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Genotype Dependent Radiosensitivity of Autotetraploids in *Trigonella foenum-graecum* L.

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Summary. Different diploids of *Trigonella foenum-graecum* L. and their corresponding autotetraploids were seed-treated with 40 krad of γ-rays, and parameters such as germination, survival, growth reduction, pollen fertility, pod setting, etc. were recorded. A stimulation of seed germination due to the irradiation could be observed. Contrary to the general rule that polyploids are more radioresistant than their corresponding diploids, one 4× strain was completely killed while the 2× version survived comparatively well. Apparently gene reduplication is not the overall protective mechanism as was once earlier believed. The importance of genotypic influence on radiosensitivity was demonstrated at both the 2× and 4× level. The limitation of interphase chromosome volume and degree of ploidy in predicting radiosensitivity is discussed.

Key words: Radiosensitivity – γ-irradiation – *Trigonella foenum-graecum* – Autotetraploids – Chromosome volume

Introduction

The study of radiosensitivity with special reference to level of ploidy is of applied significance in both plants and animals. A large proportion of domestic plants are polyploids and polyploidy is closely linked to malignant and pathological growths in animals. A number of workers have investigated the relationship between level of ploidy and radiosensitivity (Sparrow et al. 1967; Ichikawa and Sparrow 1967) however the present authors have not come across any reference which deals with comparative radiosensitivity of diploids and their corresponding autotetraploids within the same species (Raghuvanshi and singh 1977). Results from such studies will be reported here. It should also be observed that mutation breeding is of special interest in polyploids since they generally exert a higher frequency of viable mutations than comparable diploids (Mac Key 1959; Swaminathan 1964).

Material and Methods

Five strains of *Trigonella foenum-graecum*, differing in height, branching, number of nodes and internodes, flowering, fruiting period, etc., were chosen for the experiment. Four (sels. 1-4) were also represented by their corresponding autotetraploids that had reached the C_5 generation. 100 seeds per treatment were exposed to a single dose of 40 krad γ-rays (source Co^{60}, dose rate 64 krad/sec., temperature at exposure, dry 24°C, wet 19°C). The dose was chosen on the basis of previous experiments with a dose range from 10 to 60 krad, and the irradiated seeds were sown in replicates along with their respective controls.

For determination of interphase nuclear volume (INV), seeds of each strain were germinated on moist blotting paper. Root tips of equal size were fixed in Navashin's fluid A and B (1:1). Permanent preparations of paraffin sections (12μ) of root meristems were used for measurements of nuclei from the second cell layer. In all, 50 nuclei were measured from 5 root tips of each treatment and INV was calculated by assuming the nuclei to be spherical and thus applying the formula $4/3 \cdot \Pi \cdot R^3$. The interphase chromosome volume (ICV) was obtained by dividing INV with the somatic chromosome number.

Results

The effect of radiation on seed germination is reported in Table 1 and Fig. 1. Clear genotypic differences can be observed. At the 2× level, sel. 4 shows the highest radiosensitivity; at the 4× level, the newly produced strains in particular often suffer from poor seed germination, for sels. 1, 3, and 4 are evidently stimulated by the effect of the γ-rays. Such a stimulatory effect of irradiation on seed germination has also been reported for *Brassica* (Swaminathan 1964).

An improved seed germination does not necessarily imply a higher survival rate (Table 1, Fig. 2). A direct relationship between seed germination and survival cannot be found on either level of ploidy since different genotypes differ in their response. The extreme sensitivity of the 4× version of sel. 2 resulted in complete

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Table 1. Data on germination and survival alongwith different morphological parameters studied in 2x and 4x of different selections of *Trigonella foenum – graecum* L.

Selections (genotypes)	Ploidy levels and treatments	Germination			Survival		Growth (35 days cms, AM ± S.E.)	Growth (at maturity cms, AM ± S.E.)	Pollen fertility (%, AM ± S.E.)	No. of pods (AM ± S.E.)
		No. of seeds sown	No. of seeds germinated	Percent of control	No. of seedlings survived	Percent of control				
Sel. 1	2x, control	100	90	–	90	–	11.20 ± 0.20	65.00 ± 1.20	99.50 ± 2.03	36.43 ± 1.57
	2x, 40 K rad	100	90	100.0	68	75.5	3.60 ± 0.001	60.97 ± 0.80	71.80 ± 4.14	16.40 ± 0.53
	4x, control	100	73	–	73	–	8.60 ± 0.10	86.03 ± 0.03	80.80 ± 1.82	27.47 ± 1.94
	4x, 40 K rad	100	89	122.0	58	79.4	5.04 ± 0.14	52.50 ± 2.60	72.04 ± 2.22	3.70 ± 0.68
Sel. 2	2x, control	100	99	–	99	–	16.30 ± 0.20	76.60 ± 3.64	97.86 ± 3.60	32.20 ± 2.24
	2x, 40 K rad	100	97	97.8	80	80.8	6.10 ± 0.20	65.03 ± 1.00	79.20 ± 4.31	19.70 ± 1.24
	4x, control	100	46	–	46	–	10.90 ± 0.47	83.80 ± 1.00	60.60 ± 3.20	20.50 ± 1.65
	4x, 40 K rad	100	45	97.8	00	00	–	–	–	–
Sel. 3	2x, control	100	95	–	95	–	14.62 ± 0.40	60.00 ± 1.20	99.00 ± 2.01	54.20 ± 0.34
	2x, 40 K rad	100	87	91.5	57	60.0	7.15 ± 0.18	66.20 ± 0.06	83.67 ± 3.80	20.90 ± 1.10
	4x, control	100	89	–	89	–	12.64 ± 0.34	76.68 ± 1.30	83.67 ± 3.10	39.70 ± 1.94
	4x, 40 K rad	100	91	102.2	78	87.6	4.35 ± 0.18	47.80 ± 2.62	46.30 ± 4.93	4.46 ± 0.57
Sel. 4	2x, control	100	69	–	69	–	5.41 ± 0.10	40.62 ± 2.90	98.71 ± 3.21	52.80 ± 2.10
	2x, 40 K rad	100	56	81.2	48	69.5	1.77 ± 0.20	34.60 ± 2.10	57.62 ± 5.60	16.60 ± 0.85
	4x, control	100	43	–	43	–	5.33 ± 0.25	51.80 ± 2.10	70.70 ± 2.20	31.40 ± 6.30
	4x, 40 K rad	100	53	123.2	32	74.4	2.62 ± 0.20	32.20 ± 3.40	51.11 ± 4.66	6.04 ± 9.74
Sel. 5	2x, control	100	84	–	84	–	13.10 ± 0.20	87.53 ± 0.80	97.90 ± 1.30	39.90 ± 2.60
	2x, 40 K rad	100	75	89.2	46	54.7	5.99 ± 0.10	73.60 ± 2.04	80.51 ± 0.90	25.70 ± 2.00

death of all plants. This 4× strain was also the only one not showing a stimulated germination rate after irradiation. The tetraploids of sels. 1, 3, and 4 verified the rule that gene reduplication generally implies radioresistance. The total collapse of the 4× sel. 2 might be due to chromosomal damage but more likely it is due to some gene im-balance causing a physiological death.

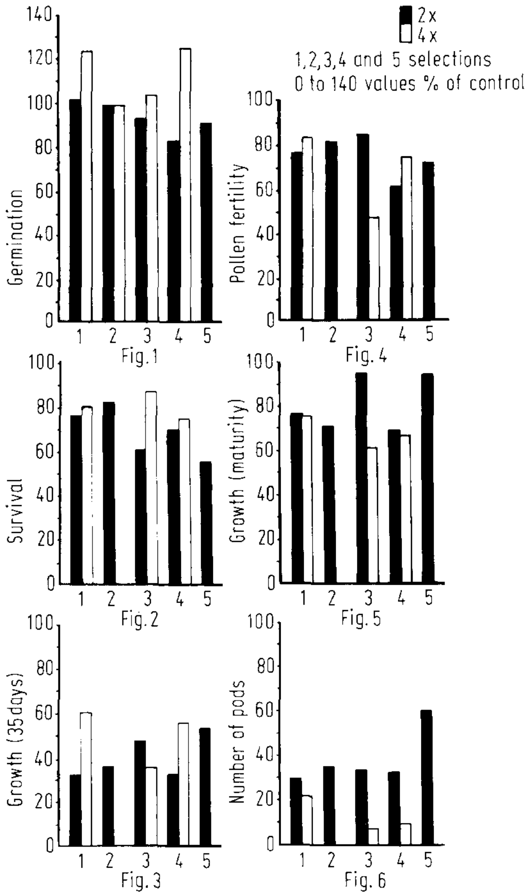
The influence of the seed irradiation on successive plant growth was first recorded 35 days after sowing (Table 1, Fig. 3). A consistent pattern could not be found for this parameter either, again emphasizing a high genotypic dependence. This impression is strengthened by the fact that 4× sels. 1 and 4 maintained the stimulatory response recorded for both germination and survival while 4× sel. 3 did not. The same trend can be observed for pollen fertility (Table 1 and Fig. 4).

It appears as if this genotypic dependence for reaction to irradiation will level off as ontogeny proceeds and plants reach maturity. Table 1 and Fig. 5 illustrate this tendency (also observed for *Melandrium* by Devies 1962) by presenting plant height at final stage of maturity.

The retarding effect of an irradiation upon successive growth over the whole vegetative period is demonstrated in Table 1 and Fig. 6 by a sharp decline in reproductive capacity, illustrated by the number of pods which developed. Having already a disturbed physiological and chromosomal balance, the tetraploids will suffer more than do the diploids. Genotypic differences can, however, still be

Table 2. Interphase nuclear and interphase chromosome volume (INV and ICV) of 2x and 4x of different selections of *Trigonella foenum-graecum* L.

Selections	INV (μ^3 ± S.E.)		ICV (μ^3 ± S.E.)	
	2x	4x	2x	4x
Sel. 1	194.80 ± 3.93	576.30 ± 6.08	12.11 ± 0.02	17.99 ± 0.08
Sel. 2	193.75 ± 4.30	592.13 ± 6.09	12.09 ± 0.06	18.26 ± 0.09
Sel. 3	192.44 ± 3.85	582.13 ± 7.03	12.05 ± 0.04	18.09 ± 0.09
Sel. 4	192.08 ± 5.12	578.18 ± 6.78	12.02 ± 0.04	18.01 ± 0.11
Sel. 5	193.14 ± 3.99	–	12.07 ± 0.01	–



Figs. 1-6. Comparative radioresponse of 2X and 4X of different selections of *Trigonella foenum-graecum* L. with regard to germination (1), survival (2), growth after 35 days (3), pollen fertility (4), growth at maturity (5), and number of pods (6)

recorded within ploidy level.

Table 2 adds information on the INV and ICV of the different diploid strains and their corresponding autotetraploids used in the present experiment. At the diploid level particularly, there are small or almost no differences in neither nuclear volume nor in chromosome volume. Also, the difference among the tetraploids is small though more apparent. The tetraploids do not only show a larger nuclear volume but also a larger chromosome volume than do the diploids. More often a higher packing density in the polyploids will result in a decreased ICV, i.e. a decreased target, which has been associated with an improved radioresistance (Sparrow 1965; Ichikawa and Sparrow 1967). The difference lies very likely in that the present measurements are made on newly produced autopolyploids while the general tendency earlier observed was based on well established and long-evolved polyploids.

Discussion

Irrespective of this difference between new and established polyploids, the impact of ICV at predicting relative radiosensitivity should not be overemphasized. The present results clearly demonstrate an interference of a genotypic dependence and such exceptions have also been found earlier (Boyle 1968; Wangenheim 1970). The experience with the tetraploid version of sel. 2 with its extreme radiosensitivity shows that genic controls can be very strong.

Three main conclusions can be drawn from the present experiment: (1) Comparative radiosensitivity at 2X level cannot be taken as measure for predicting the reaction of their corresponding autotetraploids; (2) radiosensitivity at autopolyploid level is more or less genotype dependent; (3) ploidy level and interphase chromosome volume cannot always be considered a safe guideline for predicting radiosensitivity.

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