EFFECT OF VARIOUS DOSES OF GAMMA RAYS AND FAST NEUTRONS ON HAPLOID SHOOTS OF WINTER RAPESEED IN VITRO

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Mutagenic agents may be applied in plant cultures in vitro both to multicellular structures, as embryoids, anthers, shoots, plantlets, and to single cells forming a suspension in liquid medium, originating from protoplast or callus cultures. Action of mutagens on multicellular structures is comparable with their acting on seeds or meristems of intact plants, whereas inducing mutations in cell suspensions open new prospects - avoidance of chimeras and considerable increase of selection efficiency. The use of cell suspensions in mutagenesis however is limited not only by more sophisticated methods, not always available to plant breeders, but also by a high genetic variability of such cultures, aggregation of suspensions, necessity of keeping indispensable cell density and by difficulties related to plant regeneration from a mutated cell. Selection on the cellular level is not fully reliable, because gene expression may be different from that in the intact organism, adaptation and epigenetic changes may take place, and for many agronomic traits (e.g. yield related traits, plant morphology) such selection is not manageable. Therefore we applied mutagenic agents on multicel-

were determined 1 cm over the medium. In each experiment the non-irradiated material served as a check. To each dose of irradiation four flasks at a final phase of propagating passage were subjected. They contained young shoots regenerated from axial meristems of incubated shoot. After irradiation young shoots were isolated and transferred to a fresh propagating medium. On an average 19-36 shoots were obtained from 4 flasks in genotype A, and 42-70 shoots in genotype B. Four weeks after irradiation the percent of dead shoots, coefficient of reproduction, and the size of young shoots were determined. Subsequently the shoots were passaged to a root inducing medium, and after 3-4 weeks the plants were put into pots in a greenhouse.

In experiment 1, with a higher range of gamma rays, over 50 percent shoot dying was observed in both genotypes starting from a dose of 8 krad. The doses up to 5 krad did not effect shoot dying markedly. On the other hand the coefficient of reproduction diminished proportionally to the dose, but not at the same rate in both genotypes. For genotype A its value below 10 was found at 7 krad, and for genotype B - at 9 krad, although with the size of regenerated shoots being smaller. Similar results were obtained in experiment 2. Irradiating with fast neutrons affected substantial reduction of the traits studied only at a dose of 2.333 krad. This dose is approximately equivalent to an effect of 7 krad of gamma rays in tissue cultures (Miszke et al. 1979). Genotype A was more sensitive to fast neutrons than genotype B.

Over 1300 irradiated plants from experiments 1-3 were subjected to observations. A growth stimulation affected by doses 3-5 krad was found in young plants from experiment 1.

In plants treated with fast neutrons stimulation was observed at all the doses, except the highest one. Besides growth stimulation in young plants various growth anomalies of older plants after gamma rays treatment were noted, as growth inhibition, main shoot dying, irregular shoot growth habit, deformation of leaves, and alterations of flower colour. A chimera with normal and anomalous leaves was found in experiment 1 - among plants treated with 3 krad. A smaller part of an irregular leaf was deep green and resistant to powdery mildew, and a bigger part was visibly covered with mycelium. It should be remembered that separation of different tissues of a chimera is simpler in cultures than in vivo. In some plants from treatment with fast neutrons decolorization of leaf edges was noticed.

The results of our studies show that haploid rapeseed shoots in vitro are suitable for studies of radiosensitivity. The response of several shoot traits was proportional to the dose and consistent in succesive experiments. The irradiation rate should not exceed 7 and 2.333 krad for gamma rays and fast neutrons, respectively. Considering the variation of plants observed in the M_1 generation one may assume that the doses applied will appear effective. Application of mutagenic agents to haploids cultured in vitro for breeding purposes would simplify mutagenesis in winter rapeseed which generally is radioresistant and exhibits little variability, particularly in case of improved forms.

lular structures with meristems of a very limited cell number i.e. we induced mutations on 0,5 - 3,0 cm long shoots which were designed for further in vitro propagation from axial meristems. We treated haploid rapeseed shoots considering two advantages of applying mutagens to plants of single genomes : selection of recessive mutants being easier and obtaining of homozygous mutants being faster. Our aim was to test radiosensitivity of haploid rapeseed in vitro by irradiating it with gamma rays and fast neutrons at a stage of shoot propagation for breeding purposes.

Experimental material consisted of genotype A obtained by androgenesis from a strain included in cv.Górczański (high content of erucic acid and glucosinolates) and genotype B derived from a double low strain. Both strains were obtained from a Plant Breeding Station Górka Narodowa. Androgenesis was induced according to Keller and Armstrong (1978).Haploid chromosome number was confirmed in cytological studies with the use of orcein on squash preparations.

A source of gamma rays constituted cobalt bomb (dose rate 166 rad/min). A fast neutron beam of an average energy of 5,6 MeV originated from nuclear reaction of deuterons accelerated in cyclotron U-120 and bombarding the beryllium target (dose rate 30 rad/min). The gamma irradiation rate was measured with the use of a ionizing chamber AIONEX, and the dose of neutron irradiation was determined by means of a pair tissue-like ionizing chambers. In experiment 1 (with gamma rays) the following doses were used : 3, 5, 7, 8 and 9 krad, in experiment 2 - 3, 4, 5, 6 and 7 krad, and experiment 3 (with fast neutrons) - 1, 1.333, 1.666, 2, 2.333 krad. The doses

References :

1. Keller W.A., and Armstrong K.C., 1978. High frequency production of microspore-derived plants from Brassica napus anther cultures. Z.Pflanzenzüchtg. 80, 100-108.
2. Miszke W., Skucińska B., Huczkowski J., and Cebulska-Wasilewska A., 1979. Effect of different doses of gamma rays and fast nautrons on growth of carrot callus tissue. Genetica Polonica 20, 507-515.

Results of experiment 1 - gamma rays

	dose krad	shoot no.	% dead shoots	repr. coeff.	shoot size cm.
genotype A	0	35	0.0	2.2	2
	3	31	0.0	2.3	1 - 2
	5	41	4.9	1.4	1 - 2
	7	25	16.7	0.9	0.2 - 2
	8	47	59.6	0.7	0.2 - 1
	9	38	78.9	0.6	0.2 - 0.5
genotype B	0	29	0.0	3.8	2
	3	55	1.8	3.6	0.5 - 1
	5	44	2.3	2.2	0.5 - 1
	7	42	42.9	1.4	0.2 - 1
	8	46	54.3	1.1	0.2 - 0.5
	9	60	86.7	0.9	0.2 - 0.5

Results of experiment 2 - gamma rays

	dose krad	shoot no.	% dead shoots	repr. coeff.	shoot size cm.
genotype A	0	10	0.0	4.8	1 - 2
	3	20	0.0	3.1	1 - 3
	4	25	0.0	1.3	0.5 - 2
	5	25	16.0	0.6	0.5 - 2
	6	18	22.2	0.6	1 - 2
	7	17	11.8	0.6	0.2 - 1
genotype B	0	12	0.0	6.0	1 - 2
	3	50	0.0	2.4	0.5 - 2
	4	37	2.7	1.4	0.2 - 2
	5	72	6.9	0.4	0.5 - 2
	6	39	17.9	0.6	0.2 - 1
	7	42	42.8	0.2	0.2 - 1

Results of experiment 3 - fast neutrons

	dose krad	shoot no.	% dead shoots	repr. coeff.	shoot size cm.
genotype A	0	34	2.9	2.3	2
	1	40	0.0	1.5	0.5 - 2
	1.333	46	4.3	1.5	0.2 - 1
	1.666	32	0.0	1.2	0.2 - 1
	2	29	6.9	1.1	0.2 - 1
	2.333	38	55.3	0	-
genotype B	0	16	0.0	3.9	2
	1	78	0.0	3.3	0.2 - 1
	1.333	83	10.8	1.7	0.2 - 1
	1.666	62	3.2	1.5	0.2 - 1
	2	99	7.1	1.6	0.2 - 0.5
	2.333	83	25.3	0.8	0.2 - 0.5