Bulletin UASVM Horticulture, 70(1)/2013, 137-141
Print ISSN 1843-5254; Electronic ISSN 1843-5394

Protective Effect Exerted by the AD Type Bio – Phyto – Dinamic Modulators on *Arabidopsis thaliana* Species Exposed to Stress Factors

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Abstract. *Arabidopsis thaliana* is a small plant member of the mustard (*Brassicaceae)* family, used as a model organism in plant biology. The paper aims to observe the evolution of *Arabidopsis* plant in all phenological stages, starting with germination and ending with seed growth and development under the action of AD type bio-phyto-dynamic modulators. After the full growth of plant the research continues pursuing the possible changes relating to plant genome. Seeds of *Arabidopsis thaliana* were used, divided in three variants. Two with DEA and DIEE AD type bio-phyto-dynamic modulators attached and the third representing the witness variant. After the basal wheel of leaves has been formed some plants were selected to be exposed to water stress. After a period of thirteen days without water the differences between the two categories were noticeable. The presence of bio-phyto-dynamic modulators managed to restore the balance needed to make visible the processes of growth and development of plants affected by water shortages. The presence of AD type bio-phyto-modulators had a protective effect for the plants affected by water stress, acting as a protective shield against water shortage.

Keywords: *Arabidopsis thaliana*, bio-phyto-modulators, water stress.

INTRODUCTION

Arabidopsis thaliana, known in the scientific community simply as Arabidopsis, is a small annual plant belonging to the Brassicaceae family. Several species belonging to the genus Arabidopsis, but member best known and most widely used in research is *Arabidopsis thaliana* (L.) Heyhn 2n = 10. The family includes many economically important plants such as mustard (*Brassica juncea*, *B. nigra* and others), cabbage (*Brassica oleracea*), rape (*Brassica napus*) and more widespread weeds (www.arabidopsis.org).

Arabidopsis is a popular model organism in plant biology and genetics. For a complex multicellular eukaryote, *Arabidopsis thaliana* has one of the smallest genomes among plants, of approximately 135 megabase pairs (Mbp), therefore in 2000 the genome of *Arabidopsis thaliana* was the first plant, and the third multicellular organism after *Caenorhabditis elegans* (The *C. elegans* Sequencing Consortium, 1998) and *Drosophila melanogaster* (Adams *et al*. 2000), to be completely sequenced (The *Arabidopsis* Genome Initiative, 2000). This generated a series of large-scale projects aimed at discovering the functions of the 25,000+ genes identified in *Arabidopsis thaliana* (Bevan and Walsh, 2005).

The balance between vital-metabolic processes of growth and development performed on living organism is often affected by external factors. These influences represented by temperature, humidity, light intensity and nutrients, stimulate, accelerate, slow down or even stop all qualitative and quantitative development of the plant (Narsai and Whelan, 2013).

The present paper is part of a doctoral thesis that aims to establish the influences and to identify the changes due to several physical fields, considered as stressors to the plant. The development and changes that occur in Arabidopsis plant will be observed at both macroscopic and microscopic level aiming possible mutations and changes in the genome.

Research conducted aimed to observe, at first, all of the phonological stages of *Arabidopsis thaliana* plant, starting with germination and ending with seed growth and development. After reaching maturity further analysis will be conducted by Biological Research Center at the Institute for Plant Biology Szeged, Hungary, for pursuing the possible changes relating to plant genome.

MATERIALS AND METHODS

Arabidopsis thaliana seeds were used divided into three variants. In order to determine the effect of A.D. type bio-phyto-dynamic modulators, two variants of 25 seed each were sown in two different containers having applied to the bottom, one a D.E.A. and the second a D.I.E.E. device. In a third container were sown seeds of Arabidopsis without attaching any kind of device, which was placed at a significant distance from the first two, but with similar characteristics regarding temperature, moisture and light radiation. The third version was used as a reference, witness variant (www.viatasienergie.ro).

Containers used for seed germination were filled with special soil for seedling. The top layer of soil was sieved with a set of sieves with mesh size of 0.5 mm and 0.2 mm. The necessity of obtaining a layer of soil as fine as possible was given by the very small size, of less than 1 mm, of Arabidopsis seeds. The seeds of this species need light to germinate, so it must be covered with soil at the same time, however, requires a seedbed to provide optimal conditions for germination and development of both the aerial part of the plant, but especially the root. To this end, the thin layer of soil constitutes the seedbed and provides the optimum environment for fixing and development of roots, and also of the future plant.

The variants under study were planted on 18 March 2013. Starting with the second day began the close monitoring of the containers in order to observe the germination process.

Room temperature where containers were placed ranged from 21ºC to 23ºC.

As mentioned in the specialized literature, the seed germination occurs within 3-5 days, under the conditions in which the seeds are incubated either before or immediately after the sowing, at a temperature of 4 ° C for 2-4 days, but no more than a week. It is considered that thermal shock applied to seeds leads to a quicker germination by feigning hibernation and stimulating the plant to come out of hibernation and start the vegetation of the new plant (Weigel and Glazebrook, 2002).

As mentioned above, this plant needs light to germinate. The second day after the seeds were planted the climatic conditions of Cluj area, where the experiments were performed, started to change becoming unfavorable. If in the day of seedling the maximum temperature arrived at 18 ºC, from the second day the temperature began to fall, reaching a high of 12 ºC, with the sky overcast, occurring precipitations as rain, drizzle, lightning, so a significantly reduced level of insolation. Coming days respected the same pattern, weather remaining the same until the end of March, so the exact moments when seeds needed a high level of lighting for starting and completing the germination process.

After the leaves basal rosette was formed the plants were moved in separated containers in order to allow their development and access to water and soil nutrients. A plant for each variant with bio-phyto-dynamic modulators and a corresponding witness plant, were chosen to be subject to water stress. The selected plants were in similar stages of development, without any significant differences between them. For these individuals the watering was completely stopped to observe their subsequent development.

The received results were analyzed in terms of variance and the significance of the received mean values was evaluated based on the P value and F-test for $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

The results began to appear, despite the weather conditions, since the third day after seeds planting to the variant with bio-phyto-dynamic modulator D.I.E.E. In the third day the germination to this variant arrived at 28%.

On the fourth day the variant with D.I.E.E. already had 72% of the seeds germinated. The first plantlets appear to the variant under the influence of D.E.A. modulator in the proportion of 44% from the total seeds submitted to germination.

On the fifth day the variant with bio-phyto-modulators reached maximum germination level at 92% for D.I.E.E. and 96% for D.E.A. variant.

The witness first plantlets don not appear until the seventh day from planting, that day the germination being 28%, and by day 11 succeed to germinate 94% of all seeds (Fig. 1).

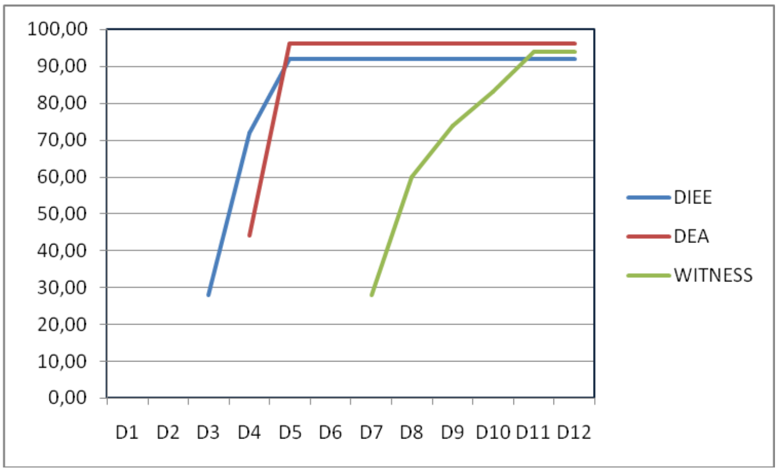

Fig.1. Speed germination in percentage for the three variants for a period of 12 days after sowing

The presence of A.D. type bio-phyto-dynamic modulators improved the speed germination. However, this result was not proved to be significantly different from the test seeds. In other words, the data didn't lead to any solid conclusions

Fig.2. Plants of *Arabidopsis thaliana* variant with D.I.E.E. and D.E.A. 30 days after sowing

Fig.3. Mature plants variants with D.I.E.E. and D.E.A. 53 days after sowing

Fig.4. *Arabidopsis thaliana* plants with flowers and siliqua with seeds

At plants subjected to water stress significant differences were observed between the variant with bio-phyto-modulator and the witness variant.

The differences are illustrated in the images below:

Fig.5. Differences between plant with D.I.E.E. and witness plant

Fig.6. Differences between plant with D.E.A. and witness plant

At the time the above comparison was made the plants were not watered for 13 days. During this period the plants have started from the same point, namely basal rosette of leaves, and in Fig.5. can be seen how the plant from the left, the witness plant, ceased to develop because of water shortage. It remained at basal rosette of leaves stage, with an undeveloped floral stem, without flowers and secondary branches.

The plant in the right was under the influence of A.D. type bio-phyto-modulator D.I.E.E. This plant continued to develop even in the absence of water, increasing by almost 9 cm longer than the witness plant.

At the variant with D.E.A. device the difference is not as great, but is visible representing 3 cm. In the case of the plant with D.E.A. mounted device, this not only grew and flourished, but even more she fructified, presenting two siliqua at the moment the photo in Fig. 6 was taken. Although they didn't manage to give additional branches, such as individuals that received regular water and nutrients, these individuals by using A.D. type bio-phyto-modulators succeed to reach maturity, to complete life cycle, to produce fruit and seeds.

After complete plant development thorough analysis of the genome of individuals will be performed at Plant Biology Institute from Szeged, Hungary, and check if the action bio-phyto-modulators left traces on the DNA sequence.

CONCLUSION

Protective effect of A.D. type bio-phyto-modulators proved to be favorable for the plants subject in this case to water stress, these acting as a protective shield against water scarcity.

The A.D. type bio-phyto-modulators acted as a mediator, a factor of balance between external factors and vital metabolic processes of the plant, so that the development continues even if the crucial factor in maintaining life, the water, is no longer available.

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