INVESTIGATION OF BIOSTIMULATION EFFECTS ON GERMINATION AND SEEDLING GROWTH OF SOME CROP PLANT SPECIES

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Dedicated to Acad. Bogdan C. Simionescu
on the occasion of his 70th anniversary

The present work investigated the effects of a combined biostimulation treatment of some agricultural crop seeds on their germination and seedling growth. The biostimulation consisted of two steps: a pretreatment of the seeds by immersion into a clay-lignocellulose solution and their laser exposure at 532 nm wavelength. Three agricultural crop species were selected for the treatment, namely, wheat (*Triticum aestivum* L.), corn (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.), which can be considered as key staple crops for most of the global human population. The study revealed that the crop seeds subjected to the combined treatment, involving 7 min laser exposure, presented the shortest germination period among all the sample series, especially as regards the beans, which exhibited a 50% reduction of the germination period from 120 h (control) to 60 h. Also, the combined treatment (with 7 min irradiation) induced obvious growth stimulation, leading to enhanced seedling growth rates, specifically, of 71.8% for wheat, of 54.5% for beans and of 47.61% for corn, as compared to the control samples measured 72 h after planting.

Keywords: laser exposure, growth stimulation, agricultural crops, ecological agriculture

INTRODUCTION

The technological revolution, which lies at the basis of modern agricultural development, has had a tremendous impact on the environment, through land and water pollution, through chemical residues affecting plant and animal life, with harmful long-term effects, which, in turn, seriously impair human health.[1] Increasing environmental awareness, in recent years, has triggered a turn towards more sustainable development in all the areas of human activity, including the agricultural sector. Sustainable agriculture aims at the conservation of resources, such as soil and water, while minimising wastes and environmental impact.[2]

In line with environmental considerations, the treatment of agricultural crop seeds by non-polluting substances and techniques that would bring qualitative and quantitative increases in agricultural production has attracted the researchers' interest. Research works in the field of environmental physics have demonstrated that biophysical techniques employed in agriculture can ensure average increases in seed germination of 20-35%, in root mass up to 24%, in leaf/stem mass between 10 and 45%, increased yield in the ranges of 10-50%, compared to plants grown under normal conditions, as well as increased resistance against diseases and pests.[3-13]

Laser treatment applied with the objective of stimulating seed germination and plant growth has already been reported in the literature. The use of laser radiation in agriculture for biostimulating seeds, seedlings and young plants is based on the synergism between the monochromatic polarized laser beam and the photoreceptors that absorb it, which triggers a number of biological reactions. Laser radiation is absorbed into biological tissues[8] and, in accordance with the Einstein-Stark law of photochemical equivalence, for every photon absorbed, a particle, such as an atom, molecule or free radical, is activated by a photochemical reaction.[12] It is a primary cellular reaction, which is followed by a secondary systemic one.[9] Low-intensity laser radiation (not exceeding 100 mW/cm^2) modifies the potential energy of the molecules, which will influence the kinetics of biochemical processes.[10,11]

A number of published studies[4,5,8] have demonstrated that laser treatment of crop seeds contributed to higher germination rate, plant height and weight. For instance, the work of J. Podleśny,[5] focusing on He-Ne laser treatment of beans, revealed that seed irradiation significantly affected the rate and the amount of dry matter accumulation of particular parts of the plants and led to accelerated germination and maturity. Moreover, the most significant results were obtained after repeated laser treatment. In addition, it has been highlighted in the literature that laser treatment can be considered a safe technique, as it does not alter the molecular bonds, has no mutagenic effect and does not cause marked morphological changes in the structure of the tissue.[10]

On the other hand, a study by Hernandez *et al.*[4] established that laser treatment of different agricultural crop seeds can have either beneficial biostimulation effects or negative ones, altering plant metabolism, or can have no effect at all on plant development, as a function of plant species and the treatment conditions applied. In their review, the authors concluded that it is necessary to investigate the parameters of laser light irradiation to produce favourable effects of biostimulation according to the seed itself and the environmental conditions. In addition, not all the crop species studied are sensitive to the same stimulus, the response being determined by the biological characteristics of each species and the applied doses. Thus, a correct use of laser biostimulation requires a preliminary experimental investigation in order to establish the optimum ranges, which will vary as a function of plant characteristics, laser intensity and time of exposure. Otherwise said, in order to obtain beneficial results, it is important to establish optimum values for each crop species.

In the search of non-polluting techniques to improve crop yields, researchers have reported that the addition of different varieties of clay, in optimum amounts, can serve as fertilizer, contributing to improved soil quality and to enhanced quality and quantity of biomass production.[14-16] For example, D. Ehret *et al.*[16] investigated the effect of adding clay to soilless media, specifically, to a sawdust medium, on the production of greenhouse vegetable and flower crops. The authors applied daily sterilized, non-swelling chlorite mica clay as a suspension in the nutrient solution. As a result, the number of marketable cucumber (*Cucumis sativus* L.) fruit increased significantly with increasing concentration of clay.

The same trend was observed in the trials with potted geraniums and hybrid impatiens: in both species, the number of flowers increased significantly with increasing clay concentration, and the size (fresh and dry mass) of the geraniums also increased.

Wheat straw is an agricultural residue resulting from wheat crop cultivation and harvesting, and is mainly composed of three polymers: cellulose, hemicelluloses and lignin. This material is commonly regarded as a waste and, in some areas, it is incinerated in the fields, which represents a serious environmental burden. However, due to its high availability and low cost, wheat straw, as well as other agricultural residues, has come into the researchers' focus as a raw material that can be valorised in a wide variety of possible applications, to name just a few, pulp and paper production,[17-19] biocomposites,[20] removal of metal ions from industrial wastewater effluents,[21] ethanol,[22] and solid biofuel production[23] *etc*.

From a sustainable agriculture viewpoint, agricultural residues are important due to their decisive role in maintaining the proportion of organic matter and in the synthesis of the compounds that form the humus. Specifically, wheat straw should be taken into consideration due to its nutritional value, which may turn out to have a beneficial fertilising effect on the soil and on plant growth.[24] Thus, wheat straw contains some of all essential plant nutrients, but nitrogen (N), phosphorus (P) and potassium (K) are the major ones found in sufficient amounts. Calcium and magnesium are also found in similar amounts. Due to its nutritional value, wheat straw is often considered for incorporation into the soil to improve soil fertility.[25]

The present study investigated the effects of a biostimulation treatment on seeds of three major agricultural crop species: wheat (*Triticum aestivum* L.), corn (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.), on their germination and seedling growth.

The biostimulation was carried out in two steps: first, a pretreatment of the seeds by immersion into a clay-lignocellulose solution, and second, their laser treatment at 532 nm wavelength.

EXPERIMENTAL

Wheat straw

The wheat straw raw material was collected manually in 2017 from a local farm. The raw material was first air-dried to atmospheric equilibrium and then ground in a blender and sieved to obtain a particle size below 1 mm. The thus-obtained powder was added to a vessel containing hot water (80 °C) and kept at room temperature for 24 hours. Then, the slurry was boiled for 3 hours under normal pressure. The obtained lignocellulose was air dried and dispersed in a clay solution prepared as described below.

Clay preparation

The clay used in the present study was obtained from an open pit in the central region of the Republic of Moldova, from uniformly coloured layers. The clay was dissolved in water at room temperature and then passed through a sieve to separate coarse particles. The container was then allowed to stand for several hours until the suspended clay particles completely settled down. After this, the water was drawn off and the residuum was left in the air until complete dehydration.

The clay sample was studied by X-ray powder diffraction on an Empyrean X-ray diffractometer in order to assess its composition.

The thus-obtained clay sample was then used to prepare a clay solution. The clay solution was mixed with lignocellulose in a ratio of 1:1 and then passed through a Fritsch Pulverisette mill to achieve uniform mixing and size reduction down to below one micron.

The thus-prepared mixture was used to pretreat the seeds of wheat, corn and beans through immersion. At the end of this procedure, it was ensured that a 0.2-0.5 mm thick layer of the mixture adhered to the seeds.

Seed preparation

Wheat (*Triticum aestivum* L.), corn (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.) were chosen as representative crop species for investigating their germination and seedling growth. 40 seeds of each species were selected for the treatment. The seeds were obtained from a local farm and were weighed and maintained in a dark, electrically shielded chamber for 24 h, at a relative humidity of 35% and a temperature of 18 °C. Then, avoiding any exposure to light, the seeds were pretreated by immersion into the lignocellulose/clay solution.

The seeds of all four species under study were divided into four series and were treated in accordance with the experimental conditions listed in Table 1.

After the treatment, the seeds were placed in separate cells, were soaked in water and the dynamics of their germination was observed. After 24 h, the seeds were removed from the water, wiped off to remove excess moisture and weighed to determine the variation in their weight related to their initial weight. As soon as deviations from the characteristic relation were observed, the measurements were done every 4 hours.

After germination, the seeds were planted and maintained under constant conditions: a temperature of 25 ± 1 °C and humidity of 65 ± 3%. For assessing early plant development, the height of the seedling stems was determined at certain time intervals.

Laser exposure

The seeds were subjected to laser exposure at a wavelength of 532 nm, using laboratory-made equipment. The equipment was fully described in an earlier study.[26] The laser equipment was provided with a conveyor belt and a vibrating device capable of communicating low-amplitude oscillations to the band at different frequencies. This allows the seeds to be repositioned in the path of the laser beam at any moment. For achieving the laser pulse irradiation of the seeds, the radiation beam passes through a system of two mirrors that oscillate in two mutually perpendicular directions, which allows obtaining Lissajous figures on the plane of the conveyor belt.[26]

Table 1
Experimental conditions used in the study

Sample series	Pretreatment solution	Duration of laser treatment, min
l1	-	-
l2	+	-
l3	+	5
l4	+	7

l1 – control series of wheat, corn and bean samples; l2, l3 and l4 – wheat, corn and bean seeds subjected to the treatment

RESULTS AND DISCUSSION

Clay analysis

For assessing the composition of the clay sample, it was studied on an Empyrean X-ray diffractometer. The obtained X-ray diffraction pattern is presented in Figure 1. The XRD pattern of the clay sample reveals the clear presence of SiO_2 (56.33-79.89% with a particle size > 0.01

mm), and traces of Na_2SO_4, Fe_2O_3, Al_6SiO_{13}, $MgSiO_2$, $(Ca, Na)(SiAl)_4O_8$ *etc*. Thus, the clay corresponds to the general compositional profile of the clays that prevail in the region, which are reported to consist of SiO_2 in the range from 57.19 to 70.18%, $A1_20_3$ ranging between 13.66 and 16.13%, Fe_20_3 in the range of 1.75-6.26%, and small amounts of MgO, K_2O, Na_2O, TiO_2 *etc*.[14]

It is known that conventional agriculture focuses on fertilizers containing the three macronutrients (nitrogen (N), phosphorus (P), and potassium (K)) largely required by plants. However, it is also known that the plants cannot reach full potential without some **s**econdary nutrients, such as calcium, magnesium and sulfur, as well as micronutrients, such as boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). Even though the quantity required is very small, these are still crucial for optimal plant function. Such secondary nutrients and micronutrients can be found in clays, and specifically, as has been noted above, some of them are present in our clay sample. Therefore, we can hypothesize that the pretreatment of the seeds under investigation with the clay mixture will have a beneficial effect on their germination, as well as on plant development.

Seed mass and germination

Hygroscopicity is one of the basic properties of seeds, which is essential for their development. Therefore, the seeds were soaked in water after the treatment, and in order to determine the variation in their weight related to their initial weight, after certain time periods, they were removed from the water, wiped off to remove excess moisture and weighed. The first weight gain was measured after 24 h, and when deviations from the characteristic relation were observed, the measurements were done every 4 hours. Figure 2 illustrates the relation between the weight variation of corn, bean and wheat seeds over time and the treatment to which the seeds were subjected.

It is obvious from Figure 2 that during the first 24 hours of water immersion, the seeds absorbed the highest volume of water, leading to the highest increase in their weight. Due to the endosmotic current, the water has penetrated osmotically into the vacuoles of the cells, leading to a significant increase in the cell volume. In this situation, the pectocellulosic cell membrane is stretched (by 10-100%) and the cell is in a state of turgescence. When it reaches maximum turgidity, the endosmosis process ceases, even if the vacuolar juice is still hypertonic to the surrounding solution.

It has been established that the minimum points on the graph coincide with the onset of the germination process, which also varies as a function of the treatment applied to the seeds. Thus, it may be noted in Figure 2 (a) that the corn seeds subjected to the combined treatment had the fastest response to the treatment with the shortest germination period – of 36 hours – related to the control seeds. Moreover, when considering weight variation, corn seeds seem to be more sensitive to all the treatments, compared to the other species used in the present study (Fig. 2 b, c).

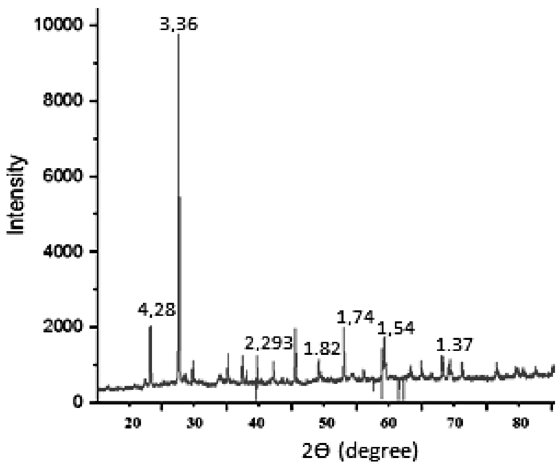

Figure 1: XRD pattern of the clay sample

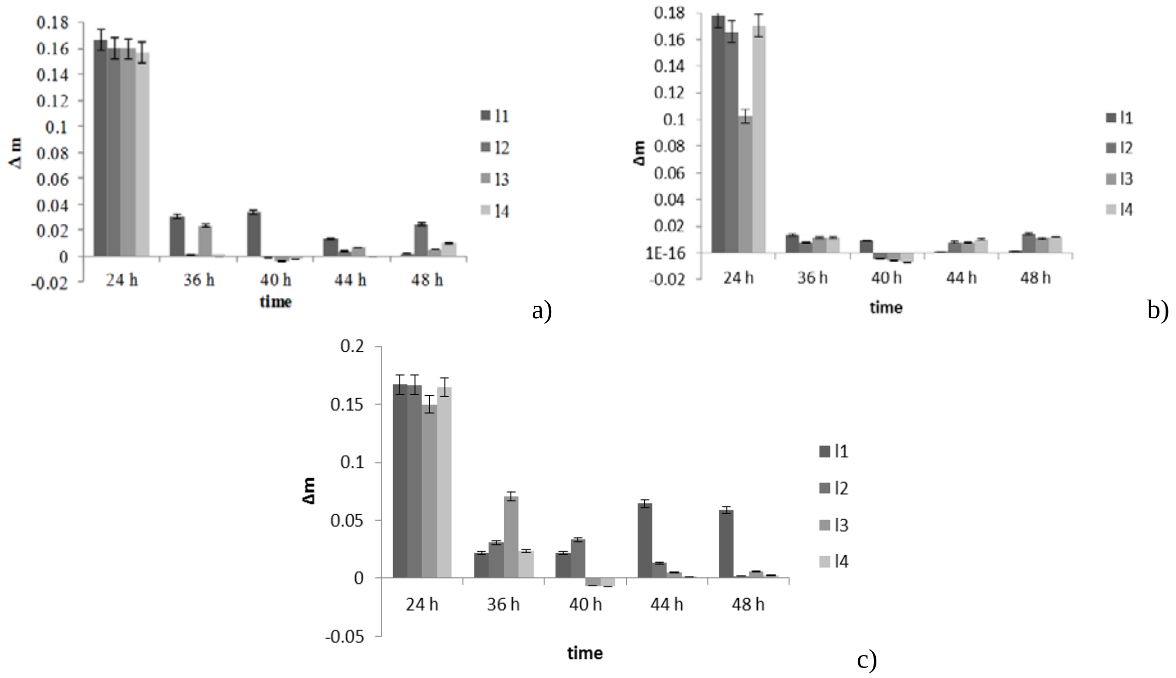

Figure 2: Weight variation of corn (a), bean (b) and wheat (c) seeds as a function of germination time and treatment conditions

Table 2
Germination rate (%) of the seeds as a function of the treatment applied

Species \ Series of samples	l1	l2	l3	l4
Corn (*Zea mays* L.)	40	75	80	80
Bean (*Phaseolus vulgaris* L)	50	85	100	100
Wheat (*Triticum aestivum* L.)	50	75	80	80

In addition, considering the effects induced by the different treatments, it may be concluded that the combined treatment involving 7 min laser treatment contributed to the most significant results.

Table 2 lists the germination rate of the seeds under study assessed after a germination period of 84 h as a function of the treatment conditions, while Figure 3 presents the evolution of the germination rate over time.

Both the data in Table 2 and the graphs in Figure 3 clearly indicate that the beans had the highest and the fastest germination rate among the species studied. Thus, when considering the different treatments applied, the beans exhibited a much higher germination rate, compared to the control, for series l2 – the seeds subjected only to the pretreatment by immersion into the clay-lignocellulose solution, and a 100% germination rate for both series subjected to the full combined treatment involving different durations of radiation exposure (5 and 7 min – l3 and l4, respectively).

In addition, it may be easily remarked that the combined treatment yielded the highest results for all the species, which can be explained by a boost of the physiological processes triggered by the irradiation, resulting in a shorter germination time and an enhanced production potential. Moreover, the combined treatment involving a longer irradiation period led to higher results, thus series l4 exhibited the earliest development in all the species. As expected, not all the species are similarly responsive to the stimuli. However, in terms of germination time, the trend was the same for all the species: l4 with the minimum germination period, followed in ascending order by l3, l2 and, finally, l1. Thus, the wheat and corn seeds did not achieve the maximum germination rate, compared to the beans, however, they also showed the beneficial effects of the treatments, and the germination rates of l4, l3 and l2 significantly exceeded that of l1 (control) (Fig. 3 a, c).

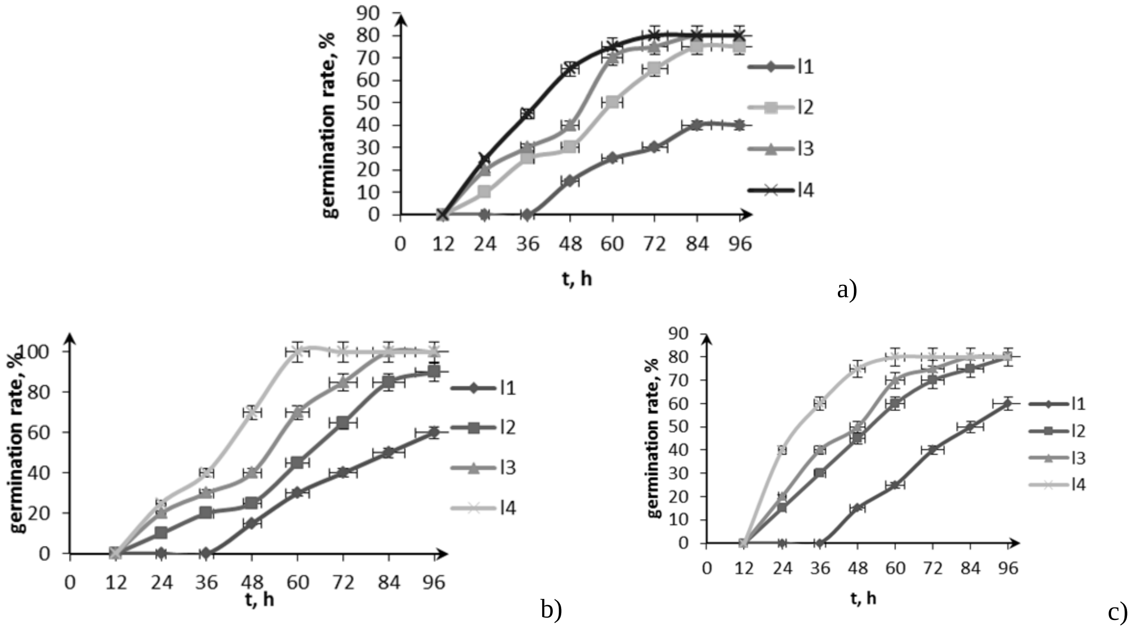

Figure 3: Germination rate of corn (a), beans (b) and wheat (c) as a function of germination time

Compared to the control, the seeds of l4 for all the species exhibited an around 50% earlier germination and development, but only the beans achieved the maximum germination rate for a decreased germination time, which was reduced from 120 h (control) to 60 h. Thus, our results are in agreement with those reported in the literature, supporting the fact that seed irradiation leads to accelerated germination.[4,5,8]

In addition, it is worth noting that series l2 (subjected only to the pretreatment) had a significantly higher germination rate for all the three species, compared to their respective control seeds. Such a great difference can be explained by the nutritional supplement provided by the pretreatment in the clay-lignocellulose mixture. It has been noted by other authors as well that wheat straw contains some of the essential plant nutrients, the major ones being nitrogen (N), phosphorus (P) and potassium (K)[25] – the macronutrients necessary for seed germination and plant development, while the clay in the pretreatment mixture provided the micronutrients, which are not less important. Thus, altogether, the pretreatment mixture served as a complete fertilizer, with a significant effect on the germination rate.

Seedling growth

For assessing early plant development, the seedlings were planted and, for the sake of comparison, all the seedlings were maintained under constant conditions: a temperature of 25 ± 1 °C and humidity of 65 ± 3%. The height of the seedling stems was determined at certain time intervals. Figure 4 displays the dynamics of seedling growth for all the three species analysed as a function of the treatment applied. Similarly with the evolution of germination rate discussed above, it may be observed that series l4 again yielded the highest results. The other series of samples presented less significant responses with regard to seedling height, still they reached values that were higher than the results for the control by approximately 25-35%.

The results illustrated in Figure 4 indicate that the treatments applied had a stimulating effect on seedling growth. Similarly to the situation discussed above regarding germination, the data in Figure 4 reveal that series l4 (the seeds subjected to the combined treatment with 7 min irradiation) showed the best results in terms of seedling height for all the crop species studied. The obvious stimulation effect of the combined treatment (l4) resulted in greater values for seedling height by 71.8% for wheat, 54.5% for beans and by 47.61% for corn, as compared to their respective controls, measured 72 h after planting the germinated seeds. It may be easily noted that although all the samples had a positive response to the treatments, there are quite significant differences in seedling height as a function of species, which can be explained by the specific biological characteristics of each species. Thus, the wheat seeds achieved the fastest seedling growth, followed by the beans and then by the corn seeds. On the other hand, it appears that the irradiation dose had the greatest impact on the wheat, as the seedling height for the beans and the corn presented very close values for l3 and l4.

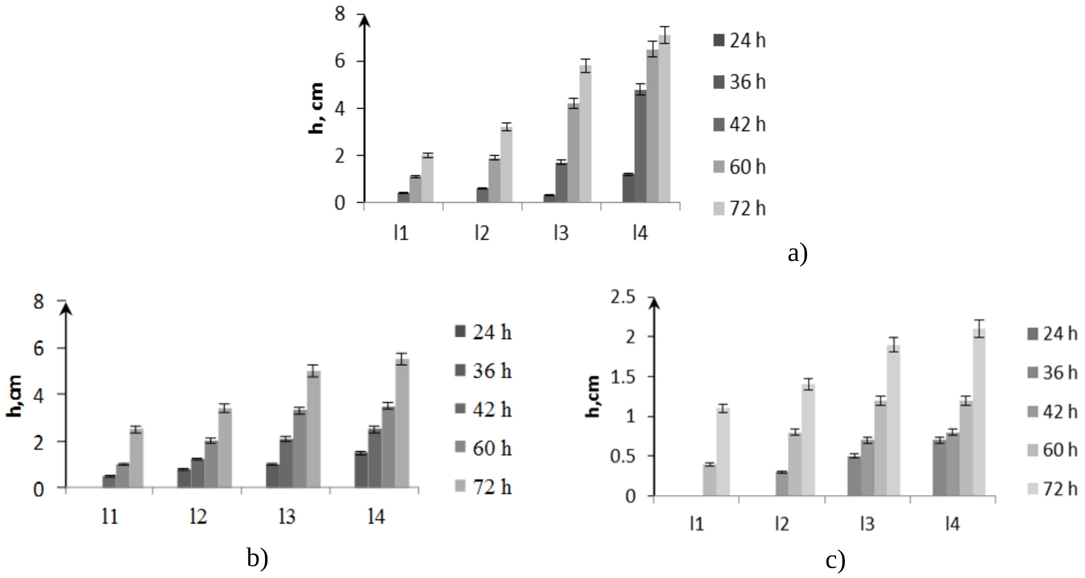

Figure 4: Seedling growth dynamics for wheat (a), beans (b) and corn (c) as a function of the treatment applied

Also, it may be noted that series l2, the seeds subjected only to the pretreatment by immersion into the clay-lignocellulose mixture, exhibited better performance regarding seedling height, compared to the control. Such an improvement confirms the fertilizing effect of this treatment, providing the nutritional input that boosted the early development of the plantlets. Although the treatment had beneficial effects on the development of all the species, it is clear that the most responsive to it were the wheat seeds, exhibiting a 71.8% enhancement in plantlet height, compared to the control, measured 72 h after planting. However, it is obvious that the combined biostimulation (pretreatment by immersion into the clay-lignocellulose mixture + laser irradiation) influences early plant development to a much higher extent than the pretreatment alone. Thus, the performance of l2 for all the species was notably weaker than that of l3 and especially l4.

CONCLUSION

The present study examined the effects of biostimulating seeds of *Triticum aestivum* L., *Zea mays* L. and *Phaseolus vulgaris* L. through a combined treatment, involving their immersion into a clay-lignocellulose solution and laser exposure at 532 nm wavelength, on their germination and seedling growth. The results obtained demonstrated that the combined treatment applied significantly improved the germination rate and shortened the germination time, as well as boosted the growth of the plantlets at the early stages of ontogenesis. Thus, the crop seeds subjected to the combined treatment (7 min laser exposure – l4) presented the shortest germination period among all the sample series. This effect was most obvious in the case of the beans, whose germination period was shortened by 50% from 120 h (for the control samples) to 60 h. Moreover, the stimulation effects could be further detected in the early development of the plantlets. Again, the combined treatment (with 7 min irradiation) induced enhanced seedling growth rates, specifically, of 71.8% for wheat, of 54.5% for beans and of 47.61% for corn, as compared to the control samples measured 72 h after the treatment.

The study confirmed the dependence of the biostimulation effects on the species and on the dose applied. Thus, in our study, only the beans achieved full 100% germination after a germination period of 60 h for series l4 and after 84 h for l3, while the other crop species reached a germination rate of only 80% after 84 h of germination time. As regards the radiation dose applied, the data on both germination and seedling growth reveal that series l4 achieve higher values for all the three species, which may suggest that 7 min irradiation time is more suitable for biostimulation.

Another finding of the study is that the pretreatment applied, involving the immersion of the seeds into a clay-lignocellulose solution, already led to an improvement in the germination time and rate, as well as in seedling height, for all the species studied, compared to their respective control. This can be explained by the nutritional supplement provided by the clay-lignocellulose mixture, offering both the macronutrients and the micronutrients necessary for plant development and, hence, it may be concluded that it served as a complete fertilizer for the young plantlets.

To conclude, the treatment investigated in the present study show very promising results and could serve as an eco-friendly and non-polluting technique to shorten the germination period and to enhance plant growth, with no negative impact on the environment. It could be used as a pre-sowing seed treatment alternative to avoid chemical fertilizers, which are both costly and harmful to the environment, and could thus have practical application in the agricultural sector. However, the mechanisms of laser biostimulation are not yet entirely understood and further research is necessary to obtain deeper insights into the effects of laser irradiation on all the stages of plant development, including fructification, as well as the nutritional quality of the food obtained from irradiated seeds.

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