Effect of Gamma Radiations on the Crossability of Wheat, Triticale and Rye and on Meiosis, Pollen Grain Germination and Pollen Tube Growth

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It is well known that crossability between wheat and rye is genetically controlled. Major genetic control of crossability between wheat and rye has been attributed to two loci, Kr_1 and Kr_2 , carried on chromosome 5B and 5A of wheat respectively (Riley and Chapman 1967). The dominant alleles at these crossability loci actively inhibit the production of intergeneric hybrids (Lange and Wojciechowska 1977). Various investigations provide evidence that the A, B and D genome of wheat as well as the R genome of rye carry crossability genes (Lein 1943, Riley and Chapman 1967, Tanner and Falk 1981, Lelley 1982). Material with about 20% crossability has been reported from India and Mexico.

The development of fertilized ovules in wheat-rye hybrids is strongly influenced by the ploidy level of the wheat parent. In tetraploid wheat-rye crosses embryo culture technique is a pre-requisite for the production of viable hybrid plantlets (Marais and Pienaar 1977) while in hexaploid wheat-rye crosses the ovules generally develop *in vivo*.

Although much has been written about wheat-rye crossability, not much is known about the mechanism causing the differences in the crossability, and the few reports that exist are contradictory. Boyes and Thompson (1937) reported a great difference in rye pollen germination on wheat stigmas between readily crossable and poorly crossable type, such difference was not observed in the studies of Tozu (1966). The growth of the pollen tubes in the poorly crossable types was found to be either slower (Boyes and Thompson 1937), or normal at the beginning and slower at later stages or not retarded at all (Tozu 1966). The dominant alleles of the crossability genes manifested themselves through retardation and eventually inhibition of pollen tube growth at the style base and in the ovary wall. However, the crossing barrier is not complete, because seed set can occur (Lange and Wojciechowska 1976).

In many cases the incompatibility between the desirable parents hampers triticale synthesis and its improvement. This paper presents the results of effect of gamma-radiations on the crossability of wheat, triticale and rye and on meiosis, pollen grain germination and pollen tube growth.

Materials and methods

The experimental material comprised of the common wheat (*Triticum aestivum*), cultivar UP 2003, a durum wheat (*T. durum*), cv. DWL 5031, a hexaploid triticale, cv. UPT 78268 and an Australian rye (*Secale cereale*). Air-dried seeds (with moisture content of $9.5 \pm 0.5\%$ in triticale, $11.5 \pm 0.5\%$ in wheat and $9 \pm 0.5\%$ in rye) were given 15kR and 30kR doses of gamma-irradiation using a ^{60}Co source.

Meiosis was studied in the pollen mother cells of the treated and control plants. The data were recorded at metaphase I and anaphase I of meiosis for univalents, laggards and bridges of chromosomes.

The treated seeds along with the controls of UP 2003, UPT 78268 and DWL 5031 and Australian rye were planted in a crossing block with three dates of sowing. The following crosses were made:

1. UP 2003 (control) $\times$ Rye
2. UP 2003 (15kR) $\times$ Rye
3. UP 2003 (30kR) $\times$ Rye
4. DWL 5031 (control) $\times$ Rye
5. DWL 5031 (15kR) $\times$ Rye
6. DWL 5031 (30kR) $\times$ Rye
7. UP 2003 (control) $\times$ UPT 78268
8. UP 2003 (15kR) $\times$ UPT 78268
9. UP 2003 (30kR) $\times$ UPT 78268
10. UPT 78268 (control) $\times$ UP 2003
11. UPT 78268 (15kR) $\times$ UP 2003
12. UPT 78268 (30kR) $\times$ UP 2003

Pollinations were performed in the last week of March. After removing the bottom and top spikelets of a spike as well as the third and higher ranking florets of each spikelet, the remaining florets were emasculated when the anthers were still pale green. Two or three days later, when the stigmas were feathery and receptive, the pistils were dusted with pollen from dehiscing anthers of the male parents. The pistils were then collected at 10 min and 4 h after pollination, and fixed immediately in a mixture of 96% ethyl alcohol and lactic acid (2: 1). Pollen grain germination and pollen tube growth were studied with normal microscopy of squash preparations of pistils in aniline blue (D'Souza 1972).

Results

i) Meiosis and pollen fertility

Meiosis in triticale was irregular with failure of chromosome pairing being the common feature (Figs. 1a–c, Table 1). The frequency of univalent chromosomes ranged from 0–9. Triticale showed 32.8% cells at control showing univalents. Though a few cells of the tetraploid wheat, DWL 5031 and hexaploid wheat, UP 2003 also showed this abnormality at control, it was very low. The frequency of univalents increased considerably with an increase in radiation dose. Similar results were obtained for laggards at anaphase I. Anaphase bridge was another major abnormality in the treated material, though a small percentage was observed in UPT 78268 even at control. Radiation increased the frequency of bridges. The maximum pollen sterility was observed in triticale, at control as well as after radiation treatment (Table 2).

ii) Pollen grain germination

A small proportion of the grains either had delayed germination or seemed not to germinate at all, and majority of the pollen grains germinated within 10 min after pollination. Among the crosses, the percentage pollen germination was almost similar in UP 2003 $\times$ rye and DWL

Figs. 1a–h. 1a, meiotic cell division in UPT 78268 (15 kR) showing univalent at metaphase I. $\times 1500$. 1b, meiotic cell division in UPT 78268 (30 kR) showing laggards at anaphase I. $\times 1500$. 1c, meiotic cell division in UPT 78268 (15 kR) showing a bridge and laggards at anaphase I. $\times 1500$. 1d, germination of pollen grains and pollen tube growth in the cross UP 2003 (15 kR) $\times$ UPT 78268. $\times 1500$. 1e, poor germination of pollen grains in the cross UPT 78268 (control) $\times$ UP 2003. $\times 1500$. 1f, abnormal pollen tube with a swelling at the tip in the cross UP 2003 (15 kR) $\times$ UPT 78268. $\times 1500$. 1g, coiling of pollen tube in the cross UP 2003 (15 kR) $\times$ UPT 78268. $\times 1500$. 1h, two pollen tubes coming out of the same pollen grain in the cross UP 2003 (15 kR) $\times$ UPT 78268. $\times 1500$.

5031 × rye. The 15kR treatment resulted in an early germination after 10 and 30 min. At 30kR there was a reduction in germination as compared to the control.

The reciprocal crosses in case of wheat and triticale showed large differences. In UP 2003 × UPT 78268 there was more pollen germination than the reciprocal cross (Figs. 1d, e). At 15 and 30kR there was a stimulation in pollen germination (Figs. 2a–d).

iii) Pollen tube growth

After penetrating the papillated stigma hairs, the tubes grew through these hairs into the style. The transmitting tissue of the style consisted of compact elongated cells and the pollen tubes grew between the cells and in the intercellular space. Next, the pollen tubes grew similar-

Table 1. Cytological abnormalities during meiotic cell division in wheat and triticale after seed irradiation

Variety	Treatment	% cells with			Average abnormality (%)
		Metaphase	Anaphase I		
		Univalents	Laggards	Bridges	
UP 2003	Control	4.8	3.0	—	3.9
	15 kR	34.7	27.7	11.6	24.7
	30 kR	57.6	58.3	19.8	45.2
DWL 5031	Control	4.2	2.0	—	3.1
	15 kR	40.6	30.5	9.9	23.7
	30 kR	53.3	50.3	17.2	40.3
UPT 78268	Control	32.8	26.0	8.3	22.4
	15 kR	51.0	47.4	18.4	38.9
	30 kR	74.6	68.9	28.6	57.4

Table 2. Effect of gamma-radiations on pollen fertility in wheat, triticale and rye

Strain		Treatment	% Fertility
Wheat	UP 2003	Control	95 ± 4.6
		15 kR	89 ± 6.5
		30 kR	71 ± 7.8
	DWL 5031	Control	94 ± 5.2
		15 kR	87 ± 8.6
		30 kR	79 ± 7.0
Triticale	UPT 78268	Control	87 ± 4.0
		15 kR	80 ± 7.9
		30 kR	59 ± 8.2
Rye		Control	94 ± 5.5

ly through transmitting tissue of ovary wall, consisting of similarly elongated yet smaller, and in particular narrower cells.

Because of the irregularity in the pollen grain germination, the pollen tube growth was irregular (Table 3). At 10 min after pollination the majority of the pollen grains had germinated, and the tubes had penetrated into the stigma hairs. Part of the pollen tubes had just started their growth in the hairs, others were already part of the way along the hairs and a few had already entered the transmitting tissue of the style. At 30 min after pollination there was still a greater differentiation in the pollen tubes. Some had already reached the base of the style or had entered the transmitting tissue of the ovary wall, but others had only just started to grow, and all intermediate stages were observed.

Table 3. Effect of gamma-radiations on pollen tube growth (μ) in various crosses

Cross combination	Treatment	Time after pollination		
		10 min	30 min	4 h
UP 2003 $\times$ Rye	Control	1.7	3.0	6.6
	15 kR	2.4	3.7	6.7
	30 kR	2.2	3.2	6.1
DWL 5031 $\times$ Rye	Control	1.7	2.8	5.6
	15 kR	2.5	3.5	6.9
	30 kR	2.4	3.6	6.2
UP 2003 $\times$ UPT 78268	Control	2.1	2.3	6.9
	15 kR	2.6	4.0	7.1
	30 kR	2.4	4.0	6.9
UPT 78268 $\times$ UP 2003	Control	2.1	2.2	3.2
	15 kR	2.2	3.1	5.0
	30 kR	2.2	3.1	4.9

Figs. 2a-d. Percentage pollen germination in different crosses after 10 min, 30 min and 4 hours of pollination. ▨ 10 min, ■ 30 min, □ 4 h.

Among the wheat and rye crosses the rate of pollen tube growth was almost similar as compared to the control. The 15kR and 30kR dose had a stimulatory effect on the pollen tube growth.

Among the triticale and wheat crosses there were greater differences in the rate of pollen tube growth in the reciprocal crosses. The pollen tubes were much larger and showed a faster rate of growth in the UP

Fig. 3. Percentage seed set in different crosses. ▨ control, ■ 15 kR, □ 30 kR.

2003 $\times$ UPT 78268 cross as compared to its reciprocal. In general, both the crosses showed a stimulation of pollen tube growth at 15kR and 30kR as compared to the control.

iv) Abnormal pollen tubes

In all the crosses abnormal pollen tubes were observed even at control, and there were more abnormalities after 4 h of pollination. The abnormal pollen tubes increased considerably from 10 to 30 min of pollination but after that there was not much of an increase. The maximum abnormalities were recorded at 30kR. The wheat and rye crosses showed more abnormalities than the wheat and triticale cross and its reciprocal.

A striking aberration was the occurrence of swelling of pollen tube tips which had the appearance of being filled with dense cytoplasm (Fig. 1f). The size of the swelling was variable. They rarely occurred in the hairs of the stigma but were most often found in the transmitting tissue of the style. Many pistills showed no swelling at all, others had one, two or several pollen tubes with swollen tips. Rarely other aberrations were observed, like for example pollen tubes growing in the wrong direction or branching of the pollen tubes. Some showed twisting on the stigma whereas some pollen grains showed more than one tube coming out from the same germ pore (Figs. 1g, h). In some cases two pollen tubes coming out from one pollen grain were observed.

v) Seed set and germination

The hexaploid wheat UP 2003 showed more seed set with rye than the tetraploid wheat (Fig. 3). The maximum per cent seed set in control was recorded in UP 2003 $\times$ UPT 78268 cross, whereas no seed was formed in its reciprocal cross. In all the crosses, seed set increased at 15kR but it was the least at 30kR. Over all, the seed setting in all the crosses was quite poor. There was 10.1 per cent seed germination in UP 2003 $\times$ UPT 78268 cross at control and 66.7 per cent at 15kR in its reciprocal cross. No seeds germinated in other crosses or with other treatments.

Discussion

Physical and chemical mutagens affect the cytological behaviour of plants in M_1 and later generations, besides inducing point mutations. Various chromosomal abnormalities are best reflected in the number of univalents, laggards and bridges etc. These abnormalities could be seen in the PMCs of the treated material.

In triticale, frequency of cells showing univalents was very high. The univalents may result either from the failure of the chromosomes to pair at zygotene (asynapsis) or of paired chromosomes separating at diplotene because chiasma formation did not occur (desynapsis). According to Chen *et al.* (1978), in triticale side-by-side associations were rare so the lack of pairing was probably asynaptic rather than desynaptic in origin, suggesting disturbances during diplotene or zygotene rather than interference with the timing of the intimacy of chiasma formation. Kaltsikes (1973) suggested that univalents in hexaploid triticale, most likely, arise from two factors a) interference with premeiotic DNA replication in rye chromosomes which results in a number of regions left unreplicated until zygotene, these regions being postulated to be necessary for chiasma formation and, b) insufficient time for rye chromosomes to accomplish pairing in triticale.

In general, radiation treatment caused a higher frequency of bridges. The possible explanation for the formation of bridges may be that the broken ends containing centromere from two different chromosomes unite, resulting in the formation of a dicentric chromosome, or the chromosomes are clumped together due to stickiness and are often unable to separate completely at anaphase and hence anaphase bridges are formed. Chromatin stickiness may be caused due to some changes in the surface properties of the chromosomes which cause them to

adhere to each other on coming in contact. This stickiness has been attributed to partial dissociation of the nucleo-proteins and an alteration in their pattern of organisation (Chowdhury and Nirmala 1976).

In the present study the hexaploid wheat and the tetraploid wheat when crossed with rye, resulted in 12.0 and 4.5% seed set, respectively. It is likely that the hexaploid wheat has a genotype Kr_1kr_2 and the tetraploid wheat Kr_1Kr_2 according to the classification of Lein (1943). When UP 2003 was crossed with the triticale UPT 78268, the seed set was 22.7% but in the reciprocal cross no seed was obtained. This suggests that the seed in the latter cross is not formed due to poor pollen germination and retardation and inhibition of the growth of the pollen in the pistils.

In all the crosses, there was an increase in the per cent seed set at 15kR as compared to the control. This shows that the inhibition barrier at the base of the style is affected by 15kR radiation allowing the pollen tubes to pass through it. This can be seen from the pollen tube growth at 15kR which is faster and the pollen tubes are the longest. Moreover, at 15kR the germination percentage of pollen grains also increased.

In the triticale $\times$ wheat cross where no seed was formed at control, the pollen germination was very poor and the rate of pollen tube growth was very slow. This may be due to incompatibility between pistil tissue and pollen tubes. It seems that the pollen tubes could not penetrate the barrier of transmitting tissue at the base of the style resulting in the lack of fertilization and seed set. In those cases where fertilization did take place, it is likely that the embryo did not develop due to post-fertilization incompatibility. But at 15kR, 1.7% seed set took place. In this case the pollen germination had increased and the rate of pollen tube growth was faster. Also, radiations might have affected the barrier to pollen tube growth resulting in some seed formation. At 30kR the seed set was less than that of control. This may be due to the formation of a higher number of abnormal pollen tubes, and also due to some damaging effect on the transmitting tissue in the style, which might be preventing enough pollen tubes to fertilize the egg to form seeds. Although the thickening of the tips of pollen tubes has only rarely been mentioned in the literature (Batygina 1974, in wheat) it seems to be of a common appearance. The present investigation on pollen tube development has shown a good number of abnormal pollen tubes in different crosses. It is not yet clear whether the enlargements are the result of a locally increased resistance to growth (Pope 1946) though that may be possible since in all the crosses except triticale $\times$ wheat where pollen tube abnormalities are more, the pollen tube growth is less. These enlargements may be due to a physiologically determined disharmony between the tissues of style or ovary wall and the pollen tubes (Batygina 1974).

This study shows that the maximum meiotic abnormalities in triticale result in poor seed set whenever triticale is used as a female parent in any cross. Moreover, the activity of Kr_1 and Kr_2 genes in the wheat-rye crosses manifests itself in a slowdown in the rate of the pollen tube growth and an increase in the number of abnormal pollen tubes and the retardation and inhibition of the growth of the pollen tubes of the rye in the pistils of wheat. This inhibition is localized at the base of the style and in the transmitting tissue of the ovary wall as reported by Lange and Wojciechowska (1976). Also, this inhibition, whose chemical and physical nature of the mechanism is not known, is affected by 15kR dose in some way resulting in an increase in the seed set.

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