Name of Organization: National Agricultural Genotyping Center (NAGC)

Project Title: Enhancement to a high-throughput genetic test for identification of herbicide resistance traits in Palmer amaranth and related pigweed species.

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Objectives of the Research
Late-season pigweeds (*Amaranthus* spp) that escaped herbicide applications are considerable threats to future crop production. Herbicide resistant (HR) populations have been rapidly emerging across the US for Palmer amaranth and waterhemp, the two out-crossing pigweed species. Palmer amaranth has been recently discovered in North Dakota, but little is known about the genetic HR potential of these populations. Likewise, the distribution of waterhemp is growing in the state as well as the number of complaints from producers about ineffective herbicides. There are several genetic markers that are linked to HR traits, particularly for glyphosate and PPO-inhibitors, the two common herbicides used to combat pigweeds in bean fields. **The objective of this project was to expand NAGC's genotyping panel by including two additional tests for genetic markers linked to glyphosate and PPO-inhibitor resistance in pigweeds.** Increasing the availability of these genetic tests will provide the opportunity to better characterize the HR potential of new and established pigweed populations in the region. Once HR characteristics of local pigweeds have been described, soybean growers can use this information to avoid ineffective herbicides and adjust their integrative pest management towards optimal pigweed control.

Completed work
Due to the need for rapid confirmation of new Palmer amaranth populations and the challenges of visually identifying pigweed species, NAGC developed an Amaranth Species ID test for leaf and seed samples. The test has already benefited the North Dakota soybean community through gaining a better understanding of the distribution of Palmer amaranth in North Dakota. The additional HR genotyping tests provide a deeper understanding of the chemical management options that soybean growers have to control pigweeds.

The genetic mechanisms for glyphosate and PPO-inhibitor resistances are fundamentally different, yet can be detected in the same DNA extracts validated for the Amaranth Species ID test. The first test for glyphosate resistance quantifies the EPSPS gene copy number in waterhemp and Palmer amaranth leaf samples. Elevated EPSPS gene copies is associated with reduced effectiveness of glyphosate in pigweeds. The second HR test is for PPO-inhibitor resistance that detects a mutation (amino acid deletion) within the *PPX2* gene, which codes for the PPO enzyme in pigweeds. By changing the shape of the PPO enzyme, this mutation reduces the ability of the PPO-inhibitor herbicide (e.g., fomesafen) to interact with its intended target. These two tests were selected for the HR panel at NAGC because multiple laboratories have used them for statewide surveys of pigweed populations over the last few years.

Most of the validation work has been completed for the HR tests at NAGC. For the glyphosate resistance test, we have optimized assay parameters to closely match the Amaranth Species ID

test. After performing specificity tests and further examined archived pigweed samples, the assay appears specific to the pigweed family. Likewise, the PPO-inhibitor test has been optimized so it can be co-amplified with the Amaranth Species ID test, and it appears to show high specificity to the pigweed family in specificity tests. Unfortunately, due to the difference in analyses between the EPSPS (copy number) and PPO-inhibitor (genotyping) assays, it is not possible to co-amplify all three tests on a single plate. We expect little delay in completing the validation under NAGC's ISO 17025:2017 standards, which provides robust quality assurance and performance.

Preliminary Results

During the validations, we tested a set of archived DNA samples of Palmar amaranth (n=26) and waterhemp (n=29) originally submitted by clients in North Dakota and Minnesota for NAGC's Amaranth Species ID test. In total, 15% (4/26) Palmer amaranth and 34% (10/29) waterhemp samples had elevated EPSPS gene copies to be considered glyphosate resistant. There were large difference in the number of gene copies between the two species, where Palmer amaranth and waterhemp had individuals with maximum EPSPS copy number of 50 and 8, respectively. This was expected because Palmer amaranth has a unique duplication mechanism that results in dozens of more gene copies than waterhemp in HR individuals. Notably, several waterhemp samples showed elevated EPSPS copies just below the threshold (four gene copies), signifying the growing potential for glyphosate resistance, which may appear in offspring from these individual plants. For PPO-inhibitor resistance, there were three waterhemp samples (10%, 3/29) containing the deletion that confers resistance. No Palmer amaranth samples in the small subset contained the deletion, but additional North Dakota populations should be tested.

Work to be Completed

Written validations for both HR tests are nearing completion. The final sensitivity and mixture analyses must be performed for glyphosate resistance test, and sequence confirmation of the expected genotypes for the PPO-inhibitor test from the archived samples. Once these analyses are completed, each validation will be critically reviewed by two NAGC scientists. If warranted, additional optimization tests may be requested and further explored. Following validation approval, two standard operation procedures (SOPs) will be created for use by scientists at NAGC. Before NAGC scientists can provide HR test results to collaborators or soybean growers, each NAGC scientist must pass a blinded proficiency test. Lastly, these HR tests will be integrated into the NAGC's ISO 17025 accreditation scope in 2021. The completion of these tests were expedited so that both test would be available to soybean growers early in the 2021 growing season.