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Pollen dimorphism and androgenesis in *Hordeum vulgare*

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Abstract

Dimorphism of binucleate pollen grains of *Hordeum vulgare* has been confirmed. It is considered, however, in contrast to the accepted opinions, that some of the large pollen grains with dense cytoplasm lying close to the tapetum are the outset forms for embryoids, and not the small pollen grains with scarce cytoplasm lying in the pollen sac centre.

INTRODUCTION

Barley pollen grains and those of several other plants exhibit features of dimorphism (La Cour 1949, Sunderland 1974, Dale 1975, Horner and Street 1978). In the binucleate stage there are, beside the predominant number of large grains with dense cytoplasm, smaller grains with a rare cytoplasm (Dale 1975, Horner and Street 1978). Only the former, considered as normal (N) stain intensively with acetocarmine, the latter, much smaller (S) stain weakly or not at all.

Study of pollen dimorphism became particularly interesting in connection with the possibility of induction in culture of androgenic embryoids. According to Dale (1975), Horner and Street (1978) only S grains are capable of producing callus differentiating to plants. The present investigations were undertaken to check this view.

MATERIAL AND METHODS

Anthers of *Hordeum vulgare* variety Alsa were taken from plants cultivated in field conditions. Part of the anthers containing pollen in the bicellular stage or in the stage of gamete division was used as outset

material for observation of dimorphism. Anthers in the mononucleate microspore stage were cultured on the medium of Murashige and Skoog (1962) with an addition of 8 per cent sucrose to inducing the process of androgenesis. After a longer period of culture the pollen grains developed to embryoids of several or a dozen or so cells. Fragments of the central part of the anthers were fixed in 6 per cent glutaraldehyde buffered with 0.1 M cacodylate, pH 6.8 for 18 h at 4°C, then washed for one hour with four changings of cacodylate buffer and fixed in 2 per cent OsO_4 in 0.1 M cacodylate buffer for two hours. The material was stained in a 2 per cent aqueous solution of uranyl acetate for one hour and dehydrated in the series: ethanol, acetone and propylene oxide and embedded in Epon 812. An LKB microtome was used for cutting semithin sections (3 μm) for observation in a light microscope. They were counterstained with uranyl acetate and lead citrate solutions (Raynolds 1963, Venable and Coggeshall 1965).

The observations were performed in an electron microscope Jeolco type 7A.

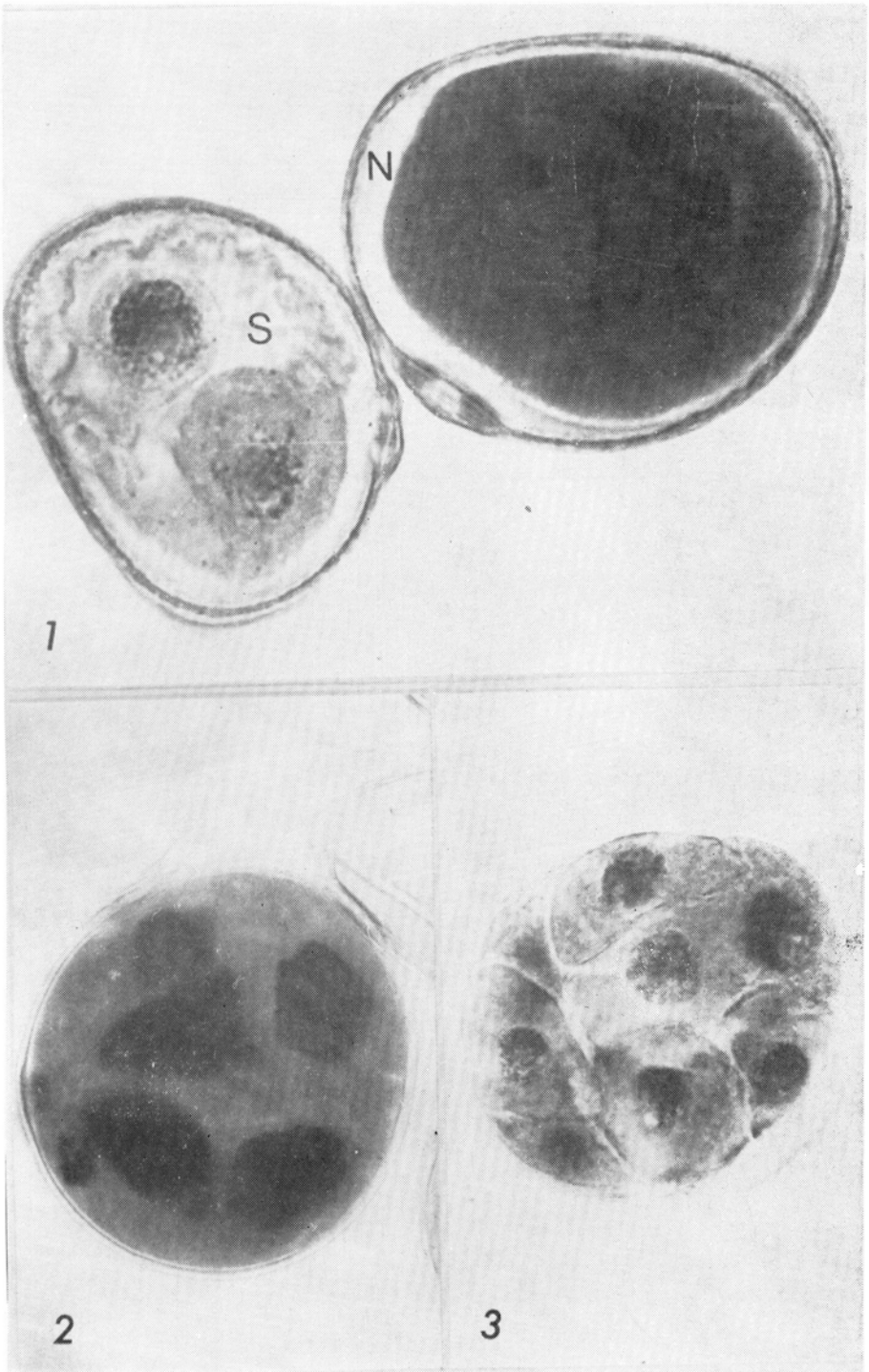
RESULTS AND DISCUSSION

Examination of squash slides of *Hordeum* anthers stained with acetocarmine confirmed the previously described diversity of pollen grains in this species (Fig. 1) as well as their ability of forming embryoids (Figs. 2 and 3).

Dale (1975) separated barley anthers into a bottom (filamentous) end and apical part and demonstrated in this way that the bottom end contains more S pollen grains than the apical one. Since there were also in the bottom end of the anther more numerous embryoids the conclusion was advanced that S grains are the source of embryoids.

The squash technique of microscopic slides, does not supply information on the distribution of the grains within the pollen sac. It results from the series of semithin epon sections from many anthers that, notwithstanding the peripheral localisation of the grains in the pollen sac (Młodzianowski and Idzikowska 1978), the grains are also scarce in the central part of the anther (Fig. 4). Medial profiles of these pollen grains were smaller than the cross sections of grains lying on the pollen sac periphery. The cytoplasm of these pollen grains was also less dense and poorer in organelles and their wall was less developed (Figs. 6 and 7). These centrally lying grains corresponded to grains S, whereas those on the periphery to grains N.

After placing the anthers on the medium, embryoids were found only in the peripheral layer (Fig. 5), whereas the grains in the central part of the pollen sac were degenerated.



Figs. 1-3. Squash slides; Fig. 1. Pollen grains stained with acetocarmine. **S** — smaller pollen grain, **N** — normal pollen. × 1700; Fig. 2. Multinucleate microspore. × 1300; Fig. 3. Multicellular embryoid (pollen callus). × 1200

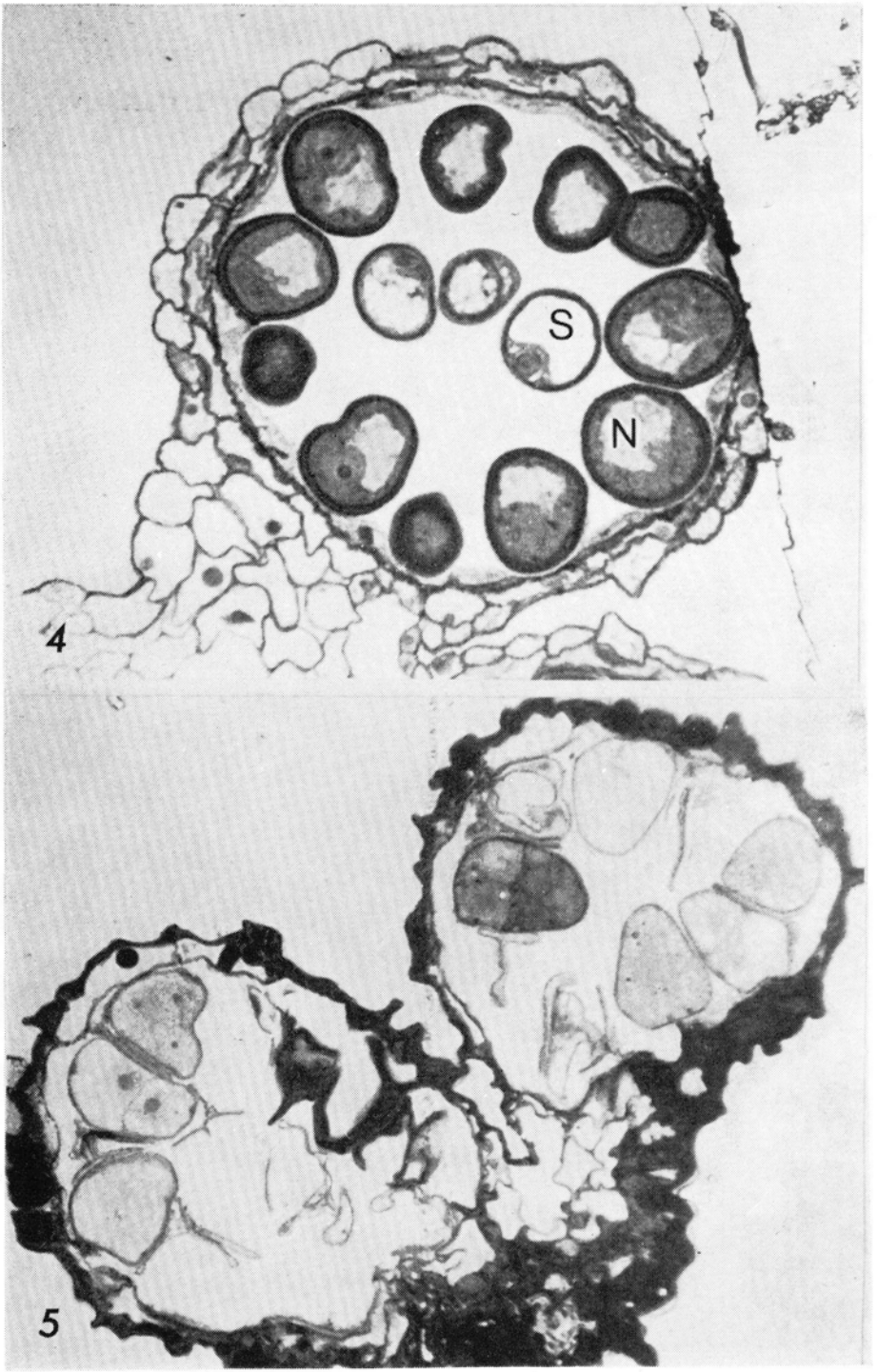
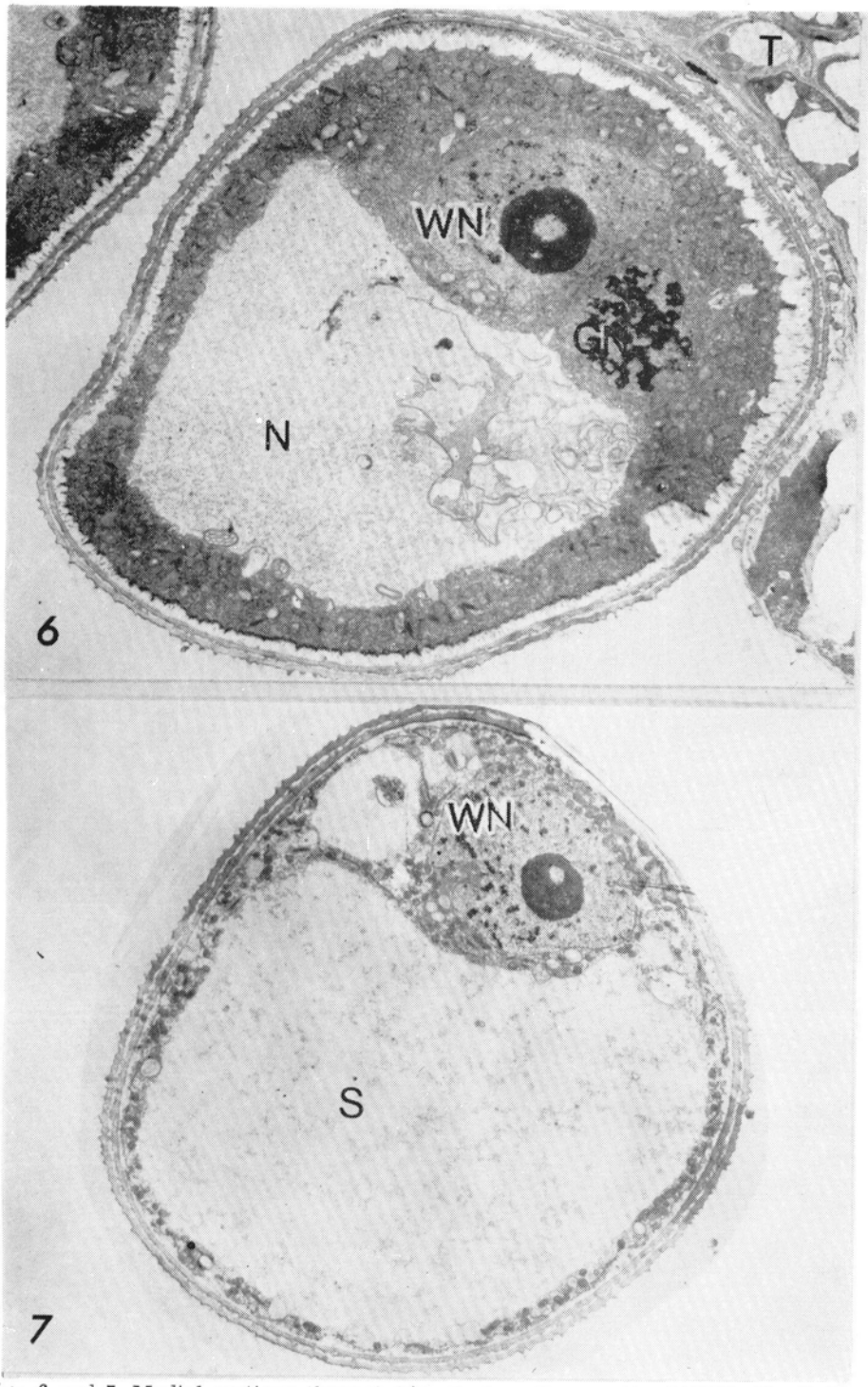


Fig. 4. Cross section through pollen sac. Dimorphism of the pollen is visible. Smaller and poorly staining grains in the central part of the pollen sac (S), larger ones with dense cytoplasm on the pollen sac periphery (N). × 530; Fig. 5. Cross section through anther with embryoids in early stage of development localised on their periphery. × 470



Figs. 6 and 7. Medial sections through binucleate pollen grains. × 3600; Fig. 6. Type N pollen grain adhering to the tapetum (T) with two nuclei: a vegetative (VN) and a generative (GN) one; Fig. 7. Binucleate pollen grain of S type with a vegetative nucleus (VN) visible on the cross section. The wall of the N pollen grain, and particularly intine is thicker, there is also more cytoplasm and organelles are more numerous than in the S pollen grains

Thus, in contrast to the accepted view, according to our opinion, there are not the S grains, but some of the N ones that constitute the outset form for embryoid formation. This is also supported by the close vicinity of N grains to the tapetum which plays an important role in nutrition of the pollen grains, to which during the androgenesis, the food supply is all the more necessary.

The view of Dale (1975), contrary to ours was based solely on the comparison of the frequency of occurrence of pollen grains S which agreed with that of embryoids. There actually is a conformity between the number of S grains and that of embryoids, but it is not causatively conditioned. S pollen grains since they degenerate are rather a source of hormones for pollen grain embryogenesis (Masahiro Mii 1980) and not a source of embryoids.

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Dymorfizm pyłków a androgeneza u Hordeum vulgare

Streszczenie

Potwierdzono istnienie dymorfizmu pyłków dwujądrowych u *Hordeum vulgare*. Jednak wbrew przyjętym poglądom uważa się, że formami wyjściowymi dla embrioidów są niektóre spośród pyłków dużych, o gęstej cytoplazmie, ułożonych przy tapetum, a nie pyłki małe o ubogiej cytoplazmie i ułożone w centrum woreczka pyłkowego.