Cytogenetics of Coenocytism in Barley (*Hordeum vulgare* L.)

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Spontaneous occurrence of coenocytes (syncytes), also called multinucleate pollen mother cells (PMCs), has been reported in many plant species, viz., *Aegilops squarrosa* L. (Kihara and Lilienfeld 1934), *Hordeum vulgare* L. (Smith 1942), *Trillium erectum* (Stern 1946), *Saccharum robustum* and *Erianthus arundinacea* (Price 1956), *Triticale hexaploide* Lart. (Kempanna *et al.* 1971), *Phacellanthus* (Matsuura, cf. Maheshwari 1950), *Saccharum bengalense* (Mehra and Kalia 1973) and *Zea mays* L. (Golubovskaya and Mashnenkov 1977). It is reported to result from premeiotic or early-meiotic fusion of variable number of cells with or without nuclear fusion. The cytogenetic behaviour of such a phenomenon, induced as well as spontaneous, in three independent lines of barley is described in this paper.

Materials and methods

During a study on the induction of polyploidy in barley, a plant characterized by coenocytic PMCs was screened in a C_2 spike-to-progeny of F_2 : (BHS 24 $\times$ Mari) irradiated with 30 kR of γ -rays before 0.2% colchicine treatment for 8 hr at $20^\circ \pm 1^\circ\text{C}$ after 12 hr presoaking in water at room temperature. A plant with similar coenocytic behaviour was also screened from the C_2 population in cultivar HBL 175 (unirradiated). Further, in a hybrid line of F_5 : (Liguleless $\times$ USDA 606) coenocytic PMCs of spontaneous origin were observed in all the nine plants studied.

For cytological studies young spikes were fixed in Farmer's fixative, 3 ethanol: 1 acetic acid, and anther squashes prepared in 2% aceto-carmin. Photomicrographs were taken from temporary slides.

Results

Coenocytism in BHS 24 $\times$ Mari

In the irradiated followed by colchicine-treated hybrid barley, "BHS 24 $\times$ Mari", meiotic studies in the C_2 generation of a plant showing tip sterility revealed characteristics of fusion of PMCs. Frequency of the fused cells (coenocytes) was 36.4%. The number of nuclei in such cells varied from two to eight, the 2-nucleate cells being the most frequent (37.5% of the coenocytes).

The coenocytes thus formed were of two types a) in which the nuclei of the fused cells remained independent (Fig. 1) and b) in which the nuclear contents of these coalesced and followed the course of meiosis as a polyploid cell (Fig. 2). The fre-

quency of these two types of coenocytes was 35% and 65%, respectively. In about 25% of the latter, fusion occurred before synapsis resulting into multivalent formation (Fig. 2), whereas in others it occurred after synapsis so that only bivalents were formed (Fig. 3). Equatorial plate of meta-I was quite wide in both of these types of the coenocytes. A few to many bivalents showed non-congression in 65% of the coe-

Figs. 1-4. 1, a coenocyte with 7 independent nuclei $\times 620$. 2, a pre-synaptic three nucleate coenocyte with coalesced nuclear contents showing multivalent formation. $\times 620$. 3, a post-synaptic five nucleate coenocyte showing bivalents only. $\times 620$. 4, unequal quartet cells as observed at the completion of meiosis in a coenocyte. $\times 620$.

Figs. 5-13. 5 and 6, coenocytes of high ploidy level. $\times 560$. 7, anaphase II in a coenocyte showing unequal distribution. $\times 560$. 8, telophase II as observed in a coenocyte and a normal PMC. $\times 560$. 9, a 2 nucleate coenocyte showing chromosomal pycnosis and chromatin lysis. $\times 620$. 10, a desynaptic cell showing 14 univalents. $\times 1320$. 11, unequal distribution of 19 : 4 at dyad stage in a 2x PMC. $\times 1320$. 12, unequal pollen grains, the larger one formed as a result of coenocyte formation. $\times 620$. 13, a pollen grain resulting from a coenocyte. $\times 620$.

nocytes. The stages of meiosis proceeded almost at the same rate in most of the coenocytes as in the normal PMCs, about 20% of the former lagging behind in the meiotic course. The different nuclei in a coenocyte showed synchronization. One to eight laggards were observed at anaphase I. The second division exhibited abnormalities like non-congression of some chromosomes and their irregular distribution, particularly in the coenocytes with high ploidy level.

As a result of cell fusion, exceptionally large quartets were observed, with microspores of varying sizes (Fig. 4). While the normal pollen grains ranged from 25 μ to 33 μ in diameter, the larger ones ranged from 40 μ to 55 μ and were of irregular shape.

Although the possibility of induction of this aberration by colchicine cannot be ruled out, yet its induction by gamma radiation appears more probable.

Coenocytism in HBL 175

In variety HBL 175 a plant from C_2 generation was screened for having tip sterility. Cytological studies on this plant showed the phenomenon of coenocyte formation in the sterile tip region and meiosis was normal in the lower fertile region of the spike. In all essential aspects, meiotic behaviour of the coenocytic PMCs was similar to that in the case of BHS 24 $\times$ Mari.

In this case, however, colchicine appears to have induced the aberration as the basic material was not irradiated prior to the colchicine treatment.

Coenocytism in Liguleless $\times$ USDA 606

Coenocytes were observed in 34% of the cells in this hybrid line (Figs. 5-8). This aberrant behaviour in this line was of spontaneous origin. As in the aforesaid case, the coenocytes had a variable number of nuclei but the range extended upto 14 nuclei in this case. Their nature and behaviour was similar to the one described in the previous case. However, at anaphase I, one to five univalents were observed undergoing equational division in about 28% of the coenocytes in this case. Bivalents were comparatively less condensed and showed elongated form in about 6% of the coenocytes. Chromosomal pycnosis and chromatin lysis was observed in a few cells (Fig. 9).

In the normal diploid PMCs, however, partial to complete desynapsis was observed in 24% of the cells (Fig. 10). At metaphase I, one to two bivalents failed to congress in about 20% of the PMCs. Unequal distribution ranging from 8:6 to 4:10 was observed at dyad stage in some PMCs (Fig. 11).

The normal pollen grains ranged from 25 μ to 33 μ in diameter, whereas the large ones formed as a result of coenocytism ranged from 40 μ to 65 μ in diameter and were of irregular shape (Figs. 12, 13).

Cytological studies on 25 plants of this line in the succeeding generation showed the absence of coenocytes in the anthers of the spikelets of the lower part of the spike, as a results of which normal pollens were formed. Only a low frequency of coenocytes was observed in the spikelets from the mid-regions, whereas the spikelets in the tip region showed high frequency of coenocytes. Table 1 shows frequency of the microspores in different regions of the spike based on 10 plants. Thus a gradual in-

crease in the frequency of coenocyte formation was observed from the base towards the tip of the spike. Although some viable pollens in the tip region were produced, yet it showed complete seed sterility, indicating that such a phenomenon might also be occurring in this region during megasporogenesis. To confirm this observation, 50 spikes of this population were emasculated and pollinated with the pollens from a

Table 1. Frequency of the normal and the coenocytic microspores in the basal, mid and tip regions of the spike

Plant No.	Spikelets of basal region		Spikelets of mid region		Spikelets of the tip region	
	Normal microspores	Abnormal microspores	Normal microspores	Abnormal microspores	Normal microspores	Abnormal microspores
1	147	0	163	2	53	45
2	111	3	27	38	14	68
3	86	1	39	51	Anthers aborted	
4	161	0	82	10	68	36
5	114	0	98	0	84	4
6	90	0	38	23	36	64
7	71	1	70	0	Anthers aborted	
8	101	6	57	29	50	72
9	17	27	Anthers aborted		Anthers aborted	
10	222	5	90	6	13	85
Total	1130	43	664	159	318	374
	Normal microspores=96.3%		Normal microspores=80.7%		Normal microspores=54.9%	

Table 2. Pollen stainability in the basal, mid and tip regions of the spike

Plant No.	Spikelets of basal region		Spikelets of mid region		Spikelets of the tip region	
	Stained pollens	Unstained pollens	Stained pollens	Unstained pollens	Stained pollens	Unstained pollens
1	156	9	165	10	0	108
2	77	11	0	65	0	79
3	69	18	0	30	Anthers aborted	
4	161	11	71	21	64	40
5	98	21	97	13	82	22
6	90	10	0	61	0	50
7	71	6	60	10	Anthers aborted	
8	103	35	86	0	6	61
9	44	0	Anthers aborted		Anthers aborted	
10	220	7	86	18	98	25
Total	1089	128	565	228	250	358
	Pollen stainability=89.5%		Pollen stainability=71.2%		Pollen stainability=39.4%	

fully-fertile cultivar HBL 87. Seed setting was observed only in 16 per cent of these spikes and in all these cases seeds were formed only in the lower parts of the spike. This lends support to the observation that the phenomenon of coenocytism occurs on the female side as well, thus causing tip sterility.

Studies on pollen fertility with aceto-carmin stainability test showed that the irregularly-shaped and large-sized pollen formed as a result of PMCs' fusion due to

coenocytism were non-stainable and hence sterile. Frequency of stainable pollens was very low in the tip region (39.4%) as compared with that in the mid region (71.2%) and base region (89.5%, Table 2).

Expression of the character of seed sterility varied from 2–3 sterile whorls of spikelets at the tip to about half of the spike being sterile. In some cases, besides tip sterility, partial sterility in the lower region of the spike was observed. However, about 80 per cent of the spikes showed tip sterility only.

Discussion

Occurrence of coenocytes has been reported in many plant species. Matsuura (cf. Maheshwari 1950) explained that these originate by the fusion of adjacent cells on the dissolution of intercellular walls. Price (1956) reported that the formation of coenocytes is frequently, but not always, followed by nuclear fusion. In the present study nuclear fusion occurred in 65% of the coenocytes in BHS 24×Mari. According to Price (1956), coenocytes were formed i) by the failure of cytokinesis in the mitotic division preceding meiosis, ii) through the passage of a nucleus from one PMC into another, and iii) through the fusion of PMCs as also reported by Mehra and Kalia (1973). However, the present studies do not support the reason of the formation of coenocytes to be due to failure of cytokinesis in premeiotic mitotic division, because in that case it becomes difficult to explain the formation of a fused pollen grain of the type shown in Fig. 13. It supports that the coenocytes were formed through the fusion of PMCs at different stages of meiosis.

Smith (1942) reported a recessive factor *mu* in barley which interferes with the normal development and behaviour of a few to most of the microsporocytes. Coenocyte formation in BHS 24×Mari was probably induced by gamma radiation as a monogenic recessive mutation. However, its mode of inheritance needs confirmation. The aberrant meiotic behaviour of the present mutants is by and large similar to that of the *mu*-mutant reported by Smith (1942). Golubovskaya and Mashnenkov (1977) reported a meiotic mutation, *pam-A344*, induced by N-nitroso-N-methylurea in maize, leading to the formation of a large number of plasmodial cells with 2 to 14 nuclei. Induction of various gross pollen abnormalities and thus sterility has been reported by Rajendra and Bates (1981) in wheat (*Triticum aestivum* L.) with the use of some gametocides. Meiosis in these plants has been reported to be similar to that in the genetic male sterile wheat.

According to Price (1956) coenocytes might have played an important role in the ploidy status of *Saccharum*, as regular transmission of 2n chromosomes in certain interspecific crosses in this genus has long been reported by Bremer (1922). However, the role of coenocytes as a source of polyploidy is disputable as the large pollen grains have little chance in competition with the normal ones in causing fertilization. The occurrence of such a phenomenon in the megasporocytes, however, might have a considerable influence in this regard.

The present studies provide an interesting observation regarding the expression of the phenomenon of coenocytism within a spike. Meiosis was normal in the base region with a gradual increase in the frequency of coenocytic PMCs towards the

tip of the spike. The authors have not come across with such an observation in the literature. The present studies also form the first report about the occurrence of the phenomenon of coenocytism during megasporogenesis too. This inference was drawn from the studies on artificial pollination. It would be worthwhile to confirm it through embryological studies in this material.

Summary

A meiotic mutant, inducing the formation of coenocytes in pollen mother cells, is reported in three lines of barley, viz., (BHS 24×Mari), HBL 175 and (Liguleless×USDA 606). The mutation appears to be of induced nature in BHS 24×Mari due to irradiation with gamma rays, of spontaneous nature in HBL 175 and of induced nature due to colchicine in Liguleless×USDA 606. It is characterised by fusion of variable number of PMCs at pre-synaptic or post-synaptic stages of meiotic prophase I, resulting into the formation of plasmodial masses or polyploid nuclei on their coalescence, irregular congression and subsequent distribution of chromatin, and formation of microspores of highly variable size and shape. The present studies establish that the phenomenon of coenocytism causes tip sterility and that the frequency of fused PMCs is higher in the tip region of the spike than in the mid or base region. These studies also indicate the occurrence of such a phenomenon during megasporogenesis too, though it needs confirmation through direct observation.

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