Plant Physiol. (1988) 88, 424–428
0032-0889/88/88/0424/05/$01.00/0

Localization of the Site of Perception of Thermoinductive Temperatures in *Thlaspi arvense* L.

Received for publication February 3, 1988 and in revised form April 25, 1988

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ABSTRACT

This paper describes attempts to localize the site of perception of low temperatures (0–10°C) during thermoinduction in *Thlaspi arvense* L. Reproductive development (stem elongation and flower formation) was observed when shoots were cooled to 4°C for 4 weeks and then returned to 21°C while maintaining the roots constant 21°C. However, chilling the roots was ineffective for initiating reproductive development. The apparent site of perception of thermoinductive temperatures was further localized to the shoot tip (apex and immature leaves) by controlling the temperature of the shoot tip independently of the rest of the plant. Furthermore, excised apices regenerated flowering plants in organ culture only if they were subjected to a 4 week cold treatment. Grafting experiments also support the notion that the shoot tip or the apex is the site of perception of thermoinductive temperatures: noninduced shoot tips grafted onto bolting donors remained as vegetative rosettes. Paradoxically, it was found that the cells of the shoot tip are not the only ones capable of being thermoinduced. Shoots regenerated from leaf cuttings excised from thermoinduced plants exhibited all signs of reproductive development, while regenerated shoots from control leaves developed into vegetative rosettes. It is suggested that many cell types are capable of being thermoinduced and that the shoot tip may appear to be the site of perception of thermoinductive temperatures because structures associated with reproductive development originate from this tissue.

Field pennycress (*Thlaspi arvense* L.) is a winter annual weed that infests cultivated fields in the Northern Great Plains of the United States and Prairie Provinces of Canada (8). This species requires a period of low temperatures (0–10°C) before the transition from vegetative to reproductive development can take place (8).

We have been interested in the process(es) by which low temperatures induce the initiation of reproductive development in field pennycress. However, reproductive development is complex, consisting of several separate, but highly integrated, processes that are initiated by a common mechanism (8). In order to simplify experimental analysis of the processes occurring during thermoinduction, work in this laboratory has focused on one specific aspect of reproductive development, namely bolting (rapid stem elongation in plants that grow as rosettes during the vegetative phase of their life cycle). Previously, we showed that thermoinduced stem elongation in field pennycress is mediated by the endogenous GAs[1] (7). Furthermore, we have identified 13 GAs native to this species as a prelude to studies on the effects of thermoinduction on the regulation of GA metabolism (10).

Obviously, knowledge of the locus for perception of thermoinductive temperatures is important in formulating a logical experimental approach in such studies. It is generally agreed that in other cold requiring species, the signal transduction chain begins at the shoot tip and/or apical meristem (1, 6, 19). Evidence presented in this paper indicates that this view is valid in field pennycress as well, although tissues other than the shoot tip can also be thermoinduced. It was concluded that the shoot tip appears to be the site of perception of thermoinductive temperatures because the visible signs of reproductive development, *i.e.* flower formation and stem elongation arise from the shoot apex.

MATERIALS AND METHODS

Localized Temperature Treatments. In preliminary experiments, the effects of subjecting the roots and shoots to different temperatures was examined. Seeds of an inbred line of field pennycress (*Thlaspi arvense* L.) (CR_1) were germinated as described before (7). One-week-old seedlings were transferred to 1 L jars and grown hydroponically in ¼-strength Hoagland solution under SD conditions (7) at 21°C. The nutrient solution was constantly aerated and was replaced weekly with fresh medium. After six weeks, selective cold treatments to either the roots or the shoots were initiated. Root systems were cold-treated by immersing the jars into a constant temperature bath at 4°C while maintaining the air temperature at 21°C. Conversely, shoots were selectively chilled in a growth chamber at 4°C while the jars containing the roots were set in a water bath at 21°C.

The cold treatments lasted for 4 weeks, whereupon the plants were transferred to a growth chamber at 21°C and LD. Stem height was measured 4 weeks later. Each treatment had five replicates and the experiment was repeated twice with similar results.

Separate temperatures were maintained for the shoot tip (apical meristem and immature leaves less than 5 mm long) and the rest of the plant with a coil of ⅛-inch o.d. copper tubing surrounding the shoot tip and crown through which polyethylene glycol solutions of different temperatures were pumped (Fig. 1). One-week-old seedlings were supported in the coil with fine vermiculite and the entire apparatus placed on a 1 L jar with the roots suspended in ¼-strength Hoagland solution. The plants were grown at 21°C with SD conditions. The temperature treatments were initiated 6 weeks later and lasted an additional 4 weeks during which time the surface temperature of the apex was determined daily with a thermocouple. At the end of the treatment period, the plants were transferred to a growth chamber at 21°C with LD conditions. Stem length was measured 4 weeks later. Each treatment had 5 replicates and the experiment was repeated twice with similar results.

Apex Culture. Field pennycress seeds were surface sterilized

[1] Abbreviations: GA(s), gibberellin(s); SD, short day; LD, long day; LDP, long day plant.

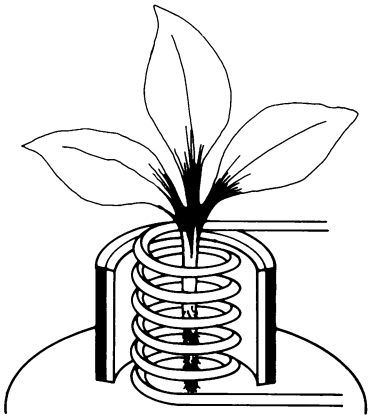

FIG. 1. Schematic drawing of the device used to selectively maintain the shoot tip at temperatures different from the rest of the plant.

by treatment for 20 min with a commercial bleach solution that had been diluted 1:5 with H_2O. The seeds were then washed five times with sterile H_2O and germinated on sterile agar (0.7%, w/v). After 1 week, apices with no more than two leaf primordia present were excised and placed on an agar (0.7%, w/v) growth medium containing mineral salts (11), sucrose (30 g L^{-1}), inositol (100 mg L^{-1}), IAA (1 mg L^{-1}), kinetin (1 mg L^{-1}), and thiamine (0.4 mg L^{-1}). Four apices were cultured on 50 ml of medium in glass jars. The cultured apices were placed in a growth chamber at 21°C or 4°C with SD conditions. After 4 weeks, all apices were transferred to a growth chamber at 21°C and LD conditions. The apices were observed for signs of reproductive development (internode elongation and flower bud formation) after 4 weeks.

Grafting Experiments. Two sets of grafting experiments were performed. In the first, transmission of a flower-inducing stimulus from an induced ("donor") to a noninduced ("receptor") field pennycress graft partner was examined while the graft partners in the second set consisted of the LDP *Sinapis alba* L. and field pennycress.

Plants of field pennycress were grown in 10-cm plastic pots containing vermiculite that was kept continuously moist by subirrigation with ¼-strength Hoagland's solution. These plants were maintained under SD conditions for 6 weeks until further use. Induced stocks were obtained by first subjecting plants to a 4 week cold treatment, followed by 2 weeks at 21°C and LD conditions as described before (7). Noninduced stocks were obtained by treating apices with 10 μg of GA_3 on alternate days for a total of three treatments. Stem elongation ceased 2 weeks after the last GA treatment resulting in the formation of an aerial rosette. No visible flower buds were observed. Shoot tips excised from field pennycress plants that had been subjected to a 4 week thermoinductive cold treatment or maintained at 21°C were used as induced and noninduced scions, respectively.

Sinapis seeds were germinated on moist filter paper in petri dishes under SD conditions and the seedlings transferred to 17-cm plastic pots with vermiculite. These plants were maintained at 21°C under SD conditions for 4 weeks and then used as stocks.

The stocks used for grafting were decapitated 1 to 2 cm below the apex, and shoot tips, cut wedge-shaped, were inserted into a cleft cut into the top of the donor stems. The scion was anchored in the cleft by tying the donor with wet raffia and then covered with a polyethylene bag lined with wet filter paper for 2 weeks following grafting. The grafted plants were placed in a growth chamber at 21°C and LD, except for the graft combination of a noninduced *Sinapis* stock and a noninduced field pennycress scion. In this instance the plants were maintained under SD conditions. The receptors were examined for signs of reproductive development (stem elongation and flower formation) 6 weeks after grafting (Fig. 2).

Twenty grafts were attempted in each experiment and roughly 75% of the graft unions were successful. Each experiment was repeated twice.

Shoot Regeneration from Leaf Cuttings. Fully expanded leaves were excised from either non-induced plants or plants that had been thermoinduced. The leaves from the thermoinduced plants were fully expanded prior to the cold treatment. One to 2 cm of the petiole was cut off to ensure no meristematic tissue from axillary buds remained on the cutting. The petiole of the cutting was then inserted into vermiculite moistened with ¼-strength Hoagland solution so that only the blade was exposed. The tray containing the leaves was covered with clear polyethylene and placed in a growth chamber at 21°C. The polyethylene cover was removed after 3 weeks when roots were established. Each treatment contained 50 leaves and the experiment was repeated twice.

RESULTS

Reproductive development was initiated only when the shoots were exposed to thermoinductive temperatures; maintaining the roots at 4°C while the shoots were at 21°C for 4 weeks caused the plants to remain as vegetative rosettes (Table I). However, stems of plants in which only the shoots received the cold treatment were consistently shorter than if the entire plant was maintained at 4°C. This effect was not due to warming of the shoot by water moving from the roots via the transpiration stream since the temperature of the shoot tip never exceeded 7°C as measured by a thermocouple. This temperature is within the optimal range for thermoinduction in field pennycress (7). Furthermore, flower buds appeared about the same time in both treatments indicating that maintaining the roots at 21°C during the cold treatment did not affect processes that occur during thermoinduction.

When the shoot tips were selectively cooled to *ca.* 4°C for 4 weeks with a small cooling coil, normal thermoinduced stem

Table I. *Effect of Selectively Cooling Roots or Shoots on Subsequent Thermoinduced Stem Elongation*[a]

Temperature		Stem Length[b]
Shoot	Root	
°C		*mm*
21	21	0 ± 3[c]
4	21	298 ± 17
21	4	0 ± 0
4	4	434 ± 24

[a] Temperature treatments lasted 4 weeks. [b] Stem length measured 4 weeks after the end of the thermoinductive cold treatment. [c] Standard deviation.

Table II. *Effect of Various Temperature Treatments Selectively Applied to the Shoot Tips on Subsequent Thermoinduced Stem Elongation*[a]

Temperature		Stem Length[b]
Shoot tip	Rest of plant	
°C		*mm*
21	21	0 ± 0[c]
4	21	420 ± 18
21	4	0 ± 0
4	4	398 ± 16

[a] Temperature treatments lasted 4 weeks. [b] Stem length measured 4 weeks after the end of selective temperature treatment. [c] Standard deviation.

Table III. *Response of Field Pennycress Receptors after Grafting onto Donors of Either Field Pennycress or S. alba*[a]

Donor	Receptor	Mean Growth of Receptor	Flowering Quotient[b]
		mm	
Induced field pennycress stock	Noninduced field pennycress scion	0	0/15
Noninduced field pennycress stock	Noninduced field pennycress scion	0	0/16
Induced field pennycress scion	Noninduced field pennycress stock	0	0/18
Induced *Sinapis* stock	Noninduced field pennycress scion	37	12/12
Noninduced *Sinapis* stock	Noninduced field pennycress scion	0	0/11

[a] Receptors assessed for signs of reproductive development 6 weeks after grafting. [b] Number of receptor plants with flower buds/number of grafted plants.

FIG. 2. A noninduced field pennycress scion remained as a vegetative rosette when grafted onto an induced stock. Photograph taken 6 weeks after grafting.

FIG. 3. Reproductive development (stem elongation and flower formation) was initiated in a noninduced field pennycress scion when grafted onto an induced *Sinapis* stock. Arrow shows position of graft union. Photograph taken 6 weeks after grafting.

growth ensued after the entire plant was returned to 21°C (Table II). Conversely, when shoot tips were maintained at 21°C while the leaves and roots were at 4°C the plants remained vegetative (Table II). This suggests that the site of perception of thermoinductive temperatures resides in the shoot tip.

The result of grafting experiments, summarized in Table III, were also consistent with this notion. Noninduced shoot tips grafted onto an induced stock remained as vegetative rosettes (Figure 2; Table III). Moreover, no signs of reproductive development were observed in axillary buds of noninduced stocks when grafted with an induced shoot tip, although the scion continued normal reproductive development and eventually produced viable seed (Table III). These results indicate that translocation of a graft transmissible stimulus from the leaves or other parts of the plant to the shoot apex is not required for the initiation of reproductive development in field pennycress.

In photoperiodically sensitive plants, the leaf is the site of perception of day length, and there is ample physiological evidence that a phloem mobile signal, usually termed the floral stimulus, moves from the leaf to the apex where it causes the transition to reproductive development (1, 6, 19). Furthermore, grafting experiments indicate that the floral stimulus is very similar in different response types (6, 19). Although no evidence was obtained to indicate the existence of a similar stimulus in field pennycress, this species is nevertheless sensitive to the floral

FIG. 4. Effect of a thermoinductive cold treatment on development of excised shoot apices in culture. The apex from the plant on the left was subjected to a cold treatment at 4°C immediately following excision, while the control was maintained at 21°C. After 4 weeks the cold-treated apex was transferred to 21°C. Photograph taken 8 weeks after excision.

stimulus from *S. alba* L. which is a LDP (2). Noninduced field pennycress shoot tips were grafted onto donor stocks of *Sinapis* and the graft combinations were either transferred to LD or maintained under SD. Only under LD (*i.e.* inductive conditions for *Sinapis*) did the field pennycress graft partner exhibit signs of reproductive development (Figure 3; Table III). Thus, the grafting procedure itself was probably not the basis for the failure of noninduced field pennycress receptors to initiate reproductive development when grafted onto induced field pennycress donors.

In total the data presented in Table II and III indicate that the shoots and/or leaves of field pennycress do not produce a floral stimulus in response to thermoinductive temperatures. On the contrary, thermoinductive temperatures are apparently perceived by the shoot tip. Organ cultures of apices were used to more precisely determine which tissues of the shoot tip are sensitive. Normal reproductive development occurred in excised apices that were subjected to a 4 week cold treatment, while apices maintained at 21°C developed into vegetative rosettes (Fig. 4) although roots and new leaves were readily produced following excision when apices were cultured at 21°C, development of these organs was not observed in apices during the 4 week cold treatment. These results suggest that it is the apex *per se* that perceives thermoinductive temperatures.

It was surprising, then, to find that the cells of the apex are not the only ones capable of being thermoinduced. Shoots were regenerated from cuttings of mature leaves excised from non-induced plants or plants that had received a 4 week thermoinductive cold treatment. In recent studies on the role of GAs in petiole growth, cell division was not observed in field pennycress petioles at the stage of development when excised (9) (JD Metzger KE Dusbabek, unpublished data). Callus development at the cut surface of the petiole was usually observed about 1 week after excision. Roots and shoots typically developed from the callus after an additional 2 and 4 weeks, respectively. Although the vast majority of the cuttings formed roots, shoot development was observed in only about 10 to 15% of the cuttings. Nevertheless, shoots that regenerated from thermoinduced leaves exhibited all

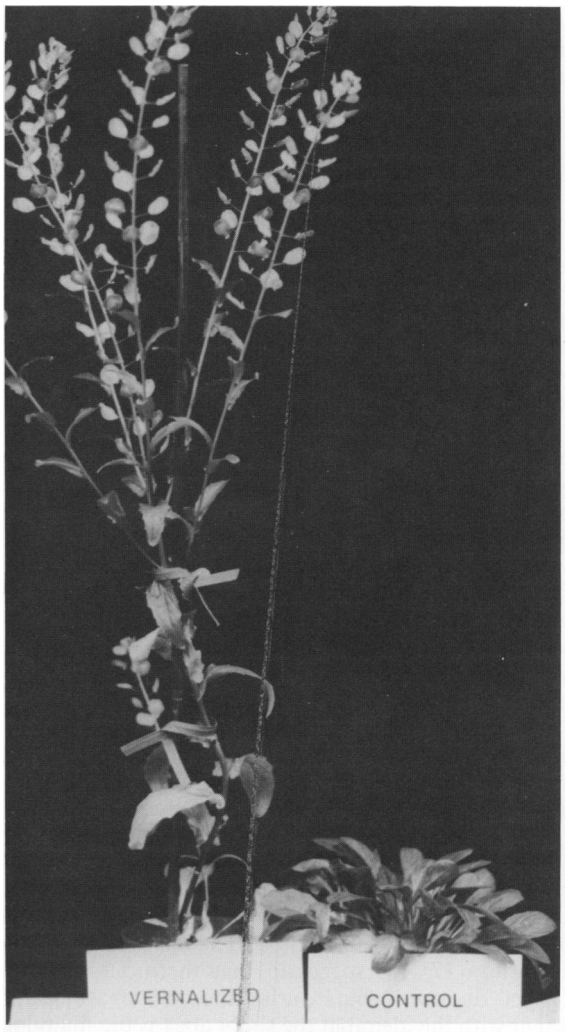

FIG. 5. Effect of a thermoinductive cold treatment on subsequent development of shoots regenerated from excised leaves. Leaves were excised from plants that had received a 4 week thermoinductive cold treatment at 4°C ("vernalized") or had been maintained at 21°C ("control"). The leaves were rooted in moist vermiculite and allowed to regenerate shoots at 21°C. Photograph taken 10 weeks after excision.

signs of normal reproductive development, while shoots regenerated from non-induced leaves always developed into vegetative rosettes (Fig. 5).

DISCUSSION

The data presented in Tables II and III as well as Figure 3 provide evidence that one locus of temperature perception in field pennycress resides in the tissues of the shoot tip, especially the apical meristem. Similar results have led others to conclude that the apex is the *only* site of perception in other cold requiring species (3, 4, 14, 15). Nevertheless, the fact that leaf cuttings from thermoinduced field pennycress regenerate into flowering plants (Fig. 5) clearly demonstrate the shoot apex is not the sole tissue capable of responding to low temperatures. Similarly, leaf cuttings from *Lunaria annua* (honesty) plants that had overwintered naturally were also observed to develop into plants exhibiting signs of normal reproductive development (5, 16). Successful *in vitro* thermoinduction of isolated leaves and even pieces of petioles from vegetative *Lunaria* plants has also been reported (12, 17). In addition, isolated root tissue from the cold-requiring plant *Cichorium intybus* (chicory) subjected to low

temperatures regenerated flowering shoots after the end of the cold treatment (13). However, in none of these examples of *in vitro* thermoinduction was it ascertained whether the meristems that ultimately gave rise to flowering shoots regenerated during or after the cold treatment. Thus, it is uncertain if in fact these tissues were thermoinduced, or if regenerated meristems were really the loci for cold perception. This problem was avoided in the present study by excising leaves for shoot regeneration only after the whole plant received the cold treatment. The possibility of meristematic tissue from axillary buds being present on the cutting was eliminated by cutting off the bottom 1 to 2 cm of the petiole. Thus the flowering shoots regenerated from thermoinduced field pennycress leaves must have ultimately been derived from non-meristematic tissues.

It is not known what role, if any, thermoinduction of tissues other than the shoot apex has in the whole plant. One possibility is that it promotes the production of a floral stimulus in some remote tissue or organ which is then translocated to the apex causing the transition to reproductive development in a fashion analogous to photoperiodically sensitive plants (1, 6, 19). However, grafting experiments in field pennycress did not indicate the existence of a floral stimulus (Table III). Nor did selective cooling of either the roots or leaves result in the transition to reproductive development (Tables I and II). Thermoinduction of tissues other than the shoot apex may be important for the integration of reproductive development over the whole plant. This may explain why maintaining roots at 21°C during thermoinduction reduced subsequent stem elongation (Table I).

It is also difficult to explain why noninduced field pennycress receptors are sensitive to the floral stimulus of another species even though evidence for an analogous signal in field pennycress is lacking (Table III). It is possible there is a floral stimulus in field pennycress that is identical or very similar to the floral stimulus of *Sinapis*, but that it is produced at or very close to the apex and is never translocated to any appreciable extent.

Wellensiek (17, 18) suggested that thermoinduction results only if cell division occurs during the cold treatment. This is consistent with the idea that the shoot apex is the main locus for the perception of thermoinductive temperatures (1, 6, 19). However, active cell division in the leaves of field pennycress is not apparently required for thermoinduction of petiole cells since flowering shoots were regenerated from excised leaves that were fully grown before the cold treatment (Fig. 5). Alternatively, it could be that many cell types can be thermoinduced, and that the thermoinduced state is perpetuated through mitosis. Organization of dividing thermoinduced cells into shoot meristems would then result in the development of shoots programmed for reproduction. Thus, the shoot apex as the apparent site for perception of thermoinductive temperatures could merely be the result of the fact that it is the apex that normally gives rise to structures associated with reproductive development.

Acknowledgments—I am indebted to Mrs. Prudence Olson for her assistance in the apex culture work. I would also like to thank Eric Worcester for the art work in Figure 1, Tom Hlavaty for the photography, and Dr. G. Bernier for the generous supply of *Sinapis* seed used in this study.

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