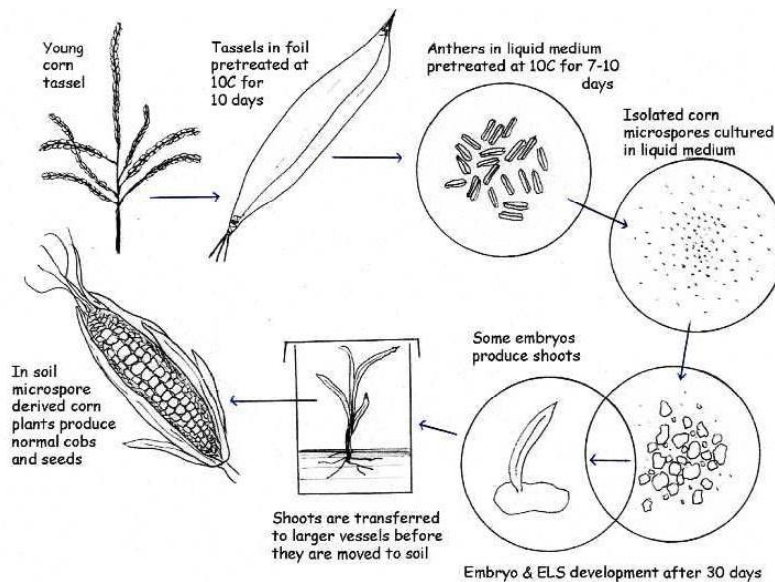


## Development of *Fusarium* Resistant Corn Genotypes for Pork Production

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### Objectives:

This research work entails 2 distinct, yet interconnected technologies. **1)** The first, involves the **development of a laboratory protocol** to induce immature corn pollen grains plated and cultured in a nutritious liquid medium, to develop into plant embryos. These embryos then can be germinated when transferred onto a solid medium to develop into corn plants (Figure 1). This type of doubled haploid production is already being used for breeding of canola, barley, wheat and other crops worldwide. Secondly, **2)** once this enabling technology has been developed and streamlined for corn, the protocol will be further modified so that during the developmental process of the pollen cells, they will be exposed to UltraViolet light in order **to induce minor genetic changes** (point mutations). As the mutagenized embryos develop, we will be able to **select those embryos that are more tolerant of *Fusarium*** attack by chemical screening in the Petri dish. The selected resistant embryos will be germinated into corn plants for seed production and the next generation will be tested for disease resistance/tolerance in the field. This method is a non-GMO technology that has been developed in our lab and is working exceptionally well for selection of canola plants resistant to *Sclerotinia*, another fungal pathogen.



**Figure 1: Pollen culture protocol for the development of corn doubled haploids**

**1) Methodology and protocol development:**

Although some progress in pollen culture of corn has been reported in the literature, to date the methods have been unreliable and very labour and cost intensive. Our commitment is to generate a relatively simple, rapid technology for corn that works well with many different genotypes. Therefore, we are evaluating and streamlining all available existing methods from the literature, which involve a multitude of trials dealing with the pretreatment of corn tassels and anthers prior to culture, various media and media inclusions (eg. hormones, sugars), durations and timing of each treatment, temperature regimes of cultured plates etc., using 9 Thompson corn inbreds and one U of Guelph line. Examples of tests involved:

*Tassel pre-treatment in cold:* temperatures of 4, 10 or 28°C  
 duration of exposure to temperatures for 4-7-10-12-14 days  
 tassels pretreated, anthers not pretreated and reverse  
 fresh tassels (not cold treated at all)

*Culture of pollen grains in liquid medium:* temperatures of 10, 28 or 32°C  
 duration of temperatures 24hr to 10 days  
 Corn medium, Konzak medium, NLN

medium

sucrose/maltose levels at 90, 130, 150 g/L

*Plating methods:* liquid medium (in 1mL, 2mL, 5mL, 10mL of medium)  
 Petri dish size (small, medium, large)  
 pollen suspension directly on solid medium  
 pollen suspension on filter paper on solid medium... etc.

To date we have eliminated or reduced the cold pretreatments, identified the best medium, carbon source and temperature regime for the rapid development of healthy embryos. We have so far produced 312 corn plants, many of which have now produced seed and will be grown out to make sure that no genetic anomalies are occurring. The development of the protocol is still ongoing as we still need to test all of the available genotypes against this system and show that this technique is repeatable under our protocol conditions.

**2) Development of the *in vitro* selection system for *Fusarium* resistance:**

Although we have worked with the mutagenesis/selection system for *in vitro* selection of various traits in canola (disease resistance, fatty acid alterations, cold tolerance etc), we have not tested this system in cereals. Since we have considerable expertise in the pollen culture of barley and wheat (which are considerably more like corn than canola), we propose to test the *in vitro* mutagenesis and chemical selection for *Fusarium* resistance in barley first. We have altered the barley system to allow for mutagenesis in the liquid phase and have already run the first mutagenesis trials. This is being done simultaneously while we are still developing the pollen culture system in corn, so that when this protocol has been adequately completed, we will be ready to screen for *Fusarium* resistance/tolerance immediately.

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