR after 10 days inoculation by P. *striiformis*. Thus, 70 samples of spring wheat from 75 mutant lines identified as APR to YR had a strong association with SR which is developed on longer time of infection. The most effective dose of irradiation to generate the genetic variation was 350 Gy with mean of 60.08 (p<0.005) according to Anova analysis. The development of an autofluorescence having the antagonistic property to formation of HMCs and therefore, it induces plant defense reactions against to rust infection.

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