Influence of He–Ne Laser Irradiation of Soybean Seeds on Seed Mycoflora, Growth, Nodulation, and Resistance to *Fusarium solani*

S.A. Ouf, N.F. Abdel-Hady

*Botany Department, Faculty of Science, Cairo University, Giza 12613, Egypt*  
Fax: +202 5727 556  
e-mail: say.ouf@frcu.eun.eg

*Botany Department, National Research Center, Giza 12311, Egypt*

Received 26 August 1998  
Revised version 7 July 1999

ABSTRACT. Laser irradiation of soybean seeds for 3 min caused a clear reduction in the number of seed-borne fungi which became more pronounced as the irradiation time was extended. Pretreatment of the seeds with methylene blue, methyl red and carmine enhanced the effect of laser. *Rhizoctonia solani, Alternaria tenuissima, Cercospora kikuchii* and *Colletotrichum truncatum* were completely eliminated when the seeds were pretreated with a dye and irradiated for 10 min. Seed germination was stimulated on exposure of the seed to 1-min irradiation. At such dose, most of the dyes were accelerators while the higher doses were inhibitory to seed germination. Chlorophyll *a*, chlorophyll *b* and carotenoid content of developed plants differed, depending on the irradiation dose and dye treatment of the seeds. In seeds irradiated for 1 or 3 min, chlorophyll *a* formation was less affected than chlorophyll *b* formation. In seeds irradiated for 10 min, both the chlorophyll contents were decreased especially in the presence of some applied dyes. On the other hand, there was an increase in carotenoid content of soybean leaves when the laser dose increased. The number and dry mass of nodules were mostly greater (as compared to the corresponding control), when the seeds irradiated for 1 or 3 min were pretreated with methyl red, chlorophenol red, crystal violet and methylene blue. Irradiation of pre-sowing seeds greatly protected soybean stands against *F. solani*. The disease incidence differed somewhat when the irradiated seeds were pretreated with dyes. The reduction in disease incidence was accompanied by accumulation of high proline and phenol levels in the infected root tissues of soybean, suggesting that these compounds have a certain role in the prevention of disease development.

Legumes constitute a major portion of food for humans. Soybean (*Glycine max* L. MERRILL) has become one of the most important crops in the world. Considering the world food problem at present and in the near future, the importance of soybean as a supplier of good plant protein is becoming more vital than ever. Storage fungi cause damage to stored grains and seeds. Their infestation results in poor germination, reduction of health and longevity, mass losses, biochemical changes, mycotoxin production, seed discoloration, heating and mustiness (Gbodi *et al.* 1989; Prasad and Prasad 1987; Prasad *et al.* 1988).

Intensive work was carried out to improve seed quality, nodulation, yield and resistance of the soybean against pathogens and seed deterioration. Several updating strategies were tried for controlling seed-borne diseases of soybean, including genetic control (Buss *et al.* 1997), physical control (Fabricius *et al.* 1997), chemical control (Jagjeet and Lodha 1997), and biological control (Pereira and Dhingra 1997). However, the use of such approaches, as indicated by some authors, may adversely affect seed quality and viability and seedling vigor. Recently, laser radiation was expanded in the field of seed science. Laser was successively used to increase seed germination of *Picea abies* (Terasmaa 1989), seed yield of *Medicago sativa* (Mandzhieva 1991), length, fresh mass and dry mass of *Vigna radiata* seedlings (Govil *et al.* 1991), fiber strength and yield of cotton seeds (Nazarov and Fatoev 1991), tillering and spike length of spring wheat (Drozd 1994), swelling rate and enzyme activity in germinating *Nepeta cataria* var. *citriodora* seeds (Kapelev 1989), rhizogenesis of green cuttings of beech (Batov and Nedelin 1990), etc.

Since the existence of a photosensitizer, such as dye, can enhance the effect of laser, it was interesting to study the role of He–Ne laser irradiation of soybean pretreated with a dye, on the occurrence of seed-borne fungi and seed germination. The evaluation of the laser effect was also extended to include different aspects of the developed plants including nodule formation, pigment content, yield and resistance of plants to the pathogenicity of *Fusarium solani* (the causal agent of root rot of soybean).
MATERIALS AND METHODS

Target plant. Seeds of soybean (Glycine max L.) Crawford used in this research were kindly supplied by the Legume Crops Research Section, Field Crops Research Institute, Agriculture Research Centre, Giza (Egypt).

Laser. The source of laser radiation was located at the National Institute of Laser-Enhanced Science (NILES), Cairo University, Giza (Egypt). The laser used was a He/Ne gas laser (NEC Corporation, Japan) with a measured power output of 7.3 mW. This emits radiation in a collimated beam (d = 1.3–10.0 mm) with a wavelength of 632.8 nm. The irradiation time of the soybean seeds was 1, 3, 6, and 10 min. The seeds were individually inserted into sterile test tubes and fixed to a rotor. The seeds were exposed to irradiation while the rotor started the motion. The test tubes were 50 mm away from the source of radiation.

Photosensitizers. Three acidic dyes (eosin Y, methylene red, chlorophenol red), one neutral dye (carmine) and three basic dyes (crystal violet, methylene blue, malachite green) were used at a dilution of 1:10^4. The seeds were soaked in the dye for 15 min before irradiation.

Seed germination. The He–Ne-irradiated seeds were germinated on moist filter paper in sterile Petri dishes (10 seeds per dish). Six dishes were used for each treatment. The dishes were kept in an incubator (in darkness) at 25 °C. A seed was considered to have germinated when the radicle emerged. Counts of germinated seeds were made daily for seven successive days. Seed germination was calculated as percentage of total seeds used.

Isolation of fungi. Three replicates for every treatment, each of 8 (about 6.0 g) irradiated and soaked seeds and pretreated with a dye (methylene blue, carmine, methyl red) were used for fungal isolation. The seeds were transferred to about 15 mL 10% sodium chloride-potato dextrose agar medium in 90 mm Petri dishes (two seeds per plate). NaCl was added to the medium to inhibit bacterial growth, retard the development of fast growing fungal species and to prevent seed germination (Mislivec and Bruce 1976). The Petri dishes were incubated at 25 °C. The fungi on the seeds were identified according to Raper and Fennell (1965), Nelson et al. (1983), Barnett and Hunter (1987), Ellis (1971), and Domsch et al. (1980).

Greenhouse experiment. Sandy loam soil infested with 0.1% inoculum of Fusarium solani was distributed into plastic pots (300 mm in diameter). Non-infested soil was used as control. The inoculum was prepared by growing a mycelial disc of the pathogen on sterilized sand corn meal for 10 d. Ten He–Ne-irradiated seeds pretreated with a dye were sown in each pot. Immediately after sowing, 2 mL of Bradyrhizobium japonicum suspension (10^7 cells per mL) was added over each seed. A 10-10-10 (N-P2O5-K2O) fertilizer was added to the soil at 0.28 g/L. A completely randomized experimental design with four replicates was used for each treatment. Pots were irrigated with tap water at regular intervals to maintain the water regime of 70% WHC. After 60 d, stand count was recorded for each treatment. Then the plants were uprooted and washed, and detached nodules were counted and examined for dry mass. The average number of pods per plant in each treatment and the dry mass of the shoot system were recorded. The experiment was initiated in summer 1995 and again in 1996 and the results were averaged.

Pigment content of leaves. After 30 d, samples of discs from the fourth upper leaf were taken at random from individuals of each treatment. The chlorophyll and carotenoid content (referred to as mg per g fresh mass) were determined spectrophotometrically according to Wettstein (1957).

Phenolics and proline contents in roots. Root samples of healthy and infected plants of each treatment were ground separately in porcelain mortar until the tissue was completely homogenized. The samples were filtered and the phenol content was determined colorimetrically with Folin–Denis reagent at 725 nm according to Swain and Hillis (1959). For proline determination, the root sample was homogenized in 10 mL of 3% 5-sulfosalicylic acid. The homogenate was filtered and the filtrate was used for the proline estimation (Bates et al. 1973).

RESULTS

Seed mycoflora. Fig. 1 shows that the samples of soybean seeds were contaminated with eleven fungal species constituting 8960 isolates per 100 g seeds. He–Ne-laser irradiation of seeds for 1 min increased the population of the seed-borne fungi even on the seeds pretreated with the dyes. Extension of the irradiation time by 3 min caused a clear reduction in the number of seed-borne fungi which