ISA Final Contract Research Progress Report

Project Title: Determine the heritability of HPPD resistance in common waterhemp and the potential for hybridization of common waterhemp and Palmer amaranth (ISU # 450-45-13)

Principle Investigator and Co-PIs: Micheal D.K. Owen

Budget Amount and Project Year: $ 75,000 (combined projects)

Brief Statement of Objectives: Determine the heritability of HPPD resistance in common waterhemp. Seed collections from fields previously identified to have evolved resistance to HPPD inhibitor herbicides will be selected recurrently so that the population is homozygous for HPPD resistance. Crosses and back crosses with HPPD sensitive common waterhemp populations will be made in the greenhouse, growth chamber and field. These crosses will determine whether or not HPPD resistance is determined by a single or multiple genes and if resistance is a dominant trait.

Determine if hybridization between common waterhemp and Palmer amaranth is possible and assess the likelihood of spontaneous hybridization where fields have populations of common waterhemp and Palmer amaranth. Crosses and backcrosses between populations of common waterhemp and Palmer amaranth with known herbicide resistance profiles will be conducted in the greenhouse, growth chamber and field. Herbicide resistance within one parent will be the marker to assess if resultant progeny represent a hybrid. Furthermore, a multivariate quantitative trait analysis will be conducted in order to describe the phylogenic relationship of the hybrid plants to the parents. Molecular comparisons will also be conducted. Seed viability of the hybrids will determine if postzygotic reproductive barriers exist which could impact the field establishment of the hybrids.

Brief Statement of Expected Deliverables: Information resulting from these studies will allow a prediction describing the speed at which HPPD resistance in common waterhemp is likely to spread in Iowa and the potential threat of Palmer amaranth hybridization with waterhemp. If the possibility of Palmer amaranth X common waterhemp hybrids is real, the project will provide information that will allow Iowa soybean growers to mitigate these threats.

These projects are important for developing management strategies for minimize the economic impact of HPPD inhibitor herbicide resistance as well as the yet-unidentified threat of Palmer amaranth and Palmer amaranth X common waterhemp hybrids. Given the increasing number of "founder" populations of Palmer amaranth that have been identified in Iowa, knowledge about the potential for hybrids between Palmer amaranth and waterhemp is important to Iowa soybean production.

The primary deliverables will be information; Iowa soybean growers will be better able to manage the most important soybean pest complex (weeds) that is evolving a new herbicide resistance and potentially a new weed species. ISU Extension and ISA collaborative publications describing the results of this research and make these available to growers in a clear and consistent manner thus facilitating better management decisions by Iowa soybean growers will

be developed. Peer-reviewed journal publications will also be written. This information will also be made available in webpages, newsletters, blogs, radio presentations, and used in grower meetings.

Brief Statement of Progress, Milestones & Deliverables:

For the HPPD resistance heritability study, see attached pedigrees. In 2013, male and females plants of HPPD-resistant and -susceptible populations were planted in isolation tents at the North Central Plant Introduction Station in Ames, IA. Reciprocal crosses were made resulting in 93 half-sib lines (F_1 populations). Seeds from each of the female plants were collected and processed individually.

In 2014, the F1 populations from the HPPD resistance project and the waterhemp and Palmer Amaranth hybridization project were planted in the greenhouse. There were a total of 166 separate F1 half-sib lines from the two projects. Each half-sib line was replicated 3 times for a total of 498 crosses established in the field. Plants growing in the field were watered and monitored throughout the summer. When flowering began on plants, six foot tall Delnet bags were placed over the top of the plants. PVC pipes were used to keep the bags from smothering the plants and allowing plants room to grow within the bag. The site was monitored daily for any breakthroughs on the bags. If a breakthrough was found, the hole was repaired or the bag was replaced. Starting in early October, we began checking plants for seeds. By the middle of October, seeds had developed and female plants were individually harvested. We finished harvest by the end of October 2014. A total of 274 full sib F2 waterhemp lines were established representing the original F1 lines which were replicated 3 times; not all lines had 3 replications. Interestingly, the crosses were more successful when the female parent carried the HPPD resistance trait. RNA sequencing to identify the genes that control HPPD resistance is underway.

In 2015, Back crosses of the F_2 lines were conducted in the field as previously described. Double reciprocal crosses were made to the parent lines and 119 BC_1 were established. Seed was collected.

All lines (F_1, F_2, and BC_1) will eventually be screened for HPPD resistance. Currently sufficient lines were evaluated for phenological response to mesotrione to allow the assessment of heritability of HPPD resistance and the number of genes involved in the resistant phenotype.

The RNA study is close to completion. This will provide specific information about the alleles that are active when HPPD-resistant waterhemp is challenged by HPPD herbicides. The compiled list includes 113,893 transcripts that are potentially expressed in waterhemp based on RNA extractions along with their annotations from 3 different databases. Of these 113,893 transcripts 72,697 transcripts were considered to be expressed in response to HPPD herbicide challenge. The data will be used to develop a list of specific genes active in the HPPD resistance response in waterhemp and support conclusions and make comparisons between genotypes and timepoints with regard to gene expression. This is an important file because when

published, other researchers that do sequencing projects with waterhemp will be able to use this as a reference to look up transcripts found in waterhemp. It is anticipated that this portion of the project will be completed fall 2016.

All data sets are available on request.

For the Palmer X Waterhemp hybridization study, see attached pedigrees. In 2013 Palmer amaranth male and female plants with susceptibility to atrazine were planted in isolation tents with waterhemp male and female plants with atrazine resistance. Reciprocal crosses were made, seeds from female plants collected and processed individually resulting in 73 half-sib lines (F_1 populations). Seeds were harvested and processed for all of the F_1 lines. Morphological data from the female reproductive structures for the Palmer amaranth and waterhemp parents and the F_1 was collected.

In 2014, 209 full sib lines (F_2) putative hybrids were established. Again, not all F_1 lines had 3 replications. Morphological data on the female reproductive structures for the putative waterhemp x Palmer amaranth hybrids was collected and seeds have been processed.

In 2015, Morphological data was collected on the female plants of the BC_1 lines and seeds of the BC_1 lines has been processed.

All data sets are available on request.

Information from these projects has been included in numerous grower presentations as well as presentations and symposia at regional, national and international scientific meetings.

Brief Statement of Plans & Next Steps:

HPPD resistance heritability in waterhemp study

The assessment of HPPD heritability of the parents, F_1, F_2 and BC_1 lines via herbicide resistance phenotyping will be completed late in 2016. This will be conducted in the greenhouse using a mesotrione herbicide challenge as previously described. Once completed, statistical analyses will be conducted that will provide definitive support to the conclusions established in the thesis and papers that are currently being written. A paper will be submitted to a high level journal and ISU Extension publications will be developed. Information will also be used in extension and outreach presentations as well as for regional, national and international scientific conferences and symposia.

A MS thesis is near completion describing in detail the heritability and the specific alleles that provide resistance to the HPPD-inhibitor herbicides in waterhemp. From the paper, two journal papers will be published in high-level scientific journals. The papers are:

Title: Inheritance of 4-hydroxyphenylpyruvate Dioxygenase (HPPD) Inhibitor Herbicide Resistance in *Amaranthus Tuberculatus* (Waterhemp)
Journal: Plant Science

Title: An RNA-Seq De Novo Transcriptome Assembly of *Amaranthus Tuberculatus* (Waterhemp) and Analyses of Differentially Expressed Transcripts Related to 4-ydroxyphenylpyruvate Dioxygenase (HPPD) Inhibitor Herbicide Resistance
Journal: BMC Genomics

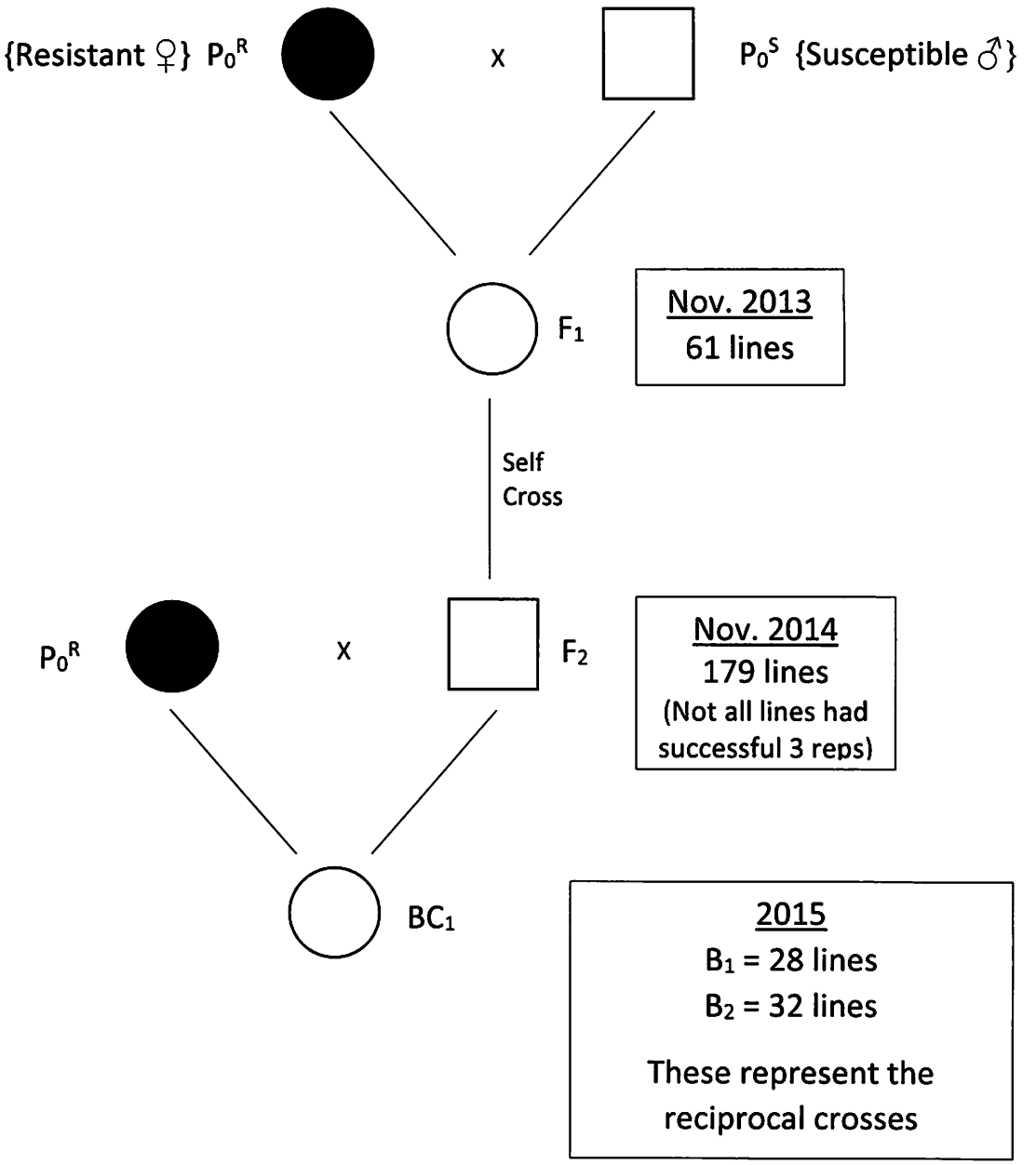
Several presentations based on these papers will be presented at regional, national and international scientific conferences and symposia. Extension and outreach articles will be developed to support Iowa soybean production and information from the project will be presented at extension and outreach meetings.

Palmer X Waterhemp hybridization study

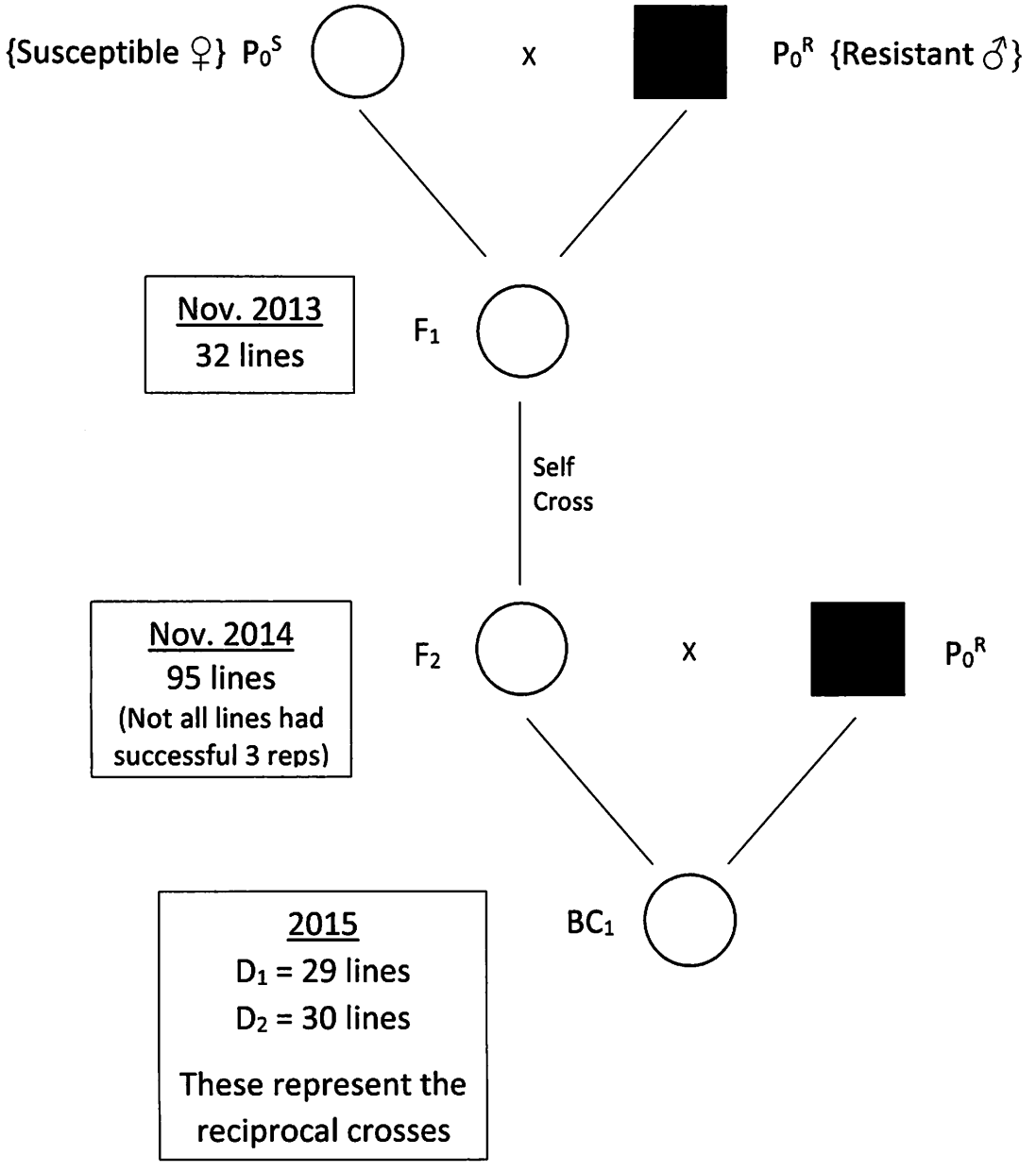
Morphological data for the putative Palmer amaranth X waterhemp F_1, F_2 and BC_1 will be analyzed in the near future. Phenological assessment of putative hybrids using an atrazine herbicide challenge will be conducted in the greenhouse as previously described. Further molecular comparisons of the parents, F_1, F_2 and BC_1 will be conducted. This information will be compiled and used as the basis for a paper that will be published in a high-level scientific journal. Presentations based on the project will be delivered at regional, national and international scientific conferences and symposia. Extension bulletins, fact sheets and popular press articles describing the results of the project will be developed. Information from the project will continue to be included in outreach and extension activities.

HPPD heritability and Palmer amaranth X waterhemp hybridization projects; establishing BC_1 lines in 2015 at the North Central Plant Introduction Station, Ames, IA.

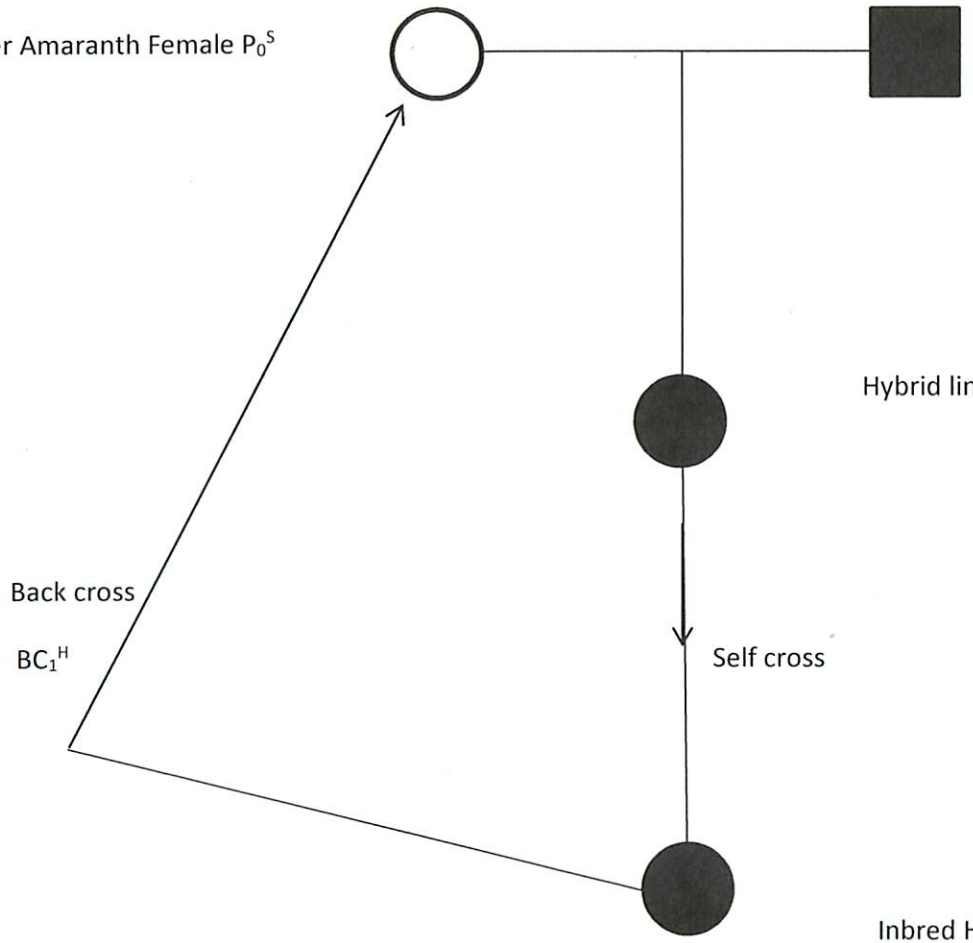
HPPD Resistance Heritability in Waterhemp



HPPD Resistance Heritability in Waterhemp

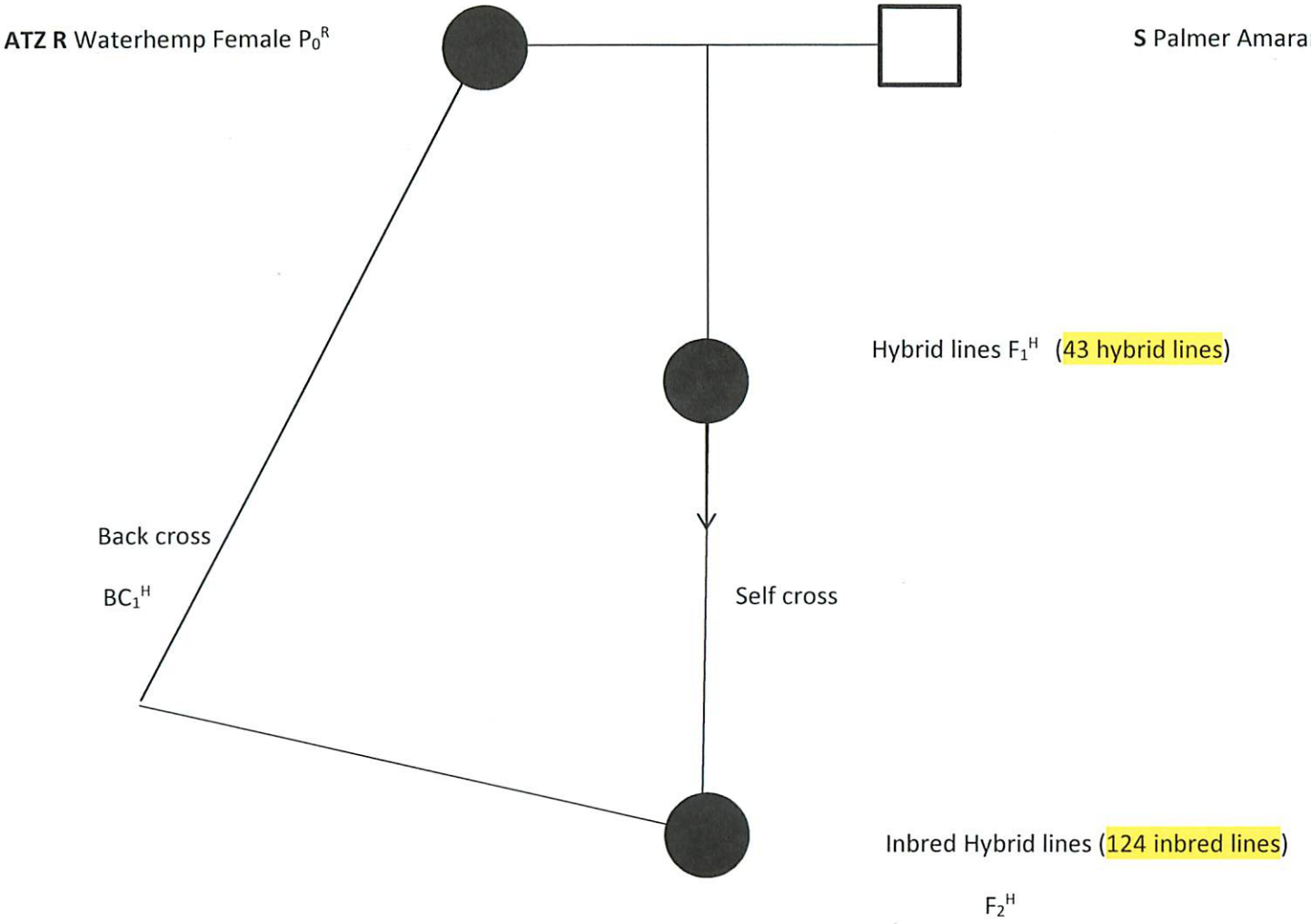


Palmer Amaranth Hybridization project



61 BC lines (Double reciprocal crosses) – 12 lines WH female to F_2, 22 lines WH male to F_2, 17 lines Palmer male to F_2 and 10 lines Palmer female to F_2

Palmer Amaranth Hybridization project



118 BC lines (Double reciprocal crosses) – 18 lines WH female to F_2, 38 lines WH male to F_2, 40 lines Palmer male to F_2 and 22 lines Palmer female to F_2