

## PROGRESS REPORT

**Project Title:** Developing Biotechnology Tools for Wheat Variety Development

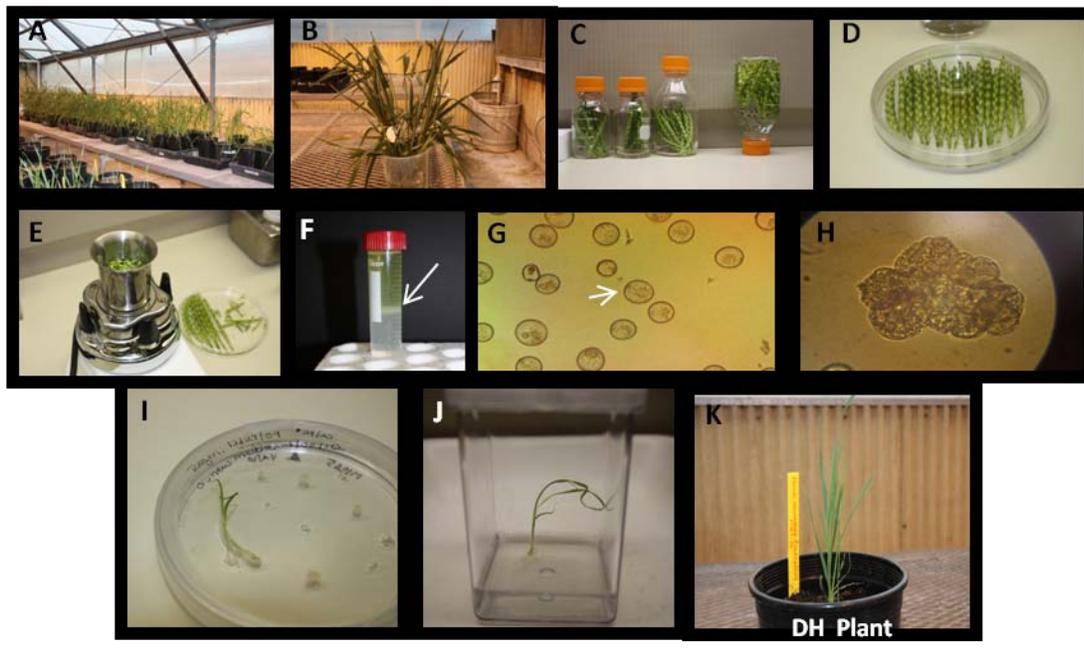
**Reporting Period:** July 1, 2009 to June 30, 2010

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Microspore culture is a specialized technique to produce double haploid (DH) plants. The DH plants produced from a single hybrid are genetically 100% identical. Application of DH technology in wheat breeding is enormous in improving breeding efficiency. The objective was to establish DH production technology for Nebraskan winter wheat varieties to support wheat breeding industry in NE and elsewhere in the USA.

### Methodology

Wheat microspore culture involves chemical pretreatment of wheat flowers (spikes) at optimum developmental stage (variety dependent) from healthy plants grown in green house (Fig. 1A-D). The immature microspores are then isolated and purified from pretreated wheat flowers (Fig. 1E, F) and incubated in tissue culture media under optimum temperature in the laboratory to induce formation of embryos (Fig. 1G, H)). Immature embryos are then induced to form shoot and root to form seedlings (double haploid plants) (Fig. Fig.1I). The seedlings were then hardened in growth chamber under controlled environment (Fig. 1J) before transferring to pots in green house for seed production (Fig. K).



## **Results and Discussion**

- Using the spring wheat variety ‘Macon’, we have standardized the staging of microspore and established different steps of the technique (pretreatment, induction, and regeneration) (Fig. 1A-J).
- Successfully produced DH plants of ‘Macon’ (Fig. 1K).
- Isolated microspores and generated embryos of NE wheat varieties ‘Anton’, ‘Pronghorn’ and ‘Millennium’.
- We are working on the following issues with these wheat varieties.
  - Staging of microspore – to identify optimum developmental stage of harvesting spike for microspore isolation of each variety.
  - Shrinking of microspore – to minimize cell shrinkage using microspore at different stage and/or modifying induction media.
  - Albino plants – to identify ways to produce more green DH plants.
- Several additional varieties are growing in green house and are being used for microspore culture at different stages.

DH production system in wheat using microspore culture is technically very sensitive and success rate very low. Success of DH plants production is determined by genotype and health of the donor plant, environmental conditions under which it is grown, staging of the microspore, pretreatment methods, and composition of induction and regeneration media. A dedicated greenhouse with specific growing conditions (day/light and temperature cycle) would be ideal for growing healthy wheat plants as donor of the spikes for the culture. The green house at Scottsbluff is used for different types of experiments under different conditions. Additionally, the green house in the summer is too hot to grow healthy wheat plants. We tried to overcome this inconvenience collecting spikes for field during summer and improving green house condition.

## **Publication**

Harvey, S. L., M. Santra, P. S. Baenziger, and D. K. Santra. “Establishment of double haploid production technique using microspore culture for US Midwest wheat varieties”. A poster presented in the US Hard Red Winter Wheat Workers’ and National Wheat genomics Conference, March 8-10, 2010, Lincoln.

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