TWIN SHOOTS FROM SPRING WHEAT SEEDS [1]

Walter Morgan, Chris Okonkwo, and Eugene Whitehead
Departments of Botany-Biology and Station Biochemistry
South Dakota State University, Brookings, 57006

Beneficial alterations have been induced in plants by irradiation. Donini et al. (1968) studied genetic changes induced in wheat and Srinivasachar and Seetharam (1971) reported the induction of rust-resistance in flax, using gamma irradiation. Useful mutations have been incorporated into commercial breeding programs, and new sources of beneficial changes are constantly being sought. The experiment to be reported hereafter describes a search for useful alterations which result from the irradiation of wheat seeds and seedlings.

MATERIALS AND METHODS

The material chosen was the Lark variety of wheat, *Triticum Aestivum*, which is described in the South Dakota Certified Seed Directory (South Dakota Crop Improvement Association 1972) as a semi-dwarf, hard, red, spring wheat. Uniform seeds of this true-breeding hexaploid variety were obtained for all of the experiments.

A ^{60}Cobalt source was employed for exposing the seeds and seedlings to gamma irradiation. The source was located in an underground chamber adjoining the greenhouse facilities at South Dakota State University. Seeds were planted in 5 x 6 cm containers with a soil depth of approximately two cm and spaced at a distance of approximately one cm. Greenhouse temperature was approximately 22 C and normal daily watering was practiced. The experiment was divided into two phases.

Phase A

Two variables were employed: (1) Pre-treatment of seed and (2) Dosage. The three pre-treatment groups were: *dry seeds* as would be normally used for commercial plantings, *wet seeds* which were submerged in water for a few minutes prior to irradiation and then removed, and *soaked seeds* which were immersed in water for 24 hours prior to irradiation and also removed immediately prior to exposure. Guided by previously reported trials (Altman and Dittmer 1966, Donini et al. 1968, and Saric 1960), dosages of 5,000, 10,000, 15,000, and 20,000 rad were administered.

Following irradiation, the seeds were individually planted as described earlier. For each of the 12 treatment groups there were two containers, resulting in a total of 40 seeds per treatment group.

[1]Approved by the director of the South Dakota Agricultural Station as Journal Series Paper No. 1196.

There were also 40 untreated, control seeds planted at the same time. In all, 520 seeds were used. The experiment was terminated two weeks after seed planting.

Phase B

As with phase A, there were replicate containers with a total of 40 plants per group in this experiment. Seedlings, instead of seeds, were irradiated in phase B. The two variables were: (1) Age of seedling, or days after planting the seed, and (2) Dosage. The seedling ages were 2, 6, 9, and 16 days. Dosages were 1,000, 2,000, 5,000, and 10,000 rad. There were 680 seeds used in this phase.

In addition to observing survival and abnormalities all plants were measured daily for the first week and on alternate days thereafter. At the end of four weeks, plants were individually taken from the soil. Following removal of the roots, the wet-plant weights were recorded.

RESULTS

Phase A

Survival of seedlings was recorded on the 14th day (Table 1). There were no gross abnormalities recognized in the living plants. The sequence of seedling appearance, as could be expected, was that the soaked seeds sprouted first and the dry seeds sprouted last.

TABLE 1

Viability of *Triticum Aestivum* Seedlings Following Irradiation of Seeds

	Seed Type					
	Dry Seed		Wet Seed		Soaked Seed	
Dosage	G	S	G	S	G	S
5,000	40	40	38	38	40	26
10,000	40	40	40	40	40	2
15,000	40	40	38	38	40	0
20,000	40	40	40	40	40	1
Totals	160	160	156	156	160	28

G — Number of seeds germinated from 40 which were treated.
S — Seedlings alive at 14 days, when experiment terminated.

TABLE 2

Germination of Lark Wheat Seedlings Which Were Irradiated While Growing in Soil

	Treatment Age				
Dosage	2 days	6 days	9 days	13 days	Total
1,000r	39	37	34	35	145
2,000r	33	35	37	36	141
5,000r	40	*40	*34	36	150
10,000r	37	36	40	34	147
Total	149	148	145	141	583

*Groups in which multiple shoots were observed.

TABLE 3

Average Weight (mg) of Four-Week-Old Seedlings Which Were Treated With ^{60}Co Gamma Irradiation

	Treatment Age				
Dosage	2 days	6 days	9 days	13 days	Unweighted Tr. Group Average
1,000r	128	135	118	143	131
2,000r	121	157	162	139	145
5,000r	88	125	191	164	142
10,000r	*50	111	125	132	105
Unweighted Age Group Average	97	132	149	145	131
Control					186

*Ten live seedlings in this group averaged 50 mg and 27 dead plants in this group weighed an average of 2 mg.

The irradiated seeds germinated approximately one day earlier than did the controls. All of the seedlings from the dry and the wet seeds appeared healthy when the experiment was terminated. The soaked seeds also germinated well, but all of those which were treated with the two highest dosages died, and 38 of the 40 which were treated with 10,000 rad also died. There were even 14 of the seedlings from the soaked group which died from the lowest dosage of 5,000 rad. In this entire population, there was only one shoot from each seed; there were no multiple shoots.

Phase B

For the experiment which involved irradiation of seedlings, the numbers of seeds which germinated, in each group of 40, are recorded in Table 2. There were also 118 control seedlings from 120 seeds in this trial. Germination of the untreated seeds in these groups ranged from 33 to 40, with a total of 583 seedlings (which were later irradiated) from 640 seeds. The terminal average weights and heights for each group are recorded in Tables 3 and 4. All plants lived to the four-week age with the one exception of plants which were treated with 10,000 rad as two-day-old seedlings. Of the 37 in that group (Table 2) 27 died. The ten survivors averaged

TABLE 4

Average Height (cm) of Seedlings When Experiment Was Terminated at Four Weeks of Age

	Treatment Age				
Dosage	2 days	6 days	9 days	13 days	Unweighted Tr. Group Average
1,000r	16.8	15.4	14.9	15.5	15.7
2,000r	16.3	16.0	13.6	16.7	15.7
5,000r	13.4	9.4	12.1	16.2	12.8
10,000r	1.7	9.7	10.9	15.7	9.5
Unweighted Age Group Average	12.1	12.6	12.9	16.0	13.4
Control					19.6

50 mg in weight (Table 3) and 1.7 cm in height (Table 4). The 27 dead plants average 1.85 mg. Relative appearance of these stunted plants, at 15 days of age, is shown in Figure 1.

An exciting phenomenon was observed in two, and only two, of the experimental groups. Twin shoots (Fig. 2) were observed in the 6-day and 9-day seedlings which had been treated with 5,000 rad. Forty-six of the 74 seedlings in these two groups had multiple shoots from a single seed. In the 6-day group, 37 of 40 had multiple shoots; 33 twin, 3 triplet and 1 quadruplet. In the 9-day group, 9 had twin shoots. For the total population of treated and control plants in phase B, there were 46 multiple shoots and 655 single shoots.

Another observation in the multiple-shoot groups was a different leaf shape and color. The control leaves were pointed (Fig. 1), twisted and a moderate shade of green. Leaves of the multiple-shoot plants were dark green, they had rounded terminal ends, and they were less inclined to twist. As may be seen in Table 4, the multiple-shoot plants at 6-day and 9-day were shorter than were the 2-day and 13-day plants in the 5,000 rad treated series.

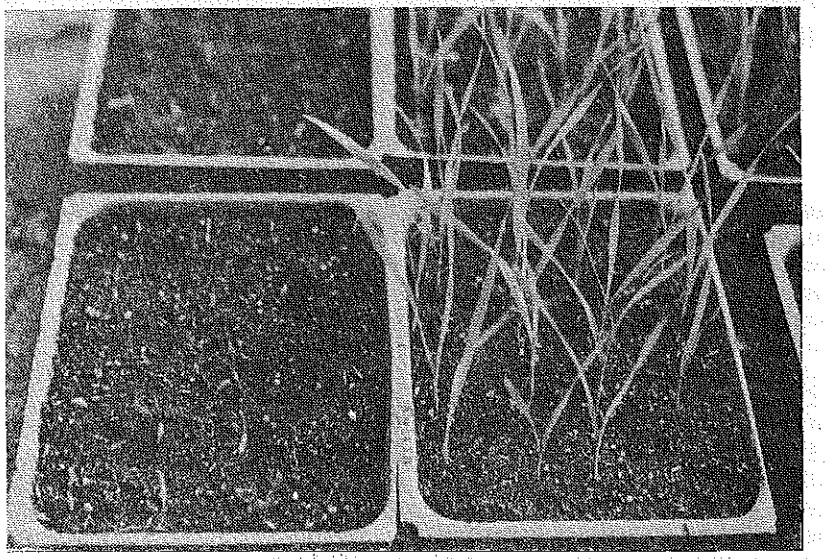

Figure 1. Stunted appearance of wheat seedlings which were treated with 10,000 rad when two days old (on left) vs. controls (on right) 20 days after irradiation.

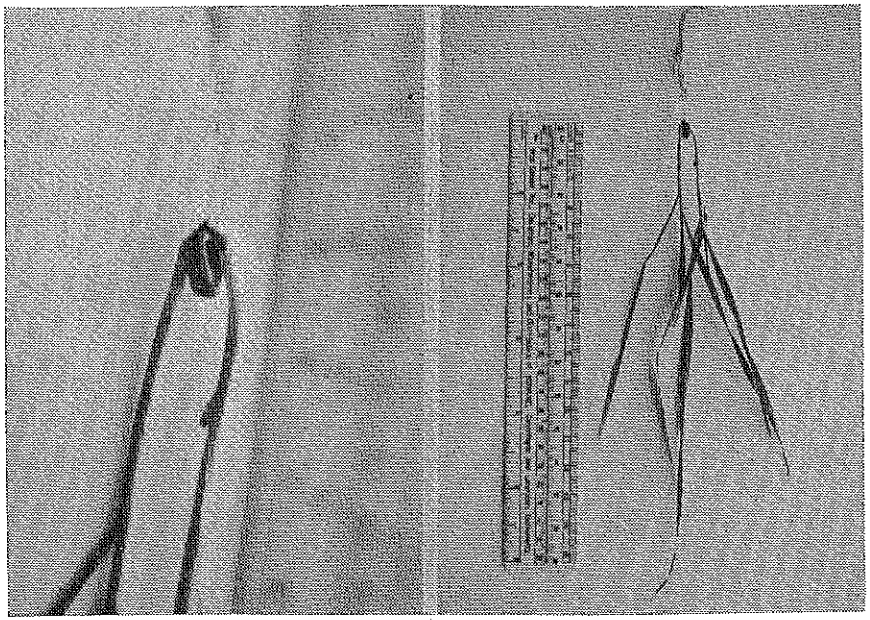

Figure 2. Twin shoots from wheat seeds three weeks after the seedlings were irradiated with 5,000 rad when six days old.

DISCUSSION

In their studies with gametophyte sensitivity in barley and durum wheat, Devreux and Donini (1972) concluded that tolerance to gamma irradiation increased as the gametophyte progressively reached maturity. Results from phase A of the present study indicated that the seeds became less tolerant as germination (soaked seeds) progressed and in phase B that the seedlings became more tolerant with age. Devreux and Donini (1972) further demonstrated that the tetraploid wheat with which they worked was capable of repair. In the present study, it is evident in phase B that all but the youngest (2-day) seedlings with the heaviest dosages (10,000 rad) were capable of repair. However, in phase A, irreparable damage resulted in most of the soaked seed series. This suggests to the authors that the greatest radiation-susceptibility is to be found in the early germination of the developing Lark embryo and seedling. Anathaswamy et al. (1971) have suggested that the endogenous amino acid pool sizes are low in irradiated seed. Amino acid destruction may account for susceptibility during early development.

Following the lethal stage, there is a stage in the growing

seedling which reacts by showing reduced growth or altered development. That is, dosages of 10,000 rad and above killed certain stages (the soaked seeds and the 2-day seedlings) but the older seedlings were only inhibited, not killed, by that dose. Killion and Constantin (1971) reported that rate of irradiation effected plant response. In Seneca winter wheat they learned that 16,000 rad when given at 40 rad/minute was lethal, but that 10 rad/minute inhibited growth to 60% of the control. Bottino and Sparrow (1971) used Opal wheat and Orbit oats for their irradiation trials. They reported that yield was more sensitive than was survival. The reduced growth in terms of height and weight is obvious in the present experiments. The average weight reduction for all treatment groups vs. control, was 55 mg, or approximately 30 percent. Although a large portion of that difference was attributable to the 2-day series, all except one of the 16 treated groups averaged less than did the controls. It is important to note that the one group exception was a group which had multiple-shoot plants. A similar comparison is true for the height data. The treated groups averaged 6.2 cm shorter than the controls and all of the 16 groups were shorter.

When seeking a possible explanation for the twinning phenomenon, two pertinent references were found. An English investigator, Davies (1968) reported the results of irradiating wheat with acute doses of 250 to 2,000 rad at various growth stages. In his report he suggested an apical meristem dependency which will be discussed forthwith. Sekiguchi et al. (1971) have recently also referred to the radiation damage in apical meristem shoots. A possible explanation of the multiple-shoot phenomenon, which is reported for the first time in our experiments, is that the apical meristem is destroyed and that there is therefore no continuing production of indole acetic acid by the plant. No bioassays or tissue cultures, such as those reported by Haber and Walne (1968) were prepared. With the destruction of IAA production, it is possible that the growth of adventitious buds is no longer inhibited and that multiple shoots result.

Induction of several shoots from the same seed could provide genetically identical plants for wheat breeding studies. If, indeed, this multiple-shoot induction is repeatable, less seed could be used and larger harvests could be realized. The commercial applications of this discovery await future research.

SUMMARY

Although multiple shoots from adventitious buds have been observed in grass seed, a similar situation is rare in the seeds of cereal grains. In a study of the effects of gamma irradiation on wheat seeds and wheat seedlings, twinning resulted from dosages

of 5,000 rad at the 6-day and 9-day ages. Concomitant growth retardation and lethality (at certain developmental stages) is also reported.

LITERATURE CITED

Altman, P. and Dorothy Dittmer. 1966. Environmental Biology, publ. Federation of American Societies for Experimental Biology, 182-189.

Anathaswamy, H. N., V. K. Vakil and A. Sreenivasah. 1971. Biochemical and physiological changes in gamma-irradiated wheat during germination. Rad. Bot. 11:1-12..

Bottino, P. J. and A. H. Sparrow. 1971. Comparison of the effects of simulated fallout decay and constant exposure-rate gamma-ray treatments on the survival and yield of wheat and oats. Rad. Bot. 11:405-410.

Davies, C. R. 1968. Effects of gamma irradiation on growth and yield of agricultural crops - I. Spring sown wheat. Rad. Bot. 8:17-30.

Devreux, M. and B. Donini. 1972. Effects radiobiologiques de l'irradiation des gametophytes chez l'orge et le ble dur. Rad. Bot. 12:19-29.

Donini, B., G. T. Scarascia Mugnozza, and F. d'Amato. 1968. Genetic studies of chronic gamma irradiation in Durum wheat. Rad. Bot. 8:49-58.

Haber, A. H. and Patricia Walne. 1968. Actions of acute gamma radiation on excised wheat leaf tissue. Rad. Bot. 8:389-397.

Killion, D. D. and M. J. Constantin. 1971. Acute gamma irradiation of wheat plant: Effects of exposure, exposure rate, and developmental stage on survival, height, and grain yield. Rad. Bot. 11:367-373.

Saric, M. 1960. Effects of ionizing radiation on seeds. Proc. Symposium, Karlsruhe IAGA Vienna.

Sekiguchi, F., K. Yamakawa and H. Yamaguch. 1971. Radiation damage in shoot apical meristems of **Antirrhinum majus** and somatic mutations in regenerated buds. Rad. Bot. 11:157-169.

South Dakota Crop Improvement Association. 1972. Hard red spring wheat. **In** South Dakota Certified Seed Directory. Pages 12-16.

Srinivasachar, D. and A. Seetharam. 1971. Induction of resistance to rust, **Melampsora lini,** in flax, by gamma radiation. Rad. Bot. 11:143-145.