**AGRICULTURAL RESEARCH FOUNDATION**
**FINAL REPORT**
**FUNDING CYCLE 2014 – 2016**

**TITLE: Creating a physical map of the barley genome using radiation hybrids**

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**SUMMARY:** A complete genome sequence of crop species offers new methods for breeders to target traits of interest. High throughput DNA sequencing has made it relatively easy to sequence the entire genome of crops, even those with large genomes like barley. However the sequence data is acquired in millions of small fragments that must be assembled into a complete sequence. Using a genetic map as a framework to assemble sequence fragments is commonly used to aid assembly, but many areas of chromosomes cannot be mapped or assembled through genetic approaches. Over the past six years we developed a Radiation-Hybrid technique to generate physical chromosome maps in wheat. The basis of the mapping technique is the generation and detection of multiple small radiation-induced deletions within a targeted chromosome. In this proposal we planned to test the utility of the Radiation Hybrid Mapping method in barley. We adapted the techniques we developed for Radiation Hybrid Mapping in wheat to barley. We tested increasing radiation dosages and developed small panels of germplasm for each dose. PCR markers required for determining the deletion rate were identified and the germplasm was screened for radiation-induced deletions. We conclude that barley lacks the ability to tolerate the level of radiation-induced deletions sufficient for Radiation Hybrid Mapping purposes.

**OBJECTIVES:**
1. Construct a radiation dosage curve to identify appropriate radiation dosages.
2. Assemble a panel of PCR markers that can be used for initial characterization.
3. Develop a mapping population of ~300 endosperm tissue samples.
4. Screen the endosperm panel with PCR markers and identify lines useful lines for mapping.
5. Genotype the mapping panel at The James Hutton Institute, U.K.
6. Generate a Radiation Hybrid map using the genotyping data.

**PROCEDURES:** Plants of barley cultivars Morex and Golden Promise were grown in individual pots in the greenhouse. Whole spikes were removed from the barley variety Morex and taken to the OSU Radiation Center and irradiated with varying dosages of γ-rays. The irradiated spikes were immediately returned to the greenhouse and used to pollinate previously emasculated spikes of Golden Promise. Twenty days after pollination the developing seeds were harvested and DNA extracted from the endosperm. The DNA from the endosperm samples was characterized for DNA marker loss (indicative of radiation-induced damage) by PCR.

**RESULTS:**
**1.** We developed a modified 'approach' system for pollinating previously emasculated spikes of the cultivar Golden Promise with pollen from irradiated spikes of Morex. Four radiation treatments were tested with radiation dosages: 0, 1, 2, and 3 krad. Seed set (%) was dramatically reduced even at the lowest radiation dosage (Table 1).

| Treatment | # Repetitions | # spikes | # florets | seeds recovered | % seed set | Mean DNA yield |
|---|---|---|---|---|---|---|
| 0 krad | 2 | 7 | 178 | 76 | 42.7 | 65.8 µg |
| 1 krad | 5 | 36 | 897 | 49 | 5.5 | 29.2 µg |
| 2 krad | 3 | 30 | 814 | 32 | 3.9 | 1.8 µg |
| 3 krad | 1 | 10 | 267 | 6 | 2.2 | 1.0 µg |

**Table 1.** Summary statistics of the effects of radiation treatments on seed set and DNA yield.

This presented a significant challenge to develop sufficient numbers of endosperm samples for the proposed mapping project. We estimate it would take over 20 radiation treatments of barley spikes to generate the 300 endosperm sample we proposed whereas our previous work with wheat required only two treatments to generate a similar number. DNA yield from the endosperm was also reduced (as expected) by the radiation treatments but remained workable at the 1 krad dosage. At higher dosages, both seed set and DNA yield indicated that no useful material could be generated.

**2.** We screened 126 PCR markers and identified 12 that were usable for measuring deletions in the DNA recovered from irradiated endosperm.

**3.** We generated independent panels of 55, 32, and 6 endosperm from the 1, 2, and 3 krad treatments, respectively.

**4.** We screened the endosperm samples with nine PCR markers to determine the level of deletions recovered. None of the 2- or 3-krad samples had sufficient paternal DNA (the contribution form the irradiated pollen in which we are interested) to generate a signal and were therefore not scorable. Of the 1-krad samples, 270 of the 402 data points were scorable. We detected seven deletions equivalent to a 2.7% deletion rate.

**5 and 6.** Our previous work with Radiation Hybrid Mapping in wheat has shown that using germplasm with deletion rates below 10% are useless in generating accurate maps. Our results suggested that further genotyping by the James Hutton Institute of the material we generated would be useless. Therefore we were unable to generate even a preliminary hybrid map.

**Summary.** Our previous success with Radiation Hybrid Mapping in wheat and the benefits the technique provided to the wheat genomics community suggested we could provide a similar benefit to the barley genomics community. It is believed that wheat tolerates a high level of radiation because it is a hexaploid and has a redundant copies of genes; despite a mutated non-functional gene the plant still grows as there are two other functional copies. Barley is a diploid and might be expected to be less tolerant of radiation. However a 1983 paper from Powell *et al*. suggests that barley tolerates radiation dosages similar to wheat and subsequently generates a high number of mutants. Our current results do not support that idea. We conclude the inability of barley to tolerate radiation-induced deletions make it unamenable to the Radiation Hybrid Mapping approach.

**BENEFITS & IMPACT:** Unfortunately, the only benefit of this project to the barley genomics community is ruling out a potential method of creating physical maps.

**ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:** A grant proposal we co-authored with Dr. Robbie Waugh (James Hutton Institute) and submitted to the Leverhulme

Trust, UK during the course of this project requested funding of $127,000 was rejected despite favorable reviews.

**FUTURE FUNDING POSSIBILITIES:** Although we had earlier considered submitting a grant proposal to the USDA, the results we achieved are insufficient to support a proposal with a realistic chance of being funded.