Plant growth regulator extracts from seaweeds confer drought tolerance in *Brassica napus* (Canola)

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Introduction

Brassica napus, commonly known as canola, is an important oilseed crop in Canada contributing over 29.9 billion Canadian dollars of economic activity annually[1]. A major challenge facing Canadian canola is drought, which has been increasingly prevalent in recent years due to the changing climate. Research investigating agronomic techniques in mitigating the drought problem is key to higher yields and sustainability in canola. One such technique is the use of seaweed extracts as bio-stimulant sprays to help offset biotic and abiotic stresses in plants[2].

Previous research has shown that the application of seaweed extracts as bio-stimulant sprays in Brassicaceae can efficiently increase drought tolerance[2]. However, this method has yet to be tested on canola. Bio-stimulant sprays can act as a novel alternative method to promote beneficial agronomic traits, independent of genetic manipulation and can lead to reduced fertilizer use. A drought-sensitive canola germplasm developed through gene-editing (*d14*) was used for drought assays.

In association with Timac Agro, we have been able to demonstrate that the Roullier extracts (RE) can help promote drought tolerance in canola. These extracts elicit responses in plants that are currently achieved only through gene editing and transgenic methodologies.

Results

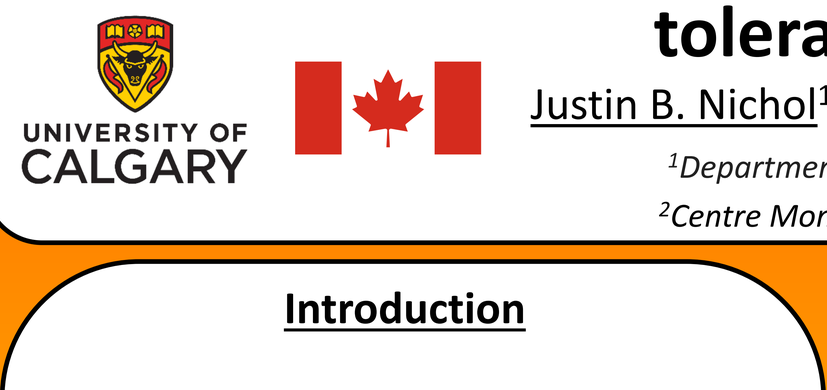

Figure 1. Seventy-two (72) plants of mutant *d14* gene-edited canola, deficient in Strigolactone response (SL) were transplanted to soil, watered, with their pot location randomized daily. At week 3, the plants were sprayed with RE at the optimal concentration with 0.01% Silwet L-77. RE1 and RE2 were sprayed at 0.5% concentration while RE3, RE4, and RE5 were sprayed at 1% concentration. Control lines were sprayed with water containing 0.01% Silwet. Plants were sprayed once a week during week 3, 5, and 6 for a total of three sprays.

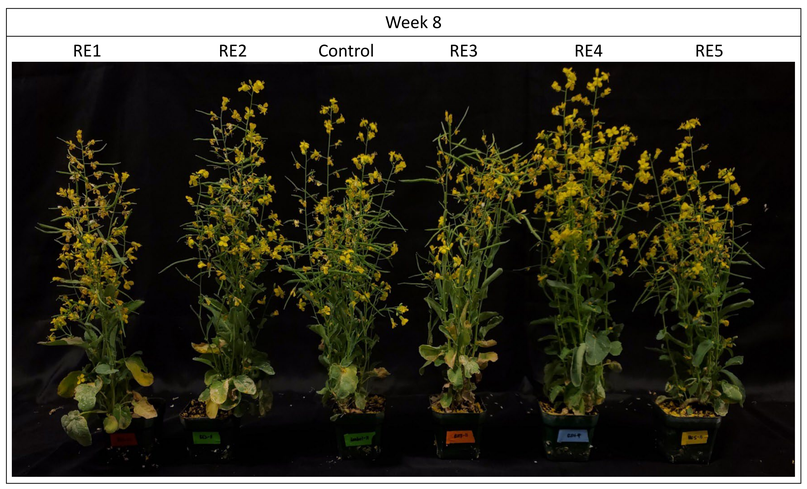

Figure 2. At week eight, 72 plants (12 plants per treatment) of mutant *d14* gene-edited canola deficient in Strigolactone response (SL) lines were imaged at maturity just before a drought assay.

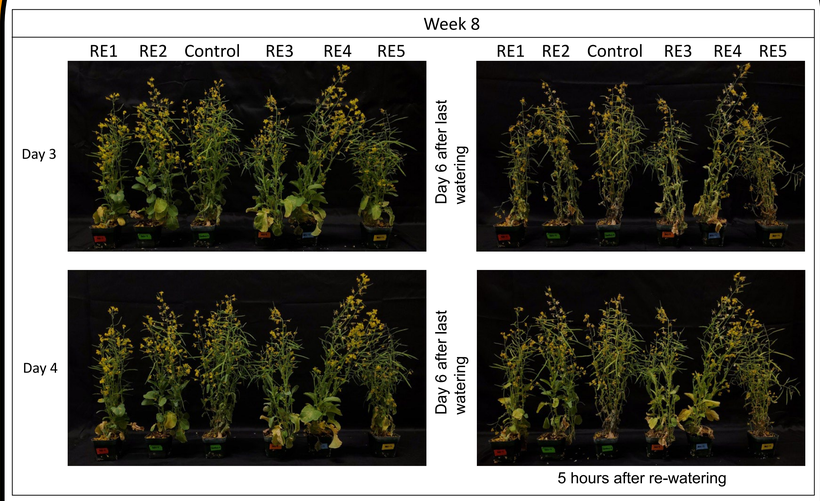

Figure 3. At week eight, 72 plants (12 plants per treatment) were subjected to a severe drought assay by withholding water for 6 days for *d14* mutant lines of canola, which is a drought-sensitive canola line. After 6 days, the plants were re-watered and plant response was captured 5h after re-watering.

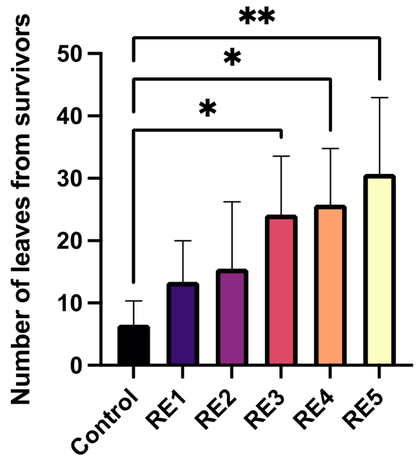

Figure 4. Number of viable leaves remaining in surviving plants (12 plants per treatment) Values were quantified and an ANOVA statistical test was performed. Error bars indicate ± SEM. Asterisks indicate significant differences (**P<0.01)(***P<0.001).

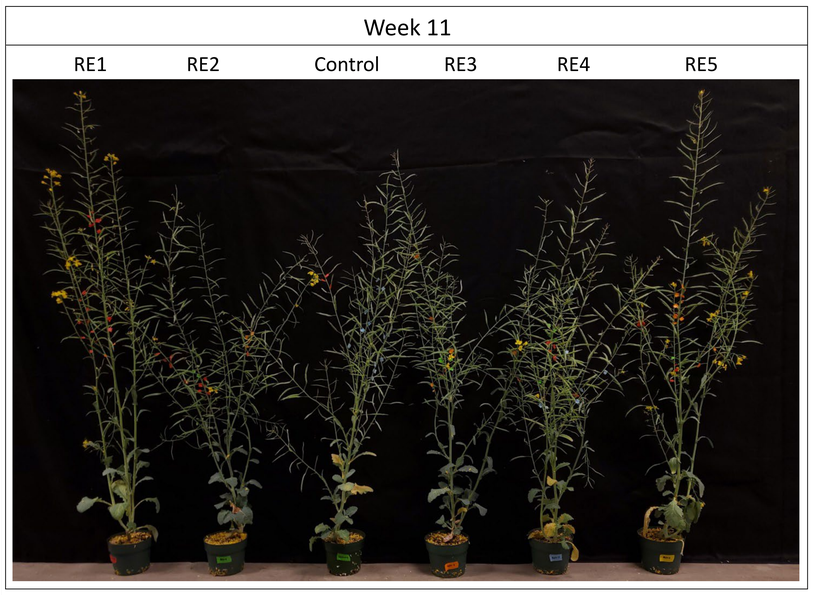

Figure 5. Phenotypic observation of wild-type canola plants treated with three sprays of RE (1-5) after week 11. At week 3, the plants were sprayed with RE at the optimal concentration with 0.01% Silwet L-77. RE1 and RE2 were sprayed at 0.5% concentration while RE3, RE4, and RE5 were sprayed at 1% concentration. Control lines were sprayed with water and 0.01% Silwet. Plants were sprayed once a week during week 3, 5, and 6 for a total of three sprays.

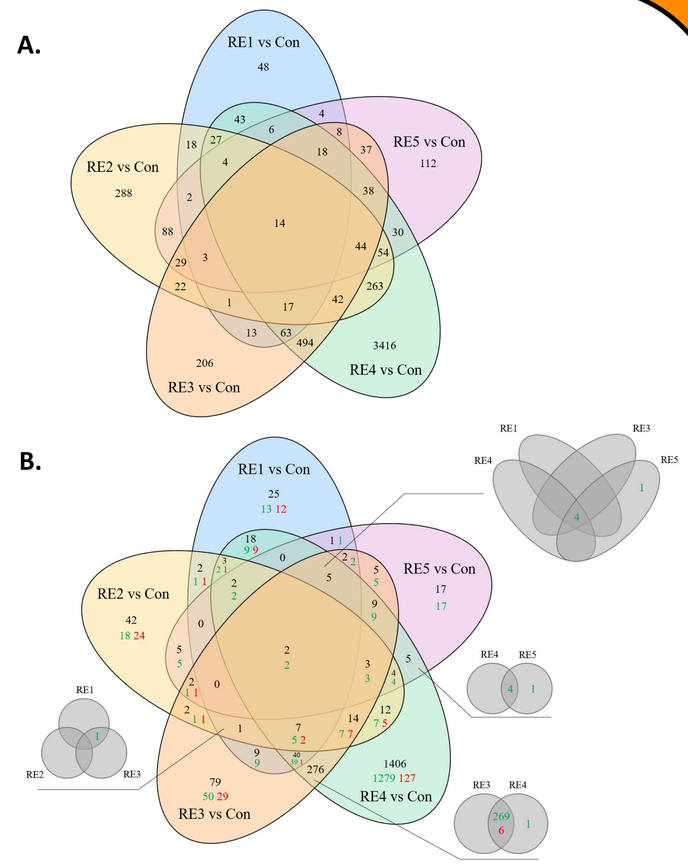

Figure 6. Whole plant tissue was collected from wild-type canola plants treated with (RE1-5) after 3 sprays each. Triplicate RNA samples were isolated from each treatment (control and RE1-5), for a total of 18 samples were subjected to RNA sequencing. **(A)** A five-way Venn diagram depicting the number of all differentially expressed genes between the treatments (RE1-5) vs control. **(B)** Absolute value of the Log2 fold change ≥ 2 Venn diagram depicting the number of all differentially expressed genes between the treatments (RE1-5) vs control with additional data depicting these gene clusters as either up or down regulated. Up-regulated genes are shown in green, and down-regulated genes shown in red. Additional Venn diagrams depict when a gene had different expression directionality under different RE treatment conditions.

Conclusion

1. Generally, plants sprayed with RE had a significantly high branching phenotype, vegetative growth, and more pods compared to the control.
2. RE4 and RE5 had the best drought tolerance and the greatest number of leaves 6 days after re-watering
3. RE4 treated plants had the highest number of differentially expressed and up-regulated genes, over 250 genes are up-regulated between RE3 and RE4 and 39 genes commonly up-regulated between RE3,4, and 5.

References

1. Canada's top canola markets | The Canola Council of Canada (2020). Available at: https://www.canolacouncil.org/markets-stats/top-markets/.
2. Ghaffar Shahriari, A., Mohkami, A., Niazi, A., Ghodoum Parizipour, M. H., & Habibi-Pirkoohi, M. (2021). Application of Brown Algae (Sargassum angustifolium) Extract for Improvement of Drought Tolerance in Canola (Brassica napus L.). *Iranian journal of biotechnology*, *19*(1), e2775.