Electrophoretic Patterns of Xanthium Leaf Extracts as Affected by Physiological Age of Leaf, Photoperiod, & Age of Plant 1, 2

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It is well known that vegetative plants of Xanthium are induced to flower when a photoperiodically induced scion is grafted onto them. However, attempts to replace this donor scion with an active extract failed (2). These negative results gave rise to an hypothesis that labile macromolecules might be involved in floral induction (2). Since it was not possible to produce an active extract, attempts were made to identify the possible nature of the inducing substance by using various unnatural analoges of natural metabolites (5, 10).

In the present investigation I seek a different approach and am concerned with those macromolecular entities in the leaf that are affected by photoperiod and by the physiological age of the leaf. This approach is supported by the knowledge that in Xanthium the physiological age of leaf plays a significant role in floral induction (6, 12).

It had been found previously, in plants other than Xanthium, that quantitative differences occur in some main protein components isolated from leaves at different stages of development (1, 3, 13) or from leaves exposed to different light intensities (7, 13).

Free-boundary electrophoresis was chosen to resolve leaf extracts into main groupings of macromolecular components. The electrophoretic patterns obtained may serve as a first clue for further investigation along these lines.

Materials & Methods

Cocklebur plants (Xanthium pennsylvanicum Wall.) were sown in March and in June of 1960. One day after germination in sand the seedlings were transferred into pots of rich soil. The plants were grown in a greenhouse with natural daylength of 12 to 14 hours. The dark period was interrupted by five hours of fluorescent light of 200 ft-c. intensity, given around midnight. When 9 days old, 40 plants were subjected to seven inductive cycles consisting of 16 hours darkness and 8 hours daylight; another lot of 40 comparable plants was maintained in the regular long-day conditions of the greenhouse. The extraction of both lots, which will be referred to as reproductive and vegetative, respectively, was performed at the end of the short-day treatment, i.e., when the plants were 16 days old. A third lot of 40 plants was subjected at the age of 35 days to six inductive cycles, as above, and subsequently returned to long-day conditions for 3 more days. The extraction of this lot, and of a comparable vegetative lot, was performed when the plants were 44 days old. The plants of the two sowings were used as replicates in the experiment. When the plants were 7 days old their primary leaves averaged 27 mm in length. By the 16th day, in both reproductive and vegetative plants, primary, first, and second leaves had appeared which were in the fully-expanded, half-expanded, and expanding stages of growth, respectively. The leaves of the reproductive plants were shorter than those of the vegetative plants, on an average by 10 to 20% (6-9 mm). By the 33rd day the plants had attained seven leaves including the expanding one. By the 44th day, both reproductive and vegetative groups attained nine leaves. The leaves of the reproductive plants were again shorter, by 3 to 7% (about 3 mm), than those of the vegetative plants.

For extraction all leaves older than the first fully-expanded leaf were excluded. The remaining leaves were divided, in the order of decreasing age, into the following classes: A, F.E., the first fully-expanded leaf; B, H.E., the half-expanded leaf; C, Exp. the expanding leaf, and D, the Apex, which included any leaf less than 15 mm long. In each replicate experiment a single extraction was performed on all the 40 leaves of each class. The average weights of single leaves for each experiment are given in table I. The extraction followed in principle the procedure of Wildman and Bonner (11). All the operations were carried out at 4°C. The sample of leaf laminae was chilled, cut into small pieces and homogenized with the equivalent of half its weight of cold 0.05 M Na2B4O7-0.05 M Na2SO4 buffer at pH 8.7 [Na2SO4 was added to inhibit browning (8)]. The homogenization was carried out with an Ultra-turrax homogenizer (working on the principle of a colloid mill and produced by Janke & Kunkel, Germany). Each sample was divided into three parts: the first part was homogenized and the homogenate
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Component C: The faster-moving component containing protein; approximate mobility -13.9 u

(about

6.5 mm).

Component D: A non-protein component. approximate mobility -17.9 u

(about

8.5 mm),

which

fluoresces in a light blue color under ultraviolet light.

Component E: A fast-moving non-protein component; approximate mobility -20.2 u

(about

10.0 mm).

On the paper electropherograms all the components except D appeared dark under ultraviolet light and had a brownish color in daylight.

Changes in Macromolecular Components During Leaf Ontogeny, as Revealed by Free-Boundary Electrophoresis. Analysis of extracts of 16 day-old plants revealed the electrophoretic patterns shown in figure 1, which presents the results for the reproductive group. The patterns obtained for the vegetative group were similar, and are not presented. Components D and E decrease rapidly as the leaf grows to its full size. When their concentration reaches low values their final patterns (figs 1 & 2) are not clear. However, the combined concentration of D+E can

Free-Boundary Electrophoresis revealed five peaks (figs 1 & 2), which represent groupings of macromolecules with similar electrophoretic mobilities. These peaks will be referred to as “components” for convenience. As the picture obtained by paper electrophoresis was similar, gross identification of protein and non-protein components on paper electropherograms served as a reference for identifying free-boundary electrophoresis components. Paper electrophoresis could not serve as an accurate tool for studying protein migration, because of protein adsorption on the paper. The characteristics of the five components were as follows:

Component A: a component containing protein close to the origin, extending to about 2.5 mm from the origin.

Component B: The slower-moving component containing protein, mobility approximately -9.8 u

(about

4.5 mm).

Component C: The faster-moving component containing protein; approximate mobility -13.9 u

(about

6.5 mm).

Component D: A non-protein component. approximate mobility -17.9 u

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be determined accurately. In the first fully-expanded leaf component C, major component in the apex leaves, decreases to a minor status and component B rises gradually to attain the status of the major component.

Extracts of 44 day-old plants revealed somewhat different patterns, as shown in (fig 2). Relative concentrations of the different components, as obtained from the two replicate experiments, are presented in table I. Components C, D, and E show the same decrease as before. Component B is very low at the apex, and increases rapidly in the expanding leaf of the reproductive plant but lags in growth in the vegetative plant. As the leaf grows, total yield of macromolecular components extracted per leaf increases, as does the absolute yield of each of the five components (see table I).

Discussion

It is understood that the electrophoretic peaks represented in figures 1 and 2 consist of mixtures of macromolecules having similar mobilities. Changes in the patterns observed indicate, therefore, alterations in the main macromolecular constituents situated at a certain region of electrophoretic mobility. It is not known exactly which specific constituents are affected, but these alterations seem to depend on the physiological age of the leaf and on the photoperiodic treatment. Thus with increasing leaf age component C, as well as components D and E, decrease while component B increases. Photoperiodic treatment affected especially components B and C in the expanding leaves of older plants. Otherwise, the electrophoretic patterns of extracts of similar leaves exposed to long and short day were practically identical.

In the cocklebur the youngest leaves (1-2 cm²), on a normal apical bud, do not respond to photoperiodic induction. The most sensitive leaf is the half-expanded one while the fourth mature leaf does not respond at all (6). On the other hand the youngest expanding leaves are known to be the most efficient in perpetuating the inductive state (12). It is tempting, therefore, to propose the following speculation: For a leaf to be induced by short-day two factors are required and they must be present in the leaf in a proper ratio. The youngest leaves contain mainly (or only) one factor, located in component C, which decreases with leaf age. The youngest leaves do not contain enough (or at all) of the second factor, located in component B. This factor increases with leaf age. The half-expanded leaf contains the two factors in the proper ratio to enable it to respond best to the photoperiodic treatment. However, the short-day treatment causes the expanding leaf to have the most favorable ratio of the two components.

In the expanding leaves of older plants the differences observed due to lengthening of the dark period resemble the differences in protein components, due to shading, reported by Zucker (13) for tobacco leaves. He suggested that the accumulation
Fig. 2. Free-boundary electrophoresis of soluble macromolecular components from leaves of reproductive and vegetative Xanthium plants 44 days old. Rep = reproductive; Veg = vegetative. (For other abbreviation and conditions of electrophoresis, see fig 1).
of the faster-moving protein in the tobacco leaves was due to inhibition in leaf growth. This interpretation does not apply in our case since the expanding leaves of reproductive plants, while having the size of the expanding leaves of vegetative plants (41 mm & 44 mm, respectively) accumulated a considerable amount of component B (fig 2). It seems, therefore, that protein accumulation (or component B in our case) is affected by the photoperiod directly and not through growth.

A flash of light in the middle of a long dark period is known to cancel the inductive effect of the dark period. The difference in the electrophoretic pattern observed in the expanding leaves of older plants due to photoperiodic induction might be merely due to lengthening of the dark period and not the result of the photoperiodic effect in the strict sense. The use of flash of light in the middle of the dark period may help to clarify that point and is at present under further investigation.

Summary

Xanthium plants were exposed to long-day and short-day conditions, and their soluble macromolecular leaf components were subsequently compared by free-boundary electrophoresis. Five components were detected, which showed a definite trend with leaf development from the apex to the fully-expanded stage. These components are not considered to be homogeneous. Photoperiod, while affecting growth rate to a very small extent, affected substantially two components of the expanding leaves of 44-day-old plants, but did not affect plants 16 days old. The results are discussed in relation to floral induction.

Literature Cited


