

The contribution of the antioxidant capacity to drought tolerance in *Medicago sativa* (Lucerne)

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Abstract

Medicago sativa more commonly known as lucerne/alfalfa is a perennial legume belonging to the Fabaceae family. It is an important forage crop used for grazing, hay, silage and as a cover crop. Drought has been shown to be one of the major environmental factors shown to impact crop yields. Plants subjected to drought tend to accumulate reactive oxygen species (ROS) which when over accumulated disrupts cellular function, causing a reduction in growth and development. This reduces lucerne yield and in the cases of severe drought, can result in crop failure. This negatively impacts food security. Plants have developed ROS-scavenging mechanisms such as antioxidants, which detoxify ROS in order to restore homeostasis. Therefore, we set out to investigate the accumulation of H_2O_2 , a ROS as well as a subset of antioxidants in contrasting lines of *Medicago sativa* grown under well watered and water-deprived conditions. In response to water-deprivation a decrease in leaf relative water content, length and weight was observed in Sardi 10 and ML99. Furthermore, water-deprivation resulted in an increase in H_2O_2 accumulation well as an increase in lipid peroxidation. Additionally, these lines proved to have both similar and varied responses in enzymatic antioxidant activity for ascorbate peroxidase and catalase. These responses differed between leaves and roots. These results suggest that the variances in the antioxidative capacity play an important role in establishing the drought tolerance in *Medicago* species.

Keywords: *Medicago sativa*, Drought, ROS, Antioxidants, homeostasis

Introduction and aims

Medicago sativa more commonly known as lucerne is a legume forage crop which is used for animal feed (grazing, hay and silage) as well as a cover crop (Kulkarni et al., 2018). Furthermore, like most legumes these plants play a role in the natural fixing of atmospheric nitrogen through symbiosis with soil-borne rhizobia (Moghaddam et al., 2015). The production of lucerne like any other crop is dependent on favourable environmental conditions. South Africa, being a semi-arid country is prone to unfavourable environmental conditions such as drought. This negatively impacts lucerne yields, compromising food security. In plants, abiotic stressors such as drought often result in the excessive accumulation of reactive oxygen species (ROS) which causes cellular oxidative stress. This limits growth and eventually results in crop failure. However, plants have developed ROS-scavenging mechanisms such as antioxidants in detoxify ROS to maintain homeostasis (Zhang et al., 2018). Therefore, this study aims at investigating the accumulation of H_2O_2 , a ROS as well as a subset of antioxidants in contrasting lines of *Medicago sativa* grown under water-limiting conditions.

Methods

- Germinate lucerne lines (Sardi 10 & ML99) in moist paper towel
- Plant germinated seed in well-watered (100 % field capacity) and water-deprived (30 % field capacity) promix
- Harvest
- Determine physiological changes (RWC, shoot weights and lengths)
- Determine biochemical changes (ROS accumulation, antioxidants)

Conclusion

Plants grown under water-deprived conditions had a decrease in RWC, length and weight. However, this decrease was larger in ML99 than Sardi 10. Furthermore, although both lines showed increases in H_2O_2 content. ML99 leaves experienced more damage to membrane lipids as indicated by MDA content. Furthermore, differential antioxidant responses were observed in shoots and roots under water-deprived conditions. Many of which suggest that Sardi 10 experienced a lesser degree of stress compared to ML99. This suggest that plants with improved antioxidant capacity would have improved tolerance to drought.

References

- Kulkarni, K. P., et al. (2018). "Harnessing the Potential of Forage Legumes, Alfalfa, Soybean, and Cowpea for Sustainable Agriculture and Global Food Security." *Frontiers in plant science* 9(1314)
- Moghaddam, A., et al. (2015). "Biological nitrogen fixation and biomass production stability in alfalfa (*Medicago sativa* L.) genotypes under organic management conditions." *Biological Agriculture & Horticulture* 31(3): 177-192.
- Zhang, C., et al. (2018). "Physiological and biochemical changes in different drought-tolerant alfalfa (*Medicago sativa* L.) varieties under PEG-induced drought stress." *Acta Physiologiae Plantarum* 40(2): 1-15.

Results

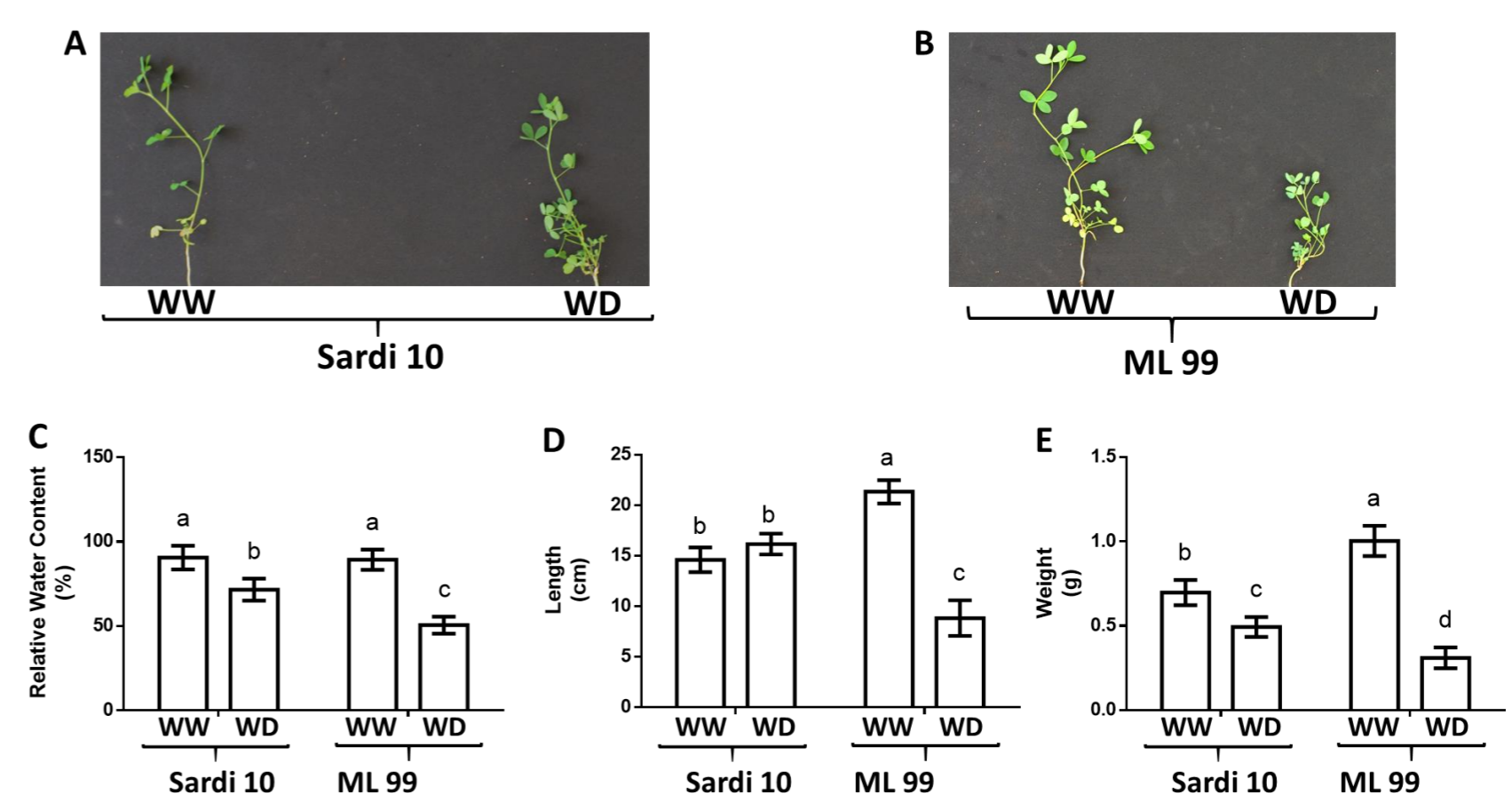


Figure 1: Images of lucerne lines (a) Sardi 10 and (b) ML 99 grown under well watered (WW) and water-deprived (WD). Graphs represent changes in (c) Relative water content, (d) length and (e) weight in shoots in response to drought. Data presented are means (\pm SE) of three independent experiments (n=3). Error bars denote standard deviation, where bars with the same letters are statistically similar and varying letters indicated statistical differences where $P < 0.05$.

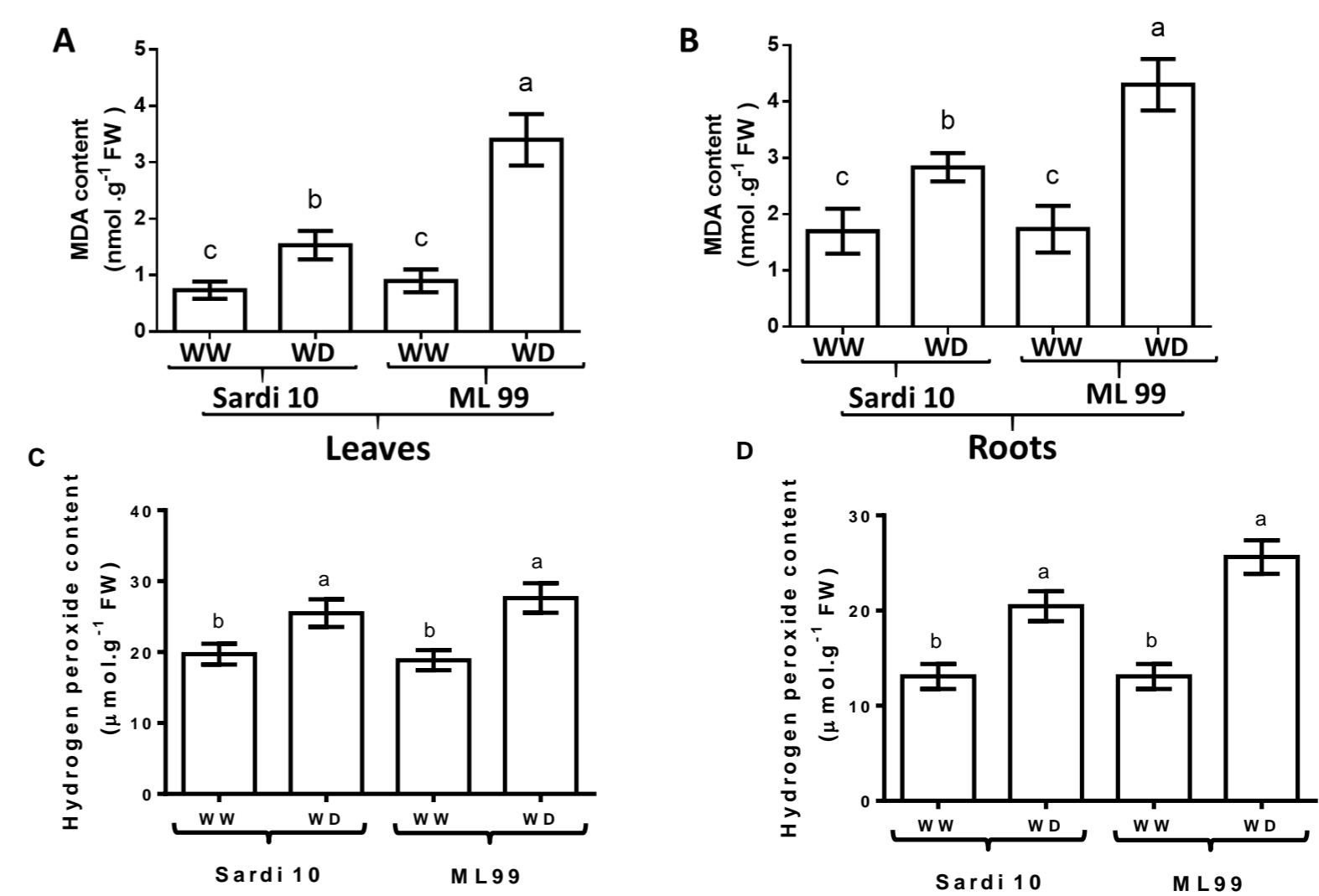


Figure 2: Malondialdehyde content (MDA) in leaves (a) and roots (b) and Hydrogen peroxide in leaves (c) and roots (d). These contents were observed in lucerne lines grown under well-water and water deficit conditions. Data presented are means (\pm SE) of three independent experiments (n=3). Error bars denote standard deviation, where bars with the same letters are statistically similar and varying letters indicated statistical differences where $P < 0.05$.

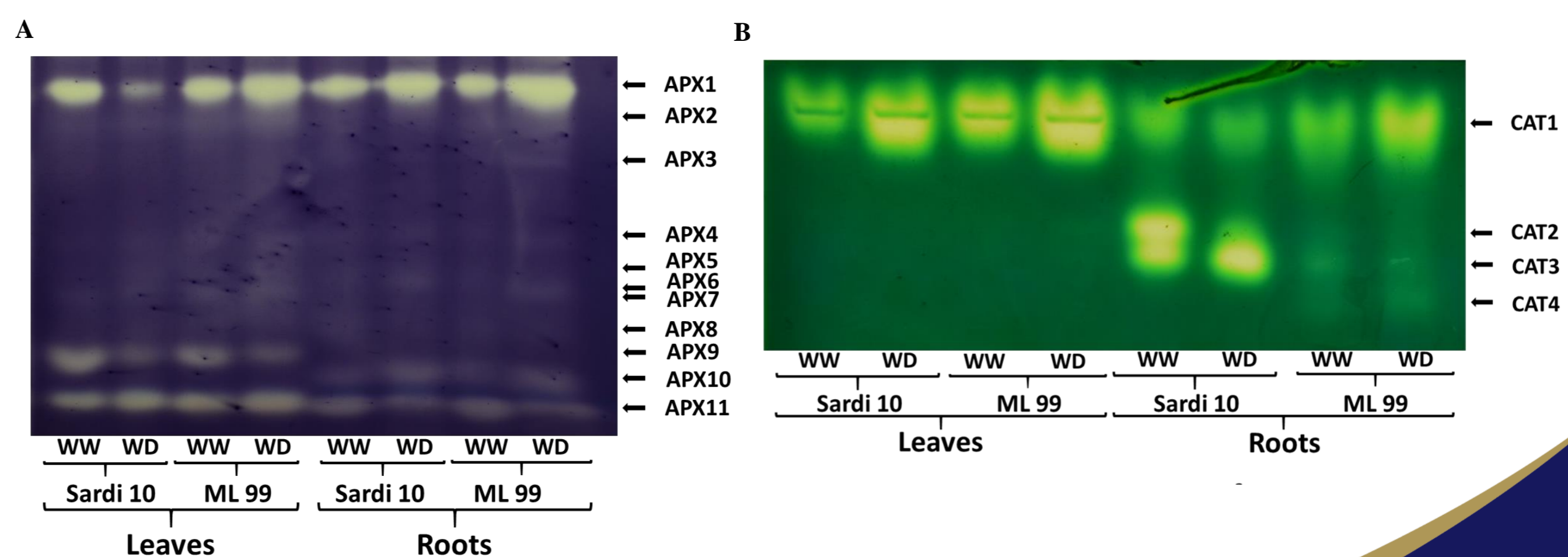


Figure 3: In-gel assay for Ascorbate peroxidase (APX) activity and Catalase (CAT) activity in contrasting lucerne lines grown under well-watered (WW) and water-deprived conditions. The in-gel assay shows different isoforms of APX and CAT in these lines in response to water-deprivation.