Int. Agrophysics, 2001, 15, 115-119

INTERNATIONAL
Agrophysics
www.ipan.lublin.pl/int-agrophysics

Influence of laser beams combined with chemomutagen (MNU) on the variability of traits and mutation frequency in spring barley

W. Rybiński

Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań

Received October 9, 2000; accepted November 10, 2000

A b s t r a c t. Laser light as a pure ecological factor started to be used as a plant biostimulator. It is especially important in the case of short exposures to laser light. However, prolongation of irradiation induces damage to genetic material in plant cells. Hence, laser can be treated as inducing mutation. As compared to ionizing radiation or chemomutagens, laser shows a markedly lower mutagenic ability and contrary to other mutagenic agents, induces low degree of biological injuries in the M_1 progeny. Specific properties of laser that provids light in red at a well-defined wavelength (630-650 nm), and strong mutagenic characteristics of chemomutagen N-methyl-N-nitrosourea (MNU) made us apply both of them in the combined treatment for biostimulation and mutation induction.

The seeds of the original material (three brewery barley cultivars) were irradiated with different doses of helium-neon laser light and MNU. The following combined treatment: laser + MNU and MNU + laser was used without application of a single treatment with laser or MNU. In the M_1 progeny, the biostimulative effect and degree of reduction were estimated. The influence of laser and MNU on the mutation frequency was analysed in the M_2 and calculated as a percentage of chlorophyll mutants in the total M_2 population.

Analysis of the M_1 plants showed that, irrespective of the kind of initial material, short exposure to laser (30 min) induced biostimulation effect on the yield parameters. Higher doses of laser (120 min) as well as MNU induced only reduction of the analysed traits. Among the analysed material, cultivar Boss showed higher susceptibility to the applied doses of MNU and laser than cv. Rambo and Rudzik.

In the M_2 progeny, the combined treatment induced higher mutation frequency than an individual treatment with laser or MNU. It was particularly true for cv. Boss and combination of MNU with 120 min of laser light. The results obtained indicated advantages of laser application in the combined treatment with MNU, as compared to a single treatment.

K e y w o r d s: combined treatment, barley, biostimulation, mutation, laser, N-methyl-N-nitrosourea, somatic damages

Corresponding author e-mail: office@igr.poznan.pl

INTRODUCTION

In the beginning of 1960, the first work on the application of ruby laser beams in biology was published [5]. This paper relates to an increase of interest in broader introduction of laser in the investigations on cell biology [2], their microsurgery and subcellular structures [29], modification of plant cell and tissues [11,27], laser induced cell fusion [28] and microdissection of chromosomes [30].

In the end of 1960, Berns [4] showed that laser beams are able to induce chromosome damage. The above gave inspiration to many investigators to use laser to induce mutation [8,23]. Other investigators showed, that although longer irradiation with laser beams damages genetic cell material, lower doses exert a biostimulative effect [13,15] markedly higher than low doses of chemomutagens [1]. With regard to this specific feature laser can be used as an effective biostimulator [10].

This specific influence of laser light on plant cell made us use a combined treatment with a potent chemomutagen N-methyl-N-nitrosourea (MNU) for the induction of biostimulation and mutation in spring barley. The aim of this work was to learn what influence could a combination of laser beam and MNU exert on somatic damage of the level of M_1 plants and mutation frequency in M_2 progeny.

MATERIAL AND METHODS

The study material consisted of seeds from three brewery cultivars of spring barley: Rudzik, Rambo and Boss. To induce mutation, a chemomutagen N-methyl-N-nitrosourea (MNU) was used at a dose of 1.0 mM. Before the mutagenic treatment, the seeds were pre-soaked in distilled water for 8 h. Mutagenic treatment was performed for 3 h.

Depending on the combination, the seeds were irradiated before or after MNU treatment for 30; 90; and 120 min. In the cases where the combined treatment was not applied, an additional, separate treatment with MNU or laser beams was performed. A helium-neon laser at the wavelength of $6328 \cdot 10^{-1}$ nm and power density of 1 mW cm^{-2} was used as a source of irradiation. The seeds not treated with MNU and laser light constituted a control combination. As a source of irradiation.

After treatment, the seeds were sown into 2.0 m^2 plots in the experimental field with three replications (M_1 progeny). During vegetation season and after harvesting, morphological and yield contributing traits were measured. The data obtained were used for the calculation of reduction (somatic damages) or stimulation effect of the analysed traits as compared to the control combination.

Seeds of the M_1 plants and control constituted material for the M_2 progeny. The harvested seeds were sown in field conditions and at a stage of seedling. Frequency of mutation was estimated. The results were calculated as a percentage of chlorophyll mutants in the total M_2 population. The mutants were classified according to the method by Gustafsson [12]. The spectrum of mutants was analysed by the contribution of individual mutant types (albina, xantha, viridis and others) in the total number of chlorophyll mutants.

RESULTS

The shortest exposure seed time to laser light (30 min) for all the used cultivars induced only a biostimulation effect (Tables 1 and 2). This effect was lower for plant height and spike length than for the parameters of yield structure. The highest value of stimulation was noted for cv. Rudzik, particularly in grain number and weight per spike and plant, respectively: 17.7, 24.7, 27.4 and 35.8% as compared to control (Table 2). Prolongation of irradiation to 90 min resulted in the appearance of stimulation or reduction effect in relation to the kind of analysed traits and cultivars. In all the cultivars, the longest exposure with laser light induced only somatic damage expressed by the reduction of value of the analysed traits.

As compared to laser light, MNU induced high increase of somatic damage and reduction of traits was similar for all the analysed cultivars. Particularly high reduction was observed for grain number and weight per plant in all the cultivars.

In the combined and reciprocal treatment (laser + MNU and MNU + laser), the reduction effect differed markedly as compared to the effect obtained after separate use of MNU or laser. Generally speaking, a decrease of reduction was observed in the combined treatment as compared to separate treatment. It was particularly visible if MNU was used in the combination with a stimulative dose (30 min) of laser light. The combined treatment of MNU with 90 min of laser exposure increased reduction level but for many yield-contributing traits this effect was lower as compared to the separate treatment of MNU. If MNU was used for 120 min of laser exposure, the level of somatic injuries was higher than after a single MNU treatment.

The degree of somatic damage depended on the sequence of treatment. Generally speaking, in the combination with pre-irradiation (laser + MNU) of seeds, the reduction effect was lower than in the combination with post-irradiation (MNU + laser). Irrespective of the cultivar used, the highest reduction was obtained in the combination of MNU 1.0 mM + laser 120 min. In the case of grain number and weight per plant for Rudzik, Rambo and Boss, reduction reached respectively 71.8 and 70.8%, 78.4 and 80.7%, 76.6 and 78.6%. In the case of the opposite combination, the value of reduction was lower and reached 34.7 and 32.7%, 50.7 and 62.3%, 48.4 and 54.8%, respectively. This result indicated also that the lowest susceptibility to the used doses of laser and MNU was observed in the cultivar Rudzik which was more sensitive than the cultivars Rambo and Boss.

Genetic effect of MNU and laser light was measured in the M_2 progeny and expressed by the frequency of chlorophyll mutants (Table 3). As compared to MNU, laser induced lower mutation rate. The highest value was obtained after exposure to laser light for 120 min, respectively for cv. Rudzik, Rambo and Boss: 0.51, 0.64 and 0.77%. The same values for MNU reached 2.88, 3.04 and 3.24%, respectively. The combined treatment (except irradiation by the stimulative dose 30 min) induced higher frequency of chlorophyll mutants as compared to separate MNU treatment. The highest frequency exceeded 4.0%. Only slightly higher mutation rate was obtained if the seeds were pre-irradiated with laser in comparison to the post-irradiation process. The most positive reaction expressed by high mutation frequency was obtained for cv. Boss, lower for Rambo and Rudzik.

DISCUSSION

The results obtained showed two different effects - biostimulation and reduction of the analysed traits. Biostimulation was observed for the seeds irradiation with short exposure to the laser (30 min). Similar effect was noticed after laser application in the study on lupin [21], French bean [25], wheat [6], beetroot [16], maize [17], faba bean [22], grasses [24], soybean [20] and tomatoes [14]. According to the study by Vasilevski [31], laser activation of plants results in an increase of their bioenergetic potential leading to higher activation at fitochrome, fitohormone and fermentative system, as a stimulation of their biochemical and physiological processes. Laser stimulation of plants is expressed in the acceleration of photosynthesis, breathing, intensity and direction of biosynthetic reaction. Hence, plants synthesise pigments, carbohydrates, vitamins and another metabolites better. One of the factors that brought about biostimulation effects in our study, was stimulative influence of laser light on a higher number of fertile spikes per

T a b l e 1. Reduction (-) or stimulation (+) of the analysed traits of the M_1 plants after application of a separate and combined seed treatment with laser light and MNU

Treatment combination	cv. Rudzik			cv. Rambo			cv. Boss		
	Plant height	Spike length	No. fertile spike/plant	Plant height	Spike length	No. fertile spike/plant	Plant height	Spike length	No. fertile spike/plant
Laser - 0.5 h	+3.0	+7.6	+24.7	+12.1	+5.5	+4.0	+3.0	+4.3	+26.6
Laser - 1.5 h	-12.4	-6.5	-1.1	+0.5	-7.8	-1.1	-1.0	+2.7	0.0
Laser - 2.0 h	-11.8	-7.7	-34.1	-1.5	-9.8	-3.3	-2.6	-0.4	-6.7
MNU - 1.0 mM	-16.6	-9.0	-39.1	-3.2	+13.4	-42.5	-10.6	-6.3	-38.6
L 0.5 + MNU	+1.7	+10.2	-9.1	0.0	-2.2	-17.2	-1.6	+7.9	-20.0
L 1.5 + MNU	+1.4	+1.0	-14.0	+0.5	-3.3	-26.0	-1.4	+5.5	-17.8
L 2.0 + MNU	-12.3	-2.6	-15.6	-4.3	-7.8	-33.3	-11.1	-0.4	-23.4
MNU + L 0.5	-12.3	+2.5	-17.7	+0.2	-11.1	-29.3	-11.9	-2.7	-37.8
MNU + L 1.5	-10.8	-2.6	-39.1	-0.9	-10.0	-36.4	-14.6	-4.0	-41.2
MNU + L 2.0	-12.0	-1.3	-45.2	-13.8	-12.2	-51.5	-26.0	-15.8	-46.7

Level of stimulation or reduction of the examined traits was calculated as a per centage of the control value.

T a b l e 2. Reduction (-) or stimulation (+)* of the yield structure traits of the M_1 plants after application of separate and combined seed treatment with laser light and MNU

Treatment combination	cv. Rudzik				cv. Rambo				cv. Boss			
	1**	2	3	4	1	2	3	4	1	2	3	4
Laser - 0.5 h	+17.7	+24.7	+27.4	+35.8	+2.6	+8.8	+4.0	+8.9	+2.6	+2.3	+29.0	+28.9
Laser - 1.5 h	-2.6	-2.0	-3.8	-1.1	-1.8	+2.6	-1.4	+3.3	-3.5	-6.4	-0.6	-6.2
Laser - 2.0 h	-3.1	-7.0	-42.4	-42.3	-4.9	-2.7	-6.2	-3.6	-4.0	-4.0	-19.2	-24.4
MNU - 1.0 mM	-19.8	-24.8	-65.5	-65.3	-14.1	-12.4	-61.0	-58.7	-19.2	-20.7	-61.9	-65.3
L 0.5 + MNU	-0.6	+6.9	-9.0	-6.6	-11.5	-12.4	-43.3	-45.6	-6.6	-15.9	-43.1	-48.6
L 1.5 + MNU	-6.6	-5.0	-29.5	-28.0	-18.9	-21.2	-47.2	-49.3	-12.7	-13.5	-49.0	-51.7
L 2.0 + MNU	-11.7	-12.9	-34.7	-32.7	-18.5	-16.9	-50.7	-62.3	-17.7	-24.7	-48.4	-54.8
MNU + L 0.5	-11.7	-15.9	-49.4	-52.8	-12.3	-16.8	-55.2	-53.4	-26.7	-36.6	-54.2	-61.0
MNU + L 1.5	-16.8	-14.9	-62.6	-64.7	-22.4	-31.0	-68.9	-71.2	-32.8	-37.8	-67.1	-70.1
MNU + L 2.0	-20.8	-19.8	-71.8	-70.8	-21.1	-33.6	-78.4	-80.7	-38.9	-53.2	-76.6	-78.6

* - Level of stimulation or reduction of the examined traits was calculated as a per centage of the control value.
** - 1 - grain numer per spike, 2 - grain weight per spike, 3 - grain number per plant, 4 - grain weight per plant.

T a b l e 3. Frequency of chlorophyll mutants in the M_2 progeny after application of separate and combined seed treatments with laser light and MNU

Treatment combination	cv. Rudzik			cv. Rambo			cv. Boss		
	No. analyzed seedling	No. chlorophyll mutants	Frequency of mutants (%)	No. analyzed seedling	No. chlorophyll mutants	Frequency of mutants (%)	No. analyzed seedling	No. chlorophyll mutants	Frequency of mutants (%)
Control	1937	-	0	2260	-	0	18.01	1	0.05
Laser - 0.5 h	1850	4	0.21	1641	5	0.30	2080	8	0.38
Laser - 1.5 h	1623	6	0.36	1795	8	0.44	2063	11	0.53
Laser - 2.0 h	1744	9	0.51	1545	10	0.64	1941	15	0.77
MNU - 1.0 mM	901	26	2.88	724	22	3.04	679	22	3.24
L 0.5 + MNU	1296	38	2.93	1295	40	3.08	1422	45	3.12
L 1.5 + MNU	1168	40	3.42	1162	39	3.35	1378	48	3.48
L 2.0 + MNU	1301	41	3.30	1012	38	3.75	1302	54	4.14
MNU + L 0.5	1216	33	2.71	1096	31	2.82	1415	43	3.03
MNU + L 1.5	1009	33	3.27	1156	37	3.20	1266	42	3.31
MNU + L 2.0	1285	44	3.42	1008	37	3.57	1210	47	3.38

plant as compared to control. Prolongation of laser exposure to 120 min reduced the value of this trait.

A reducing effect (somatic damage) occurred after using higher doses of laser beams and chemomutagen (MNU). Despite the fact that low doses of chemomutagen induced biostimulation effect [1,19] the applied MNU dose caused considerable reduction. Strong somatic injuries in the M_1 material had a negative influence on the mutation production process. According to the study by Müller [18], a direct effect of mutagenic activity in the cells of the M_1 plants is expressed by a decrease of seed emergence, delay of emergence, injuries to the chlorophyll apparatus in leaves, reduction of plant growth, decrease of the survived plants number, abnormality of morphogenesis and disorder of fertility. Part of these undesirable effects occurred in our study. From this point of view, the mutagen dose must be sufficiently strong to induce high mutation rate. However, it cannot induce big somatic damage to the M_1 plants, as a low number of harvested grain would not allow to obtain a sufficient number of plants in the M_2 population. The MNU dose used in our study induced nearly 60% reduction of grain number and weight per plant, but when combined with the stimulative doses of laser light (30 and partially 90 min), it decreased reduction of yield parameters induced by MNU. Beside the influence of laser beams on the decrease of somatic damage, application of laser combined with MNU increased the frequency of mutation. It indicates advantages of using the combined treatment of MNU with laser. The study on the use of laser with ionising rays [3], chemomutagens [7, 20] and phytohormone [8] confirmed higher efficiency of the combined treatment as compared to a single treatment.

The results obtained in this study, new mutants with improved agricultural traits [9] as well as high yields of laser cultivars [26,32] proved advantages of laser application for biostimulation processes and induction of genetic variability of traits in agricultural plants.

CONCLUSIONS

1. Irrespective of the cultivar, a short exposure to laser light (30 min) induced a biostimulation effect on the yield contributing traits in the M_1 plants. Prolongation of irradiation to 120 min caused somatic damage to plants expressed by reduction of the analysed traits.

2. Chemomutagen - N-methyl-N-nitrosourea (MNU) induced a high degree of somatic damages. This effect was markedly higher as compared to somatic damage observed after application of longer exposure to laser light. It indicates that laser light may cause genetic variability in plants with lower levels of somatic damage than MNU.

3. The stimulative dose of laser light (30 min) combined with MNU treatment reduced the degree of somatic damage induced by MNU. The results obtained were lower than after treatment with MNU only and higher than after separate seed irradiation with laser light.

4. Results of the combined treatment with laser and MNU showed that the degree of somatic injuries depended on the genotypic properties of the original material and the sequence of treatment. The cultivar Rudzik is characterised by lower susceptibility to the doses of MNU used and laser than cv. Boss and Rambo. Pre-irradiation with laser (laser + MNU) caused smaller reduction of traits than post-irradiation (MNU + laser).

5. Genetic effects of treatments expressed by the frequency of chlorophyll mutants in the M_2 progeny indicated that laser as compared to MNU showed markedly lower mutagenic activity. However, in the combined treatment with MNU, higher doses of laser light increased frequency of mutation as compared to a single MNU treatment. Particularly high mutagenic efficiency was observed for the combination laser + MNU and cultivar Boss.

ACKNOWLEDGMENTS

I would like to express my gratitude to Prof. Ludwik Pokora and staff of Laser Instruments from Warsaw for making this unique laser equipment available.

REFERENCES

1. **Adamska E. and Małuszyński M., 1983:** The stimulation of growth in shoots of *Nicotiana rustica* and *Nicotiana tabacum* after N-nitroso-N-methylurea treatment. Acta Biologica, 12, 175-184.
2. **Amy R.L. and Storb R., 1965:** Selective mitochondrial damage by a ruby laser. Science, 150, 756-757.
3. **Avremenko B.I. and Volodin V.G., 1989:** Method of obtaining breeding material of cereals by means of combined irradiation of the seeds with laser light and ionizing radiation. Thezisy Dokladov, 3-6.
4. **Berns M.W., Olson R.S., and Rounds D.E., 1969.** *In vitro* production of chromosomal lessions using an argon ion laser microbeam. Nature, 221, 74-75.
5. **Bessis M., Gires F., Mayer G., and Normarski G., 1962**. Irradiation des organites cellulaires á l'aide d'un laser á rubis. G. R. Acad. Sci., 225, 1010-1012.
6. **Drozd D., Szajsner H., and Laszkiewicz E., 1999**. The utilization of laser biostimulation in cultivation of spring wheat. Biul. Inst. Hod. i Aklim. Roślin, 211, 85-90.
7. **Dudin P., 1989.** Mutagenic action of N-diethyl-N-nitrosourea and laser radiation on spring barley. Selskohozyaitsviennaja Radiobiologia, 12, 61-68.
8. **Dudin P., 1990.** The frequency of waxy mutations in barley treated with laser radiation and phytohormones. Genetika, 26 (2), 363-366.
9. **Ehrenbergerova J., Andonov I., and Popelkova M., 1990.** Effect of laser irradiation on the degree and variability of characters under selection in spring fodder barley. Acta Universitatis Agriculturae, 38, 11-18.
10. **Gładyszewska B., Koper R., and Kolasiński D., 1997**. Effect of pre-sowing laser biostimulation of seeds of some cultivated plants. IHAR Radzików, Proc. Int. Symp., Cost, 814, 22.
11. **Greulich K.O., Monajembashi S., Cremer T., Butz P., and Wolfrum J., 1986.** Micromanipulation of biological cells by

an excimer laser pumped dye microbeam. Int. Conf. on Quantum Electronics, San Francisco Press, Proc. XIV, 74-75.
12. **Gustafsson A., 1941.** The mutation system of chlorophyll apparatus. Hereditas, 27, 225-231.
13. **Koper R., 1993.** Pre-sowing laser biostimulation of seeds of cultivated plants and its results in agrotechnics. Bonn, Proc. ICPP AM, 5, 65.
14. **Koper R., 1997.** Influence of laser pre-sowing stimulation of tomato seeds (in Polish). Owoce, Warzywa i Kwiaty, 21, 16.
15. **Koper R., 1999.** System for pre-sowing laser biostymulation of seeds. Praque, Proc. Conference TAE, 2, 187-189.
16. **Koper R., Wójcik S., Kornas-Czuczwar B., and Bojarska U., 1996.** Effect of the laser exsposure of seeds the yield and chemical composition of sugar beet roots. Int. Agrophysics, 10 (2), 103-108.
17. **Lipski S. and Koper R., 1997.** Emergence, early growth and development of maize under optimal and chilling conditions as effected by pre-sowing laser irradiation of seeds. IHAR Radzików, Proc. Int. Symp., Cost 814, 22.
18. **Müller A.J., 1964.** Mutationlösung durch Nitrosomethylharnstoff bei *Arabidopsis*. Züchter, 34, 102-120.
19. **Necas J., 1970.** Stimulating and inhibiting effects of mutagens on the growth of algae on solid medium. Arch. Hydrobiol., 39, 52-67.
20. **Plesnik S., 1993.** The evaluation of some quantitative traits in M_1 generation in soybean after laser emission and ethyleneimine. Genetica et Bilogia Molecularis, 24, 105-113.
21. **Podleśny J., 1997.** The effect of seeds laser biostymulation on the white lupin growth in differented moisture conditions (in Polish). Int. Scie. Meet., Ecophysiological aspects of plant response to stress factor, Kraków, 12-14 June, Proc., 305-308.
22. **Podleśny J., 1997.** Effect of pre-sowing seed treatment by laser irradiation on morphological features formation and faba bean yielding (in Polish). Zesz. Probl. Post. Nauk Roln., 446, 435-439.
23. **Rybiński W., Patyna H., and Przewoźny T., 1993.** Mutagenic effect of laser and chemical mutagens in barley (*Hordeum vulgare* L.). Genetica Polonica, 34, 337-343.
24. **Sawicki B., 1995.** The yielding of some grasses after irradiation of seeds with helium-neon laser (in Polish). Annales Universitatis Mariae Curie-Skłodowska, 9, 59-63.
25. **Szyrmer J. and Klimont K., 1999.** The influence of the laser biostimulation on the quality of French bean (*Phaseolus vulgaris* L.) seeds (in Polish). Biul. Inst. Hod. i Aklim. Roślin., 210, 165-168.
26. **Wang L.Q., 1991.** Induced mutations for crop improvement in China. In: Pl. Breed. for Crop Improv. Proc. of Symposium, Vienna, IAEA, 1, 9-33.
27. **Weber G., De Groot E., and Schweiger H., 1986.** Synchronization of protoplasts from *Glycine max* (L.) and *Brassica napus* (L.). Planta, 168, 488-491.
28. **Weber G. and Greulich K.O., 1988.** Manipulation of cells, organelles and genomes by laser microbeam and optical trap. Int. Rev. Cytol., 133, 1-4.
29. **Weber G., Monajembashi S., Greulich K.O., and Wolfrum J., 1989.** Uptake of DNA in chloroplast of *Brassica napus* (L.) faciliated by UV - laser microbeam. Eur. J. Cell Biol., 49, 73-79.
30. **Weber G., Stanke M., Monajembashi S., and Greulich K.O., 1991**. Microdissection of chromosomes of *Brassica napus* at 4000 x magnification with UV laser microbeam and stable transformation of higher plants. ISPMB Congress, Tucon, Abstracts, 3, 74.
31. **Vasilevski G., 1991.** By laser to healthier and cheaper food. Report of Faculty of Agriculture-Skopje, 2, 1-12.
32. **Xu M.F., 1988.** Studies on mutagenic effect of using laser in wheat. Appl. Laser, 9 , 173-175.