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EFFECT OF NO INDUCTOR ON PROLINE CONTENT OF TRITICUM DURUM UNDER DROUGHT AND SALINITY STRESS

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Abstract

The global population grows, the need for food is also rising significantly. To meet this demand, improving crop resistance to environmental stress is essential, especially for fundamental crops like *Triticum durum* (durum wheat). Drought and salinity stress are the main limiting factors for crop yield and quality. During drought and salinity stress, plant produces a lot of Reactive Oxygen Species (ROS) which can fight against stress but also can give damage to plant itself. To decrease the effects of ROS, plants produce NO radicals that activate ROS-scavenging enzymes and increase proline, an important amino acid that functions as an osmoregulator during stress. Several studies prove that sodium nitroprusside (SNP) acts as a NO^- donor and help to relieve stress. In our research, we focused on how SNP improve proline accumulation in *Triticum durum*. Our research shows that 0.2 mM SNP improves proline accumulation in Triticum durum under SNP treatment alone. However, in the combined treatments with 3% PEG + 0.2 mM SNP and 100 mM NaCl + 0.2 mM SNP, proline levels under these conditions were not significantly high compared to the SNP treatment alone. This could be explained by additional pathways in proline accumulation.

Keywords: *Triticum durum* L.*; salinity; drought stress; proline accumulation; SNP*

1. Introduction

The origins of agriculture are closely related to the cultivation of grains. Grain crops are fundamental in supporting food security all around the world. As known, there is a constantly growing need to ensure an adequate food supply. Addressing this need requires enhanced crop tolerance to environ- mental challenges, especially for fundamental plants like *Triticum durum* (durum wheat). Recent estimates indicate that, in the coming decades, extreme weather could affect several major regions, causing crop losses and a rapid rise in food prices [1, 2]. Since durum wheat is an important food source in many countries, enhancing its ability to tolerate stresses like drought and salinity is vital for sustaining food production.

Proline builds up in plants as a common response to environmental stresses like drought, salinity, cold

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and etc. It plays a special role in helping plants to manage water loss, especially during dry conditions [6]. Proline is an amino acid that plays an important role in osmoregulation and osmotolerance. It helps to protect cellular membranes and proteins from damage caused by dehydration during abiotic stress. Additionally, proline has an ability to scavenge free radicals produced under stress conditions [5, 11].

Nitric oxide (NO) regulates various physiological processes, such as seed germination, metabolism, cell death, stomatal opening and closing. NO can be produced by the breakdown of NO donor molecules like SNP, which has the chemical formula $Na_2[Fe(CN)_5NO]$ [8]. Low concentrations of NO in plants can quickly remove lipid peroxyl radicals and change the types and amounts of reactive oxygen species (ROS). This helps to prevent damage from ROS and influences the activity of antioxidant enzymes [7].

Our research focus on the effect of 0.2 mM SNP on the proline content of *Triticum durum* under drought (3% PEG) and salinity (100 mM NaCl) stress. By investigating these conditions, our purpose is to understand how SNP can enhance stress tolerance in *Triticum durum.*

2. Materials and methods

One genotype of wheat- *Triticum durum* was used in the study: *Triticum durum* L. Revan. Wheat seeds were initially treated with 0.1% potassium permanganate for 5 minutes, then they were washed with distilled water and planted in a neutral substrate - perlite. A modified Hoagland solution was used as a hydroponic solution. Beside control plant, 5 main combinations were used in order to create artificial drought and salinity stress: 0.2 mM SNP; 3% PEG- 6000; 0.2 mM SNP + 3% PEG-6000; 100 mM NaCl; 0.2 mM SNP + 100mM NaCl.

All these concentrations were given to plants in hydroponic solution.

The concentration of endogenous proline was determined by a visuali- sation test using the supernatant of fresh leaves. This method is developed by Boctor and based on the reaction between isatin and proline [3]. It is suitable for the quantitative determination of proline in protein hydrolyzate and biological fluids. Densitometric data were obtained using ImageJ program [9].

3. Results and Discussion

Our results reveal interesting patterns, as illustrated in Figures 1 and 2. Proline accumulation in control plant was similar to the level observed in the

0.2 mM control variant of proline. However, treatment with 0.2 mM SNP increased proline accumulation to a level appropriate to the 1 mM control variant of proline. The combination of 3% PEG with 0.2 mM SNP resulted in lower proline accumulation compared to SNP treatment alone. Additionally, the application of 100 mM NaCl, as well as the combination of 100 mM NaCl with

0.2 mM SNP, significantly increased proline accumulation. The proline concentrations of 100 mM NaCl and 100 mM NaCl + 0.2 mM SNP is also similar to the 1 mM control concentration of proline.

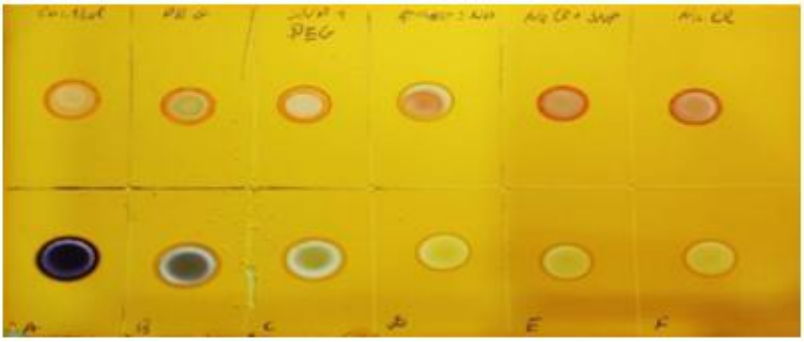

Fig.1. First row (left to right) – *Triticum durum* L. Revan: Control; 3% PEG; 0.2 mM SNP + 3% PEG; 0.2 mM SNP; 100 mM NaCl + 0.2 mM SNP; 100 mM NaCl. Second row (left to right) - A, B, C, D, E, F are control variants of proline at different concentrations (A - 10 mM, B - 2 mM, C - 1 mM, D - 0.2 mM, E - 0.1 mM, F - 0.02 mM)

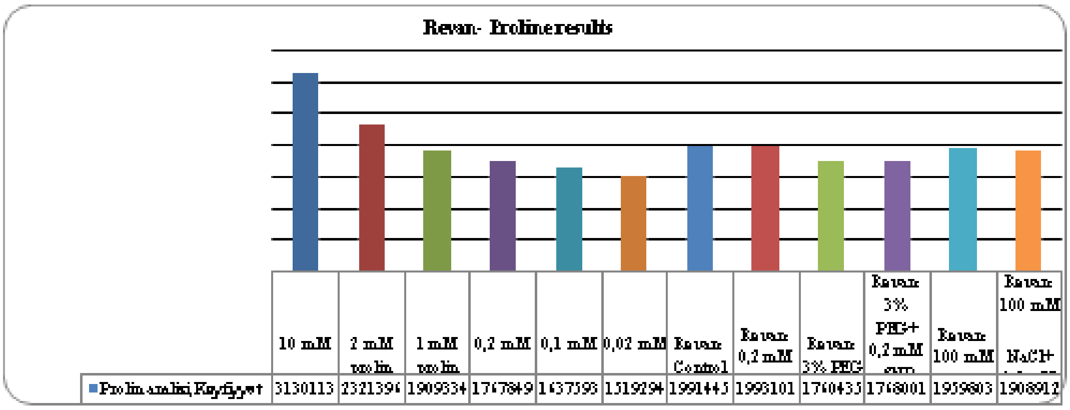

Fig. 2. The densitometric analysis results using the ImageJ program

Previous studies prove that SNP as an NO^- donor decreases effect of drought and salinity in different plants by triggering different antioxidant enzymes, as well as, by the conversion of NO^- to NO_3^- [4]. Application of signaling molecules, like SNP usually increases proline content of plant that helps in osmotic adjustment, stabilizes the biological membranes and reduces the cell acidity, which might have resulted in the better performance of wheat under drought stress [10].

Our study results show that the application of 0.2 mM SNP alone increased proline levels in compare to the control, indicating a positive effect of SNP on proline accumulation. When plant was exposed to drought stress (3% PEG), proline content rose, and this increase was even more enhanced when combined with 0.2 mM SNP, suggesting that SNP may enhance drought tolerance. Similarly, salinity stress (100 mM NaCl) elevated proline levels, but a little less amount of proline was observed in the combination of 100 mM NaCl and 0.2 mM SNP, indicating that 0.2 mM SNP might be insufficient to improve salinity tolerance by promoting proline accumulation. This may be due to the additional ionic stress of NaCl, beside the salinity stress [12].

4. Conclusions

In conclusion, our study reveals that SNP application enhances proline accumulation in Triticum durum, by increasing its stress tolerance. The combined use of SNP with drought stress increased proline levels, indicating a beneficial effect on drought resistance. However, under salinity stress, SNP's impact on proline accumulation was limited, suggesting that higher SNP concentrations might be needed to get similar results for salinity tolerance.

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