Proceedings of the third International Barley Genetics Symposium, 1975

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Abnormalities of Pollen Tetrads in Asse, a Four-Rowed Cultivar of *Hordeum vulgare*

Introduction

It is generally accepted that in order to get haploids by anther culture it is necessary to culture anthers at the uninucleate stage (Oono, 1972). During the course of detailed studies on the development of microspores at the uninucleate stage it was noticed that Asse, a four-rowed cultivar of *Hordeum vulgare* showed the following microspore abnormalities, although the frequencies were rather low (1 to 3 %).

(a) Anucleate microspores
(b) Microspores of very abnormal shape
(c) Multinucleate microspores
(d) Giant 2n-like microspores

Since microspore abnormalities will be dealt with by Mix, Foroughi, and Gaul in this Symposium, the present paper will be devoted to abnormalities of pollen tetrads and the stages before the tetrad stage.

Materials and Methods

(1) Spikes of Asse were fixed in a solution of 3 parts of 96 % alcohol and 1 part of acetic acid for a week and then the solution was renewed. The samples were kept in the refrigerator prior to observation.

(2) Acetic carmine was used for staining.

(3) Day temperature was about 20 °C and night temperature was 10 ° to 12 °C during December, 1974.

(4) Only the samples fixed on December 19, 1974 were used for the present study.

(5) Duration of the night period was 8 hours.

(6) Artificial light was used to supplement day-light.

(7) Anthers were cut into two or three pieces at the time of staining and then a cover glass was placed on the anthers in carmine. In order not to change the tetrad arrangement and the position of the nuclei in cells, care was taken to ensure that the tetrads were floating in carmine.

Results

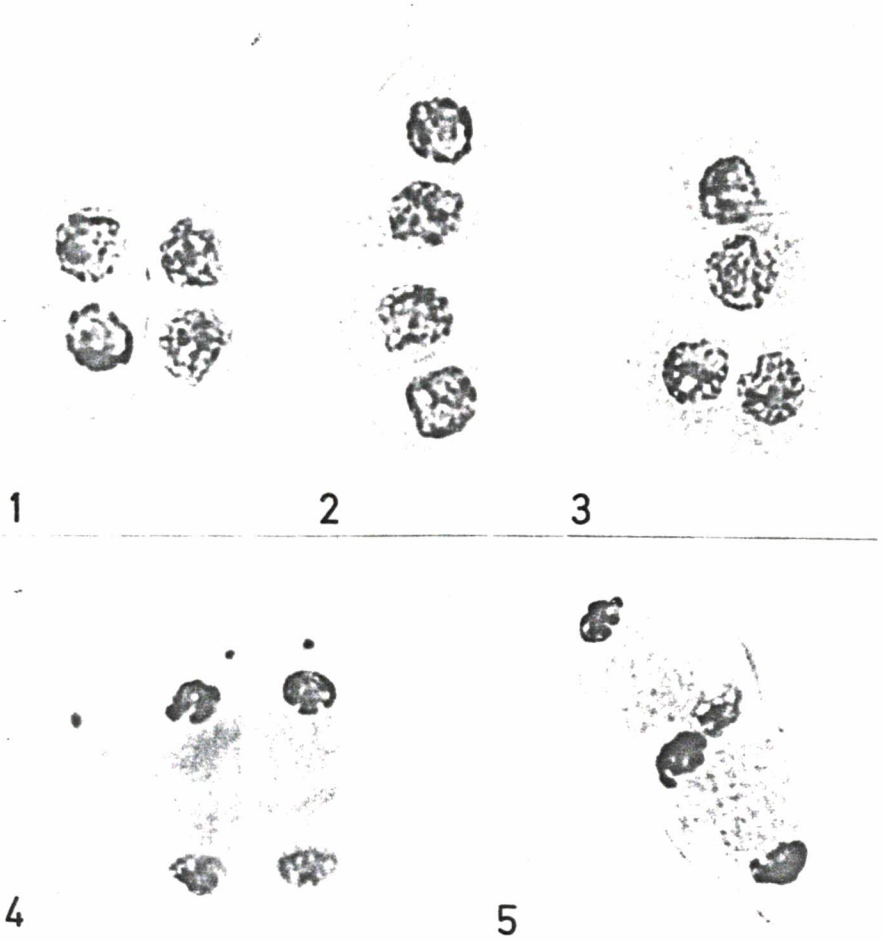
1. Pollen tetrad abnormalities

The results are summarized in Table 1.

TABLE 1. Frequencies of pollen tetrad abnormalities

Number of plants	Pollen tetrads observed			
	Total	Normal	Abnormal	
			Micronuclei	Arrangement
18	10253	9820	182 (1.77 %)	249 (2.42 %)

The pollen tetrads without any micronuclei and without any tetrad arrangement abnormalities were listed as normal (Figure 1). The most conspicuous abnormalities of tetrad arrangement were linear tetrads and semi-linear tetrads (Figures 2 and 3).



FIGURES 1-5.: 1. Normal pollen tetrad; 2. linear pollen tetrad; 3. semi-linear pollen tetrad; 4. normal 2nd telophase; 5. linear 2nd telophase (x about 2900)

18 plants showed either micronuclei and arrangment abnormalities or both. Since micronuclei formation and arrangment abnormalities must be due to spindle abnormality, those with micronuclei and/or arrangement abnormalities were classified as abnormal.

Although detailed descriptions of the abnormalities of the various plants and differences between florets are not given here, there were, in fact, considerable differences between plants and between florets. Since the percentage of abnormalities was low, attention was paid to the careful scoring of as many tetrads as possible.

2. Abnormality of 2nd telophase and linear tetrads

The axis of the 1st division of the PMC and the axes of the 2nd division must cross at right angles for the formation of the normal tetrad (Figure 4). Linear tetrads, however, must be produced by two axes, each of which is independent and parallel to the axis of the 1st division. This expectation was fulfilled by the appearance of a linear telophase figure at the 2nd division (Figure 5).

3. Relationship between pollen tetrad abnormalities and the position of the metaphase plate in the PMC

During the scoring of tetrads it was noticed that the 1st metaphase plates of the PMC's were not necessarily at the center of PMC. Thus the relationship between tetrad abnormalities and the location of 1st metaphase plate in PMC was investigated in 4 plants (A13, 15, 34, 39). The results are shown in Table 2. The Table shows that the more one-sided the metaphase plates were, the higher was the percentage of tetrad abnormalities observed. In other words, the location of the metaphase plate and tetrad abnormalities may be related.
malities may be related.

TABLE 2. Relationship between the position of the 1st metaphase plate of the PMC and pollen tetrad abnormalities

Plant No.	Position of metaphase plate				Abnormal
	Center	One-sided	?	%	
A13	25	23	6	42.5	5.61
A39	40	15	0	27.3	4.28
A34	17	3	0	15.3	1.77
A15	90	0	0	0	1.71

Discussion

(1) A question may be asked as to whether these pollen tetrad abnormalities are confined only to the Asse stock of Grünbach. In order to answer this question Asse from the University of Göttingen is now being studied. Preliminary tests suggest that tetrad abnormalities are present in both stocks.

(2) Are pollen tetrad abnormalities due to spindle abnormalities? Dover (1972) reported on pollen tetrad abnormalities and germ pore abnormalities

induced by colchicine treatment. He attributed the above abnormalities to spindle fiber abnormalities due to colchicine treatment. The present writers found almost all the abnormalities observed by Dover without any special treatment in Asse. Since Dover did not list abnormalities in the control material, it is impossible to judge how many of those he reported were due to the colchicine effect on spindle fiber organisation although the general conclusion he drew may be correct.

Not only the normal formation of the spindle fibers but also abnormal formation was observed by phase contrast microscopy. Normally spindle fibers would be formed symmetrically, but asymmetrical fiber formation was often observed although the degrees varied somewhat with spikes and with plants (Okamoto, in preparation).

The observation of one-sidedness of the metaphase plates in the PMC's in the present paper may also be interpreted as due to spindle abnormalities.

Nothing conclusive can be said at this moment as to whether pollen tetrad abnormalities are due to genetic causes or environmental conditions, but trials will be made to study progenies of plants which showed specific abnormalities. This study will give us clues as to the nature of pollen tetrad abnormalities.

(3) Relation between pollen tetrad abnormalities and the 5B effect. Avivi and Feldman (1973) reported that tetra 5B induced abnormalities of the germ apertures and ascribed these abnormalities to abnormalities of the spindle fibers due to the increased dosage of 5B. Feldman and Avivi (unpublished) have seven different disomic addition lines of *Aegilops longissima* chromosomes to common wheat, *Triticum aestivum*. They found that only one line showed high frequencies of univalent formation when subjected to a temperature of 30 °C three days prior to meiosis as was the case with tetra 5B. On this basis they supposed that this particular chromosome of *Ae. longissima* carries a 5B-like effect in the diploid condition.

Re-examination of meiotic figures of this particular line revealed exactly the same kind of meiotic disturbances observed in Asse barley (Feldman, Avivi, and Okamoto, unpublished).

On this basis it is tentatively suggested that the pollen tetrad abnormalities in Asse might be due to duplication of a 5B-like gene or genes in Asse.

Trials will be made in the future to find out whether one particular trisomic of barley shows more abnormalities of pollen tetrads than the others. If this turns out to be so, then the genetic or environmental nature of the tetrad abnormalities would be established.

(4) Tetrad abnormalities in nature

Watanabe informed the writers (personal communication) that *Typha latifolia* occasionally produces a group of four linear pollen grains. It would be of particular interest to see the meiotic stages of this species.

References

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