Influence of Laser Treatment on the Growth of Sprouts of Different Wheat Varieties

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***Abstract*—**Cereals are considered as a strategic product in human life and their demand is increasing with the growth of world population. Increasing wheat production is important for the country. One of the ways to solve the problem is to develop and implement new, environmentally and economically acceptable technologies. Such technologies include pre-sowing treatment of seed with a laser and associative nitrogen-fixing bacteria - *Azospirillum brasilense.* In the region there are the wheat varieties - Dika and Lomtagora, which are among the most common in Georgia. Dika is a frost-resistant wheat, with a high ability to adapt to the environment, resistant to falling and it is sown in highlands. Lomtagora 126 differs with its winter and drought resistance, and it has a great ability to germinate. Lomtagora is characterized by a strong root system and a high budding capacity. It is an early variety, fall-resistant, easy to thresh and suitable for mechanized harvesting with large and red grains. This paper presents some preliminary experimental results where a continuous CO_2 laser with a power of 25-40 W was used to radiate grains at a flow rate of 10 and 15 cm/sec. The treatment was carried out on grains of the *Triticum aestivum* L. var. *Lutescens* (local variety name - Lomtagora 126), and *Triticum carthlicum* Nevski (local variety name - Dika). Here the grains were treated with *A. brasilense* isolate (10^8-10^9 CFU/ml), which was isolated from the rhizosphere of wheat. It was observed that the germination of the wheat was not significantly influenced by either laser or bacteria treatment. The results of our research show that combined treatment with laser and *A. brasilense* significantly influenced the germination of wheat. In the case of the Lomtagora 126 variety, grains were exposed to the beam on a speed of 10 cm/sec, only slightly improved the growth for 38-day seedlings, in case of exposition of grains with a speed of 15 cm/sec - by 23%. Treatment of seeds with *A. brasilense* in both exposed and non-exposed variants led to an improvement in the growth of seedlings, with *A. brasilense* alone - by 22%, and with combined treatment of grains - by 29%. In the case of the Dika variety, only exposure led to growth by 8-9%, and the combined treatment - by 10-15%, in comparison with the control variant. Superior effect on growth of seedlings of different varieties was achieved with the combinations of laser treatment on grains in a beam of 15 cm/sec (radiation power 30-40 W) and in addition of *A. brasilense* - nitrogen fixing bacteria. Therefore, this is a promising application of *A. brasilense* as active agents of bacterial fertilizers due to their ability of molecular nitrogen fixation in cereals in combination with laser irradiation: choosing a proper strain gives a good ability to colonize roots of agricultural crops, providing a high nitrogen-fixing ability and the ability to mobilize soil phosphorus, and laser treatment stimulates natural processes occurring in plant cells, will increase the yield.

***Keywords*—**Laser treatment, *Azospirillum brasilense,* seeds, wheat varieties, Lomtagora, Dika.

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I. Introduction

GEORGIA is one of the oldest centres of wheat cultivation and it is distinguished by its large variety of species and their diversity. Georgia is characterized by a remarkable diversity of the domesticated wheat, and five wheat species found in Georgia, have their originate in Georgia and are local endemics. These wheat species are characterized by their taxonomic identity, morphology and the role they played in the ancient agriculture of Georgia. They are also important breeding material as they contain genes for local adaptation from the diversity of wheat that has been created in Georgia over the centuries. Georgia is the only country in the world, where as many as 15 species of wheat are harvested out of 20 recognized worldwide. There are more than 150 subspecies, forms and endemic varieties, along with other 40 subspecies of common wheat [1].

Georgia is characterized by the highest diversity and endemism of ancient hulled wheats, highest diversity of free-threshing wheats, and the presence of all tetraploid wheats and a special endemic *T. timopheevii - T. zhukovskyi* lineage of AAGG-AAGGAA genomes [2]. Archeological excavations of the ancient Neolithic farming society (Shulaveri-Shomu complex) confirmed existence of nine species of wheat in Arukhlo and eight domesticated wheat species in both, Shulaveri and Khramis Didi Gora (south-east Georgia) dated back some thousand years BC [3]. The cultivation of wheat by Georgians is mentioned in the works of Greek historians Herodotus and Xenophon [4]. The names of the ancient Georgian wheats such as Zanduri, Makha and others were first mentioned in Georgian written sources as early as the 5th century AD [4].

Increasing wheat production is an important issue on a world scale. At the present time, mineral fertilizers and pesticides are widely used to obtain high yields of agricultural crops, which creates serious environmental problems. Every year the world uses over 200 million metric tons of mineral fertilizers to increase crop yields, of which only 40-55% is used for direct purpose of growth, yet the remaining 45-60% is washed out and evaporated, causing global pollution and environmental damage [5].

In the vast majorities of countries, cereals are considered as a strategic and important product. Although Georgia is considered one of the main centres of wheat origin and domestication [6], the country satisfies only 15-20% of grain demand through its own production [7]. One of the ways to solve the problem is the development and implementation of new environmentally friendly and economically acceptable technologies. This work suggests a technique that includes pre-sowing treatment of the seed with a laser and associative nitrogen-fixing bacteria of the species *Azospirillum brasilense.*

Laser irradiation stimulates natural processes occurring in plant cells, leading to strong plant growth, enhancing the immunity, and destroying the pathogenic microorganisms which cause various plant diseases; with the laser processing of grain, the biological viability of the grain is increased and the yield rose by 25-30% [8], [9]. The inoculation of seeds with *A. brasilense* is an alternative technology showing positive results in increasing of agronomic indicators and wheat yield [10].

The bacteria of the genus *Azospirillum* are natural inhabitants of the root system of many herbaceous plants, and they have the ability to stimulate the growth and development of the host plant [11]. The presence of nitrogen-fixing microorganisms in rhizosphere of plants leads to a better assimilation of nutrients such as nitrogen and phosphorus, and thereby increasing ability of plants to adapt to variable environmental conditions. The majority species of the genus *Azospirillum* secrete plant hormones that affect growth of the plant root. The affected roots often have more branches and fine root hairs, which can help plants absorb water and nutrients more efficiently. In addition, *Azospirillum* produce antioxidants protecting roots of the plants from drought and flood stress [12], [13].

There is no doubt that nitrogen-fixing organisms create the biological reserves of the nitrogen in soil and so play an important role in obtaining environmentally friendly products, increase the yield and quality of grain crops. Application of *A. brasilense* enabled Brazil to become the second largest producer of cereals without the application of nitrogen fertilizers [14].

Abiotic factors mainly affect development of the cereal seeds, they also affect maturation of crops and synchronization flowering, while biotic factors mainly affect nutritional quality and wheat disease [15], [16].

The presented work aimed to study the joint pre-sowing treatment of wheat seeds with laser irradiation and *A. brasilense*, for the growth of seedlings of different varieties of wheat.

II. Methodology

The objects of the study were wheats of Georgian varieties: Dika (*Triticum carthlicum* Nevski) and Lomtagora 126 (*Triticum aestivum* L. var. *Lutescens*), which are among the most widespread varieties in Georgia.

The variety Dika is a frost-resistant wheat, with a high ability of adaption to the environment and resistant to falling. Therefore, it is sown in highlands. The advantages of Dika are stipulated by its persistent immunity to fungal diseases; the grains of Dika are rich in protein and lysine [17].

The variety Lomtagora 126 is distinguished by winter- and drought-resistance; it has a great ability for germination, characterized by a strong root system and a high budding capacity. It is an early variety, resistant to falling, easy to thresh and suitable for mechanized harvesting. Its grains are large, red coloured and the plant is moderately resistant to fungal diseases [18], [19].

To irradiate wheat seeds in the experiments, a continuous CO_2 laser at 2 irradiation angles was used with the following parameters: radiation power 25-40 W, grain flow rate 10 and 15 cm/sec.

Laser treated and untreated wheat grains were inoculated with *A. brasilense* isolate (10^8-10^9 CFU/ml), which was isolated by the research team from the rhizosphere of wheat (Shiraki valley, the region of Dedophlistskaro, Georgia). The inoculation was performed during 1 hour using the method described by [17]. The wheat grains were preliminarily soaked in water for 1 hour, before the treatment of grains with *A. brasilense* suspension. Different variants were used to process the wheat (100 grains for each variant of each variety of wheat), which were subsequently placed for germination in a temperature-controlled environment at 25 °C. The experimental approaches are shown in Table I.

TABLE I
EXPERIMENT APPROACHES FOR TREATING VARIETIES OF THE WHEAT

Lomtagora 126		Dika	
№	Variant	№	Variant
I-1	Original seed untreated (control)	I-1	Original seed untreated (control)
I-2	Irradiated (10cm/sec)	I-2	Irradiated (10 cm/sec)
I-3	Irradiated (15cm/sec)	I-3	Irradiated (15 cm/sec)
I-4	untreated + *A. brasilense*	I-4	untreated + *A. brasilense*
I-5	Irradiated (10 cm/sec) + *A. brasilense*	I-5	Irradiated (10 cm/sec) + *A. brasilense*
I-6	Irradiated (15 cm/sec) + *A. brasilense*	I-6	Irradiated (15 cm/sec) + *A. brasilense*

III. Results and Discussion

On the third day of the experiment, the number of germinated seeds and the length of the primary seedlings of the embryonic roots and stems were counted. The results are given in Table II and Fig. 1. It is observed that in both treated and control sets, the degree of germination is almost the same (98-100%), but the rate of germination was different in terms of roots length.

As shown from Table II and Fig. 1, the germination rate of Dika is almost the double of the Lomtagora according to the length of roots and stalks. For further investigation, the 3-day-old seedlings were transferred to the soil in plastic containers 16 × 11 cm in size. The aerial parts of the seedlings were measured on the 3rd, 14th, 21st, 32nd and 38th days. The measurement was carried out on 10 seedlings by random sampling. The results revealing the influence of different processing methods on the growth and development of Lomtagora and Dika are illustrated in Figs. 2 (a) and (b), where the data are the arithmetic mean values of the measurement data.

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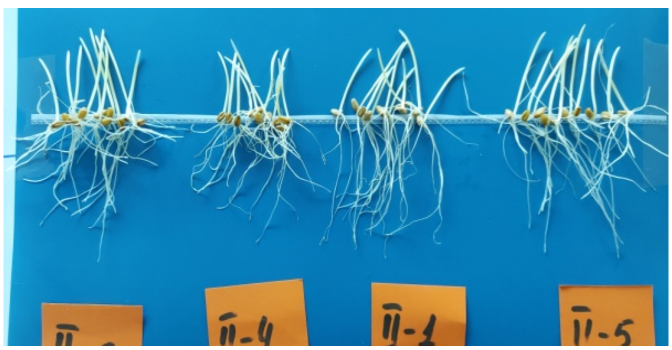
TABLE II
LENGTH OF THE ROOTS OF 3-DAY SEEDLINGS

Lomtagora	Length of the root, mm	Dika	Length of the root, mm
I-1	24.2	II-1	58.5
I-2	33.0	II-2	68.2
I-3	28.0	II-3	68.9
I-4	20,8	II-4	41.2
I-5	21.1	II-5	44.7
I-6	21.4	II-6	41.2

(a) Lomtagora



(b) Dika

Fig. 1 3-day seedlings of different varieties of the wheat

On the 14th day of development, the height of seedlings of the Lomtagora in untreated (control) (I-1) set is 7 mm less than the irradiated set (I-2); and 5 mm less compared to the set treated with both laser irradiation and *A. brasilense* (I-5 and I-6) (Fig. 2 (a)). For the unirradiated set and the set treated only with *A. brasilense* there was almost no difference. According to the results of measurements of 38-day seedlings, the difference between the control and the treated versions increased. The difference between the control and the best irradiated sets (I-4) was 53 mm, and between the control and the set with combined pre-sowing treatment with both laser irradiation and *A. brasilense* (I-6) was 65 mm.

Fig. 2 (b) shows the effect of different treatment for the Dika variety on development of seedlings. It is observed from this figure that, after three days of germination, in almost all treated sets, 7 to 10 mm improvement in growth of the aboveground part of seedlings was achieved in comparison to the control set. The difference between the height of shoots in the control and treated variants grows gradually and according to the data of 38-day-old shoots, the irradiated sets exceeded the control group by 20 mm in height. Equally, the set treated with both laser irradiation and *A. brasilense* surpassed the control by 33 mm in height. It should be noted that the development of seedlings in irradiated seeds with *A. brasilense* and without it is practically the same.

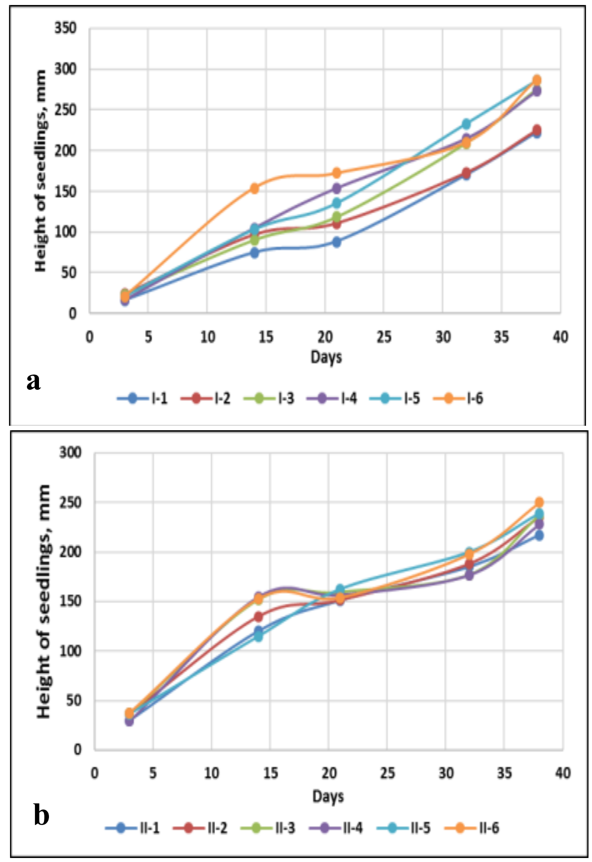
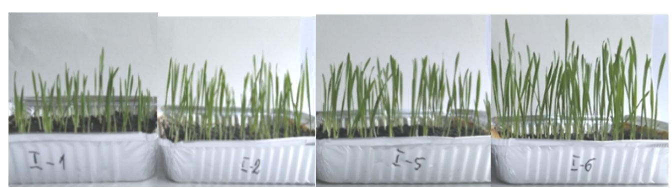
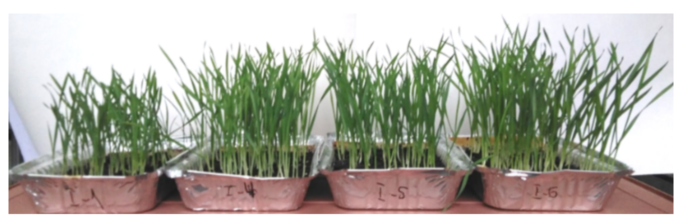


Fig. 2 Influence of different processing methods on the growth: (a) Lomtagora; (b) Dika



(a)



(b)

Fig. 3 Lomtagora seedlings: (a) 14 day, (b) 21 day; I-1 - control, I-4 – unirradiated + *A. brazilense*; I-5 - irradiated (10 cm/sec) + *A. brasilense;* I-6 - irradiated (15 cm/sec) + *A. brasilense*

The results show that treatment of wheat with both laser power and with *A. brasilense* improves the growth process in both wheat varieties. A significant improvement was obtained

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in comparison with the control set in all treated groups.

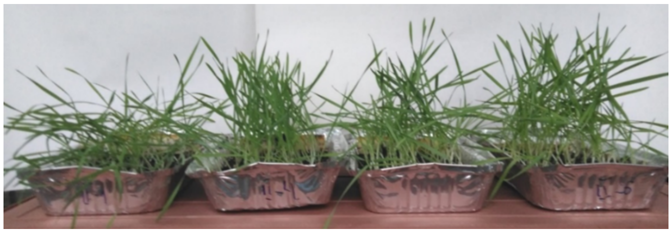

Fig. 4 21-dayes Dika seedlings: II-1 - control, II-4 – untreated + *A. brasilense*; II-5 - irradiated (10 cm/sec) + *A. brasilense*; II-6 - irradiated (15 cm/sec) + *A. brasilense*

IV. Conclusion

The results of this investigation have shown that the germination of wheat is significantly influenced by laser treatment and the treatment with bacteria.

For the Lomtagora 126 variety, the irradiation at a speed of 10 cm/sec, the growth for 38-day seedlings was slightly improved, however with the irradiation at the speed of 15 cm/sec an improvement of 23% was obtained.

The treatment of seeds with *A. brasilense* in both irradiated and non-irradiated versions caused an improvement in growth of seedlings, but in the case of treatment with *Azospirillum* alone, an increase of 22% in the growth was observed. With the combined treatment of seeds with *Azospirillum* and irradiation – the improvement reached 29%.

For the Dika, the treatment of seeds only with irradiation led to an increase in the growth of seedlings by 8-9%; in case of combined treatment of seeds with both *A. brasilense* and irradiation the seedlings' growth improvement by 10-15% was recorded, in comparison with the control set.

The best effect on the growth of seedlings of wheat seeds was obtained with the method of combined laser treatment of grains in a flow with speed 15 cm/sec (radiation power 30-40 W) + *A. brasilense*. Therefore, the promising use of a *A. brasilense* as an active agent of bacterial fertilizer is stipulated by their ability to fix molecular nitrogen in the plants. Consequently, the selection of an adequate strain, with a good ability to colonize the roots of agricultural crops, which provides a high nitrogen-fixing ability along with a good absorption of the soil phosphorus are important factors which when synergized with laser irradiation, lead to an active stimulation of natural processes in plant cells, which in it turn promote healthy growth of plant with the subsequent significant increase in the yield of cereals.

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