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Specific Proteins in Stem Meristems during Transition to Flowering*

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Abstract. Immunodiffusion tests were used for studying protein composition of apical buds of *Rudbeckia bicolor* and *Perilla nankinensis* during their transition from vegetative to reproductive state under inductive photoperiodic conditions or GA_3 treatment. In both species the induced buds differ from the vegetative ones in the presence of specific proteins (P): P1, P2, P3 appear in *Rudbeckia* apical buds 2, 8, 16 d after the start of inductive treatment; P4 appears in *Perilla* apical buds 6 d after inductive treatment. P1, P2, P4 are revealed in induced buds in the early period of apex development when morphogenetic changes are not yet present. The similarity between antigenic spectra of induced buds and of those treated by GA_3 appears only in *Rudbeckia*. These observations support the hypothesis of a change in gene expression at floral evocation.

When studying floral induction, a frequently discussed question is whether the floral transformation of shoot meristems requires a qualitative change in the expression of genetic information or whether it results from an unspecific activation of meristems. To solve this problem, the qualitative changes in the protein composition of meristems were studied during transition to flowering (ZEEVAART 1962, SALISBURY 1963, BONNER 1965, BERNIER *et al.* 1981). The occurrence of proteins specific to apical meristems of long-day plant *Sinapis alba* was found during floral evocation (PIERARD *et al.* 1977, 1980).

The aim of the present work was to look for proteins specific to apical buds of plants of two photoperiodic groups during transition to flowering using the immunological techniques.

MATERIAL AND METHODS

Growing Condition

Plants were grown in the greenhouse for 2 months under non-inductive conditions and then under inductive conditions: 2, 4, 8 or 16 long days (16 h) for *Rudbeckia*, and 4, 8 or 16 short days (8 h) for *Perilla*.

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Application of GA_3

GA_3 (4 mg per plant) was applied on the apex during 4 or 8 d under non-inductive conditions. The treatment was made at the beginning of the daily light period.

Preparation of Extracts

The apical buds (3 mm long) were collected from vegetative 2-month-old plants, as well as from induced plants and from plants treated with GA_3, 12 h after the photoperiodic or hormonal treatment, frozen in liquid nitrogen, lyophilized and stored at −20 °C. Apical buds were homogenized in 0.14 M NaCl (24 h) or 10 mM Tris-HCl buffer (pH 7.5) with 0.5 M sucrose, 5 mM $MgCl_2$, 50 mM KCl and 5 mM β-mercaptoethanol (2 h) at −5 °C. After centrifugation at 4000 *g* for 20 min the precipitate was discarded and the protein concentration of the supernatant extract was estimated by the Lowry's method. In immunodiffusion tests, the protein concentrations of extracts were 5—20 mg ml^{-1}.

Preparation of Antisera

Antisera against protein extracts of vegetative (AV) and induced apical buds (AR) were raised in rabbits by the procedure described previously (Milyaeva *et al.* 1979) with a complete Freund adjuvant. During the entire immunization cycle every rabbit was injected with 130—180 mg of protein. Immunoabsorption was carried out by incubating immunoglobulin fraction from the anti-reproductive buds antiserum (AR IgG) and heterological extract of vegetative buds for 2 h at 37 °C, then overnight at 4 °C followed by centrifugation at 5500 *g* for 40 min.

Antigenic spectra were compared by means of the double immunodiffusion agar plate and carried out according to the method described by Zilber and Abelev (1962).

RESULTS AND DISCUSSION

The antigenic spectra of the vegetative and induced apical buds of *Rudbeckia* and *Perilla* were determined by an immunodiffusion test using both homologous and heterologous antisera before and after immunoabsorption.

Table 1

Appearance of specific antigenic proteins in apical buds of *Rudbeckia bicolor* during photoperiodic induction

Induction [number of long days]	Antigens			Stage of apex development
	P1	P2	P3	
0	−	−	−	Vegetative
2	+	−	−	Floral evocation
4	+	−	−	
8	+	+	−	
16	+	+	+	Reproductive

TABLE 2
Appearance of specific protein in stem buds of *Perilla nankinensis* during photoperiodic induction

Induction [number of short days]	Antigen P4	Stage of apex development
0	−	Vegetative
6	+	Floral evocation
12	+	
16	+	Reproductive

The comparison of antigenic spectra of the vegetative and induced buds of *Rudbeckia* revealed proteins common to the buds of both types and proteins specific to the induced plants as well (MILYAEVA *et al.* 1979). The results concerning the detection of specific proteins in *Rudbeckia* buds during transition from the vegetative to reproductive state using AR IgG after immunoabsorption by extract of vegetative buds are summarized in Table 1.

Application of GA_3 to vegetative buds of *Rudbeckia* resulted in flowering. At the same time a similarity of antigenic proteins of induced buds to those treated by GA_3, using AV and AR before immunoabsorption is shown.

As in the case of *Rudbeckia*, in vegetative and induced buds of *Perilla* both common and specific proteins were recorded (KOVALEVA *et al.* 1981). When using AR IgG after absorption by vegetative antigens, only one specific protein (P 4) is assessed in *Perilla* induced buds that is absent in the vegetative ones. It is synthesized as early as 6 d after the start of induction, which period is insufficient to induce flowering (Table 2).

Application of GA_3 to *Perilla* vegetative buds did not initiate flowering and change the antigen spectra.

Thus, in the apical buds of *Rudbeckia* and *Perilla* specific proteins have been recognized during transition to flowering under photoperiodic induction. The synthesis of P1, P2 and P4 begins in the early period of apex development before morphogenetic changes are observed. These data support the hypothesis of a change in gene expression at floral evocation.

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