Pak. J. Bot., 19(1): 43–52, 1987.

THE INFLUENCE OF HYDROXYUREA TREATMENT ON VARIOUS STAGES OF THE LIFE CYCLE OF *PTERIDIUM AQUILINUM* L.

KHALIDA KHATOON

Department of Botany,
University of Karachi, Karachi-32, Pakistan.

Abstract

Hydroxyurea supplied to archegoniate gametophytes of *Pteridium aquilinum* at various stages of development was lethal at 125 ppm. Sublethal doses of hydroxyurea retarded growth and development of the sporophyte at 25 and 50 ppm whereas prolonged treatment at 100 ppm induced dormancy in the first root and leaf buds. Normal sequence of organ formation was disturbed when zygotes were exposed to hydroxyurea immediately after fertilization. Maturing eggs became non-viable. Hydroxyurea at low concentrations delayed spore germination whereas at high concentration inhibited establishment of cordate form. The archegonia initiated and developed under the influence of hydroxyurea produced normal sporophytes but were weaker in appearance.

Introduction

Maturation of egg in the life cycle of *Pteridium aquilinum* is interpreted as the time at which activation and inactivation of genes responsible for the expression of distinctive features of alternating generations takes place (Bell & Duckett, 1976). Treatment of maturing eggs with thiouracil produced inviable eggs or if viable eggs were formed upon fertilization they showed a tendency towards gametophytic growth. Use of thiouracil at post fertilization stage either induced lethality leading to abortion of zygotes or prevented embryo formation. If embryo development occurred, their growth was retarded and the ensuing sporophytes were either deformed or showed a tendency towards formation of gametophytic tissues (Jayasekera & Bell, 1972). From these results it was inferred that transcription and translation of sporphytic programme possibly takes place during maturation of egg and zygote formation. In the present study n-hydroxyurea, a specific inhibitor of DNA synthesis (Young & Hodas, 1964) was applied at various stages of the life cycle of *P. aquilinum* to obtain information about the control of embryogenesis in this plant.

Material and Methods

Gametophytes of *P. aquilinum* were grown from spores on Moore's (1903) medium solidified with 1.5% Difco agar (Khatoon, 1985). n-Hydroxyurea (B.D.H.) @ 25, 50, 75, 100 and 125 ppm was incorporated into the medium. Fully cordate gametophytes of the same age, approximately 1 cm in diam., with thick archegonial growth were selected. Fertilization was carried out by putting selected gametophytes for 3-4 h., in a thick suspension of spermatozoids, obtained from dense culture on standard medium.

The following treatments were made:

1. Fertilized gametophytes were grown for 5 days on standard medium and were then transferred to the media containing different concentrations of hydroxyurea.
2. Cordate archegoniate gametophytes, raised on standard medium, were fertilized, washed and were directly transferred to the hydroxyurea supplemented medium.
3. Fully cordate actively growing gametophytes bearing archegonia at various stages of development were transferred to the media supplemented with different concentrations of hydroxyurea. After 6 days such gametophytes were washed thoroughly with water, fertilized and were transferred to standard medium for 4 weeks.
4. In another set spores were germinated on five different concentrations of hydroxyurea (25, 50, 75, 100 and 125 ppm) to see its effect on the growth of gametophytes and sex organ formation. Archegonia produced under the influence of the drug were also fertilized with the spermatozoids produced on normal medium, to see the effect of hydroxyurea on the development and fertility of eggs thus formed.

Growing young gametophytes were sampled at intervals and examined under the microscope. Diameter of each gametophyte was measured with the help of ocular micrometer. For microscopic examination the material was fixed in formalin-propionic acid-alcohol (4:14:72) or 5% gluteraldehyde followed by treatment with 2% osmium tetraoxide for 2 h, washed with water and dehydrated through ethanol grades, embedded either in wax or epoxy resin, sectioned at 6 μm and photographed using phase contrast optics.

Results

Normal embryogeny: Five days after fertilization embryo was a homogenous mass of meristematic cells which did not show any sign of organ differentiation (Fig. 1). Seven day old embryo exhibited formation of 4 distinct organ producing regions. The anterior half, formed the first leaf and root apex and the other posterior half formed the shoot apex and foot. In 10-12 days first leaf emerged out of the calyptra and the root ruptured the calyptra after 13-14 days.

Effect of hydroxyurea on normal embryo: Fertilized gametophytes placed on standard Moore's medium for 5 days and then transferred for another 5 days on media containing 25, 50, 75, 100 and 125 ppm of hydroxyurea (HU) were fixed and sectioned. Gametophytes treated with 25 and 50 ppm of HU showed differentiation of organ forming regions similar to the normal embryo. Young embryos grown on 75 and 100 ppm of HU produced a large proportion of parenchymatous cells constituting the foot whereas a small proportion formed stem apex and root apex. At 125 ppm the gametophytes

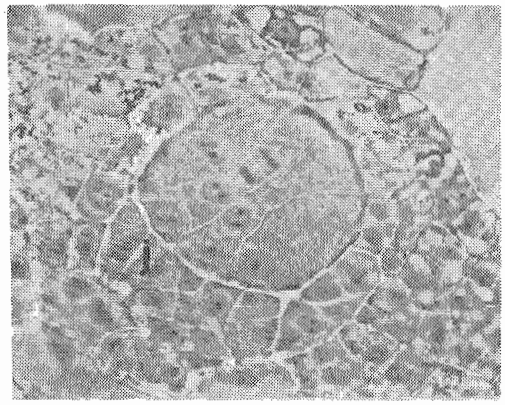

Fig. 1. Five days old normal embryo. J, Jacket; x 200.

gradually turned yellow and eventually died within 30 days. In control the embryo produced root primordia. First leaf showed considerable elongation which resulted in stretching of the calyptra near the tip.

Development of embryos on gametophytes which continued to grow on HU supplemented medium for 30 days (Table 1) did not show any marked change in their morphology at 25 and 50 ppm HU except a delay in the formation of leaves. At 75 and 100 ppm HU a decrease in size and change in shape of pinna of first leaf was observed (Fig. 3B & C). Root formation was inhibited at 75 ppm HU as compared to controls which produced elongated first root and secondary roots also developed on them (Fig. 3A). At 100 ppm the inhibitory effect was more pronounced and sporophytes produced only 1-1.5 mm

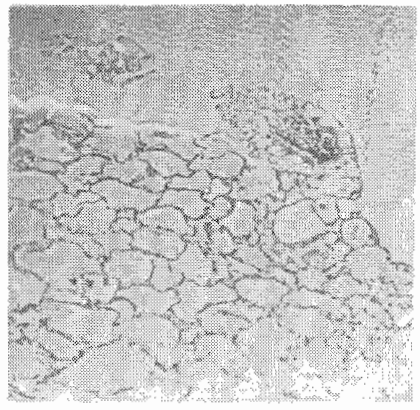

Fig. 2. An archegonium fed with hydroxyurea for six days before fertilization and then transferred to the drug-free medium. Note the remnants of degenerated egg in the venter.

Table 1. The influence of hydroxyurea treatment on the morphology of developing sporophyte of *Pteridium aquilinum* L.

Treatment Hyroxyurea conc. (ppm)	Number of gametophytes used	Duration of treatment in days	Morphological observation of sporophyte
0	15	16	First leaf fully expanded. Second leaf primordium developed.
	10	30	Morphology of plant normal. Fourth leaf unfolding.
25	19	16	Sporophyte similar to control. No apparent change in morphology.
	14	30	Retarded growth. Third leaf unfolding.
50	17	16	No apparent change in morphology of sporophytes.
	11	30	In some third and in others second leaf unfolding.
75	15	16	Second leaf unfolding. First leaf not fully expanded. Root growth stunted and smaller than the control.
	14	30	Second leaf and roots showed increase in length.
100	17	16	Roots 1-2 mm in length and turned brown. First leaf deformed and not fully expanded.
	17	30	Appearance of 2-3 leaf primordia. Dormancy of root and leaf buds.
125	20	16	Yellowing of tissues of gametophyte. Further development of sporophyte stopped.
	15	30	All gametophytes turned brown and appeared senescing.

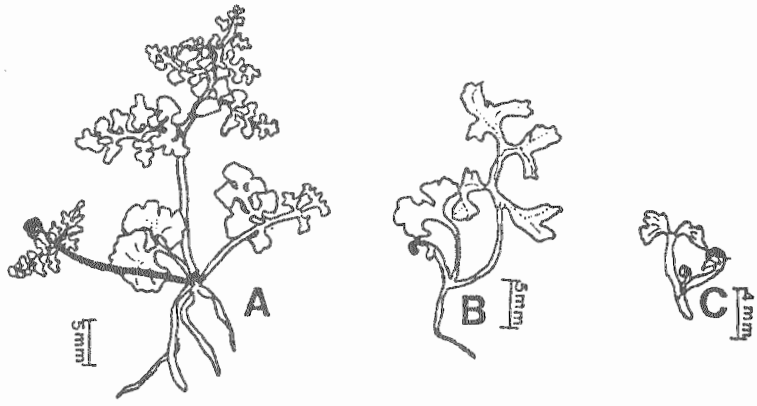

Fig. 3. *Pteridium aquilinum* Sporophytes, 35 days old, dissected away from the gametophytes. A. Control: grown for 35 days on normal Moore's medium. B. 5 days old normal embryo fed with 75 ppm hydroxyurea for 30 days. C. Five days old normal embryo fed with 100 ppm hydroxyurea for 30 days.

long roots in 21 days and 2 leaves in 30 days period. At the end of third week second leaf appeared which remained folded till the end of 30 days period. Other leaf buds remained dormant. Maceration of the dormant buds showed that the outermost peripheral layer of cells developed thick walls and very few cells in the inner mass of thin walled cells were in mitosis. Similarly, roots developed at 100 ppm HU, were 2-3 mm long, did not show increase in length, and turned brown after 30 days. Cells in such roots developed thick walls in root cap and meristem contained non dividing nuclei. Dividing cells were not seen.

Fresh weight of leaves and roots showed a decrease with an increase in HU concentration (Fig. 5 & 6) indicating increased inhibition of growth caused by HU at higher concentrations.

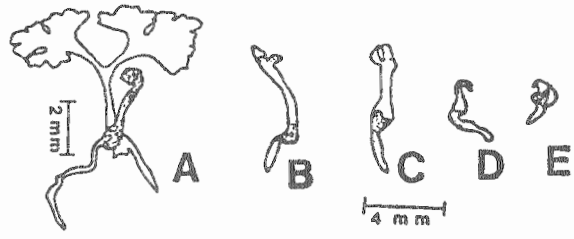

Fig. 4. *Pteridium aquilinum* Sporophytes, 16 days old, dissected away from the gametophytes. A. Control; grown for 16 days on standard Moore's medium. B. Fed with 25 ppm hydroxyurea following fertilization. C. Fed with 50 ppm hydroxurea following fertilization. D. Fed with 75 ppm hydroxyurea following fertilization. E. Fed with 100 ppm hydroxyurea following fertilization.

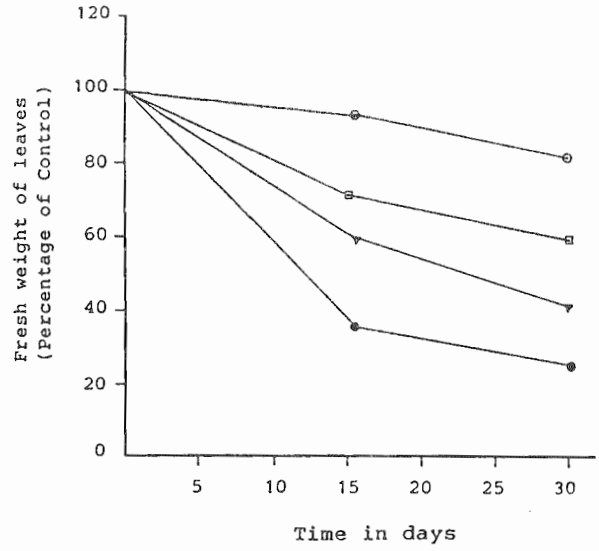

Fig. 5. The effect of hydroxyurea on the fresh weight of leaves of *Pteridium aquilinum* excised from 5 day old normal embryos and treated with different concentrations of the drug for 30 days. 25 ppm (○); 50 ppm (□); 75 ppm (△) and 100 ppm (●).

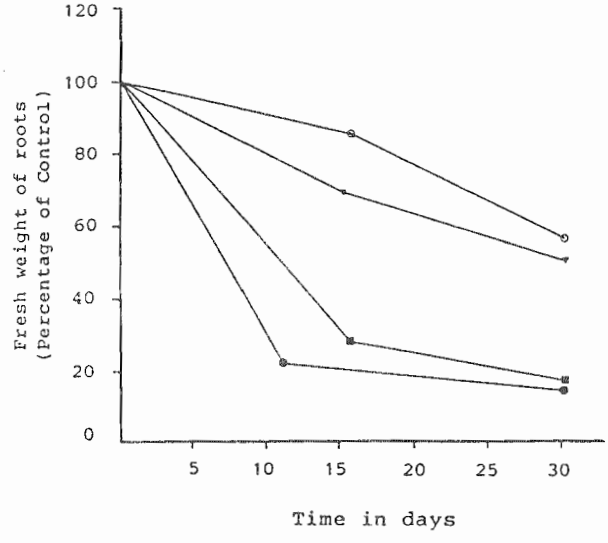

Fig. 6. The effect of hydroxyurea on the fresh weight of roots of sporophytes of *Pteridium aquilinum*, developed from 5 days old normal embryos fed with different concentrations of the drug for 30 days. 25 ppm (○); 50 ppm (▲); 75 ppm (■) and 100 ppm (●).

Effect of hydroxyurea after fertilization: Gametophytes growing on standard medium were fertilized and transferred to HU supplemented medium. At all concentrations a striking change in the course of normal embryogeny observed was the emergence of roots from the calyptrae prior to leaves. The roots emerged out of the embryos after 10 to 11 days of growth after fertilization. Emergence of leaves was delayed for 2-3 days at 25, 50 and 75 ppm. First leaf ruptured the calyptra on the 14th – 15th day of transfer whereas at 100 ppm treatment calyptra was intact at the point of emergence of leaf till the end of 16 days period (Fig. 4A-E).

Effect of hydroxyurea before fertilization: The archegonia which had been allowed to grow for 6 days on various concentrations of hydroxyurea supplemented media when fertilized and planted on hydroxyurea-free medium failed to form embryos. Sectioning of such gametophytes revealed that some of the archegonial chambers were empty whereas others contained degenerated remains of the egg (Fig. 2).

Effect of hydroxyurea on growth of gametophyte and sex organ formation: Spore germination was observed up to 100 ppm of HU whereas 125 ppm was inhibitory for spore germination. A gradual decrease in the rate of growth of gametophytes and a delay in the germination of spores was observed with an increase in HU concentration (Fig. 7). In control 3-9 celled stage was reached after one week of sowing whereas in the presence of 100 ppm HU first signs of germ tube formation were observed after 20 days of treatment when very few cordate prothalli were seen. Nearly all the gametophytes were filamentous upto 70 days experimental period.

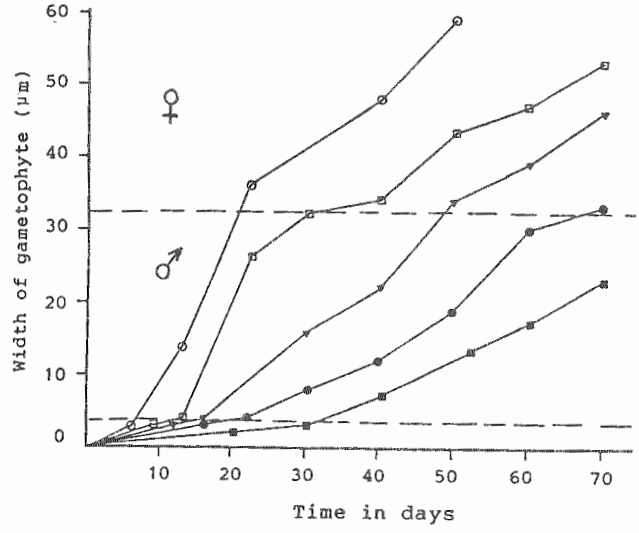

Fig. 7. Effect of different concentrations of hydroxyurea on the growth of gametophytes and sex organ formation in *Pteridium aquilinum.* Control (○); 25 ppm (□); 50 ppm (▲); 75 ppm (●) and 100 ppm (■).

Antheridia formation was observed in the gametophytes when they reached 4-6 μm in width both in the control and HU treatments. Similarly first archegonial initials were observed in the cordate gametophytes when they grew 30 μm in width. At 100 ppm none of the cordate forms produced archegonial initials upto the end of experimental period.

Behaviour of archegonia produced under the influence of hydroxyurea on subsequent fertilization: Embryo formation occurred on all the archegoniate prothalli which developed under the influence of different concentrations of HU. Bursting of the calyptrae of the developing sporophytes was very much delayed and at the end of thrid week only small parts of the enclosed sporophytes were visible. The ensuing embryos were very similar to the normal embryos except second and subsequent leaves which produced fewer pinna and their petioles were thin and much elongated. Gametophytes produced at 100 ppm HU showed browning of the tissues earlier than any other treatment and the embryos produced on them were less vigorous as compared to other treatments.

Discussion

Globular embryos when exposed to the action of HU show normal embryo development except for a delay in the emergence of organs from the bursting calyptrae and retardation of growth of the emerging sporophyte. The results suggest that the globular embryo is furnished with all the information needed for the expression of sporophytic growth and determination of organs in the embryo takes place before the embryo is globular in form. Normal development of sporophyte from the slightly older embryos (7 day old) of *P. aquilinum* treated with thiouracil has also been reported by Jayasekera & Bell, (1972).

The development of sporophytes, though normal but with altered sequence of organ emergence from zygotes planted immediately after fertilization on HU supplemented medium suggests that drug might have interfered with the physiology of the gametophyte and most probably with the IAA synthesis. The first emergence of roots instead of leaf from the embryo recalls the work of Jayasekera & Bell (1959) in which same response was observed when apical region of the gametophyte of *Thelypteris palustris* was replaced by a concentrated source of auxin. On the other hand in *Pteris longifolia*, a complete inhibition of 2nd leaf formation occurred when IAA was applied to the 1st leaf stump (Albaum, 1938). The formation of roots *in vitro* and on stem cuttings in response to the application of relatively high concentration of auxin is a prevalent phenomenon in angiosperms and has been reported in *Daucus carota* (Kamada & Harada, 1979), *Lapageria rosea* (Jordan *et al*, 1983) and *Simmondsia chinensis* (Lee & Palzkill, 1984). In similar experiments, degeneration of zygotes in *P. aquilinum* as a result of application of thiouracil has been repoted by Jayasekera & Bell (1972). In the present investigation the development of normal sporophytes from HU treated zygotes may be interpreted as a consequ-

ence to unimpaired transcription and translation, characteristic of HU treated cultured cells (Young & Hodas, 1964). The altered sequence of emergence of organs suggests that the determination of organs in the developing embryo possibly takes place in the zygote or at a slightly advanced stage when embryo is not globular in form.

The main reason for the inviability of eggs matured in the presence of 100 ppm HU cannot simply be attributed to the toxicity of drug for the process of oogenesis, since spores of *P. aquilinum* planted on the HU containing medium when allowed to grow continuously on the same medium developed normal archegonia which upon fertilization produced sporophytes. It seems likely that the gametophytes growing continuously on one medium may condition the medium for the normal development and maturity of eggs. Changing the medium at the crucial stage of development and maturity may deprive them from certain growth factors essential for their normal development and hence may lead to their inviability. In similar experiments, varied responses were recorded by Jayasekera & Bell, (1972), the major effect being the inviability of eggs at high concentration of thiouracil. The main reason for variability in their results has been suggested to be the differing amount of drug taken up by the eggs, initially being at different stages of development.

Hydroxyurea applied at 125 ppm was lethal for spore germination indicating an almost complete block of DNA synthesis. Retardation of growth exhibited by cultures grown under sublethal doses of HU appears to lead to restricted cell divisions as a result of reduced DNA synthesis. At 100 ppm HU prevented transition of gametophytes from filamentous to cordate form of growth. A similar response was observed in *Dryopteris erythrosora* (Hotta & Osawa, 1958) and in *D. Borreri* (Bell & Zafar, 1961) as a result of application of 8-azaguanine, an inhibitor of protein synthesis. In the present work, the arrest of gametophytic phase into filamentous form may be due to lesser quantity of building material avaiable in consequence to restricted DNA synthesis. Delay in the formation of sex organs with increasing concentration of HU appears to be related mainly with the slow rate of growth of the parent gametophyte. The archegonia thus formed produced normal sporophytes, upon fertilization, in contrast to the ones exposed to the drug for short period, just before fertilization, suggests that during prolonged exposure gametophytes may develop resistance against the drug or may condition the medium for the normal development of eggs.

From the present study it is conceived that the activation of genes responsible for the development of sporophyte probably takes place in the egg and the information remains untranslated till fertilization occurs. Organ determination remains untranslated till fertilization occurs. Organ determination in the embryo possibly takes place either in the zygote or at a slightly advanced stage but before the time when embryo is globular in form. Globular embryo probably develops resistance against the experimental treatments and therefore generally leads to the formation of sporophyte.

References

Albaum, H.G. 1938. Inhibitions due to growth hormones in fern prothalia and sporophytes. *Amer. J. Bot.*, 25: 124-133.

Bell, P.R. and J.G. Duckett, 1976. Gametogenesis and fertilization in *Pteridium. Bot. J. Linn. Soc.*, 73: 47-78.

Bell, P.R. and A.H. Zafar, 1961. Changes in the level of the protein nitrogen during grwth of the gametophyte and the initiation of the sporophyte of *Dryopteris borreri* Newm. *Ann. Bot. (N.S.)*, 25: 531-546.

Hotta, Y. and S. Osawa, 1958. Control of differentiation in the fern gametophyte by amino acid analogs and 8-azaguanine. *Exptl. Cell Res.*, 15: 85-94.

Jayasekera, R.D.E. and P.R. Bell. 1959. The effect of various experimental treatments on the development of the embryo of the fern *Thelypteris palustris. Planta*, 54: 1-14.

Jayasekera, R.D.E. and P.R. Bell. 1972. The effect of thiouracil on the viability of eggs and embryogeny in *Pteridium aquilinum. Planta* (Berlin), 102: 206-214.

Jordan, M., I. Cortes and G. Montenegro. 1983. Regeneration of *Lapageria rosea* plantlets by tissue culture (Family Philesiaceae). *Gartenbauwissenchaft*, 48: 97-100.

Lee, C.W. and D.A. Palzkill, 1984. Propagation of Jojoba by single node cuttings. Hort Science, 19: 841-842.

Kamada, H. and H. Harada. 1979. Studies on the organogenesis in carrot tissue cultures. I. Effect of growth regulators on somatic embryogenesis and root formation. *Z. Pflanzenphysiol.*, 91: 225-266.

Khatoon, K. 1985. The influence of hydroxyurea on the induction of apogamy in *Pteridium aquilinum L. Pak. J. Sci. Ind. Res.*, 28: 248-251.

Moore, G.T. 1903. Methods of growing pure cultures of algae. *J. Appl. Microbiology*, 6: 2309-2314.

Young, C.W. and S. Hodas, 1964. Hydroxyurea: Inhibitory effect on DNA metabolism. *Science*, 146: 1172-1174.

(Received for publication 3 March, 1986)