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Laser light and magnetic field stimulation effect on biochemical, enzymes activities and chlorophyll contents in soybean seeds and seedlings during early growth stages

Tehseen Asghar [a], Yasir Jamil [a,*], Munawar Iqbal [b,*], Zia-ul-Haq [c], Mazhar Abbas [d]

[a] Bio-Electromagnetics and Laser Laboratory, Department of Physics, University of Agriculture, Faisalabad, Pakistan
[b] Department of Chemistry, The University of Lahore, Lahore, Pakistan
[c] Department of Physics, University of Agriculture, Faisalabad, Pakistan
[d] Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan

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ABSTRACT

Laser and magnetic field bio-stimulation attracted the keen interest of scientific community in view of their potential to enhance seed germination, seedling growth, physiological, biochemical and yield attributes of plants, cereal crops and vegetables. Present study was conducted to appraise the laser and magnetic field pre-sowing seed treatment effects on soybean sugar, protein, nitrogen, hydrogen peroxide (H_2O_2) ascorbic acid (AsA), proline, phenolic and malondialdehyde (MDA) along with chlorophyll contents (Chl "a" "b" and total chlorophyll contents). Specific activities of enzymes such as protease (PRT), amylase (AMY), catalyst (CAT), superoxide dismutase (SOD) and peroxides (POD) were also assayed. The specific activity of enzymes (during germination and early growth), biochemical and chlorophyll contents were enhanced significantly under the effect of both laser and magnetic pre-sowing treatments. Magnetic field treatment effect was slightly higher than laser treatment except PRT, AMY and ascorbic acid contents. However, both treatments (laser and magnetic field) effects were significantly higher versus control (un-treated seeds). Results revealed that laser and magnetic field pre-sowing seed treatments have potential to enhance soybean biological moieties, chlorophyll contents and metabolically important enzymes (degrade stored food and scavenge reactive oxygen species). Future study should be focused on growth characteristics at later stages and yield attributes.

1. Introduction

Pre-sowing laser and magnetic bio-stimulation of seeds beneficial effect on germination, seedling growth and yield have attracted the attention agriculturists [1]. The mobilization of seed storage proteins represents one of the most important post-germination events and seedling growth. Enzymes play a central role in the metabolic pathways during germination and the accelerated enzymatic activities under the effect of laser and magnetic field pre-sowing seed treatments have been reported [2–7]. It is hypothesized that laser light can increase free radicals in irradiated seeds, which can react with oxygen and this process leads to the formation of peroxides. The activity of hydrolytic enzymes increased under the influence of laser irradiation and resultantly, fast mobilization of reserve substances accelerates the seed germination, emergence and seedling growth [8]. Vashisth and Nagarajan [9] reported enhanced enzymatic activities as a results of exposure to magnetic field and Chen et al. [10] documented that He-Ne laser has accelerating effect on enzyme activities during germination and early growth stages. Similarly, [5,11] also reported accelerated enzymatic activities along with fast germination and seedling growth. It is reported that pre-treatment of seed with laser have apparent inductions on the enzymatic activities, thermodynamic properties, altered physiological and biochemical metabolism pathways [10]. Similarly, the enhanced plant characteristics in response of magnetic field treatment have been correlated with change in ferromagnetic properties of particle, change in energy level, changes in electron spins at atom and molecular level, which collectively affect physiological and biochemical metabolic pathways [2–4,7,12–14]. Under the current scenario of environmental pollution and soil chemistry alteration [15–51], different seed stimulating techniques are used to enhance germination and related plant characteristics. However, environmental pollution cannot be limited where primary use of chemical additives is frequent, which are harmful to biological systems. Clean, eco-friendly, more efficient and less expensive techniques are viable alternative and laser and magnetic field pre-sowing seed treatments is of great interest since these are non-destructive, safer and cheap [2–7,11,14,52].

Soybean is one of valuable commodity in the global market as oil crop and is beneficial for health as it is a good source of unsaturated fatty acids and free from cholesterol [53]. The major components of

* Corresponding authors.
E-mail addresses: yasirjamil@yahoo.com (Y. Jamil), bosalvee@yahoo.com (M. Iqbal).

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soybean are proteins (40%) and carbohydrates and lipids (20%) along with important secondary metabolites (phenolic components, saponins and isoflavones). Among all legumes and cereal crops, soybean is a good source of protein for human and animals [54,55]. The fermented soybean foods are very popular in Asian countries as food [53]. Soybeans are planted worldwide and ~75 million acres are planted with soybeans every year in United States and in comparison, Pakistan, covers ~2300 to 6000 (variable and depends upon weather conditions each year) hectares/year since 1978 for soybean cultivation. Germination is considered the sensitive stage in the life cycles of soybean and under drought stress, the germination of soybean affected badly [56]. The drought stress affects soybean germination at planting stage. For normal germination, 50% water absorption is needed of total seed weight. As the water contents drops below this limit, germination decreased significantly and also mean germination time is increased [57]. In Pakistan, KPK is the major producer of soybean and 59% of total soybean is cultivated in Abbottabad and Mansehra districts (Hazara division) and small area is also cultivated in Malakand division and FATA. In Sindh, Hyderabad division (Badin and Hyderabad districts) is main cultivated area of soybean. Sanghar and Thatta districts also cover small area for soybean cultivation. In Punjab, very small area in Multan district in under the cultivation of soybean and this scattered distribution of soybean cultivation is due to weather conditions of specific area because soybean germination affected under dry conditions [58–60]. In view of low germination of soybean in Pakistan, different strategies (sowing and management) have been investigated to enhance germination and yield of soybean [56,58,61,62]. However, effect of laser and magnetic field pre-sowing seed treatments on soybean have not been investigated previously since these methods have been successfully employed to enhance the various plant and crops characteristics [52,63–68].

Therefore, present investigation was conducted to appraise the laser and magnetic field pre-sowing seed treatments on enzymatic activities, chlorophyll contents and bio-biomolecule during early growth stages. The principle objective was to compare both laser and magnetic field pre-sowing seed treatments effect on total soluble sugar, reduced sugar, protein, nitrogen content, H_2O_2, AsA, proline, phenolic, MDA contents, CAT, SOD, AMY, PRT, POD and chlorophyll contents.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemical and reagents used were of analytical grade i.e., trichloro-acetic acid, dichloroindophenol, casein, methionine, nitroblue tetrazolim, riboflavin, ninhydrin and toluene were purchased from Sigma Chemical Co. (St. Louis, Mo, USA), whereas thiobarbituric acid, Folin-ciocalteau's reagent, anthron, potassium iodide, glacial acetic acid and Rochelle salt were purchased from Merck (Darmstadt, Germany). Bovine serum albumin and Triton X and potassium diphosphate and hydrogen peroxide were purchased from Bio-Rad (USA), MP Bioforma (France) and Fluka (USA), respectively.

2.2. Laser and Magnetic Field Setup

Portable He-Ne laser was used for seed irradiation (Model No. 1508P-1256, JDS Uniphase USA, wavelength 632.8 nm and beam diameter 1.5 mm), which emits continuous laser light of wavelength 632.8 nm. During irradiation of seeds, the laser output power was measured using power/energy meter (Quantel, France). The seeds were irradiated following the procedure reported by Chen et al. [10]. A general laser set up used for irradiation is shown in Fig. 1(A). Whereas magnetic field set up is shown in Fig. 1(B). Briefly, the electromagnet used for seed treatment contained four cylindrical coils (each coil has 4000 turns of 0.41 mm enameled copper wire) and resistance of each coil was 26 Ω. Each pair of coils was wound 10 cm apart on an iron bar, which were placed vertical to each other on both sides and metallic

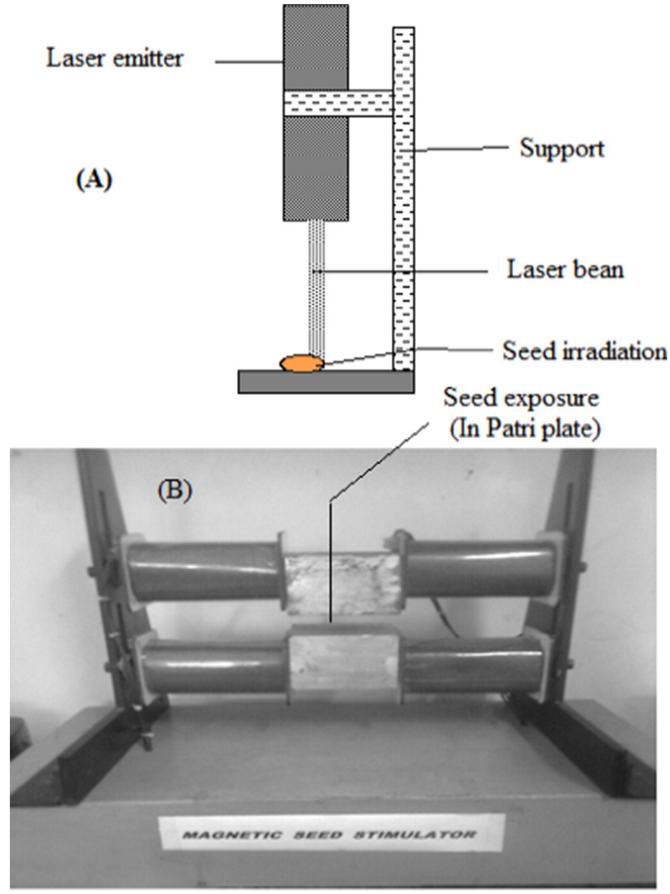

Fig. 1. Laser and magnetic field set up used for seed treatment (A) Laser and (B) Magnetic field.

support was used to hold each bar at fixed position. The coils were connected in series and fed through a variable power supply (0 to 220 V) of 50 Hz full-wave rectified sinusoidal voltage. The magnetic field strength was adjusted by passing variable voltage through coils. Magnetic flux meter (ELWE, Germany) was used for magnetic field strength measurement.

2.3. Seeds and Treatment Procedure

Healthy and uniform soybean seeds were selected (collected from ayyub Agriculture Research Institute) for experimentation. For laser, seeds were exposed to He-Ne laser of wavelength 632.8 nm, power density 1 mW/cm^2 for 3 and 5 min. For magnetic field induction, 50, 75 and 100 m Tesla (mT) for an exposure time of 3 and 5 min was used. The seeds were treated and shown in triplicate for each dose, both for laser and magnetic field. Un-treated seeds were used as control.

2.4. Measurement of Protein and Nitrogen Contents

Protein content was measured by Bredford method [69] and nitrogen contents was measured following modified micro-Kjeldhal's method [70].

2.4.1. Malondialdehyde

Malondialdehyde (MDA) was analyzed following Carmak & Horst [71] method. This method is based on the reaction with thiobarbituric acid. Briefly, fresh leaves (1.0 g) were ground properly in 20 mL of 0.1% trichloroacetic acid solution and centrifuged for 10 min at 12,000 rpm. One mL of the supernatant was reacted with 4 mL of 20% TCA solution comprising 0.5% thiobarbituric acid and heated for