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THE EFFECT OF IONIZING RADIATION ON VERNALIZATION, GROWTH AND DEVELOPMENT OF WINTER WHEAT

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The effects of gamma irradiation on the vernalization requirements, growth and development of winter wheat grown in a rainout shelter were studied during two successive growing seasons. Dry grains of winter wheat cv. Kobra were irradiated with 300 Gy radiation from a cobalt 60 gamma irradiator. Treated and control grains were pregerminated and subjected to vernalization for 0, 42 or 54 days. Morphological parameters of the plants developing from irradiated seeds (M1 generation) and the plants grown from the seeds produced by the irradiated plants (M2 generation) were measured in order to track the studied effects over two generations. Irradiation of dry grains slowed the growth and development of the plants regardless of the temperature treatment. The measured yield structure elements appeared to be lower for irradiated plants, but no clear effect of radiation on vernalization requirements was noted.

Key words: Gamma irradiation, mutation induction, vernalization, growth, development, winter wheat.

INTRODUCTION

Cold-hardiness is an adaptation which enables temperate-climate plants to survive during winter when temperatures fall low enough to be lethal to young generative organs. Cereal genotypes can be divided into winter and spring types, characterized by different growth habits. Winter cereals have increased frost tolerance, and can flower and set seed the following season only after exposure to low temperature. The processes occurring in the plant under low temperature which result in generative development are subsumed under the term 'vernalization'. The many wheat varieties present vernalization requirements ranging from those of obligatory winter plants to those of spring plants (Tóth et al., 2004). Farmers favor winter genotypes over spring types for their much higher and stable yields. Winter wheat's high yield and value have brought a steady increase in its total area of cultivation. In Poland between 1980 and 2002 its area of cultivation expanded from 1.4 million ha to nearly 2 million ha, with a yield increase from 3 to 3.8 t/ha.

The cultivation requirements of the winter genotypes include a chilling period of suitable length. Those requirements strongly depend on local climatic conditions. In oceanic temperate climate a long vernalization period is required, while a short vernalization period is favorable in continental temperate climate (Greenup et al., 2009). Studies on genetic control of vernalization show that, depending on the species, single or multiple genes and dominant or recessive genes may be responsible for vernalization requirements (Pugsley, 1983; Flood and Halloran, 1986; Distelfeld et al., 2009). The diversity of these genes makes vernalization requirements differ between winter and spring species. It is known that vernalization in cereals is controlled by a number of vernalization genes designated *VRN1*, *VRN2* and *VRN3* (Pugsley, 1993; McIntosh et al., 2003; Trevaskis et al., 2007; Distelfeld et al., 2009). Vernalization under long day results in down-regu-

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Abbreviations: ^{60}Co – cobalt isotope of 60 g atomic mass; Gy – Gray – SI unit of absorbed radiation dose

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lation of *VRN2* and up-regulation of *VRN3* and *VRN1*. Under short day, all three genes show low transcript levels, but rapid up-regulation of *VRN1* and *VRN3* is observed when plants are transferred from short to long day (Yan et al., 2006). In the longer days of spring, photoperiod genes *PPD1* and *CO* up-regulate *VRN3*, which induces *VRN1* above the threshold levels required for flowering initiation (Distelfeld et al., 2009).

Lack of vernalization results in vegetative development, but partial differentiation of apexes towards the generative phase can be observed. These changes can be seen by LM after more than 100 days of vegetation, and can be further classified according to the ten-step Kuperman scale (Kuperman, 1965). Starting from step IV of apical development the wheat plants can be seen to be developing generatively but not capable of heading.

The variation of all present forms of life has been driven by three main forces: mutation, the fundamental source of heritable variation; environment, which influences the selection of mutations that survive and reproduce; and time, during which the genotype and the environment constantly interact and evolutionary change is realized. The first important factor and the basis of the variation is mutation, which can occur spontaneously or can be induced by mutagenic agents such as radiation (ionizing and nonionizing) or certain chemicals. Mutations can be artificially induced in sexually or asexually reproducing species, and over the past 75 years it has became established as a method of creating novel alleles of genes for use in crop improvement schemes. It has also become important in revealing gene functions and discovering genes through the production of gene insert and gene knockout lines. Genes and the function of their products can be identified by isolating and studying putative mutants that are defective in specific process pathways.

The utility of mutations is best exemplified in plant breeding. Currently over 3000 mutant crop varieties are registered in a database curated by the International Atomic Energy Agency (http://mvgs.iaea.org/). Crop improvements include a wide range of traits such as enhanced yield and resistance to biotic and abiotic stresses. Induced mutations are random; this means that genes coding and regulating vernalization can be affected in unforeseen ways (Shitsukawa et al., 2007). Plant metabolism can be disturbed, and the genetic mechanisms of flowering can be blocked. In the first case the growth or development of plants may be delayed or altered; in the second case, accelerated flowering may occur after suboptimal vernalization, and plants may even produce flowers without vernalization. Consequently, induction of mutations may lead to selection of mutant genotypes that produce a high yield and do not require vernalization. Morphological changes such as early tillering would also be an advantage. A shorter vernalization requirement would benefit farmers and allow more effective use of fields for forecrops such as maize and a following crop such as winter wheat.

The material used in this experiment, winter wheat cv. Kobra, requires nine weeks of vernalization. In this work we wanted to determine whether γ-ray treatment would induce mutations affecting vernalization genes in winter wheat, and whether irradiation of the seeds would affect the growth and development of the plants. Mutations detected in early stages after mutagenesis and especially in M1 should be monitored for stability in subsequent generations, so we studied both the M1 and M2 populations of mutagenized material for the effects of irradiation.

MATERIAL AND METHODS

Grains of the winter wheat (*Triticum aestivum*) cultivar Kobra were obtained from the Plant Breeding Station in Kobierzyce, Poland. The irradiation experiment was conducted in the Plant Breeding and Genetics Laboratory of the International Atomic Energy Agency (IAEA), Seibersdorf, Austria. Prior to mutagenic treatment the grains were stored in a desiccator over a 60% glycerol/water mixture for seven days at room temperature for seed moisture equilibration. The pretreated grains were irradiated with a dose of 300 Gy from a ^{60}Co gamma source. The dose was selected based on the internal database and following radiosensitivity tests. Following the standard terminology used in induced mutation programs, seeds prior to mutagenic treatment are termed M0 and after treatment are referred to as M1. Seeds that develop on the M1 plants are therefore the M2 generation which develops into M2 plants (Forster and Shu, 2012). Mutagenized and nonmutagenized grains of winter wheat were pregerminated in moistened perlite at 26°C in the dark. The pregerminated material was vernalized for 0, 42 or 54 days. The effects of mutagenic treatment and the different vernalization times on the morphology of winter wheat were studied in two successive growing seasons, and the M1 and M2 mutant populations were observed.

DEVELOPMENTAL CHARACTERIZATION OF THE M1 POPULATION

Treated and untreated pregerminated grains of winter wheat cv. Kobra were subjected to 42 or 54 days of vernalization at 5°C under an 8 h photoperiod. At the end of vernalization the growth rates of seedlings were compared by measuring shoot and

TABLE 1. Effects of irradiation and vernalization of grains on seedling parameters (leaf length and width, root length)

Parameter [mm]	Days of growth	Non vernalized		Vernalized	
		0 Gy	300 Gy	0 Gy	300 Gy
Leaf length#	0	200 *	95 (48)	160*	67 (42)
	14	284 ns	213 (75)	282 ns	258 (91)
	28	451 ns	364 (81)	478 ns	401 (84)
Root length	0	70*	32 (46)	67*	24 (36)
Leaf width	14	8.0*	5.4 (68)	7.8 ns	7.1 (91)

#Leaf length measured from tillering node to apex of longest leaf; *difference in values between nonirradiated and irradiated plants within vernalization treatment significant at p = 0.05; ns – difference not significant (Student t-test). Parenthesized numbers give the parameter value as a percentage of the control value (nonirradiated) within vernalization treatment.

root length. Then the seedlings were placed in a growth chamber for 1 day to acclimatize. Acclimatized plantlets were transferred to pots containing garden soil, peat and sand (3:2:1; v/v/v) and placed in a rainout shelter. In the first year the physiological damage caused by gamma irradiation was evaluated in the M1 plants. To enable a comparison between vernalized and nonvernalized material, nonirradiated and irradiated grains were germinated without the vernalization step, and 10-day-old seedlings were transferred to the rainout shelter along with the vernalized material. Leaf length and width of potted vernalized and nonvernalized plants was measured at days 0, 14 and 28 after placement in the rainout shelter. These observations were supplemented by measurements performed on ear-shooting plants, when main shoot length and mass, peduncle length, spike length, and number of days until heading were recorded. Crop production parameters such as number of spikelets and grains per spike, mass of 1000 grains and mass of spike grains of main shoot were scored. Plants not subjected to vernalization did not enter the generative phase, so only measurements of the longest leaf at days 0, 14 and 28 were taken. In addition, these nonvernalized plants were observed by LM to analyze the development of isolated stem apexes and determine the development stage on the Kuperman scale (1965).

DEVELOPMENTAL CHARACTERIZATION OF THE M2 POPULATION

The M2 grains were obtained from the mutagenized M1 plants. In the second year of the experiment the heritable changes in the M2 population were analyzed. Since the results obtained in the first year of the experiment showed that 42-day vernalization was suboptimal for cv. Kobra, the M2 material was vernalized longer, for 54 days. The M2 plants were measured during their growth and development as described for the M1 population.

STATISTICAL ANALYSIS

For estimation of the damage due to mutagenic treatment, the values of the measured parameters are expressed as means of absolute values and as percentages of the control. In all treatments, 24 randomly selected plants were measured. Statistical analyses used Statistica 8.0 (StatSoft, Inc.). The significance of differences was tested by ANOVA and the means were compared by Student's t-test at p = 0.05 significance level.

RESULTS

These experiments were performed in order to identify whether and in which stages gamma irradiation would affect genes involved in the vernalization process, plant growth and development. Such effects might, for example, shorten the vernalization length requirement, alter the morphology of the plants to allow earlier tillering, or change other phenotypic characteristics such as earlier flowering time.

Gamma irradiation of grains severely weakened the growth of both vernalized and nonvernalized plants as compared with the nonirradiated control (Tab. 1).

The strongest effect of treatment on the growth habit was seen at the beginning of the vegetative phase. At day 0 the length of the aboveground part for both vernalized and control plants was half the length of the nonirradiated plants. At days 14 and 28 days of growth the differences were considerably smaller. This was the result of accelerated lengthening of the aboveground parts of plants from irradiated grains, which lengthened ~2.2 and ~3.8 times at 14 days and ~3.8 and ~6 times at 28 days in nonvernalized and vernalized plants respectively. Irradiation also strongly affected seedling root length, which was ~2 (nonvernalized) and ~2.8 (vernalized) times shorter than the control. The effect of irradiation of grains was weaker in the case of leaf width. In M1 plants the leaves of nonvernal-

TABLE 2. Effects of irradiation on plant tillering and on the final stage of generative development of shoot apexes in conditions excluding vernalization

0 Gy		300 Gy	
Number of tillers	Generative development stage[a]	Number of tillers	Generative development stage
5.51 ns	VII *	5.63 ns	V *

*difference in values between nonirradiated and irradiated plants within vernalization treatment significant at p = 0.05. ns – difference not significant (Student t-test); according to Kuperman scale.

ized were 1.5 times wider and the leaves of vernalized slightly narrower.

Some symptoms of the plants' readiness for generative development were compared. The number of side tillers usually is negatively correlated with flowering readiness, and the developmental stage of shoot apex growth is positively correlated with induction of generative development (Kuperman, 1965). Irradiation of winter wheat grains did not adversely affect the vegetative growth of nonvernalized plants: these plants from both irradiated and nonirradiated grains produced five lateral tillers on average (Tab. 2). However, irradiation did significantly slow the generative development of shoot apexes in conditions excluding vernalization (Tab. 2). For the control plants, at 120 days of growth the shoot growth apexes reached level VII of generative development on Kuperman's scale, while the plants from irradiated grains reached only level V. This suggests a specific effect towards an increase in the cold-hardiness characteristics of those plants.

At the final stage of the generative phase only vernalized plants were observed for development. Irradiation of grains reduced the mass of the main stem and the number of spikelets in the spike (Tab. 3). No statistically significant changes in other parameters of ear emergence were noted.

Irradiation decreased the number and mass of grains in the spike by ~20% (42-day vernalization) and ~70% (54-day vernalization) (Tab. 4). The mass of 1000 grains did not change in the M1 generation subjected to 42-day vernalization. In the M2 generation the decrease of the number and mass of grains in the spike was similar to the decrease in M1 after 42-day vernalization and ~40% higher than in M1 after 54-day vernalization. For both generations M1 and M2 the mass of 1000 grains decreased by ~20% after irradiation.

DISCUSSION

Common wheat (*Triticum aestivum* L.) is grown worldwide under different climatic conditions. This wide adaptability is due to the existence of varieties with different growth habits. Winter annual and biennial plants such as winter wheat require vernalization, a period of cold for induction of flowering (Curtis and Chang, 1930; Gregory and Purvis, 1938; Michaels, 2009). The shoot apex is believed to be the site of perception of low temperature (Curtis and Chang, 1930; Winfield et al., 2009). During the 2–8 weeks of vernalization, several metabolic changes are observed before morphological changes in the shoot apexes appear.

Artificial irradiation has revolutionized research in agricultural science and food technology (Sommers and Fan, 2002; Bari et al., 2003; Bari et al., 2005). Gamma irradiators containing

TABLE 3. Morphological characteristics of M1, M2 and control populations of winter wheat at maturity. Pregerminated grains were vernalized for 42 or 54 days

Irradiation dose	Length of spike [mm]	Number of spikelets in spike	Length of leaf blade [mm]	Length of peduncle [mm]	Dry mass of main stem [g]	Days to ear emergence
M1 subjected to 42 days of vernalization						
0 Gy	75	11	700	131	2.9	64.4
300 Gy	69 (92) *	10 (91) *	660 (94) ns	115 (88) ns	2.2 (76) *	63.5 (97.8) ns
M1 subjected to 54 days of vernalization						
0 Gy	84	20	580	63	1.6	55
300 Gy	69 (82) *	18 (90) *	390 (67) *	37 (59) *	0.8 (50) *	59.0 (112.4) ns
M2 subjected to 54 days of vernalization						
0 Gy	85	20	540	47	1.4	57.0
300 Gy	75 (88) *	19 (95) *	530 (98) ns	50 (106) ns	1.3 (93) ns	55.5 (97.4) ns

*difference in values between nonirradiated and irradiated plants within vernalization treatment significant at p = 0.05; ns – difference not significant (Student t-test). Parenthesized numbers give the parameter value as a percentage of the control value (nonirradiated) within vernalization treatment.

TABLE 4. Yield structure elements of M1, M2 and nonirradiated control populations of winter wheat vernalized for 42 or 54 days

Irradiation dose	Number of grains per spike	Mass of grains per spike [g]	Mass of 1000 grains [g]
M1 subjected to 42 days of vernalization			
0 Gy	27.7	1.15	42
300 Gy	21.9 (79) *	0.86 (74.8) *	42 (100) ns
M1 subjected to 54 days of vernalization			
0 Gy	30	1.5	51
300 Gy	10 (33.3) *	0.4 (27)	41 (80) *
M2 subjected to 54 days of vernalization			
0 Gy	27	1.4	52
300 Gy	20 (74) *	0.9 (64) *	43 (82.7) *

*difference in values between nonirradiated and irradiated plants within vernalization treatment significant at p = 0.05; ns – difference not significant (Student t-test). Parenthesized numbers give the parameter value as a percentage of the control value (nonirradiated) within vernalization treatment.

a radioactive isotope of cobalt are often used to induce mutations in the DNA of crop plants, In this way, desired attributes not found in nature or lost during evolution can be created. It can also increase variation in the genetic pool. Ionizing radiation can cause different levels and types of damage, however, and generates oxygen free radicals (hydroxyl radical and hydrosuperoxide radical) which can induce point mutations. Free radicals produced in areas of energy absorption can diffuse and react in other places in the tissue, becoming a source of secondary mutations. The greatest tissue damage is caused by the hydroxide radical, a strong oxidant that can change the structure of DNA, leading to functional changes in the cell and sometimes lethal damage. The mutagenic effects of radiation increase with the increase of water content in irradiated tissues; hence, dry grains are relatively resistant to those effects.

Ionizing radiation can also alter the genes determining vernalization requirements in plants, or other genes involved in the processes responsible for plant morphology and influencing the cold-hardiness of winter wheat (Brock and Davidson, 1994). In our study, irradiation strongly affected major physiological processes in M1 and M2 plants, resulting in altered development and morphology, as has been reported in other work (Konzak et al., 1972; Kozak et al., 2011; Kodym et al., 2012). The mass of both vegetative and generative organs decreased. The observed decrease in plant yield (number and mass of grains per plant) might be a secondary effect of slowed plant metabolism and development.

The M1 and M2 plant populations present an interesting source of plant variability. In further studies, both populations might provide individual plants in which the observed decrease of vegetative organ size would not be accompanied by a correspondingly large drop in the number or mass of grains. This could separate out the plants in which the vernalization genes were mutated by the γ-rays. Our results do not show that irradiation directly affected genes involved in vernalization. Advances in genomics and especially the increasing volume of publicly available sequence information for various crops add a new dimension to this type of work, and could help us determine which genes were affected. Our next study will employ that methodology.

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