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INFLUENCE OF LASER BEAMS ON THE VARIABILITY OF TRAITS IN SPRING BARLEY

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A b s t r a c t. Laser is one of the sources for inducing a biostimulation effect and genetic changes in higher plants. Lower doses of laser activate plants, resulting in increasing bioenergetical potential of the cell and higher activation their biochemical and physiological processes. Higher doses influence genetical material of the cell leading to genetic changes of plant traits. The seeds of cv. Maresi spring barley (*Hordeum vulgare* L.) were irradiated with different doses of laser beams and additionally treated with chemomutagens. For the experiment, a helium-neon laser (He-Ne) with the wavelength of 632.8 nm and power density - 1 mW cm^{-2} was chosen. In the M_1 progeny reduction or stimulation of morphological and yield-contributing traits was estimated. The frequency of chlorophyll mutations was analysed in M_2 and selection of morphological mutants was started in this generation. In the M_4 generation variability of traits in the mutants obtained was estimated. The results showed that in the M_1 progeny lower doses of laser beams (1/2 and 1.0 h) induced biostimulation effect of the analysed traits. Higher doses (2.0 h) induced reduction of the traits value in comparison to the control cv. Maresi. In addition, laser beams were able to induce chlorophyll and morphological mutations in the M_2 progeny. In comparison to the initial cv. Maresi, the mutants were characterised by higher variability of morphological and yield-contributing traits. The mutants with desirable yield structure parameters were selected. The results obtained show that the helium-neon laser with the wavelength of 632.8 nm can be an effective tool for increasing variability of traits in spring barley.

K e y w o r d s: barley, chlorophyll mutants, laser, mutants, mutations, variability, yield structure

INTRODUCTION

In recent years many investigators have used laser beam for solving various scientific problems in medicine and biology. A laser microbeam combines various tools for cell and molecular biologists in one instrument. High spatial accuracy can supply energy to fuse membranes or perform surgery or for the micromanipulation in cell, tissues, or chromosomes [15]. Using a laser one can deliver DNA into a specific cell through the cell wall and membranes. Other investigators showed that a laser can be used for the fusion of the selected cells which provides an opportunity to study physical and biological aspects of plasma membranes. The full potential of a focused laser beam becomes evident when it is used for the dissection of chromosomes. High spatial accuracy could also be used to study physical arrangement of genes on chromosomes.

Beside its use in the cell- and molecular biology, laser can be successfully use for other purposes in plant genetics and breeding. A specific peculiarity of laser beams can be used to induce a biostimulation effects, and higher doses of laser for damages of cell genetic material leading to mutation processes.

The aim of this work was to establish how far physical source of radiation - a helium-neon laser at the wavelength of 632.8 nm is able to induce a biostimalation effect and mutations in spring barley.

MATERIALS AND METHODS

For inducing a biostimulation effect and mutations the seeds of spring barley cv. Maresi were chosen. The seeds were irradiated with a laser beam at different times of exposure (1/2; 1.0; 1.5 and 2.0 h). The helium-neon laser (He-Ne) at the wavelength of 632.8 nm and power density of 1 mW cm^{-2} was applied as the source of irradiation. This type of a laser after some optical modifications was adapted for the simultaneous irradiation of a greater number of seeds preserving the same power density of the beam on each cm^2 of the irradiated surface covered with seeds. For the comparison of laser activity, the seeds were additonally treated with two chemomutagens - N-nitroso-N-methylurea (MNU) and sodium azide (SA). Before treating with both chemomutagens, the seeds were presoaked in distilled water for 12 h. The seeds that were not treated with laser beams and chemomutagens constituted a control combination.

In the M_1 progeny the level of biological injuries expressed as the value of stimulation or reduction in the level of the analysed traits were measured. Morphological and yield-contributing traits of the M_1 plants were chosen to estimate this effect.

The genetic effect of the laser and chemomutagens were analysed in the M_2 generation in the field conditions. The results were expressed as the frequency of chlorophyll mutations in comparison with the control combination. For this purposes, the method given by Gustaffson [6] was applied. Since most of the mutants are recessive, selection of morphological mutants was started in the M_2 generation.

Variability of morphological and yield-contributing traits was analysed in the M_4 generation, separately for each of the mutant groups coming from four different doses of the laser beam irradiation.

RESULTS

The level of biological injuries in the M_1 plants expressed as stimulation or reduction of the value of yield-contributing traits presents Table 1. The time of exposure to laser beams have had a marked influence on the results obtained. The lowest dose of the laser beam (1/2 h) induced the stimulation effect for all the analysed traits. It is particularly true for the parameters of yield structure - seeds number and seed weight per plant. The stimulation obtained for both traits is over 30%. The higher dose - 1.0 h. caused a similar but smaller effect than 1/2 h. Only the highest of the used doses of laser beam for all the analysed traits induced the effect of reduction (ca. 30% for the seed number and -weight per plant). Influence of the laser beam on the reduction of the number undeveloped tillers in the M_1 plants is particularly interesting.

Chemomutagens (MNU and SA) induced reduction effect in the analysed characters in

T a b l e 1. Analysis of the M_1 generation expressed as the value of stimulation or reduction in the examined traits

Treatment combination	Value of reduction (-) or stimulation (+) in %*					
	Plant height	Spike length	Number of spikes with seeds	Number of undeveloped tillers	Grain number per plant	Grain weight per plant
MNU - 0.5 mM	-8.3	-7.2	+1.1	+3.6	-25.9	-23.2
MNU - 0.9 mM	-13.0	-3.1	-2.9	+56.9	-66.2	-66.1
MNU- 1.4 mM	-28.0	-8.0	-66.7	+264.0	-91.5	-92.1
SA - 2.0 mM	-15.6	-3.1	-11.5	+20.7	-51.2	-48.2
SA - 5.0 mM	-14.0	-2.1	-11.5	+122.7	-61.0	-62.5
SA - 8.0 mM	-17.1	-5.2	-38.4	+126.8	-71.5	-73.2
Laser - 1/2 h	+4.7	+5.2	+37.6	-48.2	+32.4	+31.2
Laser - 1.0 h	+1.6	+6.4	+20.3	-15.4	+3.4	+8.6
Laser - 1.5 h	-1.1	+3.1	+1.3	-8.4	0.0	0.0
Laser - 2.0 h	-0.3	-4.0	-15.4	-3.2	-29.0	-30.2

* Level of stimulation and reduction of the examined traits were calculated as a per centage of the control value.

comparison to control. The value of this reduction is markedly stronger than the reduction obtained after using laser beams or the increase observed when higher doses of mutagens are used. Chemonutagens stimulated production of undeveloped tillers in the M_1 plants.

The genetic effect of laser beams and chemomutagens expressed as the frequency of appearance of chlorophyll mutations is presented in Table 2. Frequency of chlorophyll mutations ranged from 0.31 to 0.77%. The highest frequency (0.77%) was obtained at the dose of 2.0 h of laser beams. Chemomutagens induced higher frequency of chlorophyll mutations in comparison to laser beams. In the spectrum of chlorophyll mutations obtained after using the laser, the mutants of "others", "viridis" and "xantha" types exceeded the number of "albina" mutants. These mutants were typical in the spectrum of chlorophyll mutations obtained after treatment with chemomutagens.

In the M_4 generation variability of yield contributing traits of 92 mutants was estimated (Table 3). The highest average values of the analysed traits were obtained for the mutants derivated from the dose of 1/2 h of the laser beam. The average values decreased for the mutants obtained with an increased dose of the laser (1.0, 1.5 and 2.0 h). A broadest variability of the analysed characters (x_{min} and x_{max}) was obtained for the mutants derivated after 1.5 h of irradiation. The mutants with the most desirable parameters of yield structure (x_{max}) were found in the mutant group obtained after the use of the lowest dose of the laser beam (1/2 h). The selected mutants with improved yield parameters are presented in Table 4. Except for the desirable yield potential, part of the mutants showed improved lodging resistance and earliness.

DISCUSSION

The use of lower doses of laser beams (1/2 h) in the M_1 generation, resulted in the effect of stimulation of the yield-contributing traits was observed. Biostimulation effect is induced by many chemical compounds [11] as well as different sources of radiation [8,9,13]. Also chemomutagens at lower doses induce a process of biostimulation [1,7]. The chemical compounds compared to the physical sources of radiation show a high level of toxicity which has an unfavourable influence on the accumulation these chemicals and modification of chemical composition as well as undesirable affect on the environment. From this point of view, the laser offers a pure ecological source of energy that ensures high yield. Hence, the laser started to be used as a biostimulator in plant production [16]. In our work higher parameters of yield structure of spring barley 30% were obtained. Positive influence of laser beams on yield increase were

T a b l e 2. Analysis of the M_2 generation expressed as the frequency of chlorophyll mutants

Treatment combination	Number of the analysed seedling	Types of chlorophyll mutants				Total number of mutants	Frequency of chlorophyll mutants (%)
		albina	xantha	viridis	others		
H_2O - control	1508	-	-	-	-	-	-
MNU - 0.5 mM	1800	14	7	13	6	40	2.22
MNU - 0.9 mM	542	7	2	12	6	27	4.98
MNU- 1.4 mM	123	5	0	6	0	11	8.94
SA - 2.0 mM	936	13	1	25	0	39	4.16
SA - 5.0 mM	439	11	1	5	2	19	4.32
SA - 8.0 mM	495	16	0	9	3	28	5.65
Laser - 1/2 h	1616	0	0	2	8	10	0.62
Laser - 1.0 h	1592	0	2	2	1	5	0.31
Laser - 1.5 h	1714	0	8	1	0	9	0.52
Laser - 2.0 h	1544	1	1	5	5	12	0.77

T a b l e 3. Average value $\bar{x}$, x_{min} and x_{max} of the yield structure parameters of the mutants obtained after irradiation of seeds at different exposure of laser light

Treatment combination	Plant height (cm)			Spike length (cm)			Grain number per spike			Grain weight per spike (g)			Grain number per plant			Grain weight per plant (g)			1000 grain weight (g)		
	x_{min}	$\bar{x}$	x_{max}	x_{min}	$\bar{x}$	x_{max}	x_{min}	$\bar{x}$	x_{max}	x_{min}	$\bar{x}$	x_{max}	x_{min}	$\bar{x}$	x_{max}	x_{min}	$\bar{x}$	x_{max}	x_{min}	$\bar{x}$	x_{max}
Laser - 1/2h	72.5	85.0	106.8	7.2	8.3	10.3	20.7	23.0	26.5	0.57	0.73	0.94	108.2	162.4	219.3	3.38	4.94	7.20	27.0	31.8	38.5
Laser - 1.0 h	80.8	80.8	95.2	7.6	8.1	9.5	21.8	23.5	25.8	0.62	0.76	0.88	92.0	150.8	202.0	2.86	4.76	6.31	27.2	31.6	35.5
Laser - 1.5 h	67.8	80.4	90.9	6.7	7.8	9.2	19.3	22.2	24.6	0.46	0.64	0.85	89.0	145.8	205.1	2.45	4.25	6.51	22.1	28.9	36.8
Laser - 2.0 h	79.4	74.6	83.2	7.4	8.1	8.5	21.1	21.4	22.3	0.80	0.85	0.92	120.6	138.2	173.0	3.36	4.02	5.10	26.1	29.3	31.1

T a b l e 4. Yield structure parameters of the initial cv. Maresi and the chosen mutants obtained after using laser beams

Cv. Maresi and mutants	Parametrs of yield structure						
	Plant height (cm)	Spike length (cm)	Grain number per spike	Grain weight per spike (g)	Grain number per plant	Grain weight per plant (g)	Lodging grade (1-9)
Maresi - control	77.0	9.1	24.9	0.84	152.8	4.98	4.0
L - 10	106.8	10.3	26.5	0.94	217.2	7.20	4.0
L - 17	97.5	8.6	23.7	0.86	165.7	5.93	3.0
L - 33	78.6	8.4	23.2	0.74	174.9	6.10	1.0
L - 37	83.6	9.2	25.8	0.83	156.4	5.23	2.5
L - 46	93.0	8.6	24.6	0.85	205.1	7.09	2.8
L - 53	81.5	9.0	24.2	0.82	184.6	5.39	2.0
L - 66	89.7	9.2	22.9	0.81	183.9	6.51	3.0

also noted by Vasilevski [16] for cereals 15-20%, vegetables to 40%, tobacco 20%, poppy 20%, potatoes 30%. Higher yield and improvement of other traits in lupine [9], grasses [12], faba bean [10], wheat [4], maize[5] and barley [3] was also observed.

Laser beams are also able to induce chlorophyll mutations in the M_2 generation. Dudin [3] who worked with the same type of laser obtained 1.3-3.0% chlorophyll mutations. In our investigation, the frequency of mutations ranged from 0.31 to 0.77%. Stronger doses of the laser were more effective in inducing higher frequency of chlorophyll mutations.

Genetic effect of laser beams was also expressed in the selection of morphological mutants. They were characterised by broader variability of yield-contributing traits in comparison to the initial cultivars. The mutants obtained may be an interesting material for breeding. Using laser beams Bljandur [2] obtained new, high yielding maize mutants with markedly improved earliness. The laser mutants were successfully introduced in the breeding programme in China [14,17,18]. In the results of this work, 13 new varieties of different crops were created. It makes 5.8% all the varieties in China obtained with use of induced mutants.

The results obtained showed that He-Ne laser may be an effective tool for inducing biostimulation processes as well as broader genetic variability of traits in spring barley.

CONCLUSIONS

1. Low doses of He-Ne laser induce biostimulation effect into yielding parameters in spring barley.
2. Laser beams induced chlorophyll mutations in the M_2 generation. Their frequency was increased after using higher doses of laser light.
3. The mutants selected after using laser beams were characterised by broader variability of the yielding parameters in comparison with the initial cv. Maresi. The variability obtained allowed to identified the mutants with improved yielding ability, lodging resistance and earliness.

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