Pollen Dimorphism and Anther Culture in Barley

Philip J. Dale

Welsh Plant Breeding Station, Plas Gogerddan Aberystwyth, SY 23-3EB, U.K.

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Summary. A dimorphism is observed in barley (Hordeum vulgare L., cv. Akka) pollen when stained with acetocarmine from the mid-binucleate stage onwards. The majority of grains have staining cytoplasmcs, while the remainder have cytoplasmcs which take up little or no stain (NS grains). The staining dimorphism cannot be detected at the late-uninucleate microspore stage when anthers are normally cultured, but the evidence suggests that the microspores have already diverged at this time and it is the cells destined to become NS grains in vivo that respond in culture to become pollen calluses. Evidence comes from a comparison of the frequencies of NS grains and pollen calluses and from their distribution between and within anthers.

Introduction

Since Clapham (1971, 1973) obtained haploid barley plants from cultured anthers, work at this Station has been aimed, primarily, at increasing the yield of haploid plantlets to enable the technique to be used for the production of homozygous diploids in barley breeding. The genotype of the anther donor plant, the environmental conditions under which it is grown up to the point of anther excision and the anther culture medium all influence the success rate, but, so far, variations in these factors have not given any substantial improvement in the yield of haploids.

During routine examinations of in vivo anthers it was noticed that pollen grains could be classified into two distinct groups when stained with acetocarmine from the mid-binucleate stage onwards. The majority of grains have deeply staining cytoplasmcs while the remainder have cytoplasmcs which take up little or no stain (NS grains). Similar pollen dimorphisms have been reported in Tradescantia bracteata by La Cour (1949) and more recently in Paeonia hybrida by Sunderland (1974) who also observed the pollen dimorphism in barley. However, very little is known about the behaviour of the two types of pollen grains in culture. Results in Paeonia (Sunderland, 1974) indicate that initial divisions can be induced in the aberrant forms, but there is no information on the relative importance of different forms in culture.

In view of the fact that some species have pollen grains which are genetically predisposed to develop in culture, such as Nicotiana tabacum and Datura innoxia (see, for example, Nitsch, 1972; Sunderland, 1973) and that a form of predisposition can be induced by traumatic shocks before the anther is cultured (Nitsch and Norreel, 1972; Picard, 1973) it was considered worthwhile to study whether the two types of pollen grains would show differential response in culture. The principal aim of the work here reported was to answer two questions:
1. Are the NS grains different from the normal grains at the stage for anther culture (late-uninucleate) before they can be detected by the staining reaction and, if so,

2. What role do NS grains play in cultured anthers?

Materials and Methods

Inflorescences for fixing directly and anthers for culturing were taken from the same batch of barley plants, *Hordeum vulgare* L., cv. Akka), grown in the glasshouse during September. Inflorescences with pollen grains at the mid to late binucleate stage were fixed in 3:1 ethanol: acetic acid (v/v) for about 48 h and transferred to 70% ethanol where they remained until they were examined microscopically. Anthers were cultured when microspores were at the uninucleate and fully vacuolate stage, the stage being determined by sampling three anthers per inflorescence taken from florets at bottom, middle and top positions on the spike. If the correct stage was found at any two positions, anthers were cultured from all florets in between, on the assumption that they likewise contained anthers at the correct stage. Surface sterilization of the inflorescence was found to be unnecessary. Anthers were placed onto a Linsmaier and Skoog (1965) based medium, modified by Clapham (1973). It consisted of the following (mg/l): KNO₃ 1900, NaNO₃ 1700, CaCl₂ 2H₂O 440, MgSO₄ 7H₂O 370, KH₂PO₄ 170, NH₄NO₃ 160, MnSO₄·4H₂O 22.3, ZnSO₄·7H₂O 8.6, H₃BO₃ 6.2, KI 0.83, Na₂ Mo O₄·2H₂O 0.25, CoCl₂·6H₂O 0.025, Cu SO₄·5H₂O 0.025, Fe EDTA 36.7, alanine 400, inositol 100, glycine 1, thiamine HCl 1, pyridoxine HCl 1, nicotinic acid 1, p-aminobenzoic acid 1, indol-3 yl-acetic acid 1, 6-benzylaminopurine 1, Sucrose 30000, Sigma agar (type IV) 4400, coconut milk 100 ml/l. The pH of the medium was adjusted to 5.6 before autoclaving for 15 min at 121°C. Ten ml of medium were poured into each Sterilin Universal plastic container. Cultures were kept in the dark at 25°C for 7 days after which time the anthers were removed and fixed as described above.

For microscopic examination, cultured and uncultured anthers were cut transversely in the centre and each half was examined separately. Each part was stained with 4% w/v acetocarmine, prepared according to Sunderland and Wicks (1971), and after placement of the coverslip the slide was gently heated and left for 5–10 min before scoring. The number of pollen grains with non-staining cytoplasms was counted in the top and bottom halves of 275 uncultured anthers and, similarly, the number of pollen calluses was determined in the top and bottom halves of 249 cultured anthers. In smaller samples of anthers the proportions of NS grains and pollen calluses were determined; in some cases by counting the total number of grains present in an anther, but more commonly by scanning a sample of several hundred grains. A pollen callus is defined as a grain with four or more nuclei.

Results

Fig. 1A illustrates the staining reaction of normal and NS grains. The difference first appears during the mid-binucleate stage of pollen development and is most pronounced when the normal grains are trinucleate. NS grains are on average about three-quarters the size of normal binucleate grains and this distinction together with the staining difference, remains throughout pollen development. Apparently, microspore mitosis (uni- to binucleate condition) occurs at about the same time in grains destined to become NS and those destined to become normal types. This is suggested by the observation that when the staining dimorphism first appears, the normal staining grains and almost all the NS grains are binucleate. Conversely, a high degree of asynchrony exists at pollen mitosis (bi- to trinucleate condition) when the NS grains on average divide later. In a sample of 8 anthers 99% of the staining grains had fully formed sperm nuclei (and hence were ready for dehiscence), but 28% of the NS grains were still binucleate, 28% were in pollen mitosis and 44% were trinucleate.