IECD 2024 Conference
The 3rd International Electronic Conference on Diversity
15–17 October 2024 | Online
MDPI

Reprogramming of Proteins and modulation of Antioxidant Enzyme in Alfalfa (*Medicago sativa*) Seedlings.

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INTRODUCTION & AIM

Alfalfa (*Medicago sativa*) is considered the world's most important leguminous perennial crop belonging to the family Fabaceae, which is used for a variety of purposes, including food, medicine, hay, pasture, and silage production. Due to its high nutritional value, including vitamins, minerals, and protein contents, it is a good choice for livestock feed (Zhou, Jia et al. 2019). During seed germination, seeds use up their protein reserves to provide energy for growing and convert them into other enzymes that facilitate germination. The seed also experiences various physiological and biochemical changes; enzyme activity often increases as germination progresses due to hydration that triggers the enzymes, as well as the breakdown of stored materials in the seed that leads to increased metabolic activities. Catalase, which is an antioxidant enzyme protecting the cells from oxidative damage, is one of the most common and important enzymes produced during seedling germination, thus preventing the harmful effects of Reactive Oxygen species (ROS). The presence of ROS during seed germination triggers the activation and modulation of enzymes like catalase.

This study aims to examine how various treatments modulate protein expression patterns and enzyme activity in alfalfa seedlings.

METHOD

Alfalfa Seed C. Bridger undergoes surface sterilization using a 10% v/v solution of sodium hypochlorite for 3 minutes. Subsequently, rinse thoroughly with distilled water three times. We placed 25 seeds in 20-ml glass vials and left them in the dark for 24 hours to facilitate germination. The seeds were chased with different treatments of 1 g/l activated charcoal, 60 mL titanium dioxide, and 10 µm abscisic acid. There were also two sets of control, dry seeds and seedlings that were chased with only water. Each treatment was done three times. We collected 5 germinating seeds from each replicate, resulting in a total of 15 seedlings per treatment at 48 hrs, 96 hrs, and 144 hrs after germination. For the control, we started sample collection 24 hrs after germination. We immediately froze the collected samples in liquid nitrogen to preserve their biochemical composition. Using a mortar and pestle, the samples were homogenized with a universal phosphate buffer containing a protease inhibitor; everything was done on the ice. The samples were centrifuged at 10,00 rpm for 15 minutes at 4^0C. The supernatant was collected and aliquoted for protein quantification, catalase enzyme assay, and protein profiling via the SDS page.

RESULTS & DISCUSSION

Foot note: DS =Dry seed, S= Seedlings, AC= Activated charcoal Tio2= Titanium dioxide and ABA= abscisic acid

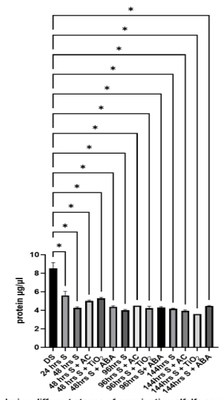

Fig 1: Total protein during different stages of germinating alfalfa seedlings treated with Activated

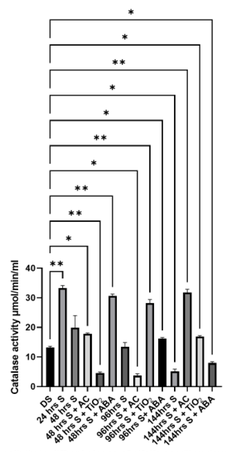

Fig 2: Catalase enzyme activity in diffrent stages of alfalfa seedling development treated with Activated charcoal, Titanium dioxide and Abscisic acid.

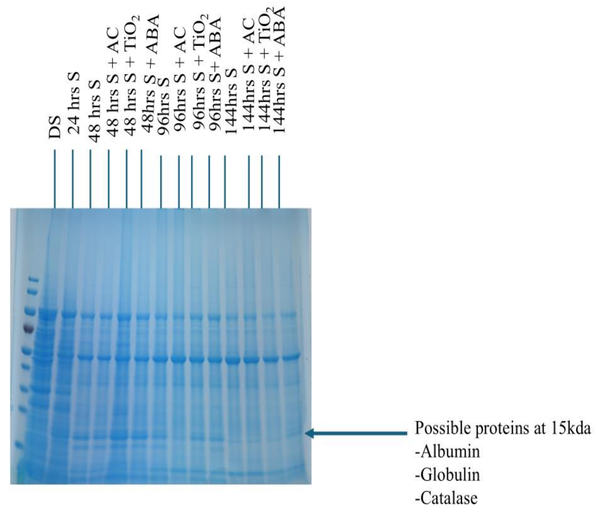

Fig: 3 SDS page gel protein profiling of alfalfa seedlings at different stages of germination and treatments

CONCLUSION

- Catalase activity increases within the first 24-48hrs in germinating seeds, with the highest activity observed between 48-96hrs of seed germination; also, more catalase activity was observed in treated seedlings, with the highest activity observed in seedlings treated with ABA; this is an indication that ABA increases enzymatic and metabolic activities of germinating alfalfa seedlings
- Protein bands disappeared with an increase in days to seed germination (144hrs after germination) seedlings at 144hrs treated with ABA still have the possible band of albumin, globulin, or catalase subunits
- The total protein in seedlings also decreases with increased days of seedlings.

FUTURE WORK / REFERENCES

*Validate the expression of the proteins expressed and other possible proteins during profiling using further analysis like mass spectrometry and protein sequencing

*Consider different parts of the seedlings for the enzyme activity, as the activity may vary from part to part

Reference

Zhou, Q., et al. (2019). "MYB transcription factors in alfalfa (Medicago sativa): genome-wide identification and expression analysis under abiotic stresses." **7**: e7714.