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Effect of Laser Priming on accumulation of Free Proline in Spring Durum Wheat (*Triticum turgidum* L.) under Salinity Stress

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ABSTRACT

Experiments were conducted during 2012 in a greenhouse of College of Abouraihan, University of Tehran, Iran. In this study the effects of salt stress and laser priming on proline content of durum wheat (*Triticum turgidum* L.) was carried out in a factorial experimental based on a randomizely complete block design (RCBD) with three replications. Seeds from two cultivars salt sensitive and salt tolerance of Durum wheat (*Triticum turgidum* L.) were exposed to neodymium-doped yttrium aluminum garnet (Nd-Yag) laser irradiation (75 mW cm^{-2}, radiated for 12 min). Salinity treatments carried out in four levels (Control, 70, 140 and 210 mM) via sodium chloride. The sampling from first leaves was carried out on four stages of growth and their proline content was measured. The result showed that free proline content in leaves increased significantly by increasing of NaCl concentration and salt tolerant variety accumulate more Proline than sensitive variety. Also proline content significantly increased with irradiation by laser beam in salinity condotion. These results indicate that the low power continuous wave Nd–Yag laser light seed treatment has considerable biological effects on plant metabolism. This seed treatment technique can be potentially employed to enhance agricultural productivity.

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1. Introduction

Environmental stresses negatively influence the plant growth, developmental stage and crops yield. Soil salinity is one of the major abiotic stresses adversely affects physiological and metabolic processes such as germination percentage, crop growth, productivity and photosynthesis in plants (Sairam *et al*., 2002). Soil salinity makes the change in plants in two ways. High concentration of salts in the soil makes it harder for roots to absorb water and leads to physiological drought, and in plant can be toxic by high concentration of Na^+. Another way is that the salts on the outside of roots have an immediate effect on cell growth and associated metabolism; toxic concentrations of salts take time to accumulate inside plants before they affect plant function (Khosh KholghSima *et al*., 2009; 2012). Because NaCl is the most soluble and widespread salt, it is not surprising that all plants have evolved mechanisms to regulate its accumulation and to select against it in favor of other nutrients commonly present in low concentrations, such as K^+ and NO_3^- (Munns, and Tester, 2008). If Na^+ and Cl^- are sequestered in the vacuole of a cell, organic solutes that are compatible with metabolic activity even at high concentrations (hence 'compatible solutes') must accumulate in the cytosol and organelles to balancethe osmotic pressure of the ions in the vacuole (Flower *et al*, 1977. Wyn Jones *et al*, 1977). The compounds that accumulate most commonly are sucrose, proline, and glycine betaine, although other molecules can accumulate to high concentrations in certain species (Hasegawa Pm *et al*, 2000. Munns, 2005), Production and accumulation of Free Amino Acids (FAA), especially proline by plant tissue during drought, salt and water stress is an adaptive response. This amino acid is widely believed to function as a protector or stabilizer of enzymes or membrane structure that are sensitive to dehydration or ionically induced damage such as Reactive Oxygen Species (ROS) and antioxidant defense. For the durum subspecies, high the level of free amino acids, especially proline in the leaf correlated well with salinity tolerance (simon-sarkadi et al, 2002). Durum wheat (*Triticum durum* Desf), which is used mainly for making pasta and macaroni is the second most important wheat, and is widely grown in Southern Europe and the Middle East, and on soils affected by salinity (Sadat Noori and McNeilly, 2000). Durum wheat compared with common bread wheat (*Triticum aestivum* L.) is known for its hardness, protein, intense yellow color, nutty flavor and excellent cooking qualities (Kneipp, 2008). Although durum wheat cultivars are more salt sensitive than bread wheat and their yield is lower under saline soils (Munns and James, 2003), for this reason breeding new cultivars of durum wheat capable of that can be grown on saline soils is of great interest. Seed dressing with various growth regulators, plant hormones, fertilizers etc. are currently considered the most efficient; the best recognized and the most often used practice. However, such substances may modify the chemical structure of the treated seeds, pollute the soil and pose a great danger to the environment. Therefore, more attention has been paid to study physical factors that favorably improve cultivated plants (Perveen, R., et al. 2010). Many studies indicate that physical methods stimulate only changes at physiological and biochemical level in the treated seeds (Aladjadjiyan, 2007; Perveen *et al.* 2010) rendering them safe and friendly to the environment. Laser is considered one of the physical methods that can be safely applied to improve the quality and yield of crop plants (Inyushin, *et al*, 1981; Ivanova, 1998; Koper, 1994; Podleoeny, 2002). The aim of these methods is the appropriate preparation of the sowing material to improve seed sprouting growth and vigor (Podlesny, and Podlesna, 2004) The laser radiation has been used by different researches (Wilczek, *et al.* 2004; Chirkova, 2002; Podlesny, Podlesna, 2004) as a physical method to improve the germination, the growth and the vigor of seeds (Salyaev *et al*. 2007). Biophysical methods can stimulate the seed and plants, through improving the energy balance and hence activating the growth and yield processes (Chen *et al*., 2005; Vasilevski, 2003). However, biophysical protocols are beneficial that enable plants to vegetate at a higher energy level. It is now evident that physical methods such as laser radiation application enhance the energy account of metabolites by internal energy transformation (Chen, *et al*. 2005). Therefore; the aim of the present study was to investigate the effect of pre sowing laser treatment on the accumulations of free proline content under salinity stress.

2. Mathematical Model

Experiments were conducted during 2011 in a greenhouse of College of Abouraihan, University of Tehran, was carried out using a factorial (salinity, laser, cultivar) based on a completely randomized block design with three replications. Factors included four salinity levels (Control, 70, 140, 210 mM NaCl), two cultivars (*Triticum turgidum* L. cv. Karkheh and Dena) and two lasers level (irradiated seeds and Non-irradiated). Seeds from two cultivars salt sensitive (Karkheh) and salt tolerance (Dena) of Durum wheat (*Triticum turgidum var.*durum L.) were obtained from Seed and Plant Improvement Institute in Karaj, Iran. Primary the selection of seeds based on their sizes was carried out and irradiated with Neodymium-Yttrium-Alminum Garnet (Nd:YAG) laser (wavelength 532nm, power intensity 75 mW cm $^{-2}$

and irradiation time for 12 minutes). The irradiated seeds sown in a 48 plastic pots that have Seven kilogram of soil composed of dried sandy loam with natural pH (pH=7.28). The pots were placed in a green house in semi-controlled conditions with a photoperiod of 16h light and 8h dark, relative humidity of 60%-75%. When seedling were in zadoks 21, salt stress treatments were imposed by adding 70, 140 and 210mM NaCl by adjustment the water content of soil to near the field capacity. The sampling from first leaves was carried out after 24 half, 3 days, 7 days and 15 days of each salinity level, then incubated in liquid nitrogen and maintained at -80°C until the measurement time.

The proline content was quantified according to Bates *et al*. (1973). Leaf samples (0.2–0.5 g of fresh weight) of frozen plant material were ground to a fine powder in a pre-cooled mortar with liquid nitrogen. The powder was homogenized in 5 mL of 3% aqueous sulfo-salicylic acid and centrifuged at 14000g for 2 minutes. Two mL of acid-ninhydrin and 2 mL of glacial acetic acid were mixed with 2 mL of the homogenate in a test tube. The mixture was incubated at 100^{0}C for an hour. Reaction was stopped by placing the test tube in an ice bath. Four mL of toluene were added to each test tube and vortexed for 15–20 seconds. The organic and inorganic phases were separated, and the absorbance at 520 nm of the organic toluene phase containing the chromophore was recorded with spectrophotometer (Perkin-Elmer, Lambda 25, USA). Concentrations of proline in plant tissue are expressed on a fresh weight basis and determined from a standard curve and calculated on a fresh weight basis as follows:

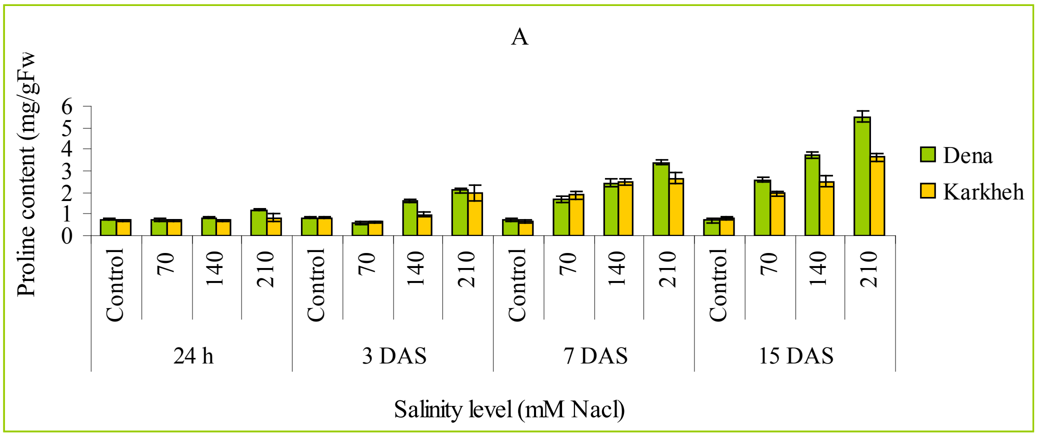
$$\mu\text{moles}\,\frac{\text{Proline}}{\text{g}}\,\text{of fresh weight material} = \frac{\left[\dfrac{\mu\text{g}\frac{\text{Proline}}{\text{ml}}\times\text{ml Toluene}}{115.5\mu\frac{\text{g}}{\mu\text{mole}}}\right]}{(\text{g sample}/5)} \quad (1).$$

3. Statistical Analyses

Data were subjected to analysis of variance (ANOVA) using the general linear model of SAS (Statistical Analysis System V.9) program. The mean differences were compared by Duncan's test at the $P \leq 0.05$ and 0.01 levels.

4. Results and Discussion

The results of analysis variance of effects of laser pretreatment and salinity stress on proline accumulation were showed in Table 1. As we can see, the interaction effect of salinity and laser on free proline content was significant in all steps on sampling ;containing 24h, 3days, 7days and 15 days after salinization (DAS).



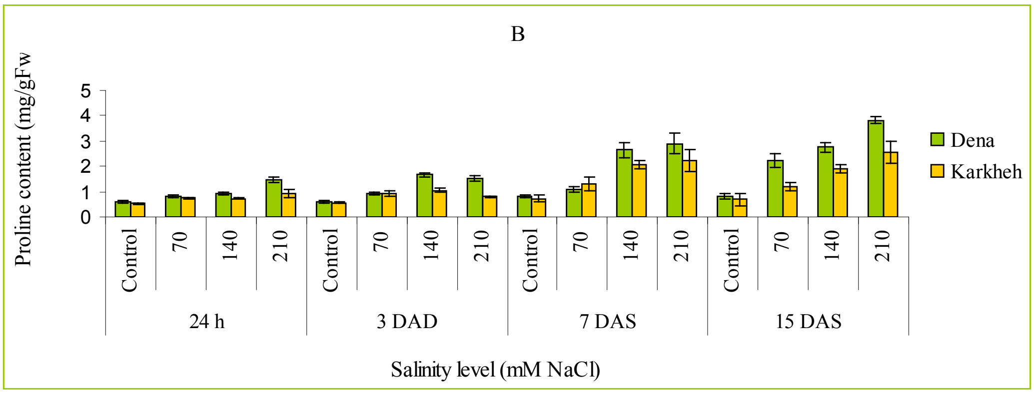


Figure 1: Effect of salinity levels on proline in durum wheat genotypes with Effect of Laser (A) and No Effect of Laser (B). Vertical bars indicate ± S.E. of mean (n=3). Data were significant at 5%probability level for days, salinity treatments and varieties.

Table 1: Analysis of variance for proline content of durum wheat (*Triticum turgidum* L.) was irradiated with laser under salinity conditions

Source of variation	df	Mean of squares			
		24h	3d	7d	15d
Replication	2	0.087**	0.192**	1.164**	0.76**
Variety	1	0.373**	0.850**	0.548**	8.97**
Salinity	3	0.440**	2.253**	10.209**	20.66**
Laser	1	0.012n.s	0.394**	0.916**	5.69**
Variety × Salinity	3	0.107**	0.292**	0.426 **	1.25**
Variety × Laser	1	0.023n.s	0.073n.s	0.068n.s	0.02n.s
Salinity × Laser	3	0.065*	0.807**	0.274**	0.95**
Variety × Salinity × Laser	3	0.003n.s	0.066n.s	0.089n.s	0.1n.s
Error	30	0.016n.s	0.029n.s	0.07n.s	0.07n.s
C. V. (%)	--	16.53	18.59	19.69	15.18

*, **significant at 5% and 1%, respectively. ns = non-significant.

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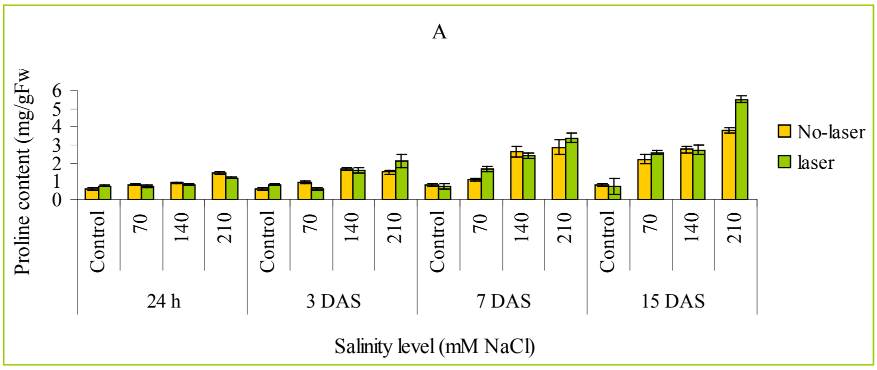
Salinity caused an important increase in proline content in both genotypes. Proline content also increased very significantly at the 3th stage as compared to the 1st and 2nd stage and then marginally at the 4th stage in both cultivars. In both conditions, laser and No-laser, two genotypes had not significant differences at low solution of salinity (70 and 140 mM) in 24h after doing of salinity stress. This revealed that differences between studied genotypes based on proline content, are obvious only after some time and in higher solution of salinity. In present of laser and at 210 mM, 'Dena' showed more proline content than 'Karkheh' statistically at all stages except of 3days after doing of salinity stress (Figure 1.A). Maximum differences between two genotypes were observed at 210 mM NaCl concentrations at the last stage of sampling. Proline content in plant cells under salt stress is a universal phenomenon that can serve as an osmotic regulator (El-Sayed *et al*. 2007) and in widely documented in the cell pressure adjustment, detoxification of injurious ions and membrane stabilization in plants under salinity conditions (Ashraf and Foolad 2007). Proline has been shown to function as a molecular chaperone able to protect protein integrity and enhance the activities of different enzymes (La´ szlo´ Szabados and ArnouldSavoure´. 2009). In No-laser condition, differences between genotypes were more obvious at starting stages of sampling, 3days, in contrast to laser condition. In study of two genotypes that were not exposed to laser, it was resulted that proline content of 'Dena' was more than 'Karkheh' statistically in all levels of salinity at 7day and 15 day except 70 level of salinity at 7day (Figure 1.B). In the 3rd stage of sampling, differences between genotypes showed that at 140 and 210 mM NaCl 'Dena' had 22% and 23% more proline content, respectively, comprising to the control. In the 4th stage and 140 mM NaCl so 'Dena' had 30% more pro than 'Karkheh' and at the 210 mM concentration 32%. During our investigation, analysis of variance showed that with the increase of salinity irrigation, proline content increased; namely 210 mM NaCl induced the highest value and control sample had the minimum extent of proline content in two varieties (Figure 1). Effect of salinity to proline content in canola, rice and wheat was reported previously (Shamseddin-Saeid and Farahbakhsh, 2008; Azizpour *et al*. 2010; Hadi *et al*. 2007). 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). Enzymes of the ROS-scavenging glutathione-ascorbate cycle showed significantly lower activities in the p5cs1 mutants compared to wild type under salt stress suggesting that Pro accumulation is implicated in the control of either stability or activity of enzymes in the glutathione-ascorbate cycle (Sze´kely et al. 2008). There are some reports showing that pretreatment of seeds by laser beams increased the quality and quantity of produced plants. According to Cwintal*et al.* (2010), presuming stimulation of seeds with laser light caused a significant increase in the content of specific proteins, phosphorus and molybdenum in dry matter of the plants, and a decrease in the content of crude fiber. In our research, laser priming caused a reduction of undesirable effects of salinity and an increase in the proline content of plants under both normal and stress conditions. With the comparison of proline content between control and plants irradiated with laser beam in the most of same salinity concentration, we observed that laser had a significant effect ($P < 0.001$) on proline content under salinity condition (Figure 2).



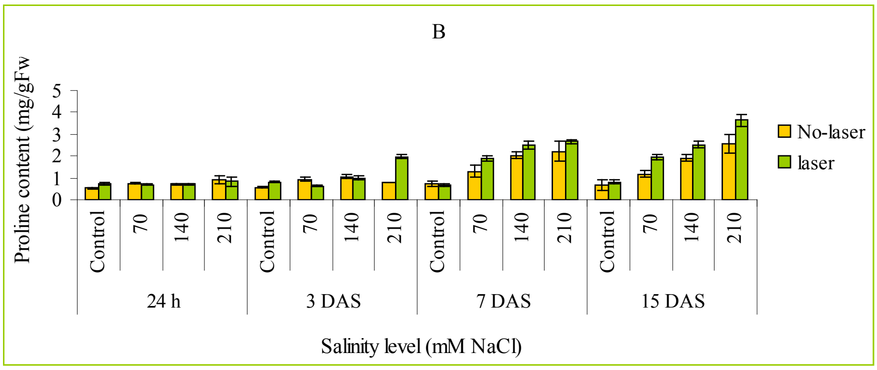


Figure 2: Effect of Laser Irradiation on proline content in durum wheat genotypes; salt tolerant (A) and salt sensetive(B). Vertical bars indicate±S.E. of mean (n=3). Data were significant at 5% probability level for days, salinity treatments and varieties.

As we can see on Figure 2A, there was not any significant difference between salinity levels at 24h stage in all salinity levels except of 70 mM. In Karkheh (a sensitive genotype), differences among using of laser and No-laser are completely visual in higher dose of salinity, especially after 7 days stage. In Dena (a tolerance genotype) and in 210 mM NaCl, using of

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laser increased the proline content statistically after 3days, 7days and 15 days of salinity stress doing, while it was reduced in 24 stage comprising to No-laser treatment (Figure 2.B). Also, using of laser in 140 mM NaCl of salinity was not change significantly the Dena's proline content in all stage except of 24h stage that it was reduced significantly in laser condition.

Proline was shown to protect Complex II of the mitochondrial electron transport chain during salt stress and therefore stabilized mitochondrial respiration (Hamilton et al, 2001).There are some reportes that shows laser pretreatment had a positive effect on proline accumulation in canola (Ashrafijou et al 2010) and common wheat (Sadat Noori et al, 2011) lead to increase salinity tolerance. One of the reasons for proline content increase can be the additional energy in plant at irradiation with laser beams. The laser Beam, as specific light, can be absorbed effectively through the macromolecules and cause some photochemical impacts (Xiang, 1995). In our research, the highest content of proline was observed in high salinity's stress, 210 mM NaCl, in Dena genotype that irradiated with laser, so proline content can a good choice for indirect selection in breeding programs for tolerance to salinity stress and also, the laser use can an alternative way for this purpose. It seems that the effects of salinity on plant growth and its osmolytes are not high in start stage of growth and sampling for examination of its effects on plants can be carry out on late period of plant's life cycle.

5. Conclusion

1. Increasing of the salinity, increases proline content and 210 mM NaCl induced the highest amount of this amino acid.
2. Salt Tolerant variety accumulates free proline content more than salt sensitive variety, so proline accumulation can be a useful parameter to breeding program for salinity tolerance.
3. Laser irradiation increased proline content in most of salinity levels and sampling steps.

The result showed that free proline content in leaves increased significantly by increasing of NaCl concentration and salt tolerant variety accumulate more Proline than sensitive variety. Also proline content significantly increased with irradiation by laser beam in salinity condotion. These results indicate that the low power continuous wave Nd–Yag laser light seed treatment has considerable biological effects on plant metabolism. This seed treatment technique can be potentially employed to enhance agricultural productivity. From this study, stimulating effect of laser radiation can be used in wheat breeding and investigating the use of laser beam on other plants is recommended.

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