Effect of salinity and radiation on proline accumulation in seeds of canola (*Brassica napus* L.)

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ABSTRACT

Since laser beam may affect plant traits, it was used to enhance accumulation of proline in rapeseed and therefore to improve its tolerance to the salinity stress. This investigation was performed to study the effect of NaCl concentration in irrigated water (0, 100, 200 and 300 mmol NaCl) on proline accumulation of Canola (*Brassica napus* L.) after laser irradiation (Red, Infra-red and Nd:YAG) at two exposure treatments. In each exposure, seeds were irradiated for three minutes once or twice by the laser set. Free proline content in leaves increased significantly by increasing of NaCl concentration. Also proline content significantly increased with irradiation by laser beam. The Red laser irradiation used once and the Nd:YAG laser used twice had the greatest effect on the proline content whereas the Infrared laser had a low effect. Double application of irradiation induced a significantly higher amount of proline in the leaves compared to only one application. This is the first report on using different lasers irradiation on proline content in a winter rapeseed.

Keywords: proline-rich proteins (PRP); osmotic; salt stress; amino acids; damage plant

Agricultural productivity is severely affected by soil salinity. Tolerance to abiotic stresses is influenced by interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Zhu 2002). Proline is the major amino acid associated with environmental stresses (salinity, extreme temperatures, UV radiation and heavy metals). When exposed to drought or a high salt content in the soil (both leading to water stress), many plants accumulate high amounts of proline, in some cases several times the sum of all the other amino acids (Ashraf and Foolad 2007). The proteinogenic amino acid-proline functions as an osmolyte, radical scavenger, electron sink, stabilizer of macromolecules and a cell wall component (Matysik et al. 2002). Increased accumulation of proline leads to the increase of enzyme activity of glutamate kinase and therefore increases biosynthesis proline (Vašáková and Štefl 1982). The plants use increased proline content for biosynthesis of physiological specific proteins and/or stress proteins (proline-rich proteins). Further increased accumulation of proline leads to inhibition of synthesis of proline. Accumulation of proline is thus given by these relationships (Štefl and Vašáková 1984). Plants utilize increased content of proline to protein biosyntheses that have specific properties. Proline and hydroxyproline are found in specific compounds. Many of these compounds have specific characteristics and these proteins help to overcome plant stress. This reason for such stress may be soil salinity. For some stress proteins rich proline content is typical (Jofre and Becker 2009, Roshandel and Flowers 2009). These compounds are known as proline-rich glycopro-

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teins, proline-rich lipoproteins, proline-rich proteins, proline-rich phosphoproteins, proline-rich polypeptides, proline-rich peptides or hydroxyproline-rich glycoproteins, hydroxyproline-rich proteins, hydroxyproline-rich phosphoproteins, hydroxyproline-rich polypeptides, hydroxyproline-rich peptides (Merchan et al. 2007). This is inconsistent with the general argument that proline is one of the major organic osmolytes. Therefore, rapid accumulation of free proline in plants is a typical response to a wide range of environmental stresses (Pavlíková et al. 2008). The results from many studies indicated that the methods of physical treatment used at optimal doses had a positive impact on plant viability (Lynikiene and Pozeliene 2003). In the national bibliography, there are many papers that indicate favorable effect of laser light on the yield performance and sometimes also on quality. Among their reports there is however a lack of any detailed particular research results showing changes in the irradiated seeds and plants grown from these seeds (Podleoeny et al. 2001). There is no clear explanation for the effect of monochromatic coherent irradiation on biological objects because of difficulties in the analysis of light-energy transformation in cells and integrated response of complex multilevel living systems to laser irradiation (Salyaev et al. 2007). Bessis et al. 1962 published the first investigation on the application of ruby laser beams in biology. Afterwards many investigations conducted about application of laser in biotechnology researches, such as effect of laser beam on *Brassica napus* about the uptake of DNA in chloroplast, microdissection of chromosomes (Weber et al. 1991), manipulation of plant cells and subcellular structures (Hoffman 1996). A positive influence of laser beams on yield increase was observed cereals up to 15–20%, vegetables up to 40%, tobacco 20%, poppy 20%, potatoes 30% (Vasilevski 1991). Lower doses of laser activate plants, resulting in increasing bioenergetical potential of the cell and higher activation of their biochemical and physiological processes. Higher dosages influence genetic material of the cell leading to genetic changes of plant traits (Rybinski 2001). Chemical compounds and physical sources were used to induce a process of biostimulation. However, it is necessary to distinguish the difference between these two procedures. The chemical compounds, compared to the radiation sources, show a high level of toxicity which has an unfavorable influence on the organisms as well as undesirable effect on the environment. From this point of view, the laser beam offers a pure ecological source of energy that may improve yield. Hence, the laser started to be used as a biostimulator in plant production and breeding (Vasilevski 1991). Results of some researches show a positive influence of laser light on alpha-amylase activity and the concentration of free radicals in the seeds of several plants (Durkova 1993). Laser increased activity of plant enzymes oxidases and catalases (Pastore et al. 2000). The results show that laser radiation affecting and activating peroxidase and catalase enzyme caused an increase of the resistance of spring wheat to water stress (Qiu et al. 2008).

Therefore, we decided to conduct this research to study the effect of laser beam in elevating proline content in canola and thus improve its tolerance to salinity.

MATERIALS AND METHODS

Plant materials and laser treatment. Seeds of *Brassica napus* L. (Canola, Hayola 308) were irradiated with two kinds of diode (semiconductor) laser, inclusive the Red, Infra-red and Neodymium-Yttrium-Aluminum Garnet (Nd:YAG) laser in two different exposures, only one exposure and two exposures with each exposure lasting for 3 min. The characteristics of set up lasers are shown in the Table 1.

After irradiation of seeds, they were sown in the pots and watered with solution of different salinity concentrations. The experiment was conducted as a factorial treatments with a completely randomized design with three replications during 2009. Factors included four salinity levels (0, 100, 200 and 300 mmol NaCl), three types of laser (Red, Infra-red and Nd:YAG laser) and two exposures of irradiation as explained above. Non-irradiated seeds were used as the control.

Proline level. Determination of free proline content performed according to Bates et al. 1973. Leaf samples (0.5 g) from each plant were homogenized in 3% (w/v) sulphosalycylic acid and homogenate filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, resulting mixture was heated at 100°C for 1 h in water bath. Reaction was then stopped by using

Table 1. The characteristics of lasers

Laser	Nd:YAG	Red	Infra-red
Wavelength	532 nm	632 nm	980 nm
Power	75 mW	110 mW	250 mW

Table 2. Analysis of variance for proline content of Canola (*Brassica napus* L.) was irradiated with different types of laser at two exposures under salinity conditions

Sources of variation	*df*	MS	*P* ≥ *F*
Laser	3	72148.6616***	< 0.0001
Exposure	1	27205.5220***	0.0001
Salinity	3	77456.3936***	< 0.0001
Laser × exposure	2	16233.3266**	0.0002
Laser × salinity	9	7743.7836***	< 0.0001
Exposure × salinity	3	3874.9428^{ns}	0.0765
Laser × exposure × salinity	6	8699.8519**	0.0002

Numbers represent *F* values at 5% level: ns – not significant; *P < 0.05; **P < 0.01; ***P < 0.001

ice bath. The mixture was extracted with toluene, and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and expressed as mg/g.

Statistical analysis. Data were subjected to analysis of variance (ANOVA), using the general linear model of SAS (Statistical Analysis System, SAS Institute Inc., 1985). The homogeneity of variance among the treatments, was tested by the Duncan's test (α = 0.05).

RESULTS AND DISCUSSION

The analysis of proline contents in leaves of canola exposed to different kinds of lasers and numbers of exposure to irradiation are presented in Table 2.

Proline accumulation may be a general response to salinity stress. Results of this study show that salinity concentrations have a significant effect on proline contents as compared to the control (Table 3).

Table 3. Mean comparisons of effect of different salinity concentrations on proline accumulation in the leaves of *Brassica napus* L.

Salinity (mmol)	Proline content (mg/g)	Duncan grouping
300	346.669 ± 13.42	A
200	302.168 ± 18.81	B
100	215.984 ± 11.14	C
Control	211.108 ± 15.93	C

During our investigation, analysis of variance showed that with the increase of salinity irrigation, proline content increased; namely 300 mmol NaCl induced the highest value and control sample had the minimum extent of proline content. Effect of salinity to proline content in canola, rice and wheat was reported previously (Shamseddin-Saeid and Farahbakhsh 2008). The accumulation of proline oxidation or diminished incorporation of proline into protein is due to impaired protein synthesis and reduced growth. Accumulated proline may supply energy to increase salinity tolerance (Girija et al. 2002). Accumulation of an osmoprotectant, proline, is enhanced in response to salinity in plants. The immunohistochemical analysis demonstrated that proline transporter (HvProT) was highly expressed in the apical region of barley roots under salt stress. On the other hand, salt stress increased proline and hydroxyproline contents in the cell wall fraction of the root apical region, suggesting an increment of proline utilization. Expression of the genes encoding cell wall proteins (proline-rich protein and extension) and cellulose synthesis was induced in barley roots by salt stress (Ueda et al. 2007).

The results indicated that the effects of salinity levels, lasers and radiation dosage are significant for proline content. For appointing the best laser, analysis of three kinds of lasers was done for each dose, separately.

Dosage of single radiation. The variation of proline content in the samples that were irradi-

Table 4. Mean comparisons of proline content in Canola exposed once to laser treatment

Laser	Proline content (mg/g)	Duncan grouping
Red laser	274.13 ± 21.31	A
Nd:YAG	254.31 ± 12.91	A
Infrared laser	205.20 ± 28.58	B
No-laser	129.27 ± 13.82	C

Table 5. Mean comparisons of proline content in exposed twice to laser treatment

Laser	Proline content (mg/g)	Duncan grouping
Nd:YAG	323.92 ± 35.07	A
Red laser	282.95 ± 19.03	B
Infrared laser	273.40 ± 14.53	B
No-laser	129.27 ± 13.82	C

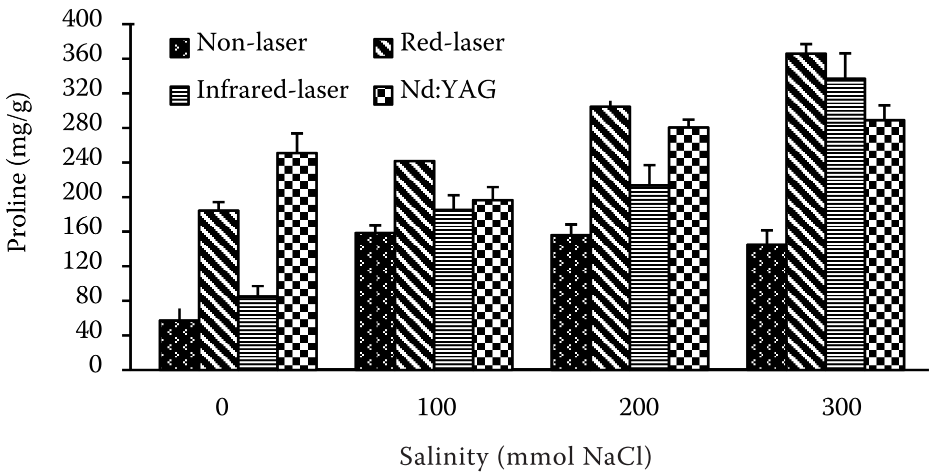

Figure 1. Effects of different lasers at single exposure on proline content in Canola (*Brassica napus* L.)

ated for 3 min in 1 day (time) was examined by the Duncan's test which is showed in Table 4.

These results suggest that for single radiation with the Red laser had the greatest effect on proline content; there was no significant difference with Nd:YAG laser. The Infra-red laser had the weakest impact on proline, but all of the lasers gave higher values compared to the non-irradiated samples. These results are shown in Figure 1.

Dosage of double radiation. For comparison of kinds of laser at the 2 times of radiation, Table 5 was presented.

Considering the results of this table, it shows that with double radiation, Nd:YAG laser had the highest values of proline content. Red laser and Infra-red laser gave higher values of proline than control samples. The results of double radiations are shown in Figure 2.

Figures 1 and 2 show that no-laser treatment gave the minimum value of proline content in leaves of canola, while Red laser at the 1 dosage and Nd:YAG laser at 2 dosages of irradiation gave the highest value of proline at the 300 mmol NaCl concentration.

The results of the Duncan grouping at the Tables 4 and 5 showed that the Red laser at 1 dosage and Nd:YAG beam at 2 dosages of irradiation had the greatest effect on proline content. The Infra-red laser had the lowest effect on the proline content.

With the comparison of proline content between control and plants irradiated with laser beam in the same salinity concentration, we observed that types of laser had a significant effect ($P < 0.001$) on proline content. One of the reasons for proline content increase can be the additional energy in plant at irradiation with laser beams. Laser beam can be used as a biostimulator factor inducing the positive effects on plant growth and development, as a result of bioenergetical structure excitement which causes 'cell pumping' with additional energy increasing the bioenergetic level in organisms (Vasilevski 2003). In higher plants, proline is synthesized in cytosol either from L-glutamic acid or from L-ornithine. On the other hand, proline is metabolized in the mitochondria to L-glutamic acid via proline dehydrogenase (Di Martino and Pizzuto 2006). The glutamic kinase requires ATP

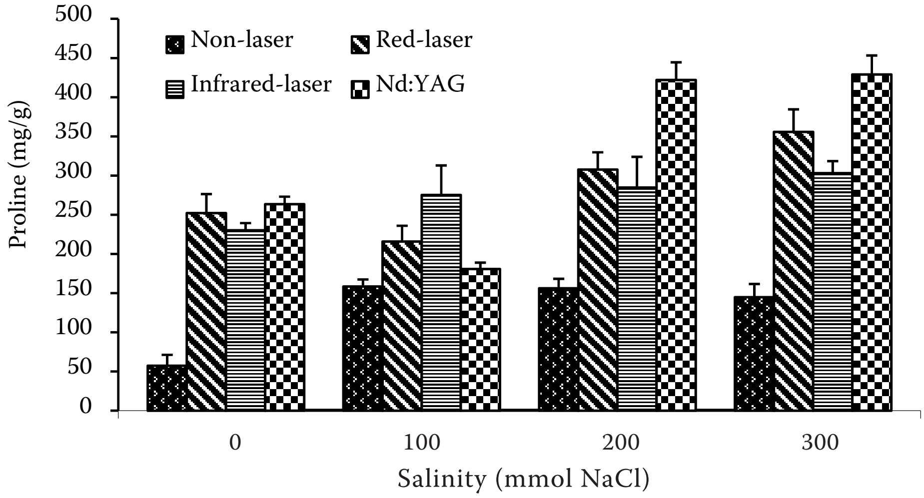

Figure 2. Effects of different lasers at double exposure on proline content in Canola (*Brassica napus* L.)

Table 6. Mean comparisons of different exposure to radiation on proline content in Canola

Dosage of laser	Proline content (mg/g)	Duncan grouping
2 period	293.421 ± 14.23	A
1 period	244.542 ± 13.21	B

for the reactions. For regulatory enzymes requiring ATP, the energy gradient, ATP/ADP thus plays an important regulatory role (Štefl and Vašáková 1982). Some researches show a physical-chemical difference in the ATP molecule after irradiation with lasers beam (Amat et al. 2004). Therefore one of the reasons for an increase of the proline content by irradiation, can be the effect of laser to activity of ATP molecules, the content of ATP was used for glutamic kinase at mitochondria. Moreover, this problem had an effect on increased synthesis of proline by glutamic kinase. As a general rule, for comparison of the effect of 2 different dosages on proline content, Duncan's grouping test was used and is presented at Table 6.

The results of Table 6 show that for 3 kinds of lasers, double exposure to laser irradiation were more effective than single radiation.

Another reason of salinity resistance increase in canola can be due to genetic process changes. Laser is one of the sources for inducing a biostimulation effect and genetic changes in plants that duration of irradiation is the forcible factor to effective of laser radiation such as our results. Lower doses activate plants, resulting in increasing bioenergetical potential of the cell and higher activation their biochemical and physiological processes. Higher dosages influence genetically material of the cell leading to genetic changes of plant traits (Rybinski 2000).

Our research showed that the dosage of radiation had the different affect to proline content as irradiated for 2 days; it had a greater effect than single radiation. Therefore, plants which were pretreated with laser for longer time, had further resistance to salinity stress. Also, different times of radiation had diverse effects on plant germination (Abu-Elsaoud et al. 2008). Starzycki et al. 2005 reported that laser beam positively affected rapeseed resistance to Blackleg disease. They used helium-neon laser at the wavelength of 632 nm at 4 different times of radiation (30, 60, 90 and 120 min). They found that irradiation for 30 min had the best effect on resistance to fungal pathogen (*Phoma lingam*) inoculation, similar results were obtained for 60 and 90 min radiation but at 120 min the effect was weaker. Thus, using too long irradiations to affect the pathogen is not necessary, and it can cause considerable damage to the seed structures. The choice of the best dosage of irradiation should have a useful effect of laser to organisms.

In general, the canola seeds irradiated by laser radiation received from Red, Infra-red and Nd:YAG lasers, showed positive changes in the proline content. These results showed that laser light irradiation can be useful for improving rapeseed yield in the field conditions, especially in those regions where salinity occurs.

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