Gametophytic abnormalities in a triploid fern *Hypodematum crenatum* (Forsk.) Kuhn

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Abstract. Himalayan populations of an advanced leptosporangiate fern, *Hypodematum crenatum* (Forsk.) Kuhn contain three cytotypes: diploid, triploid, and tetraploid. This paper describes spore germination and genotypically determined developmental and morphological plasticity of the gametophytes of triploid sporophyte. While the haploid and diploid gametophytes from the diploid and tetraploid sporophytes developed normally, those from the triploid exhibited an interesting array of morphogenetic alterations under the same culture conditions. These included formation of twin and multiple protonemata, displacement of metaphase spindle during successive mitoses, inhibition and/or delayed rhizoidal differentiation, lack of spore polarity and hypertrophy/allometric growth of protonemal cells.

Keywords. *Hypodematum crenatum*; cytotypes; triploid sporophyte; gametophytic abnormalities.

1. Introduction

The fern gametophyte, especially its cordate form, has several characteristics which make it ideally suitable for analytical and experimental studies (Nayar and Kaur 1971; Miller 1968; Brandes 1973). Its normal morphogenesis involves three distinct growth phases (one-, two-, and three-dimensional) and has been analysed by the effects of chemicals, varying intensity and quality of light. Studies on callus and tumor growth have shed light on the more general problem of gametophyte morphogenesis (Partanen 1972). For our studies on the extent and nature of morphogenetic alterations caused by the inherently disturbed nuclear and extra-nuclear contents of spore cells, we used the triploid cytotpe of *Hypodematum crenatum* (Forsk.) Kuhn, with a high degree of meiotic irregularities, both chromosomal and cytokinetic.

2. Materials and methods

Spores of the diploid, triploid, and tetraploid cytotypes were collected from different localities at Mussoorie, North-Western Himalayas. The cultures were raised on Bold's mineral nutrient medium and maintained in diffused daylight. Meiotic stages of sporogenesis were obtained from squash preparations of developing
sporangia. For mitotic chromosome counts the gametophytes were squashed in acetocarmine after fixation in Carnoy's fluid (6:3:1).

3. Observations

*Hypodematium crenatum* is well represented in the Himalayas inhabiting fully exposed cliffs at 600-2,000 m. Cytological analysis of Mussoorie populations and a few other stations showed diploid (2n=2x=82), triploid (2n=3x=123), and tetraploid (2n=4x=164) cytotypes; the diploid is by far the commonest in the localities investigated. Morphologically it was difficult to distinguish the triploid from the two other cytotypes; the chromosome number served as the only diagnostic feature.

3.1. Meiotic behaviour and sporogenesis in triploid cytotype

The archesporium is 8-celled in the three cytotypes, in sharp contrast to the well-known 16-celled in the advanced leptosporangiate ferns. In both diploid and tetraploid there was perfect pairing of the chromosomes, resulting in 32 normal spores per sporangium (figure 1A and B). In triploid, on the other hand, the meiocytes examined at diakinesis showed univalents, bivalents and trivalents, the exact associations in an exceptionally clear meiocyte being 14I, 47II and 5III. Erratic chromosomal movement, especially of the lagging univalents at anaphase II, resulted in unequal-sized micronuclei whose number varied from spore to spore. Partial or complete failure of cytokinesis was observed in practically all the sporangia examined. In consequence, unlike the normal haploid and diploid spores which contained one and two complete genomes, respectively, the spores of the triploid differed widely in their shape and size (figure 1C).

3.2. Development of the haploid and diploid gametophytes

The haploid and diploid spores showed distinct differences in size, and 70-85% germination was recorded in 6 days after sowing. After the first mitotic division two highly unequal cells resulted; the growth of the smaller rhizoidal cell and the larger prothallial cell was generally synchronous. Subsequent development leading to cordate form was of 'Aspidium-type' (Nayar and Kaur 1971).

3.3. Gametophytes of the triploid cytotype

The percentage of germinable spores varied from sample to sample. One of the samples gave 36% germination in contrast to 70-85% in haploid and diploid spores. In many cases the first division of spore cells resulted in two almost equal cells, each of which grew into a filament (figure 1D). Thus in the absence of asymmetric division, the two-celled sporelings, on further growth, produced V-shaped protonemata. Some of the germinable binucleate spores with partial cytokinesis also produced similar twin prothallial cells, each of which exhibited capacity for further growth (figure 1E and F). Interestingly, however, in sharp contrast to the axes of metaphase spindle in a straight line which controlled the normal development of haploid and diploid protonemata, the orientation of spindle rotated to the right and left through
Figure 1. A, B, C: spores of the diploid, tetraploid and triploid cytotype, small fragments seen in the triploid are plasmodial residue, ×120. D: 10-day-old protonema showing twin germ filaments, arrow pointing allometric growth of cell, ×250. E: 16-day-old protonema developed as that in D or from a binucleate spore, showing alteration of spindle axis, ×250. F: magnified, basal portion of the protonema shown in E ×800. G: 18-day-old protonema showing ascendency of the filamentous growth over the first rhizoid and aggregation of chloroplasts around the nucleus of the terminal cell, ×210.
almost 90° at each division. The disturbed axes of mitotic spindle in such cells was
accompanied by dislocation of plastids. For instance, most of the chloroplasts were
aggregated around the nucleus, a feature reminiscent of the behaviour of cells in
colchicine-treated fern sporelings (Mehra 1952; Mehra and Loyal 1956) as well as
those induced by x-rays (Palta and Mehra 1973) (figure 1: F and G).

The differentiation of rhizoidal cell was either inhibited or its growth was definitely
retarded in other cases (figure 1: G). In a few instances, the growing rhizoid tips
showed a sudden twist followed by a change in the direction of growth (figure 2: A).
Kato (1969) observed similar behaviour in rhizoidal cells derived from single cells
in callus tissue of Pteris vittata.

During the normal one-dimensional growth the similarity of cell size and form in a
given protonema indicated perfect coordination between the mitotic frequency and
cell expansion. In some protonemata, however, abnormal filamentous growth was
noticed which, when judged from cell length-width ratio, appeared to be intermediate
between protonema and rhizoid (figure 2: B and C).

Of extreme importance were the germinable spores in our cultures which showed
complete lack of polarity gradients. Such spore cells divided mitotically in various
planes (figure 2: D) and each of the resultant cells produced filamentous branches in a
rosette (figure 2: E). With prolonged cultures some of the branches switched two-
dimensional growth and eventually produced spatula-shaped and cordate-thalloid
gametophytes (figure 2: F). Generally such protonemata either failed to differentiate
rhizoids, or if any were produced, they were much too short to act as organs of
anchorage. A positive evidence of complete lack of polarity came from spore cells
which behaved much like the freely suspended cells from carrot root phloem tissue
(Steward et al 1958). First, an unorganized ‘moruloid mass’ of actively dividing
cells in various planes was formed. Later, organized growth ensued when a large
number of cells underwent one-dimensional growth, resulting in numerous filamen-
tous branches (figure 3: A). Only a few branches, on prolonged cultures, initiated
two-dimensional growth, resulting in spatula-shaped thalli morphologically similar
to the comparable stage in haploid and diploid gametophytes.

Examination of an 8-month-old culture showed a highly interesting case of twin
prothalli, presumably developed from a giant binucleate spore or from two equal-
sized germ cells (figure 3: B). Morphologically they were similar to the adult haploid
and diploid gametophytes except for the complete absence of marginal trichomes.

Table 1 summarizes the data on different categories of gametophytes observed in a
60-day-old culture. The percentage of ameristic, filamentous protonemata was the
highest and that of the cordate and 2-4-celled were the lowest. The latter ones lacked
growth potential since they were recovered as such in 8-month-old culture; obviously,
the lack of growth in such prothalli was genotypically controlled. The preponderance

<table>
<thead>
<tr>
<th>Two-four Celled</th>
<th>Filamentous</th>
<th>Ameristic formless</th>
<th>Spatula-shaped</th>
<th>Cordate</th>
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<tr>
<td>127</td>
<td>479</td>
<td>150</td>
<td>269</td>
<td>138</td>
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<tr>
<td>10.89%</td>
<td>41.18%</td>
<td>12.8%</td>
<td>23.12%</td>
<td>11.86%</td>
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of filamentous protonemata in cultures raised from different spore samples might be expected on the basis that the juvenile phases would tend to be unusually prolonged.

In 90-day-old cultures, the cordate prothalli became hermaphroditic while the filamentous ones bore antheridia only. The spermatozoids from different prothalli were alike in structural features but distinct differences in size were observed. Embryo formation was noticed in a few massive, cordate prothalli after a period of 8 months on 0.8% agar-solidified medium. The cotyledonary leaf was distinctly larger, with a high degree of lobing, than the corresponding leaf of the diploid and tetraploid cytotypes (figure 3:C).

We counted chromosome numbers in a few cases only. Preliminary results indicated a higher range of variability than in autotriploid *Osmunda* (Manton 1950). This seems to be connected with very faulty cytokinesis and the presence of micronuclei in the germinable spores. The lowest numbers, \( n = \text{circa} 28 \) and \( 44 \) were recorded in two filamentous protonemata. Although the gametophytes with such low chromosome numbers lacked two-dimensional growth at the time of observation, the question of whether they lacked genetic information necessary for achieving expansion is difficult to answer. Another hyperhaploid number was circa 54 in a spatula-shaped gametophyte (figure 3:D). The highest number recorded was \( n = \text{circa} 109 \) in an antheridial initial of a cordate gametophyte. Thus the cytological data though limited, demonstrated that the germinable spores in different cultures had discordant chromosome numbers.

4. Discussion

A large number of germinable spores showed lack of ability to differentiate the first rhizoidal cell. Two reasons can be suggested for this deficiency. First, non-differentiation of the rhizoid point during maturation of spore cells, because as demonstrated by staining techniques and electron microscopic studies, the differentiation of rhizoid point takes place at the time of development of the fern spores (Kato 1957a; Nakazawa and Tsusaki 1959). A second possible explanation is that in such spore cells the nucleus failed to move to one side of the spore cell prior to the first mitotic division. Both these possibilities are indicative of loss of polarity gradient in spore cells; and (as has been pointed out earlier) the growth pattern of the resultant germ cells was remarkably similar to free cells in suspension.

Complete inhibition or much delayed rhizoid differentiation is bound to adversely affect the establishment of such protonemata (under ordinary condition of growth) in nature. This information is important inasmuch as it explains the absence of sexually-produced progeny of triploid cytotype with fluctuating chromosome numbers in nature. In the same context, the poor competitive ability due to prolonged juvenile phase in most of the gametophytes, is worthy of note.

A concomitant effect of the rhizoid inhibition was the formation of twin germ cells and twin protonemata with equal or unequal growth potential of the two branches. Atkinson (1960) reported, though infrequently, two protonemata per germ cell in a culture of *Mohria*. Similar alteration was recorded after treating the spores of *Dryopteris erythrosora* with low concentration of tryptophane (Kato 1957b) and 80% twin protonemata were obtained from spores of *Anemia phyllitidis* with allogibberellic acid (Schraudolf 1967). The question, whether this morphogenetic change
Figure 2. A: 20-day-old protonema showing twisted growth of rhizoids, ×210. B, C: 20-day-old protonema showing branches (arrows) intermediate between protonema and rhizoid, ×200. D-F: early and late stages respectively of rosette-shaped protonema, ×190, ×90 (2F).
Figure 3. A: 40-day-old gametophyte showing initiation of two-dimensional growth from a 'moruloid mass' of filamentous growth, ×60. B: 60-day-old twin gametophytes showing identical phenotype ×60. C: first and second leaves of the sporophyte on an 8-month-old gametophyte. D: somatic metaphase showing circa 54 chromosomes, ×1900.
which is genetically determined in the present material, is mediated through the same biochemical steps at the enzyme level as are produced by the above-mentioned exogenous chemicals, needs further study on this material.

The allometric growth of individual cells in some protonemata, for instance figures 1:D, 2:C, indicates that the balance between the rate of cell duplication and cell expansion which is known to control the normal development of the gametophyte (Sobota and Partanen 1966, 1967) was completely disturbed. This may be attributed to difference in diffusion of growth factors and nucleo-cytoplasmic imbalance, but these have not been studied. Also, the presence of factors for faulty cytokinesis in meiocytes, as described earlier, is suggestive of the possibility that the intercellular plasmodesmatal connections as well as the organization of microtubules may be defective in such cells. Thus, in order to pinpoint the causal factors for the abnormal cellular behaviour in this material, perhaps fine structural studies might prove rewarding.

As regards the disposition of metaphase spindle, its complete instability in the first germ cells and their immediate derivatives resulted in rosette-shaped multiple protonemata. This observation seems to be of considerable morphogenetic and evolutionary interest. Because some similar alterations in the orientation of metaphase spindle with attending cytokinetic upsets might have occurred as a prelude to the evolution of diverse branching systems from a basic uniseriate, filamentous pattern e.g. among the eukaryotic algal cells.

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