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CHLOROPHYLL SYNTHESIS IN THE EMBRYOS OF NELUMBO LUTEA (WILLD.) PERS.

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ABSTRACT

It has been generally believed that chlorophyll synthesis takes place in the dark in the developing embryos of Nelumbo lutea (Willd.) Pers., since mature embryos are green in color. Present experiments indicate that the maturing embryos develop green plumules in the light before they are tightly enclosed by the hardened, impervious fruit wall.

The water lotus, Nelumbo lutea (Willd.) Pers., is commonly found growing in water 2-5 dm deep throughout most of Illinois. In midsummer it produces a flat-topped conical, aggregate fruit 10-15 cm in diameter that is filled with nut-like fruits which have only a small portion of their stigmatic tip exposed. Each nut-like fruit attains a diameter of approximately 16 mm before it begins to shrink by drying to about 11 mm in diameter. These large, single-seeded fruits have been of interest to Botanists for many years since chlorophyll is present in the mature embryo. Because of the hard fruit wall it has generally been assumed that light was not necessary for the synthesis of this chlorophyll. Lyon (1902) discussed the green plumules found in Nelumbo and assumed the chlorophyll was formed in the dark. Stewart and Eyster (1939) found the green pigment to be chlorophyll b. Later, Wilson (1958) found both chlorophyll a and b present, and stated that the embryo of water lotus develops chlorophyll in the absence of light.

MATERIALS AND METHODS

Experiments to verify the necessity of light for chlorophyll synthesis in Nelumbo embryos were conducted at Lake Decatur, Macon County, Illinois, and at Lake Charleston, Coles County, Illinois, during the summers of 1959, 1967, and 1968. The study was conducted by placing maturing receptacles in darkened enclosures for a period of several weeks while the receptacles were still attached to the plants. The enclosures were made by using two different sized cans, each coated with flat black paint. The smaller can, about one liter in size, was opened at the top and had a hole in the bottom to accommodate the peduncle. A slit in the side made it possible to slip this can around the peduncle and receptacle;

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* Dr. Hiram F. Thut died on December 23, 1974. This nearly completed manuscript was recently found among some of his papers and was edited for publication by Dr. John Ebinger of Eastern Illinois University.

the slit was then covered with black friction tape. A loose fitting black cloth, fastened to the peduncle with a rubber band, was used to seal the hole around the peduncle. The second can, about three liters in size, was inverted over the smaller can. It was positioned a little higher to permit air passage into the interior of the chamber. A wooden block in the top of this outer can prevented the lifting of the receptacle into a possible lighted area. The cans were secured to stakes for support. By adding two flat metal shields or reflectors on wooden blocks above and attached to the second can, it was possible to have nearly the same temperature within the cans as the air temperature outside.

RESULTS AND DISCUSSION

To prevent light from reaching the developing embryos, 32 enclosures were set up around maturing receptacles during the first week of August in 1959, 1967, and 1968. Five enclosed receptacles were harvested in 1959 after 12 days in the dark. During this 12-day period, the developing fruits and the enclosing receptacles had attained approximately one-half size and had the yellow color of the opening flowers. When the yellow carpels were opened, the immature plumules were also yellow. The maturing fruits not so enclosed were green. Most of the receptacles were harvested after 23-34 days of enclosure and had attained their maximum size, appearing quite mature and dry. One hundred fruits husked at random from several of these dry receptacles averaged 1.06 g., while a similar lot from mature receptacles not in enclosures averaged 1.2 g each.

The significant change, however, was in the plumule or epicotyl. Those growing in the light were green, but those maturing within the enclosures were yellow. Pigment extractions and chromatographs were made as described by Nutting, Voet, and Becker (1965); and these graphs were inspected under ultraviolet light. Two very distinct bands of chlorophyll were evident in the extractions from the plumules of fruits from the light; matching those from leaf extracts. The intense area of each band was removed as it fluoresced under ultraviolet light, and the pigment examined by a Beckman DBG spectrophotometer. The curves of absorption agree with those reported by Wilson (1958), with both chlorophyll a and b being present. However, the chromatographs of the pigments from the plumules maturing in darkness showed no chlorophylls under ultraviolet light. On removing areas identical with those showing the chlorophylls from the lighted fruits, no indication of pigments was recorded by the spectrophotometer. Thus it can be concluded that light is necessary for chlorophyll synthesis in water lotus plumules.

The dark, heavy, mature opaque walls of the dry fruits no doubt must have influenced the erroneous decision, as reported in the literature, that the plumules form chlorophyll in the dark. However, after the pigments are formed in the plumule before the fruit wall has hardened and turned dark, they may persist for years.

LITERATURE CITED

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