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Influence of laser light on leaf area and parameters of photosynthetic activity in DH lines of spring barley (*Hordeum vulgare* L.)

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A b s t r a c t. The initial material for performed studies was constituted by hull and hull-less DH lines of spring barley (*Hordeum vulgare* L.). The kernels were irradiated with helium-neon laser (He-Ne) in red light spectrum and at the wavelength of 632 nm. Plants obtained in greenhouse conditions were analyzed for blade area and their photosynthetic activity in flag and penultimate leaves (photosynthetic and transpiration rate, photosynthetic gas efficiency). The results indicated a biostimulation effect of laser light, causing an increase of blade area of flag and penultimate leaves. This effect was higher for flag leaves, and exposure to 180 min of irradiation was more effective as compared to 60 min. The reaction observed depended on the kind of DH lines analyzed, and two of them – R63N/14 and R58N/91 increased their blade area more effectively than others. Simultaneously, photosynthetic and transpiration rate decreased in dependence on time of irradiation and the kind of DH lines used. On the other hand, gas exchange efficiency defined as photosynthetic coefficient of water use (CO_2 assimilation / H_2O transpiration) increased for all DH lines and laser light exposure as compared to control.

K e y w o r d s: laser light, blade area, photosynthetic activity, barley

INTRODUCTION

In comparison with conventional light, laser light travels in a very coordinated manner (coherent) and is emitted almost parallel. Such parameters make laser light particularly useful for irradiation of different biological objects. Although for many years laser light has been used for different purposes in industry and medicine, its beginning in biological sciences was noticed in the sixties of the last century. The first attempts were focused on selective damage of cell structure to define their function in the cell. Laser beams were also used for perforation of chloroplasts in the depth of plant cell, transfer of DNA from cytoplasm to chloroplast (Weber *et al*., 1989), cell fusion (Wiegand *et al*., 1987) and microdissection of chromosomes (Monajembhasi *et al*., 1986). The studies of Berns *et al*. (1969) demonstrated the ability of green argon ion laser to induce damage in selected chromosomes. This effect, many years later, opened the utilization of laser beams for mutation induction (Xu, 1988; Rybiński *et al*., 1993). According to Wang (1991), 13 cultivars were released in China as direct laser mutants or derived from theirs crosses. On the other hand, short time irradiation of seeds with laser light has an influence on the course of metabolic processes in cells, as well as on their photosynthetic activity. This indicates that the cell is able to absorb, transform and use the energy of laser light photons (Szyrmer and Klimont, 1999). As results of those processes, irradiated cells increase their bioenergical potential which, in studies of many investigators, led to higher mitotic activity of meristemic cells, expressed by an increase of the mitotic index (Kobrzyński and Różanowski, 2000), better energy and ability of seed germination (Laszkiewicz, 2001), early growth and longer coleoptile (Drozd *et al*., 2001), improved and balanced seedling emergence (Dziamba and Dziamba, 2001), better growth rate of seedlings (Szajsner and Drozd, 2001), higher yielding ability and early maturity of plants (Lipski, 2001). Laser light stimulated also androgenic development of microspores in *in vitro* culture of potato (Przewoźny and Rybiński, 1994), development of non-ripened embryos in *in vitro* culture for barley DH lines production (Rybiński *et al*., 2001) as well as pollen tube growth in *Cuphea genus* (Wojciechowski *et al*., 1996).

The above mentioned biostimulation effects induced us to estimate laser light influence on the blade area of two uppermost leaves on the plant and their photosynthetic activity.

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MATERIAL AND METHODS

The initial material for the studies consisted of seeds from DH lines obtained in the Institute of Plant Genetics (Department of Quantitative Genetics) of the Polish Academy of Sciences with the use of the Hordeum bulbosum method. Two of them were hull forms (R58N/48, R63N/34) and two hull-less (R58N/91, R63N/14). Seeds were irradiated with helium-neon laser (He-Ne) in the range of red light spectrum and at the wavelength of 632 nm. For the treatments two times of exposure were used: 60 and 180 min. Seeds without irradiation constituted the control combination.

Seeds were sown in pots (3 seeds per pots) in a partly regulated greenhouse. All the pots were uniformly filled with the same weight of soil. In the first stages of plant development, the pots received water once a day, later twice a day. For uniform soil moisture, the same water amount was added to each pot. Water-solution nutrients (Florovit) was given once, before plant heading. To prolong the natural light duration, an artificial lighting system was provided with the use of sodium lamps (400 W).

The measurements were performed on fully developed flag and penultimate leaves. Both kinds of leaves were examined for blade area [(width x length) x 0.75]. The photosynthetic rate (A) and transpiration rate (E) were measured in the middle part of fully expanded leaf blade. For this purpose, the portable infrared gas analysis system LCA–4 (ADC Ltd. Hoddeson, UK) was used, equipped with a compatible PLC leaf chamber and light source PLU 2 (Tungsten 3000 K, stable 1100-1150 μmol $m^{-2}s^{-1}$ PAR at the measured leaf surface). For all genotypes and laser light combinations on each level of analyzed leaves, 6 leaves were measured. The photosynthetic coefficient of water use was estimated as the CO_2 assimilation / transpiration ratios (A/E).

RESULTS

The results of laser light influence on blade area and parameters of photosynthetic activity of leaves for all the examined DH lines are presented in Table 1. Laser light treatment significantly increased the blade area as compared to the control, and exposure of 180 min was more effective than that of 60 min. No significant differences were observed for the photosynthetic rate. In comparison to the control, laser light reduced the transpiration rate and increased the photosynthetic coefficient of water use. Laser light was particularly efficient for increasing the flag leaf area (Table 2). The flag leaf area in control plants constituted 6.88 cm^2 and with laser light exposure extended from 60 to 180 min the obtained values were 12.8 and 15.2 cm^2; for penultimate leaves - 21. 2 cm^2 (control) and 33.4 and 34.7 cm^2. The photosynthetic rate was lower for the combinations with laser as compared to the control, but the differences with relation to the control were not significant. Both doses of laser light induced a reduction of the transpiration rate of leaves, and the values obtained were higher for flag leaf than for penultimate. The photosynthetic coefficient of water use was higher for laser light combinations and increased with longer exposure to laser light, particularly for flag leaf.

The reaction of genotypes in susceptibility to laser light is presented in Table 3. The highest differences between the DH lines were observed for blade area, particularly for R63N/34. For the other parameters analyzed, the differences were smaller and not significant, except for R58N/48 which differed significantly from R58N/91 in photosynthetic rate. With respect to leaf type (Table 4), line 63N/14 was characterized by the biggest flag and penultimate leaves and the smallest leaves were observed for R58N/48. Differences between the genotypes in terms of other analyzed traits and the kind of leaf were not significant.

The values of analyzed parameters for genotypes, flag and penultimate leaves combined, and laser light exposure are presented in Table 5, and additionally for each type of leaf – in Table 6. Mean values for blade area for each DH line and for the combinations with laser were higher in comparison with the control, and irradiation with 180 min of laser light was more efficient than 60 min. Both laser light exposures increased the blade area of flag leaf as well as penultimate leaf (Table 6). An opposite reaction was observed only for R58N/48 where mean values for both types of leaves together were lower for 60 min as compared to the control, due to a reduction of flag leaf area. Except for line R58N/48, the photosynthetic rate for the remaining genotypes in combinations with laser was lower but not

T a b l e 1. Mean values for leaves area and photosynthetic activity of leaves in analyzed DH lines, together

Combinations	Leaves area (cm^2)	Photosynthetic rate (μmol $m^{-2}s^{-1}$)	Transpiration rate (mmol $m^{-2}s^{-1}$)	Coefficient of water use (mmol mol^{-1})
Control	14.06	12.67	2.72	4.88
Laser 60 min	23.11	12.08	2.08	5.37
Laser 180 min	24.87	12.31	2.19	5.74
LSD = 0.05	1.36	0.80	0.22	0.30

T a b l e 2. Mean values for flag and penultimate leaf area and photosynthetic activity in analyzed DH lines, together

Combinations	Leaf area (cm^2)		Photosynthetic rate ($\mu mol\ m^{-2}s^{-1}$)		Transpiration rate ($mmol\ m^{-2}s^{-1}$)		Coefficient of water use ($mmol\ mol^{-1}$)	
	Flag	Penultimate	Flag	Penultimate	Flag	Penultimate	Flag	Penultimate
Control	6.88	21.25	14.19	11.15	3.41	2.04	4.26	5.51
Laser 60 min	12.80	33.42	13.38	10.78	2.28	1.88	4.91	5.83
Laser 180 min	15.00	34.74	13.50	11.12	2.57	1.82	5.24	6.25
LSD $\alpha = 0.05$	1.92		1.14		0.31		0.43	

T a b l e 3. Mean values for leaves area and photosynthetic activity in each analyzed DH line and laser light exposure, together

DH lines	Leaves area (cm^2)	Photosynthetic rate ($\mu mol\ m^{-2}s^{-1}$)	Transpiration rate ($mmol\ m^{-2}s^{-1}$)	Coefficient of water use ($mmol\ mol^{-1}$)
R58N/48	17.63	11.71	2.32	5.39
R58N/91	19.23	12.90	2.52	5.28
R63N/34	19.45	12.29	2.47	5.14
R63N/34	26.41	12.51	2.39	5.52
LSD $\alpha = 0.05$	1.57	0.93	0.25	0.35

T a b l e 4. Mean values for flag and penultimate leaf area and photosynthetic activity in each analyzed DH line and laser light exposure, together

DH lines	Leaf area (cm^2)		Photosynthetic rate ($\mu mol\ m^{-2}s^{-1}$)		Transpiration rate ($mmol\ m^{-2}s^{-1}$)		Coefficient of water use ($mmol\ mol^{-1}$)	
	Flag	Penultimate	Flag	Penultimate	Flag	Penultimate	Flag	Penultimate
R58N/48	8.82	26.44	13.38	10.04	2.96	1.68	4.708	6.07
R58N/91	10.57	27.90	13.52	12.27	2.92	2.13	4.762	5.80
R63N/34	11.21	27.69	13.73	10.86	3.00	1.94	4.649	5.63
R63N/14	15.63	37.19	14.12	10.89	2.88	1.90	5.107	5.95
LSD $\alpha = 0.05$	2.22		1.31		0.36		0.50	

T a b l e 5. The values of leaf area (LA), photosynthetic rate (A), transpiration rate (E) and coefficient of water use (A/E) for both types of leaves together

DH lines and parameters	Combination		
	Control	Laser 60 min	Laser 180 min
R58N/48			
LA	17.04	16.35	19.48
A	10.60	11.57	12.96
E	2.21	2.55	2.20
A/E	4.97	5.04	6.16
R58N/91			
LA	9.46	23.71	24.51
A	13.84	12.58	12.27
E	3.00	2.38	2.19
A/E	4.89	5.30	5.65
R63N/34			
LA	12.81	21.79	23.69
A	13.05	11.92	11.91
E	2.91	2.36	2.14
A/E	4.64	5.13	5.64
R63N/14			
LA	16.87	30.57	31.80
A	13.18	12.24	12.10
E	2.77	2.14	2.26
A/E	5.04	6.01	5.62

LSD ($\alpha = 0.05$) for : LA - 2.72; A – 1.61; E- 0.44; A/E – 0.61.

significant when compared to the control (Table 5). DH line R58N/48 showed a higher photosynthetic rate for flag and penultimate leaves, but significant differences were noticed only for flag leaf and 180 min of irradiation. The highest reduction of the photosynthetic rate was obtained for R58N/91 by significant differences in flag leaf area for both laser light exposure times.

Mean values of the transpiration rate, similar to the photosynthetic rate, were lower in comparison to the control (Table 5). Only line R58/48 (line with increased photosynthetic rate) showed a higher transpiration rate in combination with 60 min of irradiation. For the remaining lines and both laser light exposure times in comparison to the control, the reduction of transpiration rate was significant. With respect to leaf type (Table 6), a significant increase of transpiration rate was noticed for R58N/48 with 60 min of irradiation. The remaining lines were characterized by a decrease of transpiration rate for flag as well as penultimate leaves and both doses of laser light. For flag leaf, the differences in comparison to the control were significant.

Irrespective of laser light exposure time, the photosynthetic coefficient of water use for the analyzed leaves together was higher as compared the to control (Table 5). For 180 min of irradiation, the differences between all the lines and the control were significant. For 60 min, significant differences were observed only in R63N/14. With respect to leaf type (Table 6), only flag leaf of R58N/48 for 60 min of irradiation showed a lower value of the above mentioned parameter. Higher values were observed for flag as well as penultimate leaves. Particularly interesting are high values of water use for flag leaf and exposure of 180 min. For penultimate leaf, significant differences when compared to the control were obtained only for R58N/48 and 180 min of laser light exposure.

DISCUSSION

Rooting ability, photosynthetic activity of leaves, allocation of plant assimilates through vegetative and generative organs, and efficiency of water and nutrient use by plant tissues, are particularly important for the release of new varieties (Górny and Wojciechowski, 1999). One of the photosynthetic aspects of plants is the assimilation area of flag leaf nod - flag leaf and spike contributed 85% carbohydrates in the total accumulation in the grain (Thorne, 1966). High correlation between yield and surface areas of green parts above the flag node of barley has been reported (Volgend and Simpson, 1967). The analysis of flag and penultimate leaves indicates an ability of laser light to

T a b l e 6. The values of leaf area (LA), photosynthetic rate (A), transpiration rate (E) and coefficient of water use (A/E) for flag and penultimate leaves

DH lines and parameters	Combination					
	Control		Laser 60 min		Laser 180 min	
	Flag	Penultimate	Flag	Penultimate	Flag	Penultimate
R58N/48						
LA	9.37	24.71	7.35	25.35	9.73	29.24
A	12.02	9.17	13.41	9.73	14.70	11.21
E	2.71	1.71	3.42	1.67	2.73	1.66
A/E	4.51	5.43	4.23	5.85	5.37	6.94
R58N/91						
LA	4.12	14.80	12.90	34.53	14.69	34.32
A	15.14	12.54	12.85	12.31	12.57	11.97
E	3.73	2.28	2.61	2.16	2.42	1.96
A/E	4.20	5.58	4.91	5.70	5.17	6.13
R63N/34						
LA	6.52	19.10	12.63	30.95	14.35	33.03
A	14.66	11.45	13.26	10.57	13.28	10.55
E	3.63	2.19	2.79	1.93	2.57	1.71
A/E	4.02	5.26	4.77	5.49	5.14	6.13
R63N/14						
LA	7.37	26.36	18.31	42.84	21.21	42.38
A	14.95	11.42	13.99	10.49	13.43	10.76
E	3.56	1.98	2.52	1.76	2.57	1.95
A/E	4.31	5.78	5.73	6.29	5.27	5.98

LSD ($\alpha = 0.05$) for LA- 3.84; A – 2.28; E - 0.62; A/E – 0.87.

increase the blade area. This effect has an undoubted connection with the biostimulating influence of short exposure to laser light, observed also in barley (Rybiński *et al*., 1993), grasses (Sawicki, 1995), sugar beet (Koper *et al*., 1996), maize (Lipski and Koper, 1997), wheat (Drozd *et al*., 1999) and faba been (Podleśny, 1997).

In the experiment performed, the blade area depended on the time of irradiation with laser light and on the kind of DH line used for laser treatment. In spite of the increase of leaf area caused by both laser doses, the exposure of 180 min was more effective than the shorter irradiation of 60 min, particularly for flag leaf. Also in maize, longer irradiation with laser (through higher water content in seeds) increased leaf area from 158 dm^2 (control) to 190 dm^2 as well as dry matter content in single plant by 10% (Lipski, 2001). In studies on tomato (Koper *et al*., 2001), laser light significantly increased the leaf area, massiveness of stem, and fruit resistance to squashing. A biostimulation effect expressed by leaf yield per hectare after laser use was also obtained in sugar beet (Wójcik, 2001).

As mentioned above, the area of flag and penultimate leaves depended on the initial material used for irradiation. Line R63N/14 was particularly effective in utilizing leaser light energy for blade area increase. Variable reaction of wheat cultivars was observed by Drozd *et al*. (2001) who selected genotypes with higher susceptibility to laser light influence. The leaf area changed in a wide range for different cultivars and years of the experiment. For 56 barley varieties, the flag leaf area in four years trials was 11.6; 9.0, 9.6 and 13.1. cm^2 (Frimm, 1981). The blade area changed also as a result of breeding progress achieved since the beginning of the 20th century. According to Górny and Garczyński (2002), older cultivars of wheat were characterized by development of uppermost leaves on the plant in the range from 41.8 to 49.9 cm^2 (harvest index – 39.6-41.1%), and newly released cultivars in the range of 37.7-43.0 cm^2 with a harvest index of 48.8-49.9%.

The photosynthetic activity of the uppermost leaves became of special importance during grain filling, when the older leaves began senescing (Górny, 2001). In the studies performed, the increase of flag and penultimate leaves was not connected with their higher photosynthetic rate per blade unite area. Among the analyzed lines, three of them, in combination with laser light, showed a lower value of this trait as compared to the control. This effect may have a connection with negative correlation between the

photosynthetic rate and the leaf blade area (Austin, 1989). The higher photosynthetic rate of leaves was obtained only for DH line R58N/48 which, as sole line, was characterized by either a reduction or the smallest increase in the leaf area. Considering the activity per unit area of wheat leaves at their full development, the smaller penultimate and flag leaves showed a markedly slower photosynthetic rate in comparison with the more vigorous lower (earlier) ones (Górny and Garczyński, 2002). According to Evans and Dunstone (1970), in cereal evolution the plants increased size of leaves and grains was connected with a lower photosynthetic rate per unit area of leaves. On the other hand, analysis of barley lines with small and large flag leaf showed that photosynthetic rates per unit leaf area were similar in small and large flag leaves, thus total photosynthetic activity per leaf was about twice as great in the larger flag leaves (Berdhal *et al*., 1972).

The increase in leaf area and decrease in photosynthetic rate in combination with laser use were accompanied by a lower transpiration rate. Similarly to the photosynthetic rate, a negative correlation was observed also between the transpiration rate and the leaf area, and the smaller leaves of modern wheat cultivars tended to be more photosynthetically active and more efficient in gas exchange per unit area than the larger leaves of older cultivars (Górny and Garczyński, 2002). Evans and Dunstone (1970) explained such a decrease in the photosynthesis of larger leaves by higher resistance to CO_2 exchange due to reduced surface area/volume ratios and larger mesophyll cells.

The majority of modern cereal cultivars are characterized by their tendency to develop leaves with higher photosynthetic activity and to be more efficient in gas exchange per unit area. The gas exchange efficient, defined in our studies as the photosynthetic coefficient of water use (CO_2 assimilation/transpiration ratios), may be considered as an important component of yield capacity in diverse environmental habitats (Van den Boogaard, 1995). Unfortunately, progress in genetic-breeding programs of barley (higher efficiency of water use) appears to be limited (Górny and Wojciechowski, 1999). In the studies performed, laser light, as compared to the control, increased the photosynthetic coefficient of water use in flag and penultimate leaves. The above mentioned effect may have a connection with the longer stay-green duration of flag leaves observed in combination with laser. This trait was closely correlated with the harvest index, both characters exhibiting a highly positive correlation with the efficiency of water use in generative matter formation, and contributed mostly to improved efficiency of water use for grain formation (Górny and Garczyński, 2002). The newly released cultivars show a tendency to longer stay-green duration of flag leaves. The ability of the uppermost leaves to remain green and active during grain filling was noticed for DH lines in combinations with laser. This effect seems to be particularly important and, according to Oosteron *et al*. (1996), may have an adaptive value.

CONCLUSIONS

1. Laser light, as compared to the control, influenced the area of two uppermost leaves as well as theirs photosynthetic activity expressed by the photosynthetic rate, transpiration rate and coefficient of water use.

2. The values of the above mentioned parameters depended on grain exposure to laser light and susceptibility of DH lines in reaction to the doses of laser light used.

3. Both doses of laser light induced a biostimulation effect for the blade area of two uppermost leaves on the plant. Laser emission was more efficient for the flag leaf area increase as compared to the penultimate leaf. It was particularly visible for longer exposure to laser light (180 min).

4. The increase in leaf area was accompanied by a decrease in the assimilation and transpiration rate of flag and penultimate leaves. Only DH line R 58N/48, as compared to the control, was characterized by a higher photosynthetic rate for both laser doses as well as flag and penultimate leaves. On the other hand, this line, in comparison to the remaining lines, R63N/34, R58N/91and R63N/14 showed the smallest leaf area increase after laser use.

5. Opposite to the influence of laser light on the photosynthetic and transpiration rate, the coefficient of water use (estimated as CO_2 assimilation / transpiration ratios) for all the genotypes and laser light doses was higher in comparison to the control.

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