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THE INFLUENCE OF HYDROXYUREA ON THE INDUCTION OF APOGAMY IN *PTERIDIUM AQUILINUM* L.

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Treatment with sublethal doses of hydroxyurea inhibited the growth of young normal sporophytes of *Pteridium aquilinum* L. and favoured the formation of apogamous sporophytes on reversal to the medium lacking the drug. The addition of sucrose during the developmental phase of the induced sporophytes seemed to be beneficial as it increased the response. Apogamous sporophytes failed to develop on a medium containing ABA during the period of induction. However, its presence during developmental phase permitted their formation but the number was less than the control.

INTRODUCTION

Since the discovery of apogamy in ferns by Farlow [6] several investigators have worked on the subject in order to establish defined conditions for obtaining apogamous development of sporophytes. In ferns apogamy occurs naturally and can also be induced experimentally. Investigations on the experimental induction of apogamy in *Pteridium aquilinum* have shown that high concentrations of phosphates [11], auxin [13] and a considerably high concentration of sugars [15, 16] induced a significant increase in the number of apogamous buds. A partial replacement of sugar with mannitol also maintained the response [14]. However, Bell [1] did not observe apogamous buds development as a result of the incorporation of sucrose in the medium. Ethylene has also been shown to induce apogamy in *Pteridium aquilinum* if supplied in combination with sucrose. However, growth of the already induced buds is shown to be independent of the ethylene but dependent on the supply of exogenous carbohydrates [5]. Formation of apogamous buds in dry old cultures [1], in cultures grown under the influence of high levels of sugars [15, 14] and ethylene [3] show that nutritional as well as environmental factors conducive to stress may be involved in the phenomenon. In the present investigation, therefore, hydroxyurea; a specific inhibitor of DNA-synthesis, was used so that the application of physiological stress may result in the formation of apogamous buds.

MATERIALS AND METHODS

Gametophytes of *Pteridium aquilinum* were obtained by the method of Bell [2] on Moore's [10] medium solidified with 1.5% Difco agar. The test medium was supplemented with η-hydroxyurea (B.D.H.). Control experiments were run on unsuplemented Moore's medium along with each treatment.

In order to maintain uniformity in the starting experimental material fully cordate gametophytes, approximately I cm in diameter, with thick archegonial growth, were selected. Fertilization was carried out by putting them in a thick suspension of spermatozoids obtained from a dense culture on standard medium, and leaving them for 3-4 hr. The gametophytes were always washed thoroughly before putting them in a suspension of spermatozoids or after fertilization when they were transferred to the experimental medium.

After a series of preliminary experiments 100 ppm hydroxyurea concentration was selected. A large number of fertilized gametophytes which had been grown for 5 days on standard medium lacking hydroxyurea were transferred to the medium supplemented with hydroxyurea. After 30 days of growth on drug-supplemented medium they were reversed to the drug-free medium, after washing. In the experiments in which sucrose was incorporated into the medium, it was always added to the hydroxyurea lacking medium during post-induction period. Two concentrations of ABA were tested both during inductive phase (extending from 6th day to 19th day) and developmental phase (extending from 20th day to 40th day) for their influence on the frequency of formation of apogamous sporophytes (Table 1). Observations were recorded either with the naked eye or under a binocular microscope.

For microscopic examination the material was fixed

Table 1. The effect of ABA on the formation of apogamous sporophytes in *Pteridium aquilinum* L.

Hydroxyurea supplemented medium (14 days) (inductive phase)		Hydroxyurea-free medium (21 days) (developmental phase)		Apogamous buds per culture (after 35 days)
ABA concentration		ABA concentration		
10^{-6} M	10^{-5} M	10^{-6} M	10^{-5} M	
–	–	–	–	6.0 ± 1.3
+	–	+	–	0.0 ± 0.0
–	+	–	+	0.0 ± 0.0
+	–	–	–	0.0 ± 0.0
–	+	–	–	0.0 ± 0.0
–	–	+	–	3.4 ± 0.9
–	–	–	+	1.6 ± 0.4

– = Absent. + = Present.

in formalin-propionic acid-alcohol (4:14:72) or 5% gluteraldehyde followed by treatment with 2% osmium tetroxide, for 2 hr, embedded in wax or apoxy resin, sectioned at 6 μm and observation recorded.

For the determination of DNA content of the nuclei of the newly developed sporophytes material fixed in acetic acid-alcohol (1:3), embedded in wax, sectioned at 10 μm, and stained with Feulgen stain, was used. Cytophotometrical measurements were made with a M85/86 scanning microdensitometer (Vickers Ltd.). Overlapping and cut portions of nuclei were ignored. Nuclear DNA from the nuclei of gametophytic cells present on the same slide was also measured.

RESULTS

Five days old normal embryo in *Pteridium aquilinum* is a homogeneous mass of cells without organ differentiation. It completes its development in 7-8 days after fertilization on standard Moore's medium. The first leaf emerges out of the calyptra after 3-4 days. The first root ruptures the calyptra 1-2 days after the emergence of the first leaf.

When five days old normal embryos were transferred to medium containing different concentrations of hydroxyurea (25, 50, 75, 100 and 125 ppm), 125 ppm hydroxyurea proved to be lethal for the growth of the young sporophytes as it induced yellowing of the gametophytic tissues leading to complete senescence of both the gametophyte and the sporophyte. Sporophytes growing on media augmented with 25 and 50 ppm hydroxyurea were similar to controls whereas at 75 ppm and 100 ppm hydroxyurea the rate of growth of the young sporophytes was slow. The effect was very much pronounced at 100 ppm. The roots of the sporophytes were 10-12 times smaller than the controls. In the leaves the elongation of rachis and the expansion of leaf blade was inhibited. Generally such sporophytes produced two leaves in 30 days. When 35 days old such gametophytes were transferred to drug-free medium, they resumed rapid growth. Their roots elongated 4-5 times and formed lateral roots. Dormant leaf buds also grew out vigorously and formed the 2nd, 3rd and 4th leaf of normal morphology. However, in some of the gametophytes which were left to grow on the same unsupplemented medium for 14 days, a very interesting phenomenon was observed, viz. some apogamous sporophytes which were comparatively weaker in morphology developed on them. The first leaves produced by them were pinnately compound and showed circinnate vernation, when young, resembling the 2nd and successive leaves formed by normal sporophyte. Roots developed at a later stage when the leaves were unfolding. A maximum of six such sporophytes were observed on a single gametophyte (Fig. 1 and 2).

In order to establish whether these sporophytes were developed from the existing archegonia which became fertilized at the time of fertilization and the resultant zygotes remained dormant for the rest of the experimental period or they were apogamous in origin, cytophotometric determination of DNA content of the cells of induced

sporophytes and parent gametophytes were made. The results are presented in Fig. 3. A majority of the nuclei

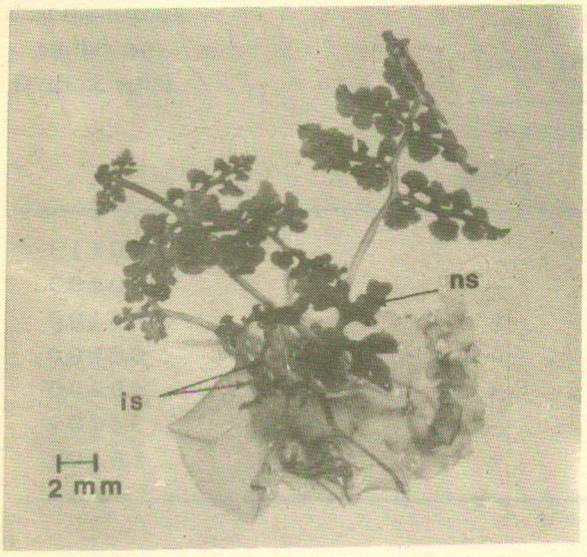

Fig. 1. *Pteridium aquilinum* Fertilized gametophytes grown for 5 days on normal Moore's medium then fed with 100 ppm hydroxyurea for 30 days and returned to normal Moore's medium for 15 days. *ns*, normal sporophyte; is, induced sporophyte.

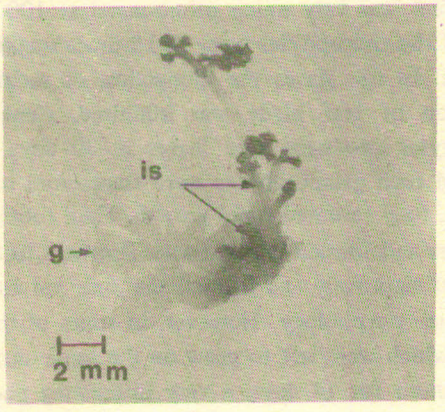

Fig. 2. *Pteridium aquilinum* A part of the gametophyte shown in Figure 1. *is*, inducedsporophyteafter the treatment; *g*, gametophyte.

from cells of the mature fully expanded gametophytes contained DNA at IC level whereas a considerable number of nuclei contained intermediate DNA content between IC and 2C level. The IC level of gametophytic nuclei shows the haploid DNA content of the gametophyte whereas nuclei containing DNA content between IC and 2C level is presumably due to the replication of DNA indicating cells being in the "S" phase of the cell cycle. Nuclei from the cells of both gametophyte and sporophyte containing 2C level of DNA appear to have already gone through the S phase and appear to have accumulated at the G_2 level of the cell cycle.

Augmentation of media with a range of concentrations of sucrose resulted in an increase in the number of apogamous buds formed per culture. Optimum concentration of sucrose which supported the maximum number of apogamous buds development was 1.5%. At higher concentrations their frequency dropped (Fig. 4).

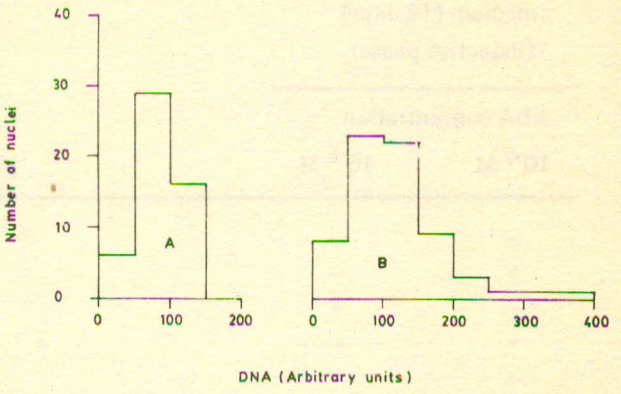

Fig. 3. Cytophotometric determination of DNA content of the nuclei of cells of gametophyte (A) and apogamous sporophyte (B).

Apogamous buds failed to develop on media containing ABA throughout the experimental period. Addition of ABA to the hydroxyurea supplemented medium during the first two weeks of culture also inhibited their formation completely. However, the incorporation of ABA during the developmental phase permitted the development of apogamous sporophytes but their frequency was less than the control which lacked ABA throughout the experimental period (Table 1).

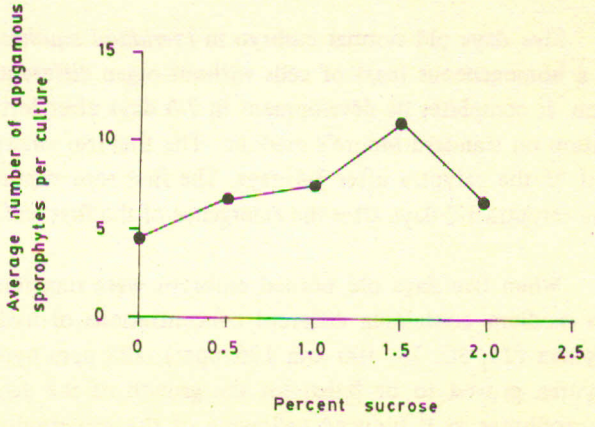

Fig. 4. The influence of various concentrations of sucrose on the frequency of apogamous buds formation in *Pteridium aquilinum* L.

DISCUSSION

Results show that prolonged exposure of *Pteridium aquilinum* gametophytes to the sublethal doses of hydroxyurea inhibited growth of the sexually produced sporophytes and led to the induction of apogamous sporophytes. Further development and growth of which remained suppressed until hydroxyurea was completely removed from the medium.

The formation of apogamous buds in *Pteridium aquilinum* has been recognized as two step process [5], both differing from each other in their requirements. In the present work too, there are indications of the existence of two distinct stages in their development. The apogamous sporophytes developed on medium containing hydroxyurea and the presence of sucrose has not been found to be essential as has been reported by Elmore and Whittier [5]. However, development and growth of the induced sporophytes proceeded on medium lacking hydroxyurea. Supply of sucrose proved to be beneficial as it increased the frequency of their formation over the control (Fig. 4). The role of sucrose at the latter stage appears to be simply that of providing nutrition to the already induced sporophytes which might have remained suppressed as a result of competition for the available substrate. Results presented in Table 1 also indicate that application of ABA during the inductive phase was detrimental for the formation of apogamous sporophytes and completely blocked their induction and subsequent growth whereas the incorporation of ABA in the medium during the developmental phase allowed the development and growth of some of the sporophytes but their number was less than the control.

Pteridium gametophytes bearing young sporophytes subjected to the influence of sublethal doses of hydroxyurea appear to have been under a condition of physiological stress due to restricted cell divisions. This check on cell division might have resulted in the production of ethylene as has been shown for other plants which show an increased production of ethylene in response to stress conditions [7 8, 17]. The role of exogenously supplied ehtylene in the development of apogamous sporophytes in *Pteridium aquilinum* is well documented [3, 4, 5]. Further, *Pteridium* gametophytes are also known to produce endogenous ethylene under normal conditions of culture [3] but the accumulated ethylene does not reach the threshold level required for the induction of apogamous sporophytes.

In the present experiments complete blockage of apogamous bud formation in response to the added ABA during the inductive phase of culture strengthens the possibility of the involvement of ethylene during the induction period. ABA is known to inhibit ethylene production in excised, wilted and turgid leaves of wheat [17] and in rose petals [9], and one of the modes of the action of ABA is reported to be the inhibition of m-RNA synthesis [12]. Therefore, it is possible that absence of certain m-RNA.s necessary for maintaining gametophytic development may thus trigger a switch in the developmental direction, namely, from gametophyte to sporophyte.

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