The effect of some environmental growth stimulators on physiological characterizations of canola seedlings under drought stress

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ABSTRACT

A key feature toward the protection of earth’s natural resources is that both scientists and engineers must come to more effective management of different fertilizers and microbiomes in soil environments. To investigate the effects of environmental factors like inorganic NPK fertilizer, organic amino acids, poultry manure, wood vinegar, biologically effective microorganisms and Spirulina algae, under drought stress, an experiment was conducted. The treatments were added to each pot of the Canola (Brassica napus L.) in two turns with an interval of 12 days. Shoot fresh weight, relative water content, chlorophyll a and b, carotenoids, proline and antioxidant content were measured. Canola seedlings grow the most with Spirulina algae treatment, it shows that Spirulina algae caused rapid growth of seedlings compared to other treatments. Drought stress increased antioxidants, and proline and decreased the relative water content. Spirulina algae, amino acids and fertilizer contain a lot of nitrogen, which increases canola seedling growth and also increases their water demand. Environmental growth stimulators including inorganic, organic and biological factors can help plant survive in normal and stressful conditions by environmental management.

Keywords: Brassica napus; Spirulina platensis; Microorganisms; Nitrogen Fertilizer; Wood Vinegar; Poultry Manure

1. Introduction

Environmental plant growth stimulators include inorganic, organic and biological factors that can help plant survive in normal and stressful conditions. A key feature toward the protection of earth’s natural resources is that both scientists and engineers must come to more effective management of different fertilizers and microbiomes in soil environments. Growing crops that require lots of mineral nitrogen and conventional activated sludge for wastewater treatment are two examples of nutrient-energy inefficient approaches that require enhancement. Drought stress is an important abiotic stress that not only limits the growth and development of plants, but also causes lower yield and is expected to spread and gradually affect or cover larger areas in the future[1,2]. In such conditions, plants respond with several physiological and morphological changes such as reduced transpiration and photosynthesis rate, osmotic adjustments, suppression of root and shoot growth, overproduction of reactive oxygen species and senescence[3].

Rapeseed oil and canola oil are derived from the same plant (Brassica napus L.) which is considered as an important oilseed crop in the world.

The growth and performance of rapeseeds are mostly limited by drought and salinity stress. Of the economic importance of canola, so
far extensive studies have been conducted to evaluate the harmful effects of abiotic stresses[4]. According to several studies, it has been shown that rapeseed highly needs nitrogen[5]. Spirulina platensis is a filamentous and multicellular blue-green microalgae[6]. S. platensis is mainly found in tropical and subtropical lakes having high pH and high salt concentration[7]. S. platensis might be used as a rich source of macronutrients, vitamins, amino acids, polypeptides, phytohormones, antioxidants and compounds with antibacterial and antifungal properties[8]. Foliar application of S. platensis on Vicia faba L. increased total protein level, N, P, K content and photosynthetic activity[9].

Effective microorganisms (EM) include microorganisms that other than being eco-friendly, help increase agricultural products. EM, as a bio-fertilizer that has received much attention, is an inoculum composed of fungi and bacteria, which coexist in the fermentation liquid medium[10] and was developed by the University of the Ryukyus, Okinawa, Japan[11].

NPK fertilizer is usually used in increasing the plant growth. The N element in NPK fertilizer plays a significant role in the production of amino acids or proteins, nucleic acids, nucleotides and chlorophylls. Phosphor element in NPK fertilizer plays a role in energy storage and transfer, and the K element in NPK fertilizer is used as an enzyme activator, ion balance and helps in the transfer of substances absorbed from the leaves to the plant tissue[12,13].

Amino acids are organic molecules that are composed of nitrogen, oxygen, hydrogen and carbon. The importance of amino acids is related to their abundant use for the biosynthesis of many types of organic compounds[14]. Free amino acids, as a form of soluble organic nitrogen, are a better source of nitrogen than ammonia and nitrate[15].

The use of poultry manure might affect the amount of released nutrients (soil chemical properties), growth and yield of maize[16]. The chemical composition of poultry manure is different. Application of poultry manure, as a source of the main plant nutrients, on the land is the most common way to use this manure which is a soil conditioner to improve soil tilth and to reduce problems related to the soil compaction[17].

Wood vinegar is a liquid byproduct obtained from condensed vapors during the thermal decomposition of biomass[18]. Wood vinegar mainly containing acetic acid, butyric acid, catechol and phenol, has a compound effect in increasing crop growth similar to plant growth regulation, and is eco-friendly; besides, it can increase biological and abiotic resistance of products[19]. Analyzes have shown that wood vinegar contains 10%–20% organic compounds and more than 200 different types of organic compounds[20].

2. Materials and methods

2.1 Cultivation conditions

To investigate the effects of inorganic NPK fertilizer, organic amino acids, poultry manure and wood vinegar and biological effective microorganisms and Spirulina algae, under drought stress, an experiment was conducted. This experiment for each treatment was performed in one kilogram pot with three replications. B. napus seeds were sown in 63 pots. For each stimulus, 9 pots were considered. After seed germination and growth, 10 seedlings of the same height were selected in each pot and the rest of the seedlings were thinned. When the seedlings reached a height of about 10 cm, the first stage of fertilization was applied, after 12 days, the second stage of fertilization was applied. Amino acids and wood vinegar were given to the plant by foliar application. Each stimulus had a concentration level and was performed under non-stress, low and high stress conditions. Pots under low and high stress were collected after 6 and 12 days of drought stress, respectively from 42 pots. The daily temperature changes of the research greenhouse during experiment were at least 24 °C and at most 32 °C.

2.2 Soil analysis

The soil in the pots contained sand (1/3 of the total volume), clay (1/3 of the total volume), and perlite and coco peat (1/3 of the total volume). The analysis of the soil is shown in Table 1.
Table 1. Analysis of soil used for the experiments

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC ms/cm</td>
<td>2.50</td>
</tr>
<tr>
<td>PH of paste</td>
<td>8.13</td>
</tr>
<tr>
<td>TNV</td>
<td>57.20</td>
</tr>
<tr>
<td>OC (%)</td>
<td>0.86</td>
</tr>
<tr>
<td>OM (%)</td>
<td>1.48</td>
</tr>
<tr>
<td>TN (%)</td>
<td>0.09</td>
</tr>
<tr>
<td>P ava (ppm)</td>
<td>13.20</td>
</tr>
<tr>
<td>K ava (ppm)</td>
<td>325.00</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>57.00</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>31.80</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>11.20</td>
</tr>
<tr>
<td>Cu ava (ppm)</td>
<td>1.14</td>
</tr>
<tr>
<td>Mn ava (ppm)</td>
<td>38.70</td>
</tr>
<tr>
<td>Fe ava (ppm)</td>
<td>6.40</td>
</tr>
<tr>
<td>Zn ava (ppm)</td>
<td>3.78</td>
</tr>
</tbody>
</table>

EC: electrical conductivity; TNV: total neutralising value; OC: organic carbon; OM: organic matter; TN: total nitrogen; P ava: available phosphorus; K ava: available potassium; Cu ava: available copper; Mn ava: available manganese; Fe ava: available iron; Zn ava: available zinc.

2.3 Shoot fresh weight and relative leaf water content (RWC) measurement

The aerial part of plants in each pot was separated and measured with a scale to the nearest thousandth of a gram. For RWC, the last developed leaf without fracture and rupture was removed from the seedling. Its fresh weight (FW) was measured and then placed in distilled water for 24 h at the temperature of 4 °C. After 24 h, the leaf was removed from distilled water and its saturation weight was measured. Then it was placed in an oven at 70 °C for 24 h. Dry weight (DW) leaf sections were measured and then calculated by placing the obtained numbers in Equation (1) the RWC. The saturated weight (SW) of the leaf was measured after being placed in distilled water.

\[
\text{RWC} (%) = \left( \frac{\text{FW} - \text{DW}}{\text{SW} - \text{DW}} \right) \times 100
\]

2.4 Measurement of chlorophyll and carotenoid content

Fresh leaf sample (0.1 g) was grounded with 80% acetone, then it was made up to 10 mL. This solution was centrifuged at a speed of 6,000 revolutions per minute (RPM) for 10 min, and the solution became two-phased. The upper solution was used to measure chlorophyll and carotenoids. The absorption in the spectrophotometer model Spekol 1300 was read at a wavelength of 663 for chlorophyll a, wavelength of 645 for chlorophyll b and wavelength of 470 for carotenoids. The following Equations (2), (3) and (4) were used to calculate chlorophyll a, b and carotenoids respectively.

\[
\text{Chlorophyll a} = \left[ \frac{12}{7} (\text{OD}_{663}) - 2.69 (\text{OD}_{645}) \right] (\mu\text{g/mL})
\]

\[
\text{Chlorophyll b} = \left[ \frac{22}{9} (\text{OD}_{645}) - \frac{4}{68} (\text{OD}_{663}) \right] (\mu\text{g/mL})
\]

\[
\text{Carotenoid} = \left( \frac{1000 (\text{OD}_{470}) - \frac{1}{82} (\text{Chl a}) - \frac{85}{02} (\text{Chl b})}{198} \right) (\mu\text{g/mL})
\]

2.5 Measurement of proline and antioxidant content

Aerial part of the plant (0.5 g) was grounded with 10 mL of 3% sulfosalicylic acid, and it was centrifuged at 1,500 rpm for 10 min. The upper solution was used as the base extract to measure proline. 2 mL of base extract plus 2 mL of ninhydrin reagent and 2 mL of glacial acetic acid (100%) was poured into a test tube. The test tube was placed in a snail at 100 °C for 1 h. After 1 h, it was removed from the test tube and transferred directly to the ice bath until the end of the reaction. Then, it was transferred to room temperature and 4 mL of the toluene was added to the contents of the test tube and was mixed for 30 seconds in a vortex machine. The contents became two-phased and after 20 min, the optical absorption of the upper solution was read at 520 nm. To plot the proline standard curve, standards of 0, 4, 8, 12, 16, and 20 milligrams per liter of proline (ppm) were prepared and their absorbance was recorded at 520 nm. Using the spectrophotometer model Spekol 1300, the proline content of the sample was calculated using the standard curve.

For antioxidant measurement, the sample leaves were placed in an oven at the heat of 40 °C for 24 h to dry completely. Then to make a completely uniform solution, 1 g of the sample was ground with 15 mL of 100% methanol. The sam-
ple was placed in a falcon foil on the shaker for 24 h. Then, the extract was placed in a centrifuge at 4,500 rpm for 12 min and the upper solution was considered as the base extract for measuring antioxidants. The plant base extract (150 μL) was added to 2,850 μL of 0.004% DPPH solution. The sample was kept in the dark for 1 h and then its absorbance was read at 517 nm. The spectrophotometer was zeroed by 100% methanol. Using the standard Trolox curve, the amount of antioxidants was calculated[24].

Trolox stock has a concentration of 1,000 micromolar. Different concentrations (0, 25, 50, 100, 200, 300, 400, 500, 600, 700, and 800 micromolar) were prepared and 150 μL of each of the concentrations was added to 2,850 microliters of 0.004% DPPH solution and was placed in the dark for 1 h, then its absorbance was read at the wavelength of 517 nm and the Trolox standard curve was plotted.

3. Results

3.1 Fresh weight shoot and RWC

Drought stress has caused a significant reduction in growth and fresh weight. The treatment of *Spirulina*, amino acid and NPK fertilizer increased fresh weight significantly at the $P < 0.05$ level compared to the control (Figure 1).

Drought has caused a significant decrease in the relative water content of canola seedlings’ leaves. All treatment seedlings shown a significant decrease in relative leaf water content in high stress. In addition, expect the treatment of *Spirulina*, all low stress has decreased the relative leaf water content significantly at the $P < 0.05$ level compared to the control (Figure 2).

3.2 Chlorophyll and carotenoid content

Treatments containing *Spirulina algae*, compared to the control, has shown a significant increase in chlorophyll a content in non-stress, low and high stress conditions. Other treatments show no difference compared to the control. Only *Spirulina* treatment has shown a significant increase in chlorophyll b content compared to the control. Pots containing amino acid and NPK that were subjected to high stress, compared to the control, showed a significant increase in carotenoid content (Figure 3).

![Figure 1](image1.png)

*Figure 1.* The effects of drought stress, *Spirulina algae*, EM, amino acid, NPK fertilizer, poultry manure and wood vinegar on shoot fresh weight of canola seedlings. Each data series is the average of three replicates. Different letters indicate significant differences at the $P < 0.05$ level. Error bars are based on standard error (± SE). D1: 6-day drought stress (low stress); D2: 12-day drought stress (high stress); S: *Spirulina algae*; E: Effective microorganisms; A: Amino acid; N: NPK fertilizer; K: Poultry manure; and B: Wood vinegar.
Figure 2. The effects of drought stress, *Spirulina algae*, EM, amino acid, NPK fertilizer, poultry manure and wood vinegar on RWC of canola seedlings. Each data series is the average of three replicates. Different letters indicate significant differences at the $P < 0.05$ level. Error bars are based on standard error (± SE). D1: 6-day drought stress (low stress); D2: 12-day drought stress (high stress); S: *Spirulina algae*; E: Effective microorganisms; A: Amino acid; N: NPK fertilizer; K: Poultry manure; and B: Wood vinegar.

Figure 3. (Continued).
Figure 3. The effects of drought stress, *Spirulina algae*, EM, amino acid, NPK fertilizer, poultry manure and wood vinegar on the content of chlorophyll a, chlorophyll b and carotenoid of canola seedlings. Each data series is the average of three replicates. Different letters indicate significant differences at the $P < 0.05$ level. Error bars are based on standard error (± SE). D1: 6-day drought stress (low stress); D2: 12-day drought stress (high stress); S: *Spirulina algae*; E: Effective microorganisms; A: Amino acid; N: NPK fertilizer; K: Poultry manure; and B: Wood vinegar.

3.3 Proline and antioxidants

In the control and the *Spirulina algae*, amino acid, fertilizer and poultry manure treatments, high drought stress has caused a significant increase in proline content compared to the non-stress condition of control but in the treatment of *Spirulina algae*, under no stress and low stress conditions, also a significant increase in proline content was observed (Figure 4).

Figure 4. The proline standard curve and proline content of canola seedlings in drought stress and treatments of *Spirulina algae*, EM, amino acid, NPK fertilizer, poultry manure and wood vinegar on the proline content of. Each data series is the average of three replicates. Different letters indicate significant differences at the $P < 0.05$ level. Error bars are based on standard error (± SE). D1: 6-day drought stress (low stress); D2: 12-day drought stress (high stress); S: *Spirulina algae*; E: Effective microorganisms; A: Amino acid; N: NPK fertilizer; K: Poultry manure; and B: Wood vinegar.

In low and high stress, all treatment except the treatment of *Spirulina*, the antioxidants have increased significantly. Under non-stress and low stress conditions, the antioxidant of *Spirulina* treatment was decreased but under high stress, no significantly changes were observed compared to the control (Figure 5).
4. Discussion

4.1 The fresh weight of the aerial part and RWC

Dryness is an important limiting factor in plant growth and might prevent plant respiration and photosynthesis, and thus lead to the damage of plant growth and physiological metabolism. When the plant is under drought stress, it responds to the stress via changing its external shape and internal structure, which gets the plant to grow slowly or even drop off[25]. Canola seedlings grow the most with *Spirulina algae* treatment, it shows that *Spirulina algae* caused rapid growth of seedlings compared to other treatments. This weight difference is high between the control and *Spirulina* treatment without stress, as well as the amino acid and NPK treatments caused growth in rapeseed seedlings. *Spirulina algae*, amino acid and NPK, which contain nitrogen, had the highest growth on seedlings. The dry weight of *Spirulina algae* has 60% to 70% protein.

Drought stress reduces leaf size and shoot development, disrupts plant water relations and reduces water use efficiency[26]. Relative water content is an important determinant of water status in plants and reflects the balance between plant tissue water storage and transpiration rates[27]. When drought stress increases, it causes a decrease in the percentage of relative water content.

4.2 Chlorophyll and carotenoid content

Chlorophyll is the most important and effective pigment in photosynthesis that reflects the state of plant growth and stress level. Chlorophyll content often decreases under drought stress conditions and the ratio of chlorophyll a, b and carotenoid changes, which causes changes in the performance of photosynthesis[26]. The reason for the decrease in chlorophyll content in leaves might be due to the direct destruction of chlorophyll by drought[28]. Based on the obtained data, *Spirulina* treatment, compared to the control in canola seedlings, has caused a significant increase in the contents of chlorophyll a and b. The content of chlorophyll a was not significantly different in the treatment of *Spirulina* under stressed and non-stressed conditions, which shows that *Spirulina* increases photosynthetic performance.

4.3 Proline and antioxidant

In many plant species, proline increases under different stress conditions[29]. Proline performs a role in plant tolerance to stress[30]. Increasing proline helps the rapeseed plant to maintain its water balance and to increase tolerance to drought.
stress in the plant[31]. A significant increase in proline content is observed in treatments compared to the control.

The function of antioxidants and antioxidant enzymes, under environmental stress conditions, ceases the uncontrolled oxidation process caused by ROS in plants[32]. Each treatment in low and high stress conditions causes an increase in antioxidant content compared to the same treatment in no stress conditions. The treatment of Spirulina algae, under non-stress conditions, has had a significant decrease in antioxidant content compared to the control, which shows that Spirulina treatment has played the role of antioxidant regulator in the plant and has gradually increased with the onset of stress; thus, it can be concluded that the plant responds quite well to stress.

In general, according to this article and other researches, water deficit stress and plant growth stimulators have significant effects on the remobilization of nutrients from the soil to plant and following metabolism synthesis changes[33,34]. The treatment of Spirulina algae was the best stimulator in this way. Spirulina is filamentous, blue-green cyanobacteria that are found widely in soil, marshes, freshwater, and seawater but now it is cultivated in little pools of greenhouse in many countries so can be used in agriculture and horticulture[35].

5. Conclusion

Environmental growth stimulators include inorganic, organic and biological factors can help plant to survive in normal and stress conditions by environmental management. Dryness is an important limiting factor in plant growth and might prevent plant respiration and photosynthesis, and thus lead to the damage of plant growth and physiological metabolism. Canola needs growth stimulators for better growth like biological effective microorganisms, Spirulina algae, amino acids and fertilizer contains a lot of nitrogen and wood vinegar which increases canola seedling growth and also increases their water demand. Canola seedlings grow the most with Spirulina algae treatment, it shows that Spirulina algae caused rapid growth of seedlings compared to other treatments. In addition, Spirulina treatment, in canola seedlings, has caused a significant increase in the content of chlorophyll a and b compared to the control. Also, Spirulina treatment has played the role of antioxidant regulator in the plant and has gradually increased with the onset of stress; thus, it can be concluded that the plant responds quite well to stress.

Conflict of interest

The authors declare that they have no conflict of interest.

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