Shonda, E.A.

Lydia Ann

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STEM AND STRIPE RUST RESISTANCE IN WHEAT INDUCED BY GAMMA RAYS AND THERMAL NEUTRONS

Elpis A. SKORDA
Plant Breeding Institute,
Thessaloniki,
Greece

Abstract

STEM AND STRIPE RUST RESISTANCE IN WHEAT INDUCED BY GAMMA RAYS AND THERMAL NEUTRONS.

Attempts were made to produce rust-resistant mutants in wheat cultivars. Seeds of G-38290 and G-58383 (*T. aestivum*), Methoni and Ilectra (*T. durum*) varieties were irradiated with different doses of γ-rays (3.5, 5, 8, 11, 15 and 21 krad) and thermal neutrons (1.7, 4, 5.5, 7.5, 10.5 and 12.5 X 10^{12}) and the M_1 plants were grown under isolation in the field. The objective was mainly to induce stripe, leaf and stem rust resistance in G-38290, Methoni and Ilectra varieties and leaf rust resistance in G-58383. Mutations for rust resistance were detected by using the "chimera method" under natural and artificial field epiphytotic conditions in M_2 and successive generations. The mutants detected were tested for resistance to a broad spectrum of available races. Mutants resistant or moderately resistant to stripe and stem rusts but not to leaf rust, were selected from G-38290. From the other three varieties tested no rust-resistant mutants were detected. The frequency of resistant mutants obtained increased with increased γ-ray dose-rate, but not with increased thermal neutron doses. Some mutants proved to be resistant or moderately resistant to both rusts and others to one of them. Twenty of these mutants were evaluated for yield from M_5 to M_8. Some of them have reached the final stage of regional yield trials and one, induced by thermal neutrons, was released this year.

INTRODUCTION

Breeding for resistance to stem, leaf and stripe rust is becoming increasingly difficult in Greece because of the rapid evolution of ever more widely virulent races, owing to the presence of *Berberis cretica* throughout the country and its geographical location, which favours the flow of rust from many directions (Skorda [1]).

The most representative example was the rapid appearance and distribution of the new stripe rust race 20A, which rendered susceptible almost all wheat varieties grown in Greece and most of the promising breeding material. The

problem was further complicated because known varieties such as Hope, Thatcher, Newthatch etc., used so far as sources for resistance to stem rust, were susceptible to race 20A of stripe rust.

A major effort has been undertaken at the Plant Breeding Institute to develop new wheat cultivars with general (horizontal) resistance to leaf, stripe and stem rusts. To this end mutagen-induced genetic diversity is being used extensively along with new resistant genes from various sources (Skorda [2]).

MATERIAL AND METHODS

Cultivars G-38290, G-58383 (*T. eastivum*), Methoni and Ilectra (*T. durum*) were used. Dry seeds from a single plant of each cultivar were irradiated with γ-rays (3.5, 5, 8, 11, 15 and 21 krad) and thermal neutrons (1.7, 4, 5.5, 7.5, 10.5 and 12.5×10^{12} n/cm^2) through the courtesy of the AERC "Democritos".

The choice of cultivars used was based on their productivity, adaptability, and susceptibility to rusts. G-38290 especially was the leading variety (half the wheat crop area) and was limited to 1/10 of the area two years later when the new rust races prevailed.

Five hundred treated and same number of untreated seeds from each parent plant, per cultivar and treatment, were sown and grown to maturity in an isolated field. To avoid cross-pollination, spikes from each plant were bagged at flowering. Newly arisen mutants were distinguished from contaminants by the "chimera method" in the mutated M_1 plant (Jørgensen [3]).

Thus, from each M_1 plant three to four spikes were harvested and grown in strips in isolated fields bordered by a susceptible cultivar. At the five-leaf stage they were naturally inoculated with leaf and stripe rust. At the jointing stage the stem rust susceptible border was artificially inoculated by the injection method with the prevalent races 14, 21, 17 and 34. A good natural and artificial epidemic of leaf, stripe and stem rusts, respectively, was initiated. Plants were examined at the dough stage and those resistant to stripe or to stem rust were selected. All M_2 plants from a mutated M_1 plant were tested in order to determine the frequency of homozygote and heterozygote mutants along with the wild-type homozygotes in the M_3 generation. The selected plants were grown the following year to confirm the resistance one more year and to multiply the seed, and the next year some mutant lines with reduced incidence of stripe and stem rust infection were isolated. The mutant lines, when true breeding, were further evaluated for their agronomic performance, i.e. lodging resistance, and primarily rust resistance etc. Promising ones were successively tested and considered for selecting new commercial varieties directly, or for use as donors of a rust resistance gene(s). The selected material was advanced to M_4 and tested in preliminary field trials. The most promising mutants were tested from M_9 to M_8 in several locations, using randomized complete block designs.

TABLE I. FREQUENCY OF INDUCED MUTATIONS RESISTANT TO *P. Striiformis* AND *P. Graminis* OF THE WHEAT VARIETY G-38290

Mutagenic agent and dose	No. of plants M_1	No. of resist. plants to *P. Striiformis* No. (%)		*P. Graminis* No. (%)		No. of spikes M_1	No. of resist. spikes to *P. Striiformis* No. (%)		*P. Graminis* No. (%)	
γ-rays (krad)										
0.0	82	0	0.0	0	0.0	291	0	0.0	0	0.0
3.5	68	2	2.9	0	0.0	229	2	0.9	0	0.0
5.0	72	4	5.5	1	1.4	265	4	1.5	1	0.4
8.0	72	7	9.7	4	5.6	236	8	2.9	4	1.7
11.0	73	10	13.7	6	8.2	214	12	5.6	7	3.2
15.0	59	12	20.3	13	22.0	195	14	7.2	16	8.2
21.0	42	13	31.0	7	16.7	149	16	10.7	10	6.7
Thermal neutrons ($n/cm^2 \times 10^{12}$)										
0.0	62	0	0.0	0	0.0	221	0	0.0	0	0.0
1.7	56	2	3.6	1	1.8	199	2	1.0	1	0.5
4.0	79	4	5.1	4	5.1	178	4	2.2	4	2.2
5.5	76	4	5.3	2	2.6	231	5	2.1	2	0.9
7.5	70	6	8.6	9	12.9	235	6	2.6	9	3.8
10.5	74	2	2.7	3	4.1	132	2	1.5	3	2.3
12.5	73	5	6.8	4	5.5	121	6	5.0	5	4.1

RESULTS

Only from G-38290 did we detect resistant or moderately resistant mutants to stripe and stem rusts, but not to leaf rust. From the tested cultivars no resistance to rust mutants was found. Fifty-four out of 986 M_1 plants examined in G-38290 appeared to be resistant or moderately resistant to *P. gaminis,* and seventy-one plants to *P. striiformis* (Table I). Some of these resistant plants were segregated, while others were homozygous.

The frequency of resistant mutants increased with increased γ-ray dose-rate, while the difference was low between the several rates of thermal neutrons. Gamma rays produced a higher frequency of moderately resistant mutants than

TABLE II. MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF SOME INDUCED MUTANTS IN *Triticum Aestivum var.* G-38290 (THREE-YEAR RESULTS)

Mutant lines	Grain yield (kg/ha)	(%)	Culm length (cm)	Heading time[a]	*P. Striiformis*	*P. Graminis*
G 90443	2905	107	118	29	18 MR	60 S
G 90444	3035	111	122	27	22 MR	70 S
G 90448	2847	105	116	29	36 MS	70 S
G 90454	2927	108	116	29	34 MS	70 S
G 90460	2915	107	122	26	38 MS	70 S
G 90462	2372	87	104	26	26 MR	70 S
G 90491	2550	94	110	26	54 S	70 S
G 90495	2602	96	115	26	38 MS	70 S
G 90496	2557	94	112	27	26 MR	70 S
G 90522	3035	111	116	27	38 MS	70 S
G 90523	2820	104	107	27	40 MS	70 S
G 90524	2345	86	111	27	38 MS	70 S
G 90536	2447	90	114	27	36 MS	60 S
G 38290	2722	100	122	26	88 S	80 S
LSD 5%	293	10.8				
LSD 1%	392	14.4				

[a] Number of days from 1 April.

that obtained with thermal neutrons. Thus, γ-radiation is considered to be a better tool for the plant breeder interested in the production of viable mutations. The frequency of a mutant moderately resistant to stripe rust was higher than that to stem rust, while it was almost the same with thermal neutrons.

Although the number of spike progenies tested for each neutron dose-rate was low and consequently diplontic selection increased the variability in mutation rates, the observed rates indicated that they were lower than those obtained with gamma radiation. It has been reported that thermal neutrons produce a higher frequency of chromosome aberrations than γ-radiation, which indicated that the mutations of rust resistance are more point mutations than chromosome aberrations (Sparrow [4]).

TABLE III. MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF SOME INDUCED MUTANTS IN *Triticum Aestivum var.* G-38290

Mutant lines	Grain yield (kg/ha)	(%)	Culm length (cm)	Heading time[a]	*P. Striiformis*	*P. Graminis*
G 01370	1820	59	125	15	30 MR	20 MR
G 01477	2410	79	128	15	40 MR	60 MS
G 01483	1390	45	135	22	40 MR	30 MR
G 01489	1140	37	125	20	40 MR	30 MR
G 01585	1430	47	125	16	50 MS	60 MS
G 01594	2640	86	128	14	40 MS	60 MS
G 01724-1	3580	117	125	14	40 MS	30 MR
G 01725-3	3400	111	130	16	40 MS	30 MR
G 01726-1	3920	128	130	16	40 MS	50 MS
G 01728-1	3060	100	120	15	40 MS	50 MS
G 01730-1	3150	103	127	12	40 MS	50 MS
G 01753	900	29	125	14	00	30 MR
G 01781	1270	42	95	16	40 MS	15 MR
G 01782	2360	77	122	14	10 MS	70 MR
G 01849-1	4860	109	92	20	5 MR	5 R
G 01849-2	3860	126	90	20	30 MS	5 R
G 01851-5	2640	86	125	15	00	80 MR
G 01858	2360	77	122	14	70 MS	60 MS
G 38290	3060	100	120	16	80 S	60 S

[a] Number of days from 1 April.

In the two lower doses only one spike of each mutated plant was mutant, whereas in the three higher doses there were two to three cases in which more than one spike of the mutated plants was mutant. This is evidence that with higher doses the two to three spikes of each M_1 plant originated from the same meristem of initial cells in the M_1 embryo. Because of the heavy mutagenic treatment only one of the initial cells in the embryo survived.

Genetic analyses of mutants are not yet finished. Also, testing for resistance to broader spectra for races continues.

Results presented in Tables II and III suggest that there has been considerable improvement in most mutant lines for resistance to stem rust and also to stripe rust. The parent variety G-38290 was susceptible, whereas the mutants appeared

TABLE IV. GRAIN YIELD OF THE WHEAT MUTANT VARIETY G-07783 ISOLATED FROM THE VARIETY G-38290 (1 to 4 years in different locations)

Location	Number of years in trials	Grain yield (kg/ha)			Per cent of:		LSD (0.05
		G-07783	Generoso	G-02763	Generoso	G-02763	
Thessaloniki (PBI)	4	3780	3140	3120	120	121	200
Thessaloniki (UF)	3	3520	3345	3089	105	114	195
Nea Zoi	1	3630	3020	3310	120	110	524
Giannitsa	2	5125	4732	4991	108	103	214
Larisa	2	4640	3794	3883	122	119	254
Serre	2	4565	3603	4475	127	102	346
Aliartos	2	5565	4463	5551	125	100	250
Vardates	1	5300	4490	5260	118	101	450
Mean		4515	3824	4207	168	108	152

to be resistant or moderately resistant. Most of these mutants were not utilized directly as an improved cultivar, but will be used as breeding material, because of concomitant deleterious effects chiefly in quantitative traits.

The resistant mutants with good agronomic traits were tested in comparative yield trials with the parent variety and other wheat varieties grown widely in the country (Tables II-III). These mutant lines were significantly more resistant to rusts than the respective parent variety. The yielding ability of some mutants tested was better than their parent variety but not as high as new varieties released in the meantime. Only one of them, G-07783 (thermal neutrons, 12.5×10^{12} n/cm^2) was better in yield than these new varieties. This variety was tested for a further two years under different environmental conditions in trials conducted throughout the country during 1974–1976 and it performed, on the average, better in Generose, G-84865 and G-02763 (selection of C. Cerros), all the most popular high-yielding wheat varieties grown at present on a large scale in Greece (Table IV). In addition, G-07783 has a moderate resistance to all rusts and mildew. Because of its superior performance in regional trials this variety was released this year.

Several other promising mutant strains are now in advanced evaluation tests. A sister variety of G-07783 and G-01849-I was tested in the Fifth Preliminary Observation Nursery (Bread Wheat) 1975–76, and gave 0.2 average coefficient of infection (a.c.i.) to stem rust (out of 16 locations) while Mexipak, which was considered as relatively resistant to rusts in Greece, gave an a.c.i. of 18.8 to stem rust. This variety is also moderately resistant to other rusts.

The phenotypic frequency distribution curves for stripe rust indicated that the major part of the induced genetic variability tended towards susceptibility. From these curves it appears that the radiation induced both resistance and susceptibility. The same is true for the other rust.

DISCUSSION

The prime objective of the present work was to produce rust-resistant mutants in two bread and two durum wheat varieties. For the durum and one of the bread varieties the project was not successful. No mutants for either leaf, stripe and stem rust resistance were obtained. In the G-38290 material mutants for stem or stripe rust resistance were found.

The results of this study showed that mutants resistant to rusts in wheat, which can successfully compete with a parent variety, or even surpass it on other important characters including grain yield, are very rare.

Furthermore, utilization of induced mutations might be time-consuming and uncertain compared with other varieties (sources of resistance genes). This is primarily because of the simultaneous presence of deleterious mutants, which calls for producing as many induced mutants as possible in order to be able to screen for promising material. However, considering that in cereals the varietal change becomes more and more urgent, and parent varieties are soon outyielded by new cultivars, much more effort should be devoted to detect resistant or tolerant mutants that are agronomically satisfactory and can be used directly by farmers (e.g. G-07783).

The reason why the mutant line G-07783 yielded, on an average, more than the highest yielding variety in the country, was probably not only because of its improved resistance to rusts but also due to some other better agronomic characteristics. The adaptation analysis was especially interesting since its yielding capacity was stable over a wide range of environments in different years. Also, the permanent resistance of the mutant sister line G-01849-1 in 16 locations, in which there were different races of rusts in different years in Greece, showed that this resistance might be general (horizontal).

Genetic studies of this mutant line to detect if the alleles are identical with those existing in collections have not been finished. But even if the alleles of this mutant are identical to the existing ones, they are very useful because they have been induced in a variety with a completely different genetic background, which developed into a new variety.

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DISCUSSION

K.J. FREY: With regard to Table IV in your paper, was the high yield of wheat mutant variety G-07783 compared with that of Generoso due entirely to stem and stripe rust resistance?

Elpis SKORDA: No, this mutant also had a higher yield at sites where there was no rust infection.

K.J. FREY: What mutated traits apart from greater rust resistance contributed to the higher yield?

Elpis SKORDA: Greater resistance to lodging and more spikes per square metre among other things.

K. MIKAELSEN: I think the fact that you did not find any mutants in three of the four mutagen-treated varieties may be due to the smallness of the screened populations. How big were the M_2 and M_3 populations?

Elpis SKORDA: They consisted of over 4000–5000 plants for each treatment of the four varieties. In the case of the three varieties for which no disease-resistant mutants were found, we detect other types of mutation – for example, lower plant height, compactoid types and vaviloid types.

K.J. FREY: One oat variety, CI 7555, has an interesting mutability pattern. It gives no measurable spontaneous mutations, but has a very high mutation rate for any trait when treated with EMS or thermal neutrons.

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