In Vitro Formation and Development of Floral Buds on Tobacco Stem Explants

EFFECTS OF KINETIN AND OTHER FACTORS

ABSTRACT

Stem segments were excised from plants of Wisconsin 38 tobacco (Nicotiana tabacum L.) in three regions differing in their distance below the inflorescence. They were cultured in vitro in 8- or 16-hr days. After 8 weeks, floral and vegetative buds were counted, and extent of floral development was assessed. Kinetin at $10^{-3}$ M inhibited formation and development of floral buds regardless of indoleacetic acid concentration. Supplied at this concentration with adequate auxin, kinetin stimulated vegetative bud formation and may have caused floral bud abortion. Indoleacetic acid ($10^{-4}$ M) inhibited vegetative and floral bud formation when supplied with low kinetin concentration ($10^{-3}$ M) but did not affect floral development. When supplied with high kinetin concentration ($10^{-4}$ M), it inhibited floral bud formation and stimulated vegetative bud formation. More floral buds were formed in 16-hr days than in 8-hr days. Few formed on explants other than those derived from the region nearest the inflorescence regardless of other treatment.

Chouard and Aghion (2) first demonstrated the presence of a "floral gradient" in flowering plants of Nicotiana tabacum cv. Wisconsin 38. Stem explants were excised from flowering plants and cultured in vitro. Those excised from near the inflorescence formed more floral buds than those excised at some distance from it. Stem explants from vegetative plants formed only vegetative buds regardless of origin. This floral gradient was believed to be caused by a gradient of endogenous floral stimulus in the stem. This in vitro system seemed to provide an opportunity for studying factors affecting the morphological expression of this stimulus.

Aghion-Prat (1) studied separately the effects of kinetin and IAA and the effects of daylength on floral bud formation. Concentrations above $10^{-3}$ M of either kinetin or IAA inhibited floral bud formation. The IAA inhibition could be partly overcome by kinetin. More floral buds were formed in long days than in short days. Wardell and Skoog (10, 11) also examined the effects of IAA and kinetin on floral bud formation in Wisconsin 38 tobacco stem explants. At a constant kinetin concentration of $7.5 \times 10^{-8}$ M, there was a reversion from floral to vegetative bud formation as IAA concentration increased from $10^{-6}$ to $10^{-4}$ M. On the basis of two experiments in which IAA and kinetin concentrations were varied together so as to maintain constant IAA to kinetin molar ratios of 1.2 and 120 they concluded that kinetin played no determinative role in floral bud formation and only caused a loss of apical dominance in floral shoots developing on the cultured explants.

To study further the effects of these factors on floral bud formation and to examine for interactions with the floral gra-
Mean number of floral buds (□) and of total buds (■) per callus as influenced by kinetin concentration, IAA concentration, daylength, and explant origin.

experiments. The experimental unit was a flask containing three stem explants. Flasks containing the same IAA and kinetin concentrations served as the basis for randomization. Within each hormone treatment group equal numbers of explants of A, B, and C origin were randomly assigned to flasks; within each explant origin subgroup, flasks were randomly assigned to 8- or 16-hr days. A single plant could not provide enough explants for all hormone treatment groups. Therefore, each plant was selected and assigned randomly to two or three such groups. Each experiment was treated to an analysis of variance. Treat-
ment means were compared using the least significant difference (LSD) test (8).

RESULTS AND DISCUSSION

The extent of floral bud formation depends on the origin of explants in the "parent" plant, as illustrated in Figure 1. The decrease in number of floral buds between A and B explants was statistically significant ($P \leq 0.01$). This further illustrates the floral gradient reported by Chouard and Aghion (2), Aghion-Prat (1), and Wardell and Skoog (10). There was a slight decrease in the total number of buds formed, both floral and vegetative, with increasing distance of explant origin from the inflorescence (Fig. 1). Previous workers did not report such an effect on total number of buds. Due to the paucity of floral buds formed on B and C explants, effects of the other treatments on floral bud formation were observed almost exclusively on A explants.

Daylength had a significant effect on floral bud formation by A explants ($P \leq 0.01$); fewer buds were formed in 8-hr than in 16-hr days (Fig. 1). Nutritional differences due to the longer period available for photosynthesis in 16-hr days may have accounted for this effect. Aghion-Prat (1) observed that glucose would in part substitute for the light requirement in floral bud formation.

Kinetin at concentrations below $10^{-5}$ M had no effect on the number of floral buds formed on A explants (Fig. 2). At $10^{-5}$ M kinetin, floral bud formation was inhibited (Figs. 1 and 3). Floral buds appeared sporadically (Fig. 3) and only when a high concentration of IAA (about $10^{-4}$ M) was also present in the medium. In contrast, the number of vegetative buds increased sharply at $10^{-5}$ M kinetin when auxin was in sufficient supply (Figs. 1 and 3). The inhibitory effect of kinetin on floral bud formation is consistent with results obtained by Aghion-Prat (1) and Paulet (7), although they found inhibition occurring at lower concentrations (between $10^{-7}$ and $10^{-3}$ M kinetin). Tran Thanh Van (9) found a similar inhibition of floral bud formation by $10^{-3}$ M kinetin on explants consisting of only epidermal and cortical cells. The reported lack of kinetin effect by Wardell and Skoog (10) may have resulted from their use of constant ratios of IAA to kinetin. In no case did the kinetin concentration exceed the IAA concentration in their experiments.

The inhibition of bud formation by kinetin when supplied with low IAA concentrations (Fig. 1) is probably due to kinetin inhibition of callus growth. On such media callus formation was sporadic and many explants died. Those which survived formed callus from which a few vegetative buds arose. The inhibition of floral bud formation by kinetin supplied with high IAA concentration is of more interest as it was accompanied by a striking stimulation of vegetative bud formation. These effects of kinetin may be due to its stimulation of vegetative growth and a consequent inhibition of floral stimulus expression. This is supported by the findings of Wittwer and Aung (12) showing that kinetin inhibits flowering in whole plants of tomato, another day neutral member of the Solanaceae, while it stimulates vegetative growth. Moreover, Migniac (5) found that flowering by cotyledonary buds of Scrofularia arguta was inhibited by kinetin whereas vegetative growth was stimulated in vitro.

The development of floral buds, as determined at the end of the 8-week culture period, was similarly inhibited by $10^{-4}$ M kinetin (Fig. 3). In most cases this resulted in death of the floral buds after formation of all floral parts. Axillary buds developed on these shoots with aborted floral apices, but remained vegetative. Such aborted floral buds were observed in two of three

![Fig. 2. Stem explants after 8 weeks in culture showing open flowers, flower buds, and vegetative buds. These were a part of the experiment of Figure 1.](www.plantphysiol.org)
experiments (the experiment of Fig. 1 was the exception). They occurred only on media containing $10^{-8}$ M kinetin in combination with IAA at or near $10^{-6}$ M (Fig. 3). Their abortion may have resulted from an interaction of IAA, kinetin, and time in culture. Initially these combinations of kinetin and IAA allowed some floral bud formation. After a few weeks, IAA in the medium probably diminished in concentration as it is known to degrade during this early part of the culture period. Kinetin may then have become toxic to floral buds while remaining innocuous to the numerous vegetative buds.

Although endogenous auxin may be necessary for floral bud formation, an exogenous supply is not always required. Aghion-Prat (1) found that floral buds formed and developed normally in the absence of exogenous IAA, while Wardell and Skoog (10) stated that it was required for normal development of floral buds. In one of our experiments in which few floral buds were formed, none were formed in the absence of exogenous IAA; in another, a large number of floral buds were formed (Fig. 1) and developed normally in its absence. This difference must derive from unknown differences in plants used as sources of explants in the two experiments.

In A explants, IAA had no effect on floral bud formation when supplied at concentrations below $10^{-6}$ M. At $10^{-6}$ M, IAA inhibited floral bud formation (Fig. 1). Similar results in tobacco stem explants were reported by Aghion-Prat (1) and Paulet (7), and in Cichorium intybus stem explants by Margara (4).

Vegetative bud formation was similarly inhibited by $10^{-6}$ M IAA except when accompanied by a high concentration of kinetin. With high kinetin concentration, IAA increased vegetative bud formation as shown in Figure 1 and as observed in one other experiment. Although not examined in these experiments, a transition from floral to vegetative bud formation may occur when even higher concentrations of IAA are accompanied by low kinetin concentration (10).

In conclusion, kinetin at high concentration inhibits floral bud formation irrespective of auxin status. At the same concentration it may strongly promote vegetative bud formation but its effectiveness in this is dependent on auxin status, locus of explant origin, and possibly other factors.

**LITERATURE CITED**


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