The Effect of Sucrose on Apogamy in Cyrtomium falcatum Presl[1]

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The phenomenon of apogamy provides an excellent opportunity to study the direct origin of a vascular plant from a non-vascular fern gametophyte. Besides the investigation of the change from the gametophytic to the sporophytic developmental pattern, apogamy which is a deviation in the normal life cycle provides a site to study the factors controlling the alternation of generations.

Studies by Whittier and Steeves (1960, 1962) have demonstrated a new method for controlling induced apogamy, i.e. the formation of a sporophyte directly from the vegetative cells of a gametophyte which is able to form sporophytes by fertilization under other conditions. Several species of fern gametophytes grown in sterile culture on nutrient media containing suitable concentrations of sugar produce apogamous sporophytes. In the absence of sugar no apogamous plants are formed by the gametophytes. The availability of supplementary sugar to the gametophytes has a direct relationship to the formation of the apogamous plants.

Obligate apogamy, i.e. the formation of a sporophyte directly from the vegetative cells of a gametophyte which is unable to form sporophytes by fertilization, has been described in many ferns, but this type of apogamy has been the subject of few experimental investigations. In one such study with *Pteris cretica* L., Bell (1959) reported apogamy occurred sooner on prothalli supplied with sugar than others without sugar. In view of this report and the effect sugar has on induced apogamy, this study was undertaken to determine if sugar influences obligate apogamy other than accelerating its appearance.

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MATERIALS AND METHODS

Cyrtomium falcatum Presl. was chosen for this study because the spores of this ferns were readily available and the development of the apogamous plant had been described (DeBary, 1878; Allen, 1914).

The spores were wetted with a 0.1% solution of Tween 80 and exposed to a 15% Clorox solution for two minutes to sterilize them. The sterile spores were collected on filter paper, washed several times with sterile water and suspended in sterile water. The spores were innoculated into square one ounce bottles containing 15 cc of nutrient medium. The nutrient medium was composed of Knudson's solution of mineral salts, minor elements and 0.5% agar. This medium differed from the one reported by Whittier and Steeves (1960) in that the iron was supplied as ferric sodium ethylenedianime tetra-acetate (Fe-EDTA) instead of ferric citrate. In a preliminary experiment the fastest growth took place on a medium containing 2.5% sucrose, therefore this concentration along with 0, 0.5 and 6.0% were employed in this investigation. The cultures were maintained at a temperature of 24 $\pm$ 1° C with 12 hours of illumination every 24 hours from Sylvania Gro-Lux lamps at an intensity of ca. 100 foot-candles. The remaining sterile culture techniques were those employed by Whittier and Steeves (1960).

The area of the gametophytes was found with a microprojector and a polar planimeter. Tracings of the projected prothallial images were measured with the polar planimeter. The mean area of twenty gametophytes was employed as a measure of prothallial growth. The size of the gametophytes undergoing initial apogamy was determined by calculating the mean area of twenty prothalli with early stages of apogamous development. The mean cell size of the gametophytes was found by dividing the cell number of twenty similar sized gametophytes into the area of these gametophytes. A T—test was employed to determine if the differences between the responses on the concentrations of sucrose were significant.

RESULTS

The spores on all concentrations of sucrose germinated the seventh day after innoculation. The least growth took place on the medium without sugar and the largest growth on the 2.5% sucrose medium (Table I). The differences between the growth on the various media were significant at the 5% level.

TABLE I. THE EFFECT OF SUCROSE ON APOGAMY AND THE GAMETOPHYTE OF CYRTOMIUM FALCATUM.

Sucrose %	Mean Prothallial Size in Sq. mm 37th Day	Initial Apogamy	Day First Apogamy	Mean Cell Size Sq. mm.
0.0	0.43±0.02[1]	2.15±0.04[1]	41st	0.0021±0.0001[1]
0.5	1.45±0.09	1.85±0.04	35th	0.0021±0.0001
2.5	1.89±0.08	1.46±0.02	30th	0.0022±0.0001
6.0	0.80±0.05	1.05±0.03	36th	0.0023±0.0001

[1] Standard error of mean.

The prothallial development proceeded normally from the filamentous to the cordate stage. Some time after the cordate shape was attained a pale green area, which later turned brown, appeared a short distance behind the sinus. This pale green region formed behind the sinus after the cordate prothallus had become more than one cell thick in that area. Although this light region was not initially apogamous, it was considered the first stage leading to apogamous development because the apogamous sporophyte originated from the cells of this area. The initiation of the apogamous plant occurred once the pale green area had become a few cells thick.

On the 30th day the apogamous development was initiated on the 2.5% sucrose medium (Table I). This was five or six days earlier than on the other sucrose media and eleven days before apogamy started on the medium lacking sugar. At the time the apogamous plants were being initiated on the 2.5% medium, none of the prothalli without exogenous sugar were more than one cell thick or beyond the early cordate stage.

The decrease in the size of the prothalli undergoing initial apogamy with the increase in the sucrose concentration was sig-

nificant at the 5% level (Table I). The mean cell size of the prothalli on the various sugar concentrations did not vary significantly from each other (Table I). Since the prothallial size is determined by the cell number and cell size, the decrease in prothallial size was due to a reduction in the number of cells per gametophyte because the mean cell sizes were not significantly different.

DISCUSSION AND CONCLUSIONS

The growth rate of the prothalli was increased significantly above the rate on no sugar by the addition of sucrose to the medium, although at 6.0% sucrose the growth decreased significantly from that on 2.5% sucrose. This decrease in growth on the high concentration of sugar probably was due to the high osmotic potential of the medium interfering with the growth. This has been found to be true in other studies on the growth of gametophytes in sterile culture (Mitra & Allsopp, 1959; Whittier, 1962).

Besides the change in growth rate the size of the prothalli undergoing initial apogamy decreased significantly as the concentration of sugar increased. This observation does not support Bell (1959) who reported no change in gametophyte size undergoing apogamy on different concentrations of sugar. This difference in size was due to a reduced number of cells in the gametophytes because the mean cell sizes on the various concentrations of sugar were the same. Consequently, less growth and fewer cell divisions were required for the prothalli to initiate apogamy on the media containing sugar.

In terms of the number of days for the occurrence of apogamy, it was initiated on the 2.5% sucrose medium about 15% sooner than on the other sucrose media and 25% sooner than on the medium without sugar which is in agreement with Bell's report on *P. cretica* (1959). This was due to the gametophytes on the 2.5% sucrose medium having the fastest growth and a small prothallial size which would produce apogamy. It can be concluded that the time necessary for obligate apogamy is de-

termined by the growth rate and the prothallial size undergoing apogamy on any concentration of sucrose.

The sugar brought about the requisite conditions for obligate apogamy in the gametophyte sooner with fewer cell divisions and less prothallial growth. One of the required conditions is the thickening of the gametophyte which always precedes the development of the light green area behind the sinus and the apogamous plant. In other species the thickening of the prothallus before the initiation of the obligate apogamous plant has been reported (Duncan, 1943). Also, a thickened prothallial growth is necessary for induced apogamy (Whittier, 1962; Whittier & Steeves, 1960). Thus, the thickening of the gametophyte is a requisite condition for both types of apogamy. In sterile culture the thickening necessary for induced apogamy, which is many times the thickening required for obligate apogamy, is controlled by the sugar in the medium. Without sugar the necessary thickening of the gametophyte for induced apogamy is absent, and apogamy fails to occur. The supplementary sugar causes the thickening required for induced apogamy and accelerates the normal thickening preceding obligate apogamy probably by modifying the carbohydrate metabolism of the prothallus.

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Ferns and Fern Allies of Pine Hills Field Station and Environs (Illinois)

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This is one in a series of studies which have emanated from the Southern Illinois University Field Station. A check-list of vascular plants has been prepared (Mohlenbrock & Voigt, in press). The study of the ferns and their allies of the area was completed during the summer of 1961 when the junior author participated in a National Science Foundation Research Participation Program.[1,2] The authors are grateful to the graduate council of Southern Illinois University for assistance in this study.

THE PINE HILLS AND ENVIRONS

Two representative areas were chosen for exhaustive study of the fern and fern ally groups native to the Pine Hills and its environs. These areas were the Pine Hills Recreation Area and the adjoining Union County Forest Preserve. Both areas are located in the Shawnee Hills of Southern Illinois.

Pine Hills Recreation Area is approximately 37 miles from Carbondale, Illinois, off of Illinois Route 3. The territory covered in the survey extended from the second set of railroad tracks on the Aldridge Levee Road to Hutchins Creek, and from the Pine Hills Recreation Area entrance to the edge of Otter Pond. All of the roads, trails, picnic areas, and the penetrable interior of the Recreation Area were covered.

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[1]N. S. F. Grant 16,180.

[2]Contribution from the Pine Hills Field Station and the Department of Botany, Southern Illinois University.

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