2.5
Anther culture for doubled haploid production in barley
(Hordeum vulgare L.)

I. Szarejko
Department of Genetics, University of Silesia, Jagiellonska 28, 40-032 Katowice, Poland

Introduction

In barley, haploids can be produced both from female and male gametophytes, through a number of techniques, including: chromosome elimination following inter-specific crosses with Hordeum bulbosum, androgenesis using in vitro culture of anthers or isolated microspores, and gynogenesis using ovule culture in vitro. Among these systems, only two have been developed for large-scale production in barley: androgenesis including anther and microspore culture, and wide hybridisation followed by chromosome elimination. The isolated microspore culture system, often referred to as microspore or pollen embryogenesis, provides the most efficient and uniform route to mass scale production of haploid plants. It is, however, technically demanding and requires controlled environment for donor plant growth. Using anther culture and the protocol outlined below, we were able to produce green regenerants from donor plants grown in a greenhouse or in field conditions, for a wide range of barley genotypes. Among many factors, composition of the induction medium has been found to be important or even critical for androgenic response. In barley anther culture, major improvements have been achieved by replacement of sucrose by maltose as a carbon source, the balance of organic and inorganic nitrogen compounds and the replacement of agar by Ficoll or agarose in the induction medium. In the presented protocol, liquid induction medium BAC3 (Szarejko and Kasha, 1991; Cai et al., 1992) supplemented with Ficoll 400 (a high molecular weight sucrose polymer which increases medium density) gave a higher androgenic response than other media tested, due to better embryo formation. We have routinely used BAC3 medium (Szarejko and Kasha, 1991; Cai et al., 1992) for anther culture of barley varieties, hybrids, mutants and other breeding materials.
Protocol

Donor plant growth
Donor plants should be grown in controlled environments. It is very important to ensure that plants are grown under optimal conditions, as factors which introduce stress upon the donor plants, have a profound effect on androgenic response. The vigour of donor plants is influenced by several parameters, such as temperature, light intensity, photoperiod, nutrition, water relations and application of pesticides.

Seedlings of winter genotypes, 2 weeks after sowing into flats are vernalized at 4°C, 8 h photoperiod, light intensity 100 µE m⁻² s⁻¹ for 8 weeks. After this period they are transferred to 15 cm diameter pots with a mixture of soil, peat and perlite (3:1:1). Seeds of spring genotypes are sown directly to pots with the same soil mixture.

The highest efficiency of barley anther culture is achieved when donor plants are grown in a controlled growth room, at relatively low temperature, 15°C during the day and 12°C during the night, or alternatively, at 12°C constant, 16 h photoperiod, light intensity at a pot rim level about 350-450 µE m⁻² s⁻¹, humidity 60-80%. We also grow donor plants during winter/spring season in a semi-controlled greenhouse, at a temperature 10-15°C during the day and 8-12°C during the night, 16 h photoperiod. Additional light is supplied by a mixture of high pressure sodium and mercury lamps (HQI-T 400W Planta and HQL 400W DE LUXE, Osram), to obtain the final intensity of 350-450 µE m⁻² s⁻¹. During the whole vegetation period, plants should be properly watered and fertilized weekly to maintain a vigorous growth. Fungicides and insecticides may be applied, if necessary, during plant vegetation but such treatments should be avoided 2 weeks before spike collection. It is possible to use field grown material as donor plants but lower efficiency should be expected. The more proper and uniform the growth conditions of donor plants are, the more uniform is the development of microspores, and more green plants are produced in the culture.

Spike selection and microspore staging
The stage of microspore development is the other critical factor for successful induction of androgenesis. In barley, the development of microspores within florets and florets within the spike is generally synchronous, with apical and basal florets being slightly delayed. In 6-row genotypes, anthers in the side florets are also lagging behind in their development as compared to the main florets. Tillers containing spikes at the desired developmental stage can be pre-selected on the basis of their morphology. To confirm the initial staging of microspores, anthers from the central part of the spike are excised and squashed on a microscope slide in a drop of 4% acetocarmine. The stained microspores are examined under the light microscope. The stage of microspore development can be determined on the basis of the size and the position of nucleus and vacuole in the cell (Fig. 2.5-1a-h). The highest androgenic response is achieved when the collected spikes contain microspores at mid to late uninucleate stage of development (Fig. 2.5-1d-e).

In barley, the distance between the flag leaf and the penultimate leaf indicates the proper stage of microspore development. For most barley genotypes, tillers should be collected when the distance between the flag and the penultimate leaf is 3-6 cm (Fig. 2.5-2a). For some genotypes, the flag leaf may be emerged 0-1 cm above the penultimate leaf and for others, the tips of awns should be visible above the flag leaf. The plant morphology indicating the proper developmental stage of microspores depends both on plant genotype and growth conditions. Only those spikes with anthers at the mid and mid-late uninucleate stage should be used for pretreatment (Fig. 2.5-2b, c).

Cold pretreatment
Many barley genotypes require a 3-4 week cold pretreatment before culture. The collected tillers are surface sterilized with an aerosol of 75% ethanol. Spikes containing microspores at