Production of Barley Doubled Haploids using Anther Culture

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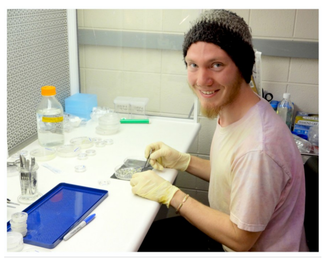
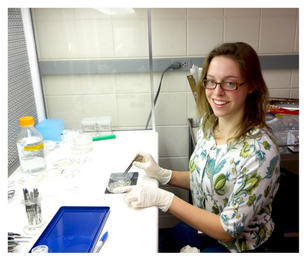
http://barleyworld.org






Breeding self-pollinated crops is a long-term process that requires multiple years to achieve complete homozygosity. Homozygosity is an essential requirement for maintaining the purity of a variety. A plant resulting from a cross contains two gene sets, each one potentially slightly different and leading to variability among progeny. In doubled haploid (DH) plants, the sets are identical so plants always breed true. DH production is an alternative which bypasses the necessity of multiple cycles of self-pollination: complete homozygosity can be obtained in a single generation. This shortens the time required to produce homozygous plants in comparison with the conventional breeding methods that employ several generations of selfing (Devaux and Kasha, 2009). DH production has additional advantages: rapidly fixing different traits in desirable combinations, speeding up the development of mapping populations and discovery of marker/trait associations, and phenotypic selection with greater accuracy and confidence.

Currently there are many different methodologies for producing DH plants. Anther culture (AC) and isolated microspore culture (IMC) are among the most common (Castillo et al. 2000). AC and IMC are based on a same principle but employ different protocols during the initial steps. IMC is based on isolating the microspores from the anther prior to culture, whereas AC involves culturing the whole anther.

Both techniques utilize *in vitro* androgenesis to produce a plant from a single pollen grain (Maraschin et al. 2005a). At early developmental stages the male gametic cell, or microspore, can be diverted from the normal developmental pathway to a sporophytic pathway and subsequently induced to regenerate an entire plant.

The microspores are produced in large numbers (around 2000-4000 per anther). The switch of cultured microspores from a gametophytic to a sporophytic pathway can be induced by various stresses (Touraev et al. 1997), including cold shock and sugar starvation. These stresses are also related to chromosome doubling efficiency, which in barley ranges from 70-90% (Cistué et al. 2003).

Genetics, donor plant growth conditions, microspore viability, culture conditions, media components and procedures for induction are among the factors affecting androgenic response (Cistué et al. 1995; Touraev et al. 1996a, 1996b; Oleszczuk et al. 2006; Shahinul et al. 2012). There can also be large differences in genotype response to AC and IMC (Castillo et al. 2000).

Although AC and IMC have been used extensively for many purposes, albinism among regenerated plantlets has been a limiting factor. According to Caredda et al. (2000) and Muñoz-Amatriaín et al. (2009), expression of genes related to plastid development is associated with the frequency of albino plant regeneration. Albino plant frequency is correlated with culture composition and length of time in culture. The number of regenerated green plantlets can be improved by increasing the Ficoll concentration during the embryo induction culture period (Cistué et al. 2003). The annual cycle/clock and the position of the spike on the plant can also affect the yield of AC culture and the frequency of albino plantlets (Jacquard et al. 2006).

In barley, IMC is becoming an important method for producing DHs, but so far this technique has been used less frequently in breeding programs than anther culture (AC) or the *Hordeum bulbosum* method.

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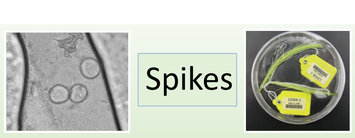
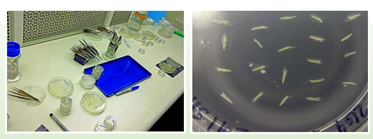
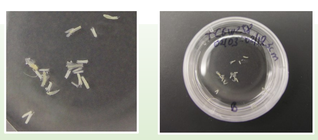





70% EtOH

Sterilization

Spikes

Petri dishes

+4°Cdark

2-3 days

Cold stress

30 anthers

6 cm Petri dishes

+24°C dark

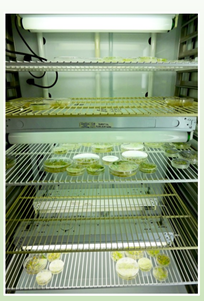
4 days

Pretreatment

15 anthers

3 cm Petri dishes

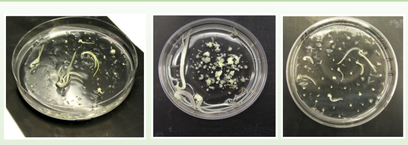
Induction, Stage I



+24°C
16/8 light/dark

10-18 days

Regeneration



Responsive Anthers

3 cm Petri dishes

Induction, Stage II

+24°C dark

15 anthers

3 cm Petri dishes

7-14 days

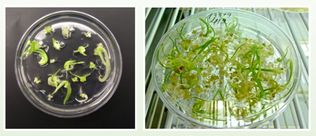
+24°C dark

12-14 days

15 anthers

3 cm Petri dishes

Induction, Stage I



Embryos


Green plantlets, 9/box

Petri dishes

Magenta boxes

Albino plants are discarded

4 weeks

Vernalization

Magenta boxes

+4°C
8/16 light/dark

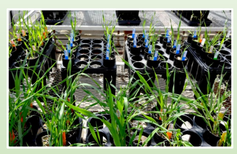
Green plantlets

+16°C
12/12 light/dark

+16°C
12/12 light/dark

1 week

Plant

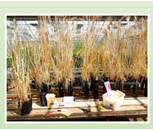


Pots with soil/vermiculite mix

+16°C
16/18 light/dark


Harvest of DH seeds

80-90% are DH

2-3 months


Plant growth

Plant



2 weeks