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THE EFFECT OF BIOFORGE ON TOMATO SEEDLINGS SUFFERING FROM HEAT STRESS

Crops face a multitude of adverse situations throughout their growth cycle, with abiotic stress in general, and heat stress in particular, being an ever-growing determinant factor of annual production losses worldwide. It is estimated that by the year 2050, the global human population will grow until reaching 9.7 billion people. With the increasing population, food security becomes a major concern, especially in countries whose populational growth exceeds their agricultural production. Therefore, any adverse effect on crop production can directly affect food security, having dire consequences on the population of those areas.

To this end, it is of great interest to develop products that can efficiently combat stress conditions in crops at a physiological level, so as to reduce the negative effects produced on crop development and ultimately on the yields, thus keeping production losses at a minimum. Stoller, which is a worldwide leader in the production of highly technological products based on plant physiology, has developed a unique product against stress, called **BIOFORGE**. In this trial, the effects of the exogenous application of **BIOFORGE**, which contains patented Stoller Technology, were studied on improving the growth of tomato seedlings grown in a hydroponic system and subjected to high temperatures (40°C).

3 treatments were made based on the application of **BIOFORGE** using different doses (0.5 mL/L, 1mL/L and 2mL/L as well as 2 controls, (normal control and heat stress control). During the trial, several evaluations were made mainly based on the plant's physiological behavior.

The treatments were distributed as follows:

Nº	TREATMENTS	CONDITIONS	EQUIVALENT DOSE (Liters/Ha)	APPLICATION METHOD	APPLICATION MOMENT
C	**CONTROL**	NORMAL	-	-	-
CT	**CONTROL**	STRESS	-	-	-
B1	**BIOFORGE 1**	STRESS	0.5 L/Ha	WATERING	**Every 7 days from the transplant, applied directly to the nutrient solution.**
B2	**BIOFORGE 2**	STRESS	1.0 L/Ha	WATERING	
B3	**BIOFORGE 3**	STRESS	2.0 L/Ha	WATERING	

GROWTH CONDITIONS:

Tomato seeds were germinated in a substrate consisting of silica sand and was watered with a nutrient solution mix containing essential micro and macronutrients. A week after germination, the seedlings were transplanted into 200 mL plastic containers, where they were individually grown in a common growing chamber. It is in this moment, when the first application of **BIOFORGE** was made, being able to help with the initial root growth in the treated plants (B1, B2 and B3). One week after the transplant, the heat stress was applied to the plants by transferring the treatments to be subjected to the stress into another chamber that regulated the temperature conditions during the course of the trial.

Image 1: Seedlings in the growing chambers.

Image 2: Tomato seedlings transplanted into hydroponic containers.

Both growing chambers had the same light and humidity levels, with the humidity levels being maintained at 70%. While the light intensity was adjusted according to the simulated time of day. The temperature in normal conditions oscillated between 18ºC at night and 25ºC during noon. In the case of the chamber in stress conditions, the nocturnal temperature was 18ºC whereas the **maximum diurnal temperature was of 42ºC** during noon. The maximum temperature was maintained for **3 hours daily**, throughout the course of the trail.

EVALUATIONS:

After subjecting the different theses to stress for 20 days, the different evaluations were carried out.

As mentioned earlier, the evaluations were based on physiological evaluations, with different sub evaluations being carried out under this category.

In brief, we have:

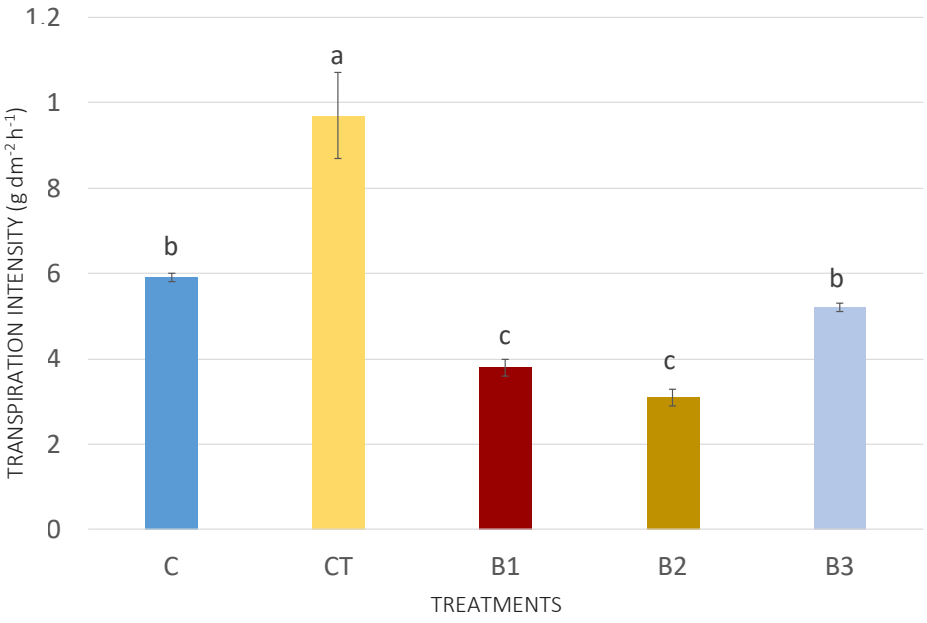
EVALUATIONS { **PHYSIOLOGICAL** { ***General state***: *Transpiration, membrane integrity and chlorophylls.*
Oxidative stress: *stress indicators, ROS and enzyme activity.*

PHYSIOLOGICAL EVALUATIONS:

These evaluations are essential to get an insight into the plant's physiological behavior. Stress first affects the plant's physical barriers and then its physiological balance, altering different chemical reactions that would adversely affect plant development in diverse and complex ways. The epitome of plant physiological stress is oxidative stress that ultimately leads to cell death. Oxidative stress is produced when Reactive Oxygen Species (ROS) are produced in large amounts due to the cell's inability to eliminate them, thus causing irreversible damage to different cell organelles ending in cell apoptosis. When produced in large amounts, oxidative stress inhibits the vital physiological processes to be taken place, thus adversely affecting plant development.

Here, several factors that affect the plant's general physiological behavior including transpiration, membrane integrity and chlorophyll content are measured. Beyond this, oxidative stress is measured by analyzing stress indicators, ROS molecules and finally antioxidative enzyme activity.

In summary we have:



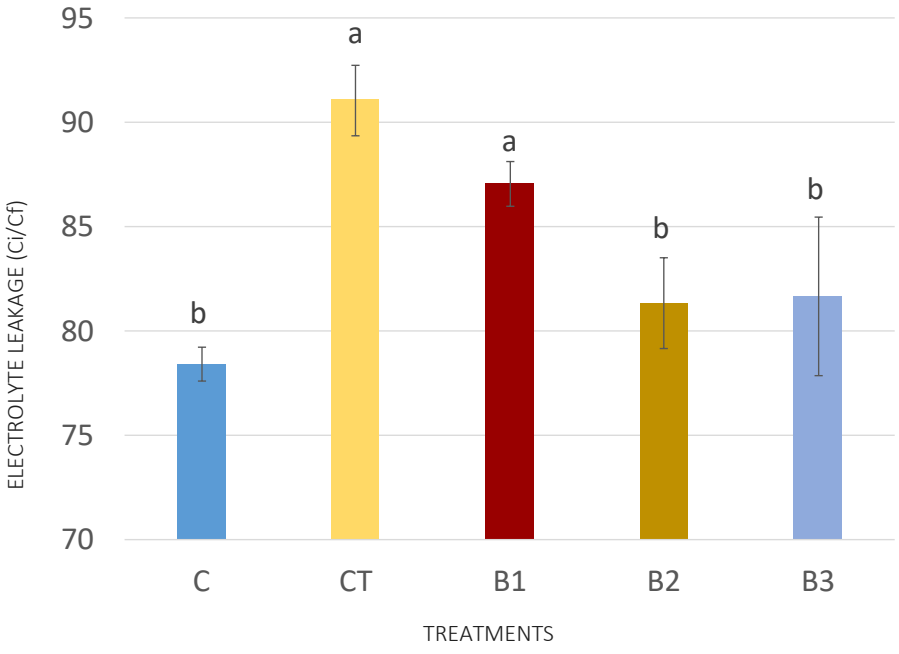
Graph 1: Transpiration intensity of the different thesis.

All the theses treated with BIOFORGE (B1, B2 and B3) show statistically significant differences against the heat stress Control (CT). In B3, similar results to those of the normal control (C) were obtained, while in B1 and B2, a reduction of the transpiration rates were detected in comparison to the normal Control (C), with the differences being statistically significant. Therefore, the addition of **BIOFORGE produced a significant decrease of the transpiration values compared to the heat stress Control (CT).**

In normal conditions, a regulated level of transpiration exists, as is observed in the normal Control. In heat stress conditions, the plant opts for increasing water loss as a cooling mechanism to avoid tissue damage owing to this type of stress. **The seedlings treated with BIOFORGE** had a lower transpiration rate probably due to the fact that they **did not suffer the consequences of heat stress, and thus they developed a similar behavior to the Control (C) seedlings**.

ELECTROLYTE LEAKAGE

Electrolyte leakage is a measurement of membrane integrity, a more damaged membrane presents a higher leakage and thus more damage to the cell, rendering it ineffective. The analysis made shows the following:



Graph 2: Electrolyte leakage in the different thesis.

The results in graph 2 show that **there is a lower electrolyte leakage in the membranes of thesis B2 and B3, achieving significant differences against the heat stress control (CT)** while obtaining similar values to the normal Control (C).

B1 treatment, attains the lower values to the heat stress Control without having statistically significant differences between them. Therefore, in view of the results, there is a clear reduction in the electrolyte leakage in the treatments with **BIOFORGE**, with the most notable effects being in the theses with higher doses of the products (B2 and B3).

In normal conditions, a good ion Exchange between the cell and its medium exist, while in cases of stress, this Exchange can be affected due to damages in the membrane, causing ion leakage. The membrane in stress situations can have its fluidity affected, giving way to a higher ion leakage from its interior as can be seen in the following diagram.

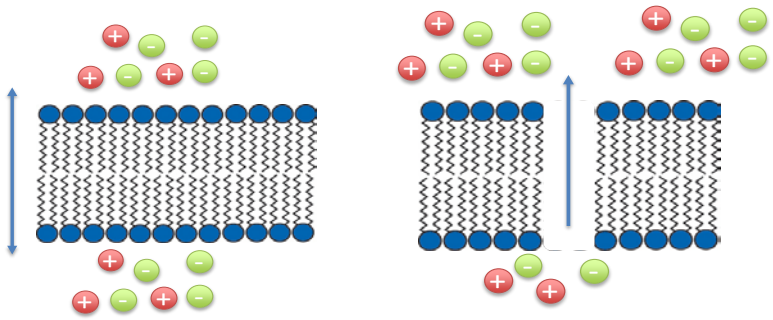
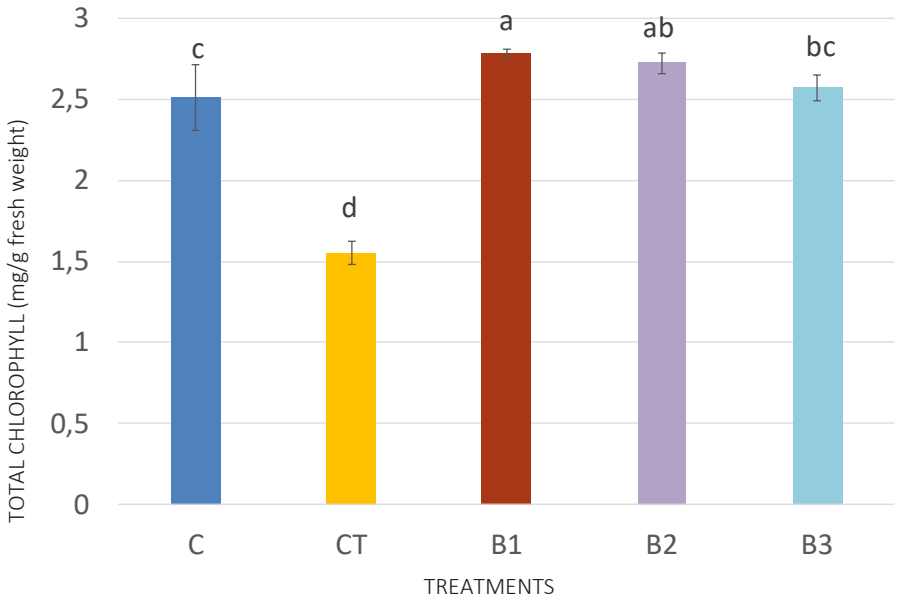


Diagram 1: Ion Exchange in the membrane in good state (left) and damaged membrane (right).

PHOTOSYNTHETIC PIGMENTS

In this case, the chlorophyll content was analyzed in fresh leaves so as to determine the photosynthetic capacity of the plant. Therefore, a spectrophotometric study was made in a solution containing chlorophyll extract, with the results being presented in the following graph:



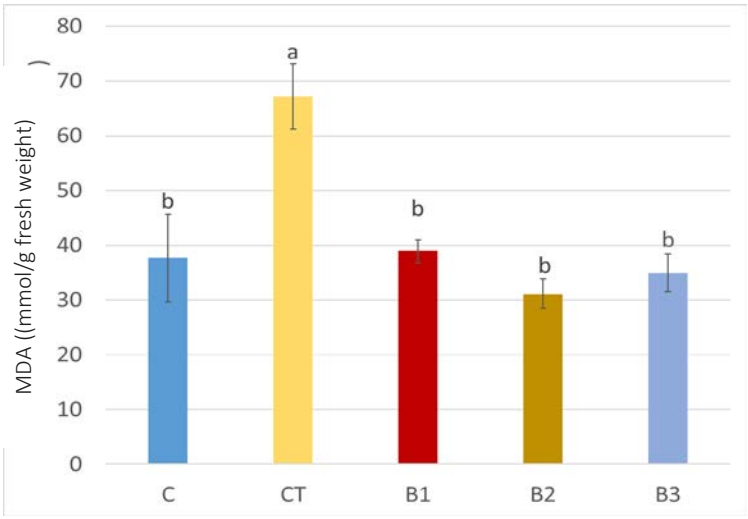
Graph 3: Chlorophyll content from the different theses.

Image 3: Chlorophyll extract from each thesis.

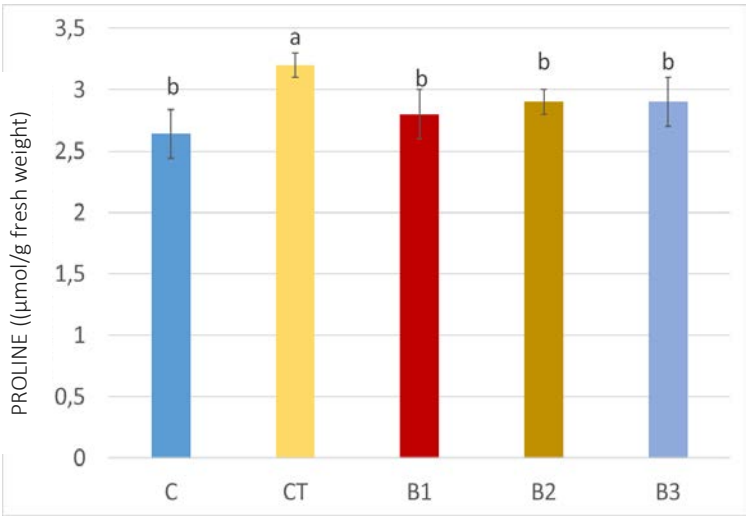
The data in graph 3 show that **there is a considerable increase in chlorophyll content in the theses treated with BIOFORGE**, obtaining **statistically significant differences in comparison to the heat stress Control (CT)**. Thesis B1 obtains higher values tan the normal Control (C) with statistically significant differences. The results obtained, confirm that **BIOFORGE** stimulates photosynthesis thanks to its influence on the expression of photosynthetic genes, thus increasing the accumulation of photosynthetic pigments, specifically chlorophyll.

INDICATORS OF OXIDATIVE STRESS:

In oxidative stress conditions, the accumulation of some substances able to alleviate the damage is produced. The osmolytes as they are called, can be from sugars up to amino acids. In this trial, the concentrations of Malondialdehyde (MDA) a dialdehyde produced during stress and an indicator of membrane damage is studied. Proline, an amino acid that accumulates during oxidative stress due to its protective nature against damages produced from this stress is also studied.



Graph 4: MDA content in the different theses.



Graph 5: Proline content in the different theses

The data obtained in this study are in accordance with the results obtained in the membrane integrity analysis. MDA is known to be an indicator of membrane damage, hence an increase in its accumulation signifies more damage in the membrane. In both cases, the treated theses with **BIOFORGE**, present a membrane in good state, a fact reinforced by the concentration of MDA present in the measured theses. In the heat stress Control (CT) a higher accumulation of MDA is observed and thus representing more damage in the membrane as can be seen in graph 2.

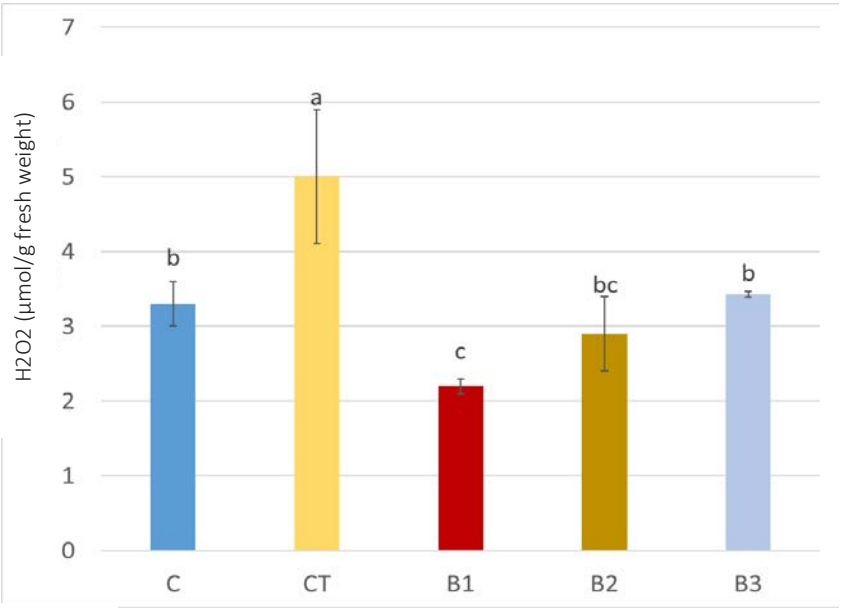
As observed in the case of MDA, the quantity of proline accumulate is statistically inferior in the treated theses with **BIOFORGE** against the heat stress Control (CT) while equaling the results of the normal Control (C) (Graph 5). Once more, the results provide proof that the treated seedlings were not in oxidative stress.

To corroborate the lower incidence of oxidative stress in the treated seedlings, a ROS molecule is measured as is the case of hydrogen peroxide (H_2O_2) y and the activity of enzymes responsible for the degradation of ROS such as Catalase and Ascorbate Peroxidase.

HYDROGEN PEROXIDE (H_2O_2):

Hydrogen peroxide is considered as a reactive oxygen species (ROS), so in high concentrations, it forms part of the phenomenon known as oxidative stress, thus, being toxic when in excess

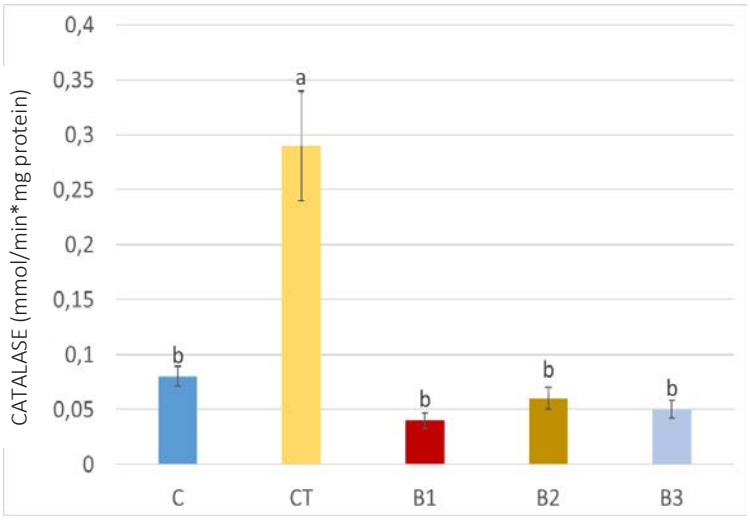
As can be observed in graph 6, there is an important reduction in the presence of hydrogen peroxide in the theses treated with **BIOFORGE.** All of the treated theses present statistical differences against the heat stress control, which has a high concentration of this ROS molecule. **Theses B2 and B3 achieve values similar to the normal Control**, without significant differences between them. **Thesis B1, presents the lowest concentration of the molecular with statistical differences compared to the normal Control**.

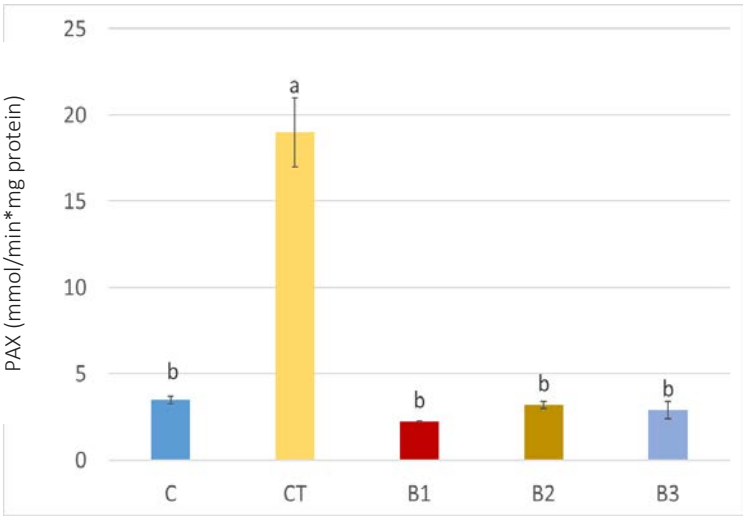


Graph 6: H_2O_2 concentration in the different theses.

In summary, the lower presence of ROS molecules can be verified in the treated theses with BIOFORGE and thus indicating that these seedlings are absent of oxidative stress. BIOFORGE, was indeed able to inhibit the synthesis of the Stress hormone, Ethylene, so that the synthesis of ROS molecules is reduced and thus, the stress damage is also reduced.

ENZYME ACTIVITY (CATALASE and ASCORBATE PEROXIDASE):



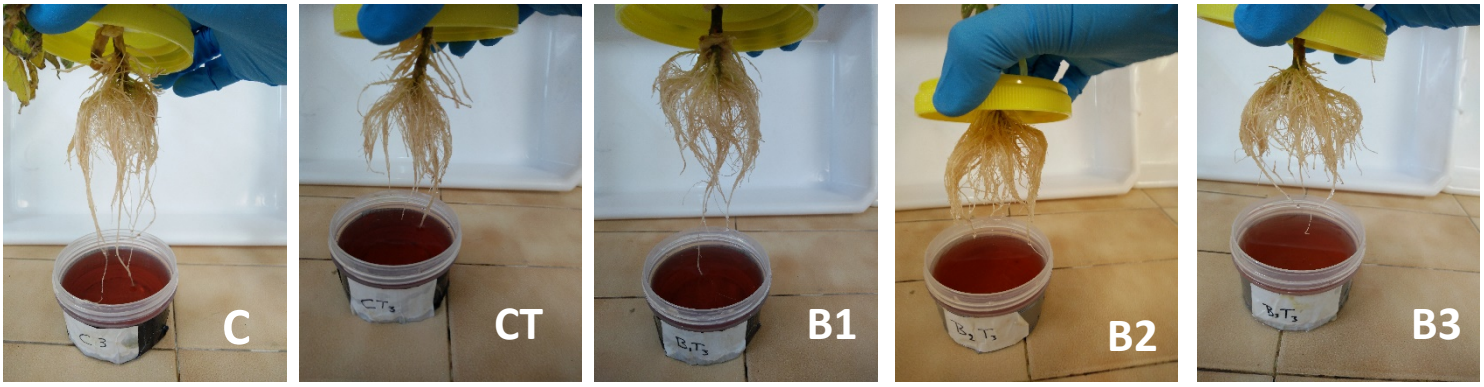


Graphs 7 and 8: Enzyme activity in the samples of each theses.

The heat stress Control seedlings show high levels of antioxidant enzyme activity with statistically significant differences in comparison to the rest of the theses. This is due to the fact that the seedlings are in oxidative stress (Graphs 4, 5 and 6) and therefore, require high activity of these enzymes to avoid any damages in their tissues. The Stoller technology present in **BIOFORGE**, **aids the seedlings in avoiding this situation as is observed in all the oxidative stress parameters measured. In all the measured parameters, the results indicate that indeed the treated seedlings were absent from oxidative stress.**

ROOT GROWTH

A visual evaluation of root growth was made, observing significant increments in root mass in the BIOFORGE treated seedlings compared to CT.



Images 4 - 8: Root growth in the different theses.

In conclusion, all the results from this trial verify the positive effect of **BIOFORGE** on the global physiology of the seedlings, by inhibiting the synthesis of ethylene and reducing the presence of stress, thus restoring the seedlings to a physiologically balanced state, despite being in an adverse medium, all thanks to the unique and patented Stoller technology in **BIOFORGE**.