Investigate the Effect of Nd-Yag Laser Beam on Soybean (Glycin max) Leaves at the Protein Level

Noha S. Khalifa (Corresponding author)
Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt
E-mail: Nohakhalifa@hotmail.com

H. El Ghandoor
Department of Physics, Faculty of Science, Ain Shams University, Cairo, Egypt
E-mail: helghandoor@gmail.com

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Abstract

The effect of pre-sowing laser treatment on soybean seeds was investigated, using neodymium-doped yttrium aluminium garnet (Nd-Yag) laser source at wave length ʎ = 532nm. Soybean uniform seeds (Glycin max L. cv. Giza 111) were exposed to laser irradiation for 5, 10, 30, 60 and 120 minutes. The growth potential of 4 - week-old seedlings was progressively enhanced with the increase in time durations of laser treatment. In this study, protein banding patterns of the leaf pools of the irradiated and non-irradiated control plants were investigated to elucidate the role of laser at the molecular level. These patterns showed proportional increased levels of a ~ 55 KD protein in alliance with the pre - sowing exposure time to laser irradiation. This protein was gel eluted and identified using de novo protein sequencing analysis of Liquid Chromatography - Mass Spectrometry and Tandem Mass Spectrometry (LC/ MS/MS) technique. The database search of the results led to identification of this protein as the large subunit of the enzyme 1,5 ribulose bisphosphate carboxylase/ oxygenase (RubisCO). The concomitantly enhanced growth potential of soybean seedlings with the elevation of the level of RubisCO will be discussed from different points of views.

Keywords: Nd-Yag laser, Soybean (Glycin max), Leaves, Liquid chromatography

1. Introduction

Improving the criteria of crop plants by applying physical factors such as laser has the advantage over other physiological and chemical methods which are currently used for this purpose. 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 (Barbatni *et al.*, 2007; Perveen *et al.*, 2010). Many studies indicate that physical methods stimulate only changes at physiological and biochemical level in the treated seeds (Podleoeny, 2000; 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). Presewing Laser treatment of seeds was found to achieve biostimulation in many aspects of plant growth and development. In this respect, laser treatments enhanced the rate of germination, seedling growth, in some cereals (Inyushin *et al.*, 1981; Koper, 1994), vegetables (Wilde *et al.*, 1969) and Legume plants (Podleoeny, 2001; 2002). Treated plants were also less susceptible to disease and other unfavorable environmental conditions (Chen *et al.*, 2010).

Weber *et al.* (1990) stated that Laser primarily resulted in the perforation of the plant cell wall and consequently facilitated the internalization of nutrients essential for plant growth and development; which is likely the apparent reason for the increased growth in the treated plants. Karu (1988), suggested that low power laser mechanism is based on the electronic excitation of chromophores in cytochrome c oxidase which modulates α

redox status of the molecule and enhances its functional activity. Therefore, upon laser treatment cytochrome c oxidase becomes more oxidized and oxidative metabolism is increased (Sarcina *et al.*, 2006).

As far as the authors are aware, the exact molecular mechanism of laser effect on higher plants is not investigated at all. Therefore, the aim of the present study was to investigate the effect of presewing laser treatment on higher plants as general. For this purpose, soybean was selected as a model plant particularly due to its significant economic value as world wide cultivated crop for its high oil and protein content. In this respect, protein patterns of 4 - week- old seedlings that previously pre-sewed with laser beam at different time intervals were investigated. Our findings indicated that Laser enhanced the expression level of RubisCO in treated plants.

To be fully active and be able to fix carbon dioxide, RubisCO necessitates the binding of 2 subunits, the chloroplast encoded large subunit and the nucleus encoded small subunit inside the chloroplast. Many recent research studies aimed to replace RubisCO in some plants with more efficient ones in order to stimulate the plant's growth at both individual and crop levels. For instance, trials had been made to replace RubisCO in C3 plants with the more efficient variants from other plants (Whitney *et al.*, 2001; Andrews and Whitney, 2003; Zhu *et al.*, 2004; Zhu *et al.*, 2007). This has prompted significant research into engineering the existing RubisCO, finding better RubisCOs and elucidating ways to effectively transplant these improvements into crop plants. However, experiments done in this respect indicated that engineering of RubisCO is likely to require complementary changes to both subunits (Read and Tabita, 1992b; Spreitzer *et al.*, 2005). Although modifications in L-subunit gene (*rbcL*) by homologous recombination in the model plant tobacco (*Nicotiana tabacum*) is routine (Whitney and Sharwood, 2008), yet there is no appropriate means for efficiently engineering the small subunit genes (*rbcS*) with comparable precision. This is mainly due to the presence of multiple *RbcS* copies in the nucleus that precludes targeted mutagenic or replacement strategies (Whitney and Andrews, 2001; Zhang *et al.*, 2002; Dhingra *et al.*, 2004). For all the above mentioned reasons, laser application to increase the amount and the efficiency of RubisCO will be highly beneficial in this respect. However, this is the first work among our awareness to study the effects of laser at the molecular level in plants.

2. Materials and method

2.1 Plant materials

Soybean seeds were kindly provided by the agricultural crop institute, National research center, Giza, Cairo. Soybean seeds (Glycin max, Cv Giza 111) were used in this study. Giza 111 is the most cultivated variety in recent years in different regions across Egypt (as stated by the ministry of agriculture, Cairo, Egypt).

2.2 Laser device

Nd-Yag laser source with green beam was used in this experiment. The device parts and position of treated samples are shown in Figure 6.

2.3 Time course experiment

Dry seeds were stored in the dark for at least a month before treatments. Treatments were carried out using 532 nm Nd-Yag laser "green" beam for 5, 10, 30, 60 and 120 minutes, each at a time. Well developed seeds with intact seed coat were carefully selected to have the same size and colour. Twelve seeds were subjected to laser treatment for each time interval. In the same day of laser treatment, seed sowing was carried out in the green house at the botanical garden, Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt. The seeds were planted in pots (30 cm diameter) filled with a mixture of equal portions of sand, clay and peat moss (1: 1: 1 v/ v/ v). 6 seeds were sown per pot and were irrigated with the same amount of water every other day. After emergence, thinning was done so that three seedlings of uniform growth were left per pot. The same was done with the untreated control plants. Each of the control and either of the applied treatments were represented by twelve replicates. Four weeks after emergence, The growth criteria of 12 seedlings of either the control or each treatment were measured (length of seedling, average number of leaves/ seedling, average leaf sizes, and other characters as wilting, erection were also observed. Foliage leaves were also taken from the 12 seedlings of each treatments and their pooled protein was used for protein analysis.

2.4 Protein extraction

Leaves were harvested from 4- week- old seedlings as mentioned above in the methods and prepared for protein extraction in the same day. Cotyledonary and first foliage leaves were excluded from the analysis. The leaves were ground in liquid N2 and homogenized in Trizma buffer pH =8 (W/V) Sigma-Aldrich (St. Louis, MO, USA); 0.1 mM protease inhibitor phenylamethyl sulfonylfluroide (PMSF) as protease inhibitor and 100 mM dithiothreitol (DTT) as a reducing agent (instead of mercaptoethanol). The extraction was done on ice. Total soluble proteins were collected by centrifugation at 4 ^{0}C at 15,000 rpm for 15 min. The supernatant was mixed

with activated charcoal to eliminate pigments. Protein concentrations of the different samples were measured spectrophotometrically using pierce commassei protein assay kit (thermoscience, USA) in relation to BSA standard.

2.5 SDS-PAGE protein electrophoresis

Proteins were separated on 15% polyacrylamide gel in the presence of sodium-dodecyle sulphate (i.e. SDS-PAGE). Preparation of the gel was made as described by Laemmli (1970).

Equal amounts of protein extracts were loaded onto the gel (~20 µg / lane). Electrophoresis was performed at 100 V overnight onto a maxi size vertical slap mould (PROTEAN II xi | XL Vertical Electrophoresis Cells, Bio-Rad, CA., U.S.A., measuring 16 × 20 × 0.15 cm) to retrieve high band resolution. The bands were stained over night with Coomassie brilliant blue (CBB) solution then destained using the same solution without CBB until the gel background became stain free.

2.6 LC/ MS/ MS protein sequencing analysis

Mass spectrometric analysis was conducted at the Protein Sciences Facility, Carver Biotechnology Center, University of Illinois, Urbana, IL 61801 using a Waters Q-ToF API-US mass spectrometer**.** Prior to LC/MS/MS analysis, protein band in gel slice was crushed, destained and dehydrated in 50% acetonitrile containing 25 mM ammonium acetate. The protein was digested using proteomics grade trypsin (G-Biosciences, St. Louis, MO) and a CEM Discover Microwave Reactor (Mathews, NC) for 15 minutes at 55 C at 50 Watts. Digested peptides were 3 times extracted using 50% acetonitrile containing 5% formic acid, pooled and dried using a Speedvac (Thermo Scientific). The dried peptides were suspended in 13 microliters of 5% acetonitrile containing 0.1% formic acid, 10 microliters were injected for LC\ MS.

HPLC for the trypsin digested peptides was performed with a Waters nanoAcquity ultra performance liquid chromatography (UPLC) using a Waters Atlantis dC18 nanoAcquity column (3 micron beads, 75 micron inner diameter x 150 mm length), solvents were water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) at a flow rate of 250 nanoliters per minute. Gradient was from 100% A to 60% B in 60 minutes. The effluent from the UPLC was infused directly into a Waters Q-ToF using a Waters nano-ESI ion source.

Control and data acquisition of the mass spectrometer was done using Waters Mass Lynx 4.1 under data dependent acquisition mode. After an initial full scan, the top four most intense ions were subjected to MS/ MS fragmentation by collision induced dissociation.

2.7 Database search

The raw data were processed by Waters Protein Lynx Global Server (version 2.2.5) for noise filtering and deisotoping and then by Mascot version 2.3 (Matrix Sciences, London, UK, www.matrixscience.com). The result was searched against NCBI NR Protein database. The search was made specific to the green plants.

2.8 Protein densitometric analysis

Bands corresponding to RubisCo large and small subunits are cropped from the original gel image, each at a time. Each band set image was converted into gray scale and saved into image- pro plus program for analysis. The densitometric scan for protein bands was manipulated manually by drawing a line in the middle of each band to determine the colour intensity that corresponds to protein concentration. Values retrieved from each band are integrated in a graph to represent the relative electrophoretic protein spectra/concentration for each sample.

3. Results

3.1 Determination of laser power per unit time

The power of the green laser source used in this study is 25 milli Watt with wavelength 532 nm. The energy during seed exposure can be measured according to the following equation:

Exposed energy in Joules = power in milliWatt × time in seconds

Laser exposure energy for the treatments used are calculated in Joules and shown in Table (1).

In our experiment, the accumulative effect of heat during higher exposure time was neglected because the low power of the laser source was used.

3.2 Laser treatment effect on germination and other growth criteria

Treated seeds showed earlier emergence during germination, compared to corresponding untreated controls. A significant growth potential, in response to laser treatments, was expressed as stimulated seedling (4- week- old) length, average number of leaves per seedling for longer treatment times. with laser application for 30- 120 minutes, and obvious seedling erection in 60 and 120 minute treatments (Table 2 and Figure 1). Although at

early stages of seedling there were no significant effect of the longer treatments on leaf sizes, except for the 5 minute treatment, (Figure 2-A), Three month old plants exhibited larger leaf sizes with darker green colour that increased with the laser treatment time (Figure 2-B).

3.3 Protein electrophoresis and quantification of protein using densitometer

Soluble proteins were isolated from soybean leaf and fractionated as outlined in the material and method section. Those proteins remaining in solution were combined 1:1 with SDS–PAGE sample loading buffer, quantified and adjusted volumetrically to achieve an equal protein load for all 1-DE samples. Early fractionation trials using 1-DE analysis demonstrated an incresed amount of ~ 55 KD protein. This protein concentration was very high in plants treated with 532 nm laser for 120 min when compared to the control (Figures 3 and 4). Densitometric analysis indicated that this particular protein significantly showed a concomitant increase with laser treatment time as shown in Figure 4.

3.4 Identification of protein using LC/MS/MS

Protein bands in figure 4 that separates at ~55 KD were eluted from the gel and placed in incubation buffer. This was sent for analysis. Analysis was done by Q-ToF as explained in method section. Protein bands were identified as RubisCO large subunit. The matched peptides retrieved from the Q-ToF analysis are shown in Figure 5.

4. Discussion

The results obtained herein (Figures 1& 2 and Table 2) demonstrated clearly that the vegetative growth of soybean seedlings (4- week- old) was significantly enhanced following Nd-Yag laser treatment. The magnitude of the achieved stimulation of seedling growth was generally in alliance with the exposure time of laser treatment. This conclusion is consistent with those of previous studies (Rybiñski and Garczyñski,2004; Chen *et al.*, 2005; Chen, 2008; *Cwental et al., 2010)* dealing that laser induces large number of morphological and physiological changes in higher plants. In those studies, exposure to laser beam had shown a linear increase in growth, including dry mass gain, leaf size and consequently the yield of many crop plants (Cwintal and Olszewski, 2007; Cwintal *et al.*, 2010**)**. Our present work, aimed to investigate possible molecular mechanism(s) by which laser induces growth and development in higher plants. For this target, the proteins of the seedling leaves were extracted and then separated using the SDS-PAGE electrophoresis method. After achieving a high band resolution, the most significant result was the linearly increased level of a ~ 55 KD protein concomitant with the increased applied level of laser rays. This protein was gel eluted and de novo protein sequencing analysis (LC/ MS/ MS) technique was followed for its identification. The results obtained indicated that this respective protein represents the large subunit of the enzyme 1,5 ribulosebisphosphate carboxylase/ oxygenase (RubisCO). To our knowledge, direct influence of laser on *RUBISCO* gene activity was not reported before. Thus, this finding may represent a lead that can explain the vigorous and rapid seedling growth in a linear manner with the increase of the applied laser dose.

The enzyme ribulose 1,5- bisphosphate carboxylase/ oxygenase (RubisCO) (EC4, 1, 1, 39) is found in most autotrophic organisms (ranging from prokaryotes to eukaryotic algae and higher plants) and is conceivably the most abundant and important protein on earth (Khrebtukova and Spreitzer, 2010). The enzyme is known to catalyze the actual primary CO_2 fixation and is widely accepted as the ultimate rate- limiting step in this photosynthetic reaction. O_2 competes with CO_2 at the same active site initiating photorespiration (Andrews and Whitney, 2003). The holoenzyme, localized in the chloroplast of plants and green algae, contains eight nuclear-encoded small subunits and eight chloroplast- encoded large subunits (Spreitzer and Salvucci, 2002).

Since RubisCO is often rate limiting for photosynthesis in plants, it may be possible to improve photosynthetic efficiency by modifying *RubisCO* genes in plants to increase its catalytic activity and/ or decrease the rate of oxygenation (Parry *et al.*, 2003, 2007). This process faces many difficulties since the manipulation of RubisCO and the mechanism of its activation within higher plants is complicated by the different genomic locations of the large (L; *rbcL*) subunit and the coding of the small subunit by a family of two or more *rbcS* nuclear genes synthesized as 20 KD precursors in the cytoplasm, processed to 15 KD during transport into the chloroplast, where the enzyme becomes a catalytically active molecule and does fix CO_2 when the two subunits bind together (Dedonder *et al.*, 1993). Furthermore, RubisCO is dominantly present in its inactive form and gets activated by the enzyme RubisCO activase, which modulates RubisCO activity by kinetically increasing the dissociation rate of sugar phosphates from its active site in a process requiring the hydrolysis of ATP (Portis *et al.*, 2008).

In the present work, we drew attention to the protein concentration of the large subunit of RubisCO, since research work focused on this subunit because it contains the active site, whereas small subunits are thought to be just regulatory (Spreitzer, 2003). In other studies, the potential of RubisCO activity was also determined by

the amount of RubisCO protein inside the cell. This was encountered by the relative rate of biosynthesis and degradation which is controlled by gene expression (Sheen, 1990; Krapp *et al.*, 1993).

Herein, we assumed that the pre- sowing laser treatments of soybean seeds affected the amounts rather than the activity levels of RubisCO. In this respect, other research workers (Zhang *et al.*, 2002) stated that both photosynthesis and the activity of RubisCO showed variation with light intensity, which supplied the energy for regeneration of the substrate (RuBP). This means that RubisCO is alternating between different activities states, but if laser mode of action was to affect such activity we would then expect to see the same amount of RubisCO large subunit on the SDS-PAGE gel. Therefore, it is more likely that laser rather upregulates the expression of RubisCO large- subunit at the gene level in a way or another. However, this finding needs further investigation.

With all the above combined, it could be concluded that presowing laser treatments of seeds led to increased concentration of the RubisCO large subunit and concomitantly enhanced growth and productivity of soybean plants. Such an increase in the large subunit concentration of the enzyme in the chloroplast might act as a positive feedback signal that increases the expression level of other RBCs genes in the nucleus. One other possible mechanism is that laser treatments were found to increase immobilization of thyllakoid membrane proteins (Weber *et al.*, 1989; Kasahara *et al.*, 2002; Kajiyama*et al.*, 2008). This immobilization might facilitate the entrance of more cytoplasmic RubisCO small subunits and activase into the chloroplast. This would then enhance the formation of more active RubisCO, more CO_2 fixation and accelerated plant growth. However, such proposed model needs further investigation.

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References

Aladjadjiyan, A. (2007). The use of physical methods for plant growing stimulation in Bulgaria. *J. Cent. Eur. Agric.*, 8(3), 369-380.

Andrews, T.J. & Whitney, S.M. (2003). Manipulating ribulose bisphosphate carboxylase/ oxygenase in the chloroplasts of higher plants. *Archives of Biochemistry and Biophysics*, 414 (2), 159-169.

Barbanti, L., Monti, A. & Venturi, G. (2007). Nitrogen dynamics and fertilizer use efficiency in leaves of different ages of sugar beet (Beta vulgaris) at variable water regimes. *Annals of Applied Biology*, 150, 197–205.

Chen, Y.P., Liu, Y.J., Wang, X.L., Ren, Z.Y. & Yue, M. (2005). Effect of Microwave and He-Ne Laser on Enzyme Activity and Biophoton Emission of *Isatis indigotica* Fort. *J. of Integrative Plant Biol.* Formerly *Acta Botanica Sinica*, 47 (7), 849−855.

Chen, Y.P., Jia, J.F. & Yue, M. (2010). Effect of CO_2 Laser Radiation on Physiological Tolerance of Wheat Seedlings Exposed to Chilling Stress. *Photochemistry and Photobiology*. 2010, 86: 600–605.

Cwintal, M. & Olszewski, J. (2007). Effect of pre-sowing laser stimulation of seeds on the intensity of photosynthesis and transpiration and on the yielding of alfalfa (in Polish). *Acta Agrophysica,* 147, 345-352.

Cwintal, M., Dziwulska, H. & Wilczek, M. (2010). Laser stimulation effect of seeds on quality of alfalfa. *Int. Agrophysics,* 24, 15-19**.**

Dedonder, A., Rethy, R., Fredericq, H., Van Montagu & M., Krebbers, E. (1993). Arabidopsis *rbcS* genes are differentially regulated by light. *Plant Physiol.*, 101, 801-808.

Dhingra, A., Portis, A.R. & Jr Daniell, H. (2004). Enhanced translation of a chloroplast-expressed RbcS gene restores small subunit levels and photosynthesis in nuclear RbcS antisense plants. *Proc Natl Acad Sci USA*, 101, 6315–6320

Inyushin, W.M., Iliasor, G.U., & Fedorowa, N.N. (1981). *Laser Light and Crop. Kainar Publ.*, A³ma-Ata.

Ivanova, R. (1998). Influence of pre-sowing laser irradiation of seeds of introduced flax varieties of linseed oil on yield quality. *Bulgarian J. Agric. Sci.*, 4, 49-53.

Kajiyama, S.I., Joseph, B., Inoue, F., Shimamura, M., Fukusaki, E., Tomizawa, K. & Kobayashi, A. (2008). Transient gene expression in guard cell chloroplasts of tobacco using ArF excimer laser microablation. *J. of Bioscince and Bioengineering*, 106 (2), 194-198.

Karu, T. (1987). Photobiological fundamentals of low power laser therapy. IEEE J. quantum electron, 23, 1703-1714.

Kasahara, M. *et al*. (2002). Chloroplast avoidance movement reduces photodamage in plants. *Nature*, 420, 829–832.

Khrebtukova, I. & Spreader, R. (2001). Elimination of the *Chlamydomonas* gene family that encodes the small subunit of ribulose-1, 5-bisphosphate carboxylase/ oxygenase**.** *Nat. Acad. Sci.*.www.pnas.org/content/93/24/13689.full

Koper, R. **(**1994). Pre-sowing laser biostimulation of seeds of cultivated plants and its results in agrotechnics. *Int. Agrophysics*, 8, 593-596.

Krapp, A., Hoffman, B., Schafer, C. & Stitt, M. (1993). Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for sink regulation of photosynthesis**.** *The Plant Journal*, 3, 817–828.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227 (5259), 680–685.

Parry, M.A., Andralojc, P.J., Mitchell, R.A., Madgwick, P.J. & Keys, A.J. (2003). Manipulation of Rubisco: the amount, activity, function and regulation.. *J. Expt. Bot.,* 54, 1321-1333.

Parry, M.A.J., Madgwick, P.J., Carvahlo, J.F.C. & Andralojc, P.J. (2007). Prospects for increasing photosynthesis by overcoming the limitations of Rubisco. *Journal of Agricultural Science*, 145, 31–43.

Perveen, R., Ali, Q., Ashraf, M., Al-Qurainy, F., Jamil, Y. & Raza, A.M. (2010). Effects of Different Doses of Low Power Continuous Wave He–Ne Laser Radiation on Some Seed Thermodynamic and Germination Parameters, and Potential Enzymes Involved in Seed Germination of Sunflower (*Helianthus annuus* L.). *Photochemistry and Photobiology*, 86, 1050–1055.

Podleoeny, J., Misiak, L. & Koper, R. **(**2001). Concentration of free radicals in faba bean seeds after the pre-sowing treatment of the seeds with laser light. *Int. Agrophys*, 15, 185-189.

Podleoeny, J. (2002). Studies on influence of laser light on seeds, growth, development and yielding of the white lupine (*Lupinus albus* L.) plants (in Polish). *Monografie I Rozprawy Naukowe, IUNG, Pu³awy*, 3, 1-59.

Portis, A.R.Jr, Li, C., Wang, D. & Salvucci, M.E. (2008). Regulation of Rubisco activase and its interaction with Rubisco. *J. Expt. Bot.*, 79(7), 1597- 1604.

Read, B.A. & Tabita, F.R. (1992). A hybrid ribulosebisphosphate carboxylase/ oxygenase enzyme exhibiting a substantial increase in substrate specificity factor. *Biochemistry*, 31, 5553–5560.

Rybiński, W. & Garczyński, S. (2004). Influence of laser light on leaf area and parameters of photosynthetic activity in DH lines of spring barley (*Hordeum vulgare* L.). *Int. Agrophysics,* 18, 261-267.

Sarcina, M., Bouzovitis, N. & Mullineaux, C.W. (2006). Mobilization of Photosystem II Induced by Intense Red Light in the Cyanobacterium Synechococcus sp. PCC7942. *The Plant Cell*, 18, 457–464.

Sheen, J. (2003). Metabolic repression to transcription in higher plants. *The Plant Cell*, 1990 (2), 1027–1038.

Spreitzer, R.J. (2003). Role of the small subunit in ribulose- 1, 5- bisphosphate carboxylase/ oxygenase. *Archives of Biochemistry and Biophysics*, 414, 141-149.

Spreitzer, R.J. & Salvucci, M.E. (2002). Rubisco: interactions, associations and the possibilities of a better enzyme. *Annual Review of Plant Biology*, 53, 449-475.

Spreitzer, R.J., Peddi, S.R., Satagopan, S. (2005). Phylogenetic engineering at an interface between large and small subunits imparts land-plant kinetic properties to algal Rubisco. *Proc Natl Acad Sci USA*, 102, 17225–17230.

Suetsugu, N. & Wada, M. (2007). Chloroplast photorelocation movement mediated by phototropin family proteins in green plants. *Biol Chem.,* 388, 927–935.

Weber, G., Monajembashi, S., Wolfrum, J. & Greulich, K.O. (1990). Genetic changes induced in higher plant cells by a laser microbeam. *Physiologia Plantarum*, 79, 190–193.

Whitney, S.M. & Andrews, T.J. (2001). Plastome-encoded bacterial ribulose- 1,5-bisphosphate carboxylase/oxygenase (Rubisco) supports photosynthesis and growth in tobacco. *Proc Natl Acad Sci USA*, 98, 14738–1474

Whitney, S.M. & Sharwood, R.E. (2008). Construction of a tobacco master line to improve Rubisco engineering in chloroplasts. *J Exp Bot.*, 59, 1909–1921.

Wilde, W.H.A., Parr, W.H. & McPeak, D.W. (1969). Seeds bask in laser light. *Laser Focus*, 5 (23), 41-42.

Zhang, X.H., Ewy, R.G., Widholm, J.M. & Portis, A.R. Jr. (2002). Complementation of the nuclear antisense RbcS-induced photosynthesis deficiency by introducing an rbcS gene into the tobacco plastid genome. *Plant Cell Physiol.*, 43, 1302–1313.

Zhu, X.G., Portis, A.R. & Long, S.P. (2004). Would transformation of C-3 crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. *Plant Cell Environ.*, 27, 155–165.

Zhu, X.G., de Sturler, E. & Long, S.P. (2007). Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. *Plant Physiol.*, 145, 513–526.

Table 1. Measurement of laser energy used at different treatment intervals

Exposure time in minutes	Energy gained by seeds during exposure
5 min.	7.5 J
10 min.	15 J
30 min	45 J
60 min	90 J
120 min	180 J

Table 2. Influence of laser light on different morphological and developmental features of soybean seedlings. Results shown are the average of 12 replicates per treatment.

Treatment	Leaf number	Plant total lenght in cm	Other morphological characters	
			Wilting	Erection
Control (No laser)	5± 4.5	24.5 ± 0.13	No	No
5 min. Laser exposure	6 ± 2.2	26.6 ± 3.05	Yes	No
10 min laser exposure	8± 0.46	31.5 ± 2.98	No	No
30 min laser exposure	8± 1.2	35 ± 1.66	No	intermediate
1hr min laser exposure	8 ± 2.3	38 ± 2.19	No	Yes
2hrs min laser exposure	10 ± 1.45	42 ± 3.6	No	Yes

Table 3. Effect of laser pre-treatment on the plant yield. There was an increase in the number of seeds bearing pods/plant and this increase was proportional to laser treatment length.

Treatment	Number of pods/plant
Control	9 ± 0.57
5 min	10 ± 2.12
10 min	10 ± 0.77
30 min	20 ± 1.29
60 min	30 ± 2
120 min	32 ± 3.11

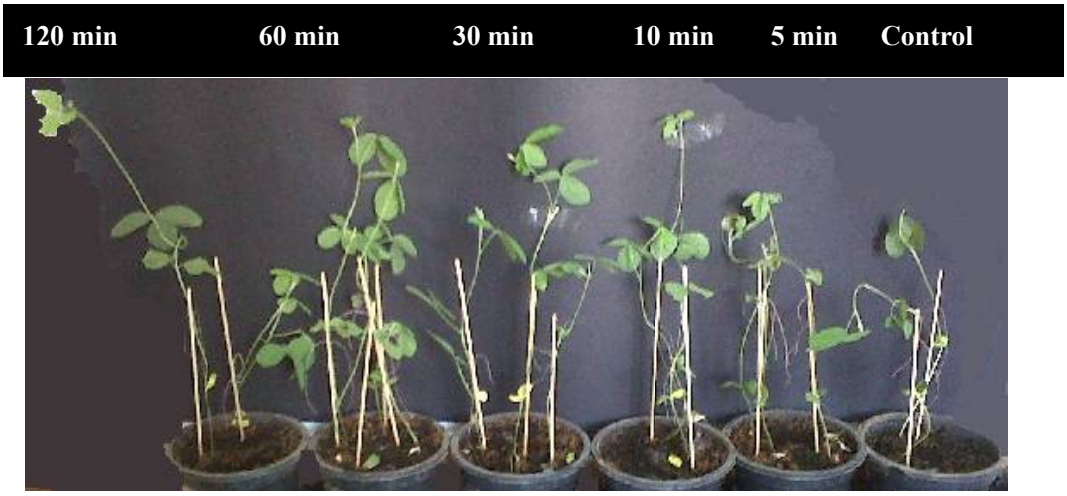

Figure 1. Influence of laser pre- sewing treatment on plant growth rate. Picture shows representative plants from each treatment. Note that seedling length is proportional with the exposure laser time of treatment. Plants irradiated with laser for 120 minutes were longer and more erect when compared to the control.

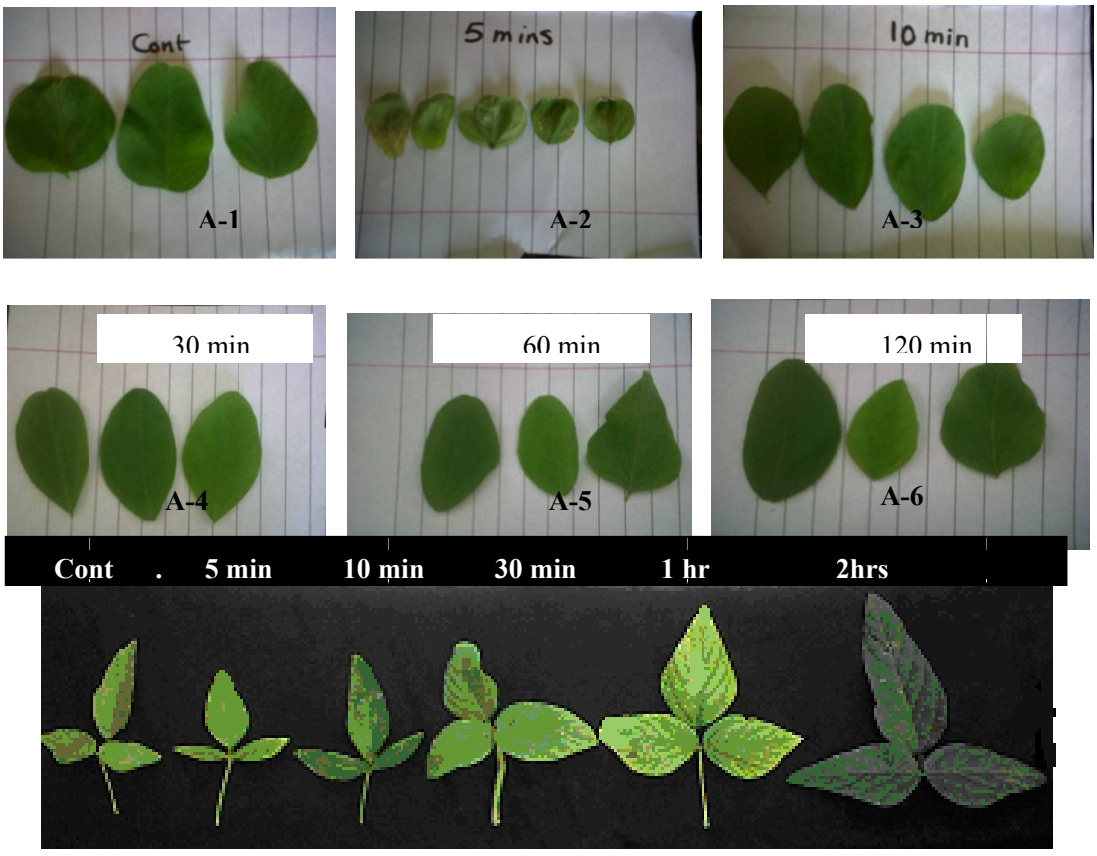

Figure 2. Effect of laser pre-treatment on leaf size of soybean seedlings. A: In 4 week old plants, leaf number increased at longer treatments time but leaf sizes are more or less the same to the untreated plants (control). The leaves shown are representative simple foliage leaves collected from different replicates that correspond to each treatment. It is noteworthy to mention that 5 minutes treatments caused severe reduction in leaf size and this result needs further investigation. Protein extract from leaves shown were electrophoresed to determine if there is any difference in protein patterns between these plants. The experiment was repeated twice to make sure that data are reproducible. B: Last fully expanded leaves were collected from 3 month old plants. The size and green colour intensity increased in longer treatment with laser.

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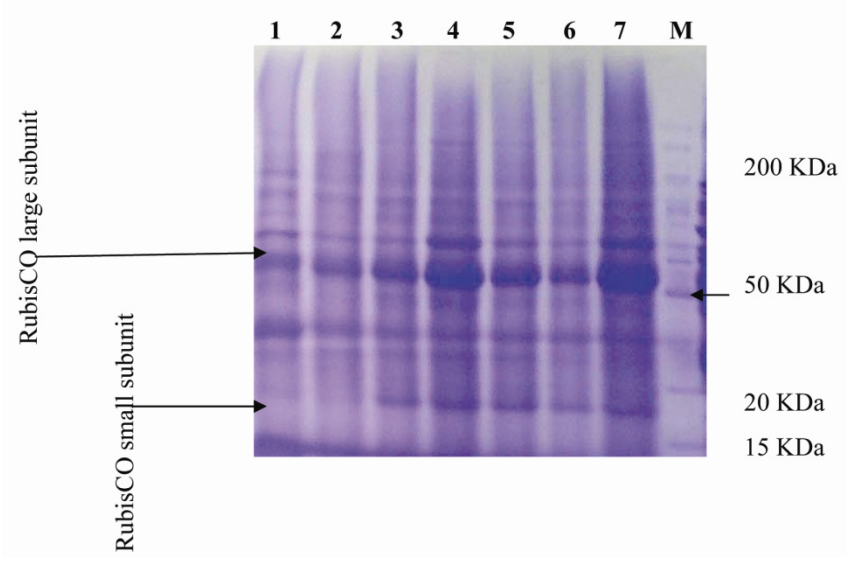

Figure 3. Gel image of SDS PAGE Protein profile pattern. SDS-PAGE soluble protein of four weeks old soybean seedlings pre-treated with laser light. Proteins were extracted from leaves and run on and 15 % Polyacrylamide gel. Note that protein marked with arrows (~ 55 KD and ~20 KD) increases with pre-treatment time and it is significantly over expressed in plants irradiated with laser for 120 minutes when compared with control. Lane 1: control (untreated with laser), pre-treated plants for: 5 min, lane 2; 10 min lane 3; 30 min, Lane 4; 60 min unbranched seedling, Lane 5; 60 min (branched seedling); lane 6 and 120 min, lane 7. M is the molecular weight protein marker (Pageruler unstained protein ladder from fermentas).

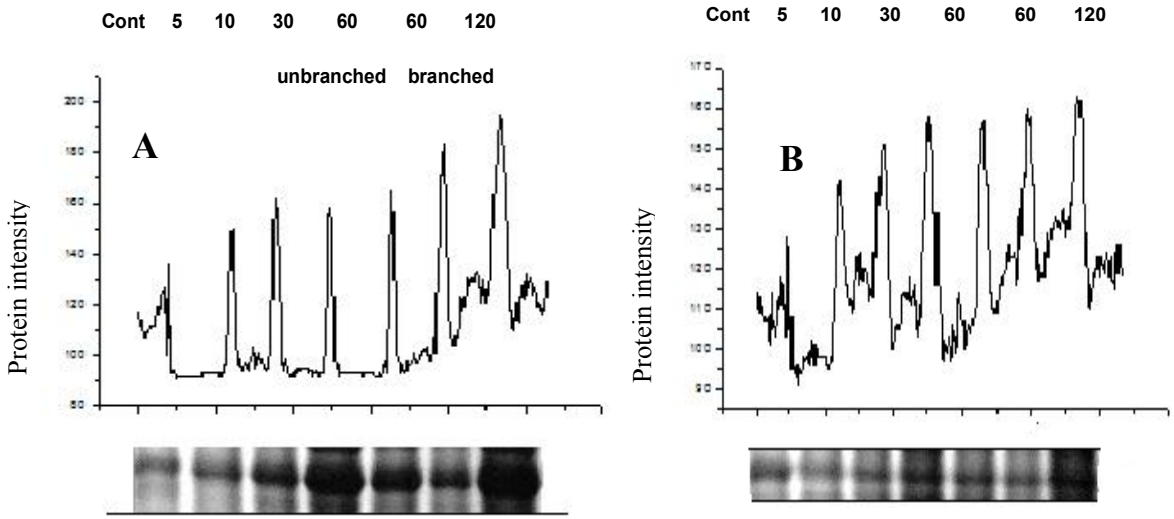

Figure 4. Densitometric determination for the intensity of RubisCO at different laser time treatments using image –pro plus software. Rubisco Large subunit (A) and small Subunit (B) showed increased band intensity at all laser treatments when compared to the untreated control.

1 VGFKAGVKDY KLTYYTPDYE TKDTDILAAF RVTPQPGVPP EEAGAAVAAE
51 SSTGTWTTVW TDGLTSLDRY KGRCYGLEPV AGEENQYIAY VAYPLDLFEE
101 GSVTNMFTSI VGNVFGFKAL RALRLEDLRI PTAYIKTFQG PPHGIQVERD
151 KLNKYGRPLL GCTIKPKLGL SAKNYGRAVY ECLRGGLDFT KDDENVNSQP
201 FMRWRDRFLF CAEAIFKSQA ETGEIKGHYL NATAGTCEEM MKRAVFARFL
251 GVPIVMHDYL TGGFTANTSL AHYCRDNGLL LHIHRAMHAV IDRQKNHGMH
301 FRVLAKALRL SGGDHVHAGT VVGKLEGERE ITLGFVDLLR DDFVEKDRSR
351 GIYFTQDWVS LPGVLPVASG GIHVWHMPAL TEIFGDDSVL QFGGGTLGHP
401 WGNAPGAVAN RVALEACVQA RNEGRDLARE GNEIIRELAN GVLN

Figure (5): Underlined amino acids are those recovered from the ~ 55 KD recovered protein. Cleavage by Trypsin: cuts C-term side of KR unless next residue is P Protein was identified as ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit "rbcL" [Glycine max] (AAB67932.1)161/444= 36% sequence coverage.

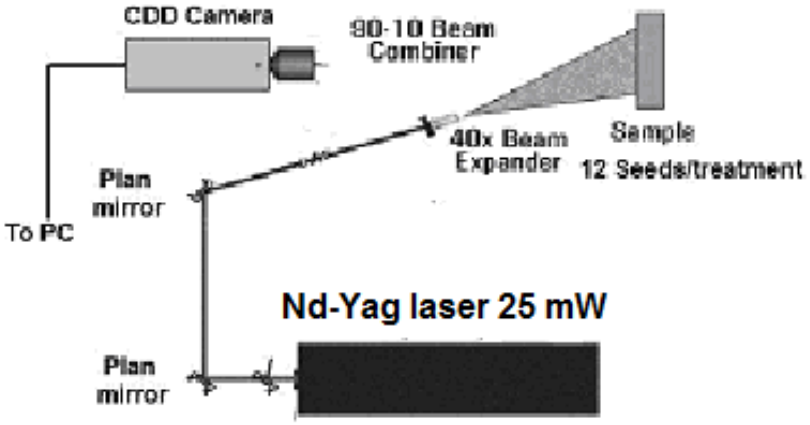

Figure 6. The schematic diagram of the experimental set up used in this work is shown in the above figure. Nd-Yag laser of power 25 mW was used as a light source. A microscope objective lens had been used to obtain a diverging spherical wave that illuminates the plant seeds. The plant seeds were irradiated with laser light wave for 5,10, 30, 60 and 120 minutes time intervals. This likely resulted in an energy gain by the treated seeds ranged from 7.5 J, 15 J, 45 J, 90 J and 180 J, respectively.