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# Experimental Induction of a Normal Morphological Phenocopy of an Inflorescence in a Zea. Mays L. Mutant

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# Experimental Induction of a Normal Morphological Phenocopy of an Inflorescence in a Zea. Mays L. Mutant

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*Abstract. FASCICLED EAR (Fa),* a dominant *ZEA MAYS* L. Mutant effecting the form of the inflorescence, was treated with aqueous solutions of α-naphthaleneacetic acid, indoleacetic acid, gibberellic acid, maleic hydrazide and triiodobenzoic acid every three days from the seedling stage until tassel emergence. The mutant responded to α-naphthaleneacetic acid and indoleactic acid in such a manner that certain treated plants developed a normal-appearing ear and tassel. The idea that an ear and tassel shoot meristem is a "plastic" system genetically programmd at specific points in development through a hormonal balance is discussed.

Understanding the development of a multicellular organism is a major challenge to biologists today (Stebbins, 1964). The sequence of morphological changes that occur as a zygote is transformed into a complete multicellular organism appears to be an ordered pattern of events. To understand the processes underlying these morphological changes we must be prepared to investigate the relationships between form, metabolism and hereditary information (Stebbins, 1965).

Apical meristems in plants are generally in a sustained embryonic condition. Morphogenesis continues in an active shoot apex thus providing an excellent experimental system for the study of plant development. A special problem which has attracted a number of investigators is the transformation of a vegetative shoot apex to a reproductive shoot apex. Various experimental techniques may disturb the normal metabolic activities of apical shoot meristems. For example, surgical operations (Snow and Snow, 1947; Wardlaw, 1959, 1963; Ball, 1960, 1963), x-ray treatments (Stein and Steffensen, 1959), tissue culture (Petru and Retovsky, 1957), and application of natural and synthetic growth substances (Phinney, 1956, 1961; Nickerson, 1960; Anderson, 1963), indicate that many cells in an embryonic condition can be deflected from their normal destiny.

Another useful technique has been to substitute a mutant allele for its normal counterpart. Suitability of mutant genes for experimental studies of development was aptly explained by Phinney and West (1960). Experimental alteration of events leading to normal development of *Zea mays L.* inflorescence by gene substitution has lead to the idea that an indispensable relay system of gene action must occur at specific stages in maize inflorescence development (See Postlethwait and Nelson's Figure 1, 1964). A similar hypothesis was also proposed by Heslop-Harrison (1961) on the basis of studies involving inflorescense development of *Zea mays.*

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It seems possible that if a relay system of gene action does underly morphological events in the development of a maize inflorescence it might, in part, function through a hormonal system. The substitution of a mutant gene may conceivably alter the hormonal balance to the extent that a mutant inflorescence would arise. Therefore, sustained treatment with certain growth substances might modify the expression of mutant genes so that treated plants would develop morphologically normal inflorescences.

## Materials and Methods

Mutant (*Fascicled Ear*) *ZEA mays* L. seeds were planted and grown under a 16-hour photoperiod during 1962-1964 in the greenhouse of the Department of Biological Sciences, Purdue University. Treatments of various growth substances (Table 1) were initiated to 13-day-old seedlings and maintained throughout the early development of both the tassel and the ear. Growth substances were applied every three days above the growing point by placing a 0.2 ml. molar solution in the central leaf whorl. Control seedlings were simultaneously treated with 0.2 ml. of an aqueous solution lacking the growth substances. Fifteen tassels per treatment were collected and the data summarized by the Duncan analysis of variance (Steel and Torrie, 1960). Appropriate samples of both tassels and ears were photographed to illustrate effects of the growth substances on the phenotypic expression of the *Fascicled Ear* gene.

**Table 1**

Molar (M) Concentrations of Growth Substances Administered to Individual Plants

| NAA | IAA | GA | MH | TIBA |
|---|---|---|---|---|
| $.5 \times 10^{-4}$M | $10^{-4}$M | $10^{-4}$M | $10^{-4}$M | $10^{-4}$M |
| $.5 \times 10^{-3}$M | $10^{-3}$M | $10^{-3}$M | $10^{-3}$M | $10^{-3}$M |
| $.5 \times 10^{-2}$M | $10^{-2}$M | $10^{-2}$M | $10^{-2}$M | $10^{-2}$M |

## Results

*Fascicled Ear (Fa)*, a branched-ear mutant of maize, was first reported by Weatherwax (1917). Hessler (1963) described the floral development of the ear and tassel. Branches which characterize the mutant ear form (Figure 2-28) apparently arise as a simple alteration during transition of the vegetative meristem to an ear meristem (Hessler, 1963). Elongation of the apex is suppressed and a broadened apex is gradually produced. Two apparently independent meristematic areas are formed and the apex bifercates. The tassel is similarly effected by the *Fa* gene. A tassel with a bifercated central spike develops on the terminal apex (Figure 1-12).

*Tassel.* Bifercation of the central spike was completely suppressed in several mutant plants treated with $\alpha$-naphthaleneacetic acid (NAA), gibberellic acid (GA) and maleic hydrazide (MH) (Table 2) and

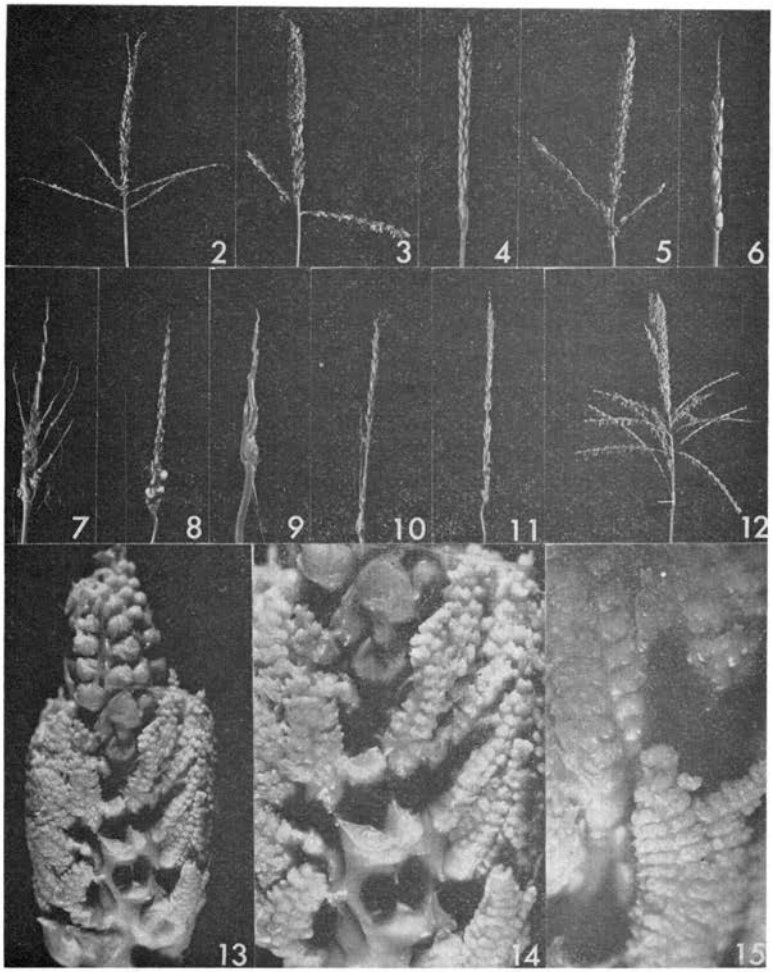


Figure 1. Some effects of certain growth substances on *Fascicled ear (Fa)* tassels and ears.
2- 4 Tassels treated with .5 × $10^{-3}$M NAA.
5- 6 Tassels treated with .5 × $10^{-2}$M NAA.
7- 9 Tassels treated with $10^{-2}$M GA.
10-11 Tassels treated with $10^{-3}$M GA.
12 Tassels treated with $H_2O$ (control).
13-15 Ears showing lateral branching from the floret position that were produced on plants treated with $10^{-2}$M and $10^{-3}$M GA.

(Figure 1). GA at higher concentrations was the most effective. Indoleacetic and 2,3,5-triiodobenzoic acid proved ineffective at suppressing the bifercation of the central spike.

Table 2

Percent of Tassels Without bifercation of Central Spike of the *Fascicled ear (Fa)* Tassel

| Treatment | Concentration (molar)[a] | | |
|---|---|---|---|
| | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ |
| NAA | 25.0 | 6.5 | 0 |
| IAA | 0 | 0 | 0 |
| GA | 50.0 | 50.0 | 25.0 |
| TIBA | 0 | 0 | 0 |
| MH | 13.3 | 13.3 | 13.3 |

[a]Concentrations of α-naphthaleneacetic acid (NAA) are $.5 \times 10^{-2}$M, $.5 \times 10^{-3}$M and $.5 \times 10^{-4}$M. The control group showed 100 percent bifercation of the central spike.

Several other tassel characteristics were observed for their possible modifications by the growth substances (Table 3). Means and analysis of variance are given in Table 3 for (a) numbers of primary branches, (b) length of peduncle, (c) height of tassel, (d) area over which tassels underwent branching, and (e) number of fertile "male" spikelets. NAA ($0.5 \times 10^{-2}$M) and GA ($10^{-2}$M and $10^{-3}$M) significantly reduced the numbers of primary branches compared to the control by 66 percent and 43 percent. The peduncle length was significantly reduced 50 percent with MH ($10^{-3}$M) and 92 percent with NAA ($0.5 \times 10^{-2}$M) compared to the control. Total tassel height was significantly reduced by 52 percent with MH ($10^{-2}$M) by 36 percent with NAA ($0.5 \times 10^{-3}$M) and by 30 percent with GA ($10^{-2}$M). Notable is the significant increase in tassel height with GA ($10^{-4}$M). NAA ($0.5 \times 10^{-2}$M and $0.5 \times 10^{-3}$M) significantly reduced the area over which branches arise by 50-66 percent. An interesting effect was the significant reduction in numbers of fertile staminate spikelets by treatments with GA, MH, and NAA. GA showed a 90 percent reduction in fertile spikelets.

*Ear.* The significant results of two experiments are recorded in Figure 2. The first was carried out with NAA, GA, MH and TIBA. Branching which characterizes the *Fa* ear form was lacking in the topmost ear on those plants receiving treatments of NAA ($0.5 \times 10^{-2}$M, $0.5 \times 10^{-3}$M, and $0.5 \times 10^{-4}$M). However, only 3 plants in the total populations treated with NAA responded in this way. Ears located at the next lowest node were examined and found to be partially reduced in the degree of branching. A second treatment series was conducted using the same molar concentrations of NAA and a second auxin, IAA (Indoleacetic acid) at $10^{-2}$M, $10^{-3}$M and $10^{-4}$M concentrations. Effects of NAA were similar to the first experimental trial. IAA ($10^{-4}$M) was effective in reducing branching of the topmost ear (Figure 2-26). Branching on the next lowest ear was also eliminated with IAA treatments.

 

Table 3

Some Effects of α-Naphthaleneacetic Acid (NAA), Maleic Hydrazide (MH), and Gibberellic Acid (GA) on the Tassel of *Fascicled ear (Fa)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Primary branch number* | | | | | | | | | |
| NAA $.5 \times 10^{-2}$ | GA $10^{-2}$ | GA $10^{-3}$ | NAA $.5 \times 10^{-3}$ | GA $10^{-4}$ | Control | MH $10^{-2}$ | MH $10^{-3}$ | MH $10^{-4}$ | NAA $.5 \times 10^{-4}$ |
| 5.82 a | 8.36 ab | 9.86 ab | 12.47 bc | 15.64 cd | 17.33 cde | 17.60 bcde | 19.69 de | 21.53 e | 21.87 e |
| *Peduncle (centimeter)* | | | | | | | | | |
| NAA $.5 \times 10^{-2}$ | MH $10^{-3}$ | MH $10^{-2}$ | NAA $.5 \times 10^{-3}$ | NAA $.5 \times 10^{-4}$ | GA $10^{-2}$ | MH $10^{-4}$ | GA $10^{-3}$ | Control | GA $10^{-4}$ |
| 1.07 a | 7.48 b | 8.60 bc | 8.67 bc | 10.30 bc | 11.32 bc | 11.47 bc | 13.46 cd | 14.70 cd | 16.86 d |
| *Tassel height (centimeter)* | | | | | | | | | |
| MH $10^{-2}$ | NAA $5. \times 10^{-3}$ | GA $10^{-2}$ | NAA $.5 \times 10^{-2}$ | NAA $.5 \times 10^{-4}$ | MH $10^{-3}$ | MH $10^{-4}$ | GA $10^{-3}$ | Control | GA $10^{-4}$ |
| 9.40 a | 12.20 ab | 13.36 abc | 14.41 abcd | 15.50 bcd | 16.41 bcd | 17.13 cd | 17.21 cd | 19.00 d | 23.21 e |
| *Tassel branch area* | | | | | | | | | |
| NAA $.5 \times 10^{-2}$ | MH $10^{-2}$ | NAA $.5 \times 10^{-3}$ | GA $10^{-2}$ | MH $10^{-3}$ | NAA $.5 \times 10^{-4}$ | GA $10^{-3}$ | MH $10^{-4}$ | Control | GA $10^{-4}$ |
| 3.77 a | 4.90 ab | 6.87 ab | 6.95 ab | 8.16 bc | 9.03 bc | 9.18 bc | 9.53 bc | 11.44 c | 11.50 c |
| *"Male" fertile spikelet number* | | | | | | | | | |
| GA $10^{-3}$ | GA $10^{-2}$ | MH $10^{-2}$ | NAA $.5 \times 10^{-3}$ | NAA $.5 \times 10^{-4}$ | NAA $.5 \times 10^{-2}$ | MH $10^{-4}$ | MH $10^{-3}$ | Control | GA $10^{-4}$ |
| 34.64 a | 52.73 ab | 78.60 abc | 144.53 abc | 178.47 bcd | 179.36 bcd | 191.16 bcd | 275.15 cde | 309.56 de | 333.21 e |

*Within a row, means followed by different letters are significantly different at the 5 percent level by the Duncan (1955) test. Means followed by the same letter are not significantly different.

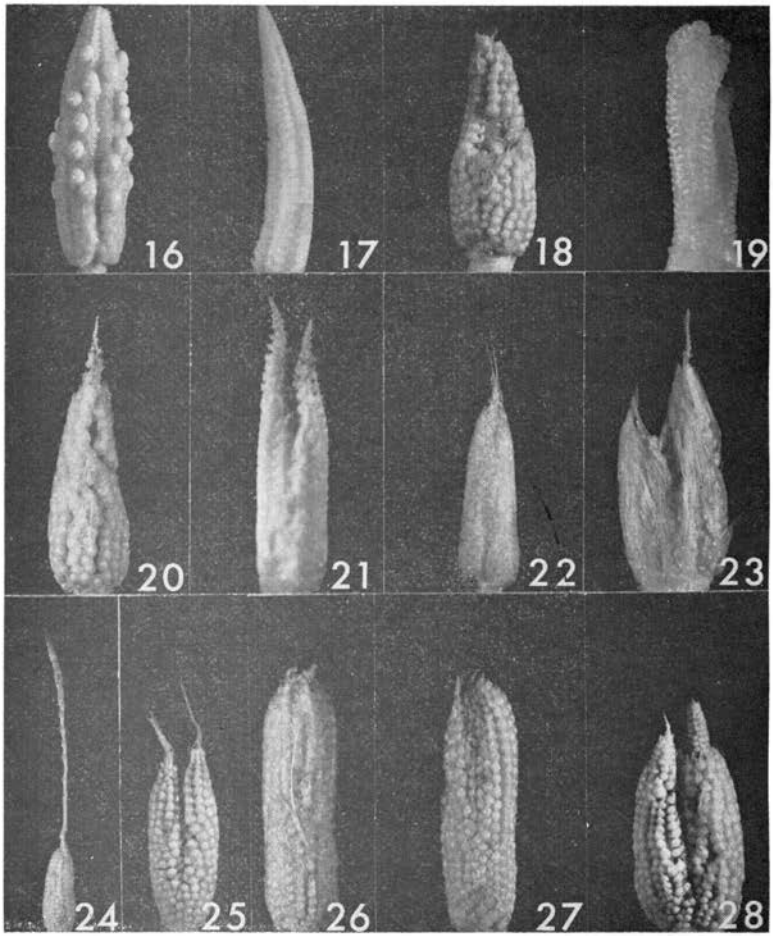


Figure 2. Some effects of certain growth substances on a few *Fascicled ear (Fa)* ears. Odd numbers represent the topmost ear on the plant. Even numbers represent the ear second from the top.
16-17 Ears treated with .5 × $10^{-4}$M NAA.
18-19 Ears treated with .5 × $10^{-3}$M NAA.
20-21 Ears treated with .5 × $10^{-2}$M NAA.
Second treated series with NAA.
22-23 Ears treated with .5 × $10^{-3}$M NAA.
24-25 Ears treated with .5 × $10^{-4}$M NAA.
26-27 Ears treated with $10^{-4}$M IAA.
28 Ear treated with $H_2O$ (control).

An interesting modification of the *Fa* ear occurred with treatments of GA ($10^{-2}$M and $10^{-3}$M). Positions on the ear normally occupied by a floret were replaced with a lateral branch (Figure 1-13, 14, 15) on the lower two-thirds of the ear. These branches displayed the

morphological characteristics one expects to find on the branches of *Fa* ears. In fact, several of these branches in the floret position showed a bifercation at the tip of the branch (Figure 1-15).

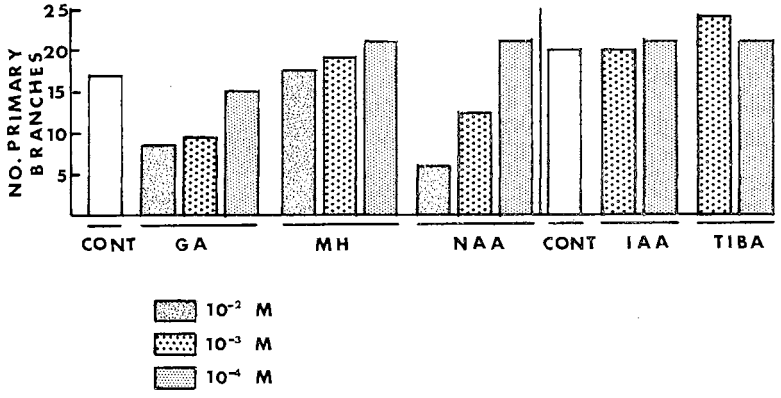


Figure 3. Comparative effects of certain growth substances on the number of primary branches formed on the *Fascicled ear (Fa)* tassel. First treatment series: control ($H_2O$), gibberellic acid (GA), maleic hydrazide (MH), α-naphthaleneacetic acid (NAA), .5 × $10^{-2}$M, .5 × $10^{-3}$M, and .5 × $10^{-4}$M. Second treatment series: ($H_2O$), indoleacetic acid (IAA), and 2,3,5-triiodobenzoic acid (TIBA). Twelve to fifteen plants per treatment. Differences in the number of primary branches formed from treatments with gibberellic acid $10^{-2}$M, gibberellic acid $10^{-3}$M, α-naphthaleneacetic acid .5 × $10^{-2}$M, and α-naphthaleneacetic acid .5 × $10^{-3}$M are significant at the 5 percent level.

## Discussion

The concept of programming and switch points is common in animal embryology and has been applied, recently, to the maize inflorescence by Heslop-Harrison (1961) and Postlethwait and Nelson (1964). Heslop-Harrison's study involved various environmental treatments to normal maize plants and revealed alternatives in the pathway at the shift of the meristem from vegetative to floral and at the shift for determining whether stamens or carpels were to continue development. He suggested that other alternatives should exist and outlined a developmental scheme to indicate where these alternative events may receive a developmental signal.

Postlethwait and Nelson (1964) re-examined the normal development pathway of the maize inflorescence in the context of their switch point concept by a detailed analysis of the ontogenetic events of maize mutants, Polytypic and Ramosa-1. They superimposed the developmental pathway of these mutants over the developmental pathway of the normal. They proposed additional switch points in the developmental scheme of the normal inflorescence where signals must be obtained by the meristem for initiation of morphological events.

Floral morphogenesis in maize may be modified by treatment with certain growth substances (Nickerson, 1959, 1960a, 1960b; Nickerson and Embler, 1960; Heslop-Harrison, 1961; Anderson, 1963). These investigations suggest that the prospective fate of a floral primordium can be altered by various environmental treatments. The question asked in this study of maize floal primordia is whether a genetic block by the *Fascicled Ear (Fa)* gene can be altered by sustained treatment with certain plant growth substances known to modify plant development.

In several cases of this study, it was possible to circumvent the genetic block imposed by the *Fa* gene on the development of the tassel and ear. Treatments with a $\alpha$-naphthaleneacetic acid, gibberellic acid, and maleic hydrazide resulted in the *Fa* tassel becoming normal in phenotype. Similar results were obtained for the ear development by treatment with $\alpha$-naphthaleneacetic and iodoeacetic acid. Apparently the mutant block to normal development has been circumvented. Perhaps a hormonal imbalance is created in the *Fa* shoot apex by the presence of the *Fa* gene which blocks the transition from vegetative to floral development and treatments restored this balance. Perhaps a specific hormonal balance is critical for the normal sequence of floral initiation.

Recently demonstrated is the maize mutant gene *branched-silkless (bd)* effect on the normal sequence of inflorescence development (Anderson, 1963). A genetic block is imposed by the *bd* gene at a point where the spikelet meristem gives rise to two floret meristems. Anderson has partially circumvented the mutant block by sustained treatment with maleic hydrazide and gibberellic acid.

Induction of lateral branches in some of the *Fa* ears with gibberellic acid occurred in the position normally occupied by the floret meristem. Apparently gibberellic acid has blocked the normal switch from a spikelet meristem to a floret meristem in *Fa* ears. Perhaps the normal development of a floret meristem is supported by a balance of hormones. Furthermore, it is possible that branching, itself, is partially regulated by gibberellic acid.

The later idea is supported from studies of several types of normal and mutant maize plants (Nickerson, 1959, 1960a, 1960b). Nickerson proposed that gibberellic acid treatments cause a suppression or simplification of branching in the tassel. Statistical analysis of gibberellic acid effects on the amount of primary branching in the *Fa* tassel do show a significant reduction in branching. However, perhaps a modification of the position taken by Nickerson (1960b), Brian (1957, 1959), and others regarding the relationship of branching and gibberellic acid should be acceptable in view of the lateral branching induced on the *Fa* ear by gibberellic acid treatments.

These experiments and others of a similar kind carry an important implication: That in the normal sequencial development of an inflorescence, a native hormonal metabolism is critical at several of the switch points proposed by Postlethwait and Nelson (1964). Obviously, it must now be demonstrated that gene systems are activated or deactivated at specific times in the developmental pathway through hormonal regulation.

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