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MPSG FINAL EXTENSION REPORT

PROJECT TITLE: Enhancing water stress tolerance in soybean through phytoglobin manipulations

PROJECT START DATE: 1 March 2016 **PROJECT END DATE: 31 December 2019**

DATE SUBMITTED: 12 February 2019

PART 1: PRINCIPAL RESEARCHER

PRINCIPAL

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PART 2: EXECUTIVE SUMMARY

Outline the project objectives, a summary of the activities and results, and their relevancy to pulse and soybean farmers.

This project consists of several related objectives:
(1) Develop soybean plants with altered expression of phytoglobin (Pgb). (2) Use of Pgb as a molecular marker to estimate flooding tolerance.

The ongoing research will test the potential of using Pgb as a powerful molecular marker to select soybean varieties tolerant to flooding and waterlogging. This will assist pulse and soybean farmers in choosing varieties suitable to grow in these environments.

Year 2016-2017. We have generated transgenic soybean lines over-producing or under-producing Pgb. In addition, preliminary experiments were conducted to estimate the relationship between Pgb expression level and tolerance to flooding in 20 commercial soybean varieties.

Year 2017-2018. We have characterized the behavior of the transgenic soybean lines (developed in 2016-2017) to submergence and waterlogging, and further established the link between Pgb expression and tolerance to excessive moisture in the 20 commercial soybean varieties.

Year 2018-2019. We have measured the expression of several aquaporins, repeated the flooding experiment and conducted preliminary studies on the effects of Pgbs during water stress.

PART 3: EXPERIMENT DESCRIPTION & RESULTS

Concisely describe the experimental methods and results to date. You may include up to 3 graphs/tables/pictures in the Appendix.

1. Development of transgenic lines with altered levels of Pgbs.
Soybean lines up-regulating or suppressing Pgb were generated (See Fig. 1 and 2 of the 2017 Extension Report).

2. The behavior of the transgenic lines to waterlogging
To evaluate the behavior of the transgenic lines to waterlogging the following parameters were measured: photosynthetic rate, stomatal conductivity, intercellular CO2 level, transpiration rate, and number of adventitious roots. As shown in Fig. 1-5 of the 2018 extension report, over-production of Pgb enhances all these parameters, while the under-production of Pgb has negative effects on all the parameters measured.

3. The behavior of the transgenic lines to submergence
The percentage of plants able to recover and grow (recovery rate) after exposure to one week of full submergence in the dark was used as an estimation of submergence response. As shown in Fig. 6 of the 2018 Extension Report, relative to wild type (WT) with a normal "natural" level of Pgb, plants over-producing Pgb have a much higher recovery rate while those under-producing the same protein exhibit reduced recovery rate. This behavior correlates well with the level of Pgb expression measured in these plants.

4. The response of the 20 commercial soybean varieties to waterlogging and submergence was also further investigated by closely looking at the correlation between Pgb expression level and tolerance to each stress. For waterlogging a weak correlation (0.49) was observed between Pgb in the roots (Fig. 7 of the 2018 Extension Report) or shoots (0.54, data not shown) and photosynthetic rate. This was in contrast to the submergence experiments where the correlation between recovery rate and Pgb expression was 0.94 in the roots (Fig. 8 of the 2018 Extension Report) and 0.78 in the shoots (Fig. 9 Extension Report).

5) Expression levels of aquaporins (Tonoplast intrinsic proteins, TIPs) and alcohol dehydrogenase (ADH) during submergence in the dark or light of the soybean lines with altered levels of Pgb was measured. TIPs facilitate movement of water between cells while ADH is a marker of the fermentation pathway operating during anaerobiosis. Among the several TIPs measured, in lines up-regulating Pgb the expression level of TIP1.1 and TIP2.7, known to increase in lines tolerant to excessive moisture were induced after 2 days of full submergence. This was in contrast to the expression of TIP1.5, known to be low in plants able to withstand excessive moisture, which declined in the soybean line over-producing Pgb. Alteration of Pgb had no noticeable differences on the expression of ADH.

6) The response of plants over-producing or suppressing Pgb was examined in response to drought, simulated by applications of polyethylene glycol (PEG). Plants over-expressing Pgb had increased root length and higher number of lateral roots (Fig. 1); this was in contrast to plants suppressing Pgbs which displayed a reduced root length and lower number of lateral roots. These results suggest a better capacity of plants over-producing Pgb to cope with water stress.

Collectively, these studies demonstrate that over-production of Pgb represents a strategy to tolerate conditions of submergence and drought, and that the natural variations of Pgb can be expoited to select lines able to cope with both types of stress.

PART 4: RELEVANCE TO FARMERS AND FUTURE RESEARCH

Describe how the project results can be captured to benefit pulse and soybean farmers (production recommendations, innovation items, marketing plans, commercialization of technology etc). Identify any future research opportunities.

Besides providing fundamental knowledge on the molecular mechanisms of plant responses to stress, this study provides evidence that Pgb could be used as a marker to predict plant behaviour to conditions of water stress (excess moisture and drought). The simple measurement of Pgb could in fact be utilized as a rapid screen to select varieties tolerant to water stress. Furthermore, knowledge from this work could be exploited by plant breeders to introgress this trait, i.e. high Pgb level, in desirable varieties suitable for growing in Manitoba.

PART 5: COMMUNICATION

List extension meetings, papers produced, conference presentations made, project materials developed.

A summmary of the results of this work have been released https://www.manitobapulse.ca/wp-content/uploads/2018/12/Pulse-Beat_85_December-2018_Final_WR.pdf

The PI and Dr. Huang, the Post. Doc. involved in the project have had frequent connections with representatives of MPSG

APPENDIX

Include up to 1 page of tables, graphs, pictures.

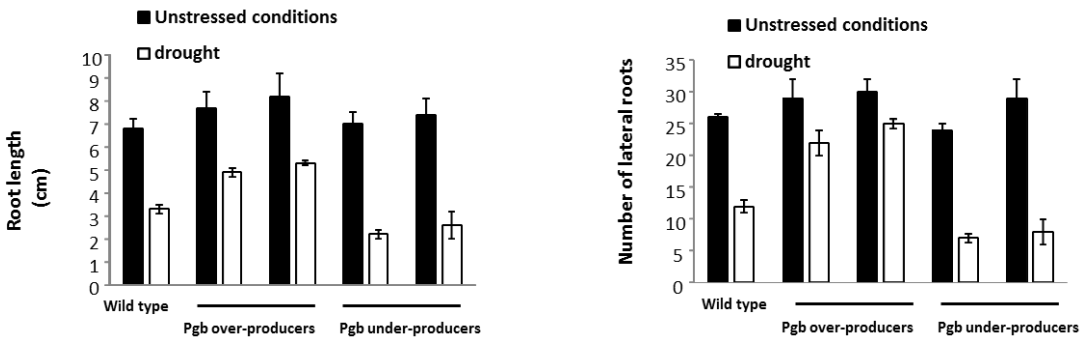

Fig. 1. Root length and number of lateral roots measured in 2day-old seedlings exposed for 5 days to water stress