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Polyamine metabolism, floral initiation and floral development in chrysanthemum (Chrysanthemum morifolium Ramat.)

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ABSTRACT

MARTIN-TANGUY, J., M. ARIBAUD, T. GASPAR, C. PENEL & H. GREPPIN (1996). Polyamine metabolism, floral initiation and floral development in chrysanthemum (Chrysanthemum morifolium Ramat.). *Saussurea* 27: 67-81. In English, English and French abstracts.

In the short-day plant chrysanthemum (*Chrysanthemum morifolium* Ramat. variety Pavo) putrescine and spermidine conjugates appeared in the apical bud before the first observable transformation of the meristem into floral structures. These compounds accumulated on floral initiation and well before floral evocation. Spermidine conjugates were predominant during floral initiation whereas free amines did not accumulate to any significant extent. Different associations of amides were observed during floral initiation as compared with the reproductive phase. 3,4-Dimethoxyphenylethylamine conjugates (water-insoluble compounds) were the predominant amine conjugates observed during flower development. These compounds decreased drastically after fertilization. In vegetative buds from plants grown in long days polyamine conjugates were very low and appeared as plants aged. Under short days, the activity of ODC was much higher than that of ADC at all stages of development, reaching maximal activity before floral evocation. ADC activity was not found during floral initiation. ADC and ODC activities remained low in shoot tips cultivated under long days, with ODC detected only as plants aged. We present evidence that ODC regulates putrescine biosynthesis during floral initiation and floral development. When ODC action was blocked by DFMO, a specific, irreversible inhibitor of ODC, flowering was inhibited, and free and conjugated polyamines were not detected. This treatment led to a slight enhancement of ADC activity. When putrescine was added, polyamine titers and flowering were restored. A similar treatment with DFMA, a specific, irreversible inhibitor of ADC did not affect flowering and the polyamine titers. The results suggest that ODC and polyamine conjugates are involved in regulating floral initiation in *Chrysanthemum*. DAO activity was found only under short-day conditions. During floral initiation and floral evocation DAO followed the same pattern as ODC. DAO could be involved in the regulation of putrescine concentrations and/or transport at the subcellular level, depending on the physiological stage of floral development. This result might suggest that putrescine catabolism is needed for floral initiation and/or floral development. Under short-day treatment PAO and TGase levels were low, no change was observed. In contrast in vegetative buds from plants grown in long days PAO and transglutaminase activities appeared exponential throughout the whole period of development.

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RÉSUMÉ

MARTIN-TANGUY, J., M. ARIBAUD, T. GASPAR, C. PENEL & H. GREPPIN (1996) Métabolisme des polyamines, initiation et développement floral chez le chrysanthème (Chrysanthemum morifolium Ramat.). *Saussurea* 27: 67-81. En anglais, résumés anglais et français.

Chez le chrysanthème (*Chrysanthemum morifolium* Ramat. var. Pavo), plante de jour court, des conjugués de la putrescine et de la spermidine apparaissent dans le bourgeon apical avant la première observation de la transformation du méristème en structures florales. Ces composés s'accumulent pendant l'initiation florale bien avant l'évocation. Les conjugués de la spermidine sont prédominants pendant l'initiation florale tandis que les amines libres ne s'accumulent pas de façon significative. Pendant l'initiation florale on observe des associations d'amides différentes de celles de la phase de reproduction. Ce sont les conjugués de la 3,4-dimethoxyphenylethylamine (composés insolubles dans l'eau) qui prédominent pendant le développement floral. Ces composés diminuent fortement après la fécondation. Dans les bourgeons végétatifs de plantes cultivées en jours longs, la concentration des conjugués polyaminés est très faible et augmente quand la plante vieillit. En jours courts l'activité de l'ODC est beaucoup plus élevée que celle de l'ADC à tous les stades de développement, atteignant un maximum d'activité avant l'évocation florale. On ne trouve pas d'activité ADC pendant l'initiation florale. Les activités ADC et ODC restent faibles dans les extrémités des pousses cultivées en jours longs, l'ODC n'étant détectée que lorsque les plantes sont agées. Nos résultats démontrent que l'ODC régule la biosynthèse de la putrescine pendant l'initiation et le développement floral. Quand l'action de l'ODC est bloquée par la DFMO (inhibiteur spécifique, irréversible de l'ODC), la floraison est inhibée et les polyamines libres et conjuguées ne sont pas détectées. Ce traitement conduit à une légère augmentation de l'activité ADC. Quand on ajoute de la putrescine, le taux de polyamine est restauré ainsi que la floraison. Un traitement semblable avec la DFMA, un inhibiteur spécifique, irréversible de l'ADC, n'affecte ni la floraison, ni le taux de polyamines. Les résultats suggèrent que l'ODC et les conjugués polyaminés sont impliqués dans la régulation de l'initiation florale du chrysanthème. On ne trouve d'activité DAO que dans les conditions de jours courts. Pendant l'initiation et l'évocation florale(s) la DAO suit le même modèle que l'ODC. La DAO pourrait être impliquée dans la régulation des concentrations de putrescine et/ou son transport au niveau subcellulaire, selon l'état physiologique du développement floral. Ce résultat suggèrerait que le catabolisme de la putrescine est nécessaire à l'initiation et/ou au développement floral. En jours courts les niveaux de PAO et TGase sont bas et on n'observe pas de changement. Au contraire dans les bourgeons végétatifs de plantes en jours longs les activités de la PAO et de la TGase augmentent de façon exponentielle tout au long de la période de développement.

Abbreviations : ADC = arginine decarboxylase ; DAO = diamineoxidase ; ODC = ornithine decarboxylase ; DFMA = α-DL-difluoromethylarginine ; DFMO = α-DL-difluoromethylornithine ; PAO = polyamine oxidase ; TGase = transglutaminase.

Introduction

Polyamines (PAs) are now generally recognized as necessary for the orderly patterns of growth and development in plants, animals and microorganisms (BACHRACH & HEIMER, 1988; GALSTON & SMITH, 1985; TABOR & TABOR, 1984). Recent studies with higher plants have also implicated PAs in such varied processes as response to stress (ORMROD & BECKERSON, 1986), senescence (GALSTON & KAUR-SAWHNEY, 1987), regulation of cell cycle (BAGNI, 1988), embryogenesis in tissue culture (FEIRER & al., 1984) and floral initiation (MALMBERG, 1983; PERDRIZET & PREVOST, 1981).

Amine conjugates (polyamine and aromatic amine conjugates), covalently bound to hydroxycinnamic acids have also been found in high levels in plants (BENDECK DE CANTU & KANDELER, 1989; KAUR-SAWHNEY & al., 1988; MARTIN-TANGUY, 1985) and are thought to be correlated with developmental phenomena. These compounds do not normally occur in leaves or other vegetative shoot tissues of plants. They accumulate in shoot apices upon floral initiation (see review (MARTIN-TANGUY, 1985)). The amides appear before the first observable transformation of the meristem into a floral structure. When the plants flower, the amides are found in abundance in the inflorescence, mainly in the sex organs of the flower, but practically disappear from the leaves (see review (MARTIN-TANGUY, 1985)). These compounds are absent from sterile reproductive organs, and they appear to constitute biochemical markers for pollen and ovule fertility (see review (MARTIN-TANGUY, 1985)). The concentration of the amides in flowers decreases quickly and drastically following fertilization, while free amines do not accumulate to any significant extent at any time (see review (MARTIN-TANGUY, 1985)).

It appears that in tobacco plants free polyamines derived through ADC may be involved in vegetative development (BURTIN & al., 1991), while conjugated polyamines derived through ODC may be required for floral initiation and sexual differentiation. The irreversible suicide inhibitors DFMA (KALLIO & al., 1981) and DFMO (METCLAF & al., 1987) specifically inhibit plant ADC and ODC activities. They are not metabolized in plants.

In this context, attention has been mostly drawn to the regulation of polyamine levels and biosynthetic enzyme activities during particular physiological events or in response to external stimuli (RASTOGI & DAVIES, 1991). In contrast, polyamine catabolism has received little attention and, only recently, the biochemical characterization and the physiological modulations of enzymes involved in these processes have been studied in detail. Far from being only a means of eliminating cellular polyamines, the enzymes involved in polyamine catabolism, and the products derivating from their action, have been demonstrated to be involved in important physiological research, opening up an exciting new area of biochemical and physiological research (FREDERICO & ANGELINO, 1991; HAUSMAN & al., 1995; MALINSKI & al., 1965; PERIN & al., 1985; RINALDI & al., 1986; SERAFINI-FRACASSINI, 1991). Enzymes oxidizing diamines occur sporadically throughout the plant kingdom but are particularly active in the *Leguminosae* (SMITH, 1980). They all contain copper (Cu-amine oxidases) and mainly oxidize diamines (putrescine, cadaverine). Sometimes they also oxidize spermidine but their specificity varies widely (SMITH, 1980, 1985). The oxidation of diamines leads to corresponding aldehyde, hydrogen peroxide and ammonium ions. In the case of putrescine oxidation the resulting γ-aminobutyraldehyde spontaneously cyclises to form Δ^1-pyrroline and γ-aminobutyric acid (FLORES & FILNER, 1985).

Polyamine oxidases oxidize spermidine and spermine. The oxidation of spermidine leads to hydrogen peroxide, diaminopropane and γ-aminobutyraldehyde. The resulting aldehyde cyclises to form Δ^1-pyrroline and γ-aminobutyric acid (FLORES & FILNER, 1985).

Polyamines, owing to their chemical nature, can form hydrogen, ionic, or covalent linkage with other molecules. In some cases, polyamines linked to particular proteins have been isolated (BALESTRERI & al., 1987; ROCH & al., 1983; RUSSELL, 1981). In animals, post-translation covalent linkages of polyamines to numerous proteins have been demonstrated. They are solely catalyzed by transglutaminases (TGases) (R-glutaminyl-peptide: amide-γ-glutamyl-transferase) and form cross-linked and noncross-lin-

ked complexes with two or one peptide-bound glutaminyl residues respectively (reviewed in FOLK, 1980). TGases have been investigated extensively in animals where they are widely distributed in various tissues, organs, and extracellular fluids (FOLK, 1980; DELCROS & al., 1984). A number of biological functions involving protein cross-linking have been proposed for extracellular TGases in animal systems including formation of the fibrin clot as a terminal step in the bloodclotting cascade (LORAND & CONRAD, 1984), coagulation of seminal plasma in rodents (WILLIAM-ASHMAN, 1984), ionophore induced hardening of the erythrocyte membrane (LORAND & al., 1976) and participation in receptor-mediated endocytosis (LAEMMLI, 1970). In only a few cases specific physiological protein substrates for TGases have been identified and the function of the intracellular form of TGase is unknown. Covalently δ bound polyamine-protein complexes have been reported in tubers of *Helianthus tuberosus* (DINNELLA & al., 1992; GRANDHI & al., 1992; SERAFINI-FRACASSINI & al., 1988) in thin-layer tobacco tissues culture (APPELBAUM & al., 1988), in oat protoplasts (MIZRAHI & al., 1989), in young tobacco internodes, leaves and ovaries, especially in meristematic areas (SAWHNEY & APPELWHITE, 1992). Recently presumptive evidence was reported for the occurrence of intracellular TGases in photosynthetic (MARGOSIAK & al., 1990), emergent sprout (SERAFINI-FRACASSINI & al., 1988), and etiolated plant tissues (ICEKSON & APPELBAUM, 1987). Although the function of this enzyme in plants has not been established, the apparent induction of its enzymatic activity at wounded sites of excised or bruised leaves suggest a potential role in wound repair (MARGOSIAK & al., 1987). Alternatively, the discovery of polyamines linked to cell wall polysaccharides or membranous fractions indicates prospects for a function in formation and anchoring of the plant cell wall polysaccharide net to the plasma membrane, or in coupling membrane proteins to cytoplasmic structural protein (SERAFINI-FRACASSINI & MOSSETTI, 1986). Recent papers report results on identification of a major *in vivo* substrate protein for TGase in the soluble fraction of alfalfa *(Medicago sativa)* meristematic floral tissue. The substrate protein which has been identified is Rubisco, the catalyst for primary carbon dioxide fixation *via* the reductive pentose phosphate cycle. Moreover, evidence is presented which shows that only the L, and not the S, serves as a substrate for TGase (MARGOSIAK & al., 1990).

One possible mechanism through which polyamines might regulate cell division and related growth processes involves their binding to specific regulatory proteins.

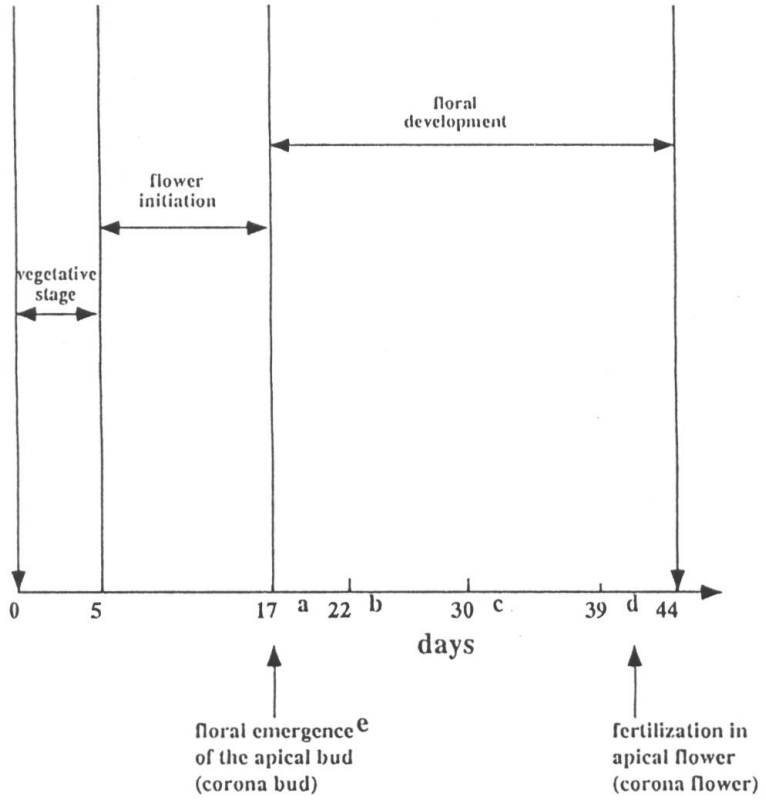
The function of polyamines in cell division and morphogenetic processes in plant systems has been studied using two kinds of experiments: (1) seeking correlations between polyamine levels, activities of their biosynthetic enzymes, the enzymes involved in polyamine catabolism and the enzymes involved in post-translation covalent linkages of polyamines to proteins on cell division or morphogenetic processes and (2) studying the effects of inhibitors of polyamine biosynthesis, with and without exogenous polyamines, on the morphogenetic process. We are presently using this approach in chrysanthemum focusing on two morphogenetic processes in which cell division is involved: floral initiation and floral development. Chrysanthemum plants grown under 8 h light/16 h dark periods will flower, while they remain vegetative under a 16 h light/8 h dark regime in our controlled condition growth rooms.

Material and methods

Cuttings from chrysanthemum plants (*Chrysanthemum morifolium* Ramat. var. Pavo) cultivated under long days, were planted in clay pot containing peat and gravel

and fed with a nutrient solution. They were cultivated under controlled conditions; with either a 10 h or 16 h light period (300 µmol $m^{-2}S^{-1}$ PPFD from Philipps TLF 110 fluorescent tubes and 40 W incandescent lamps), 80% RH and 20°C. When grown in short days (10 h light periods) complete development was observed and two chronological reference points were identified. The first occurred at 5 days of culture. 3-day old cuttings were grown under short days (10 h light periods), and then placed under long days (16 h light periods). These plants, never initiated flowers, but 5 day (or older) cuttings cultivated under short days and then placed under long days flowered. Thus, the long day inhibitory effect on flowering does not exist in the latter case. The other reference point is situated at 17 days and corresponds to emergence of the floral apical bud. The different stages of development are presented in Figure 1.

The first day of treatment was designated as day 0. During the first week of treatment, inhibitors were applied to the soil of pot-grown plants three times per week in a volume of 20 ml. Controls were treated with deionized water. During the following weeks treatment was reduced of twice per week. On days of no treatment each plant received 20 ml of nutrient solution (ARIBAUD & MARTIN-TANGUY, 1994).



a apical bud (length = 6 ± 2 mm, diameter = 5 ± 2 mm

b apical bud (length = 15 ± 3 mm, diameter = 8 ± 2 mm

c apical bud (length = 22 ± 3 mm, diameter = 14 ± 3mm

d apical flower before fecondation

e meiosis takes place during floral emergence in the corona bud

Fig. 1. – Developmental stages of chrysanthemum plants under short days. From ARIBAUD & MARTIN-TANGUY, 1994.

Amine contents and the activities of the related enzymes were quantified in shoot tips of chrysanthemum cultivated under short and long days. A sample of the apex including the first 1 to 1.5 cm fragments of the foliar stem before floral evocation is termed the shoot tip. Under short days measurements were made at different stages of floral development. In the present study we investigated changes in amines and related enzymes only in apical buds (corona buds) and flowers (corona flowers).

Results

Free amine titers

The main free amines detected in *Chrysanthemum* var. Pavo were putrescine (Put), spermidine (Spd), spermine (Spm), phenylethylamine (Phe), tyramine (Tyr) and 3,4-dimethoxyphenylethylamine (3,4-Phe). Amine conjugates were of two types: water-soluble, having a primary amine function (polyamine conjugates) and water-insoluble,

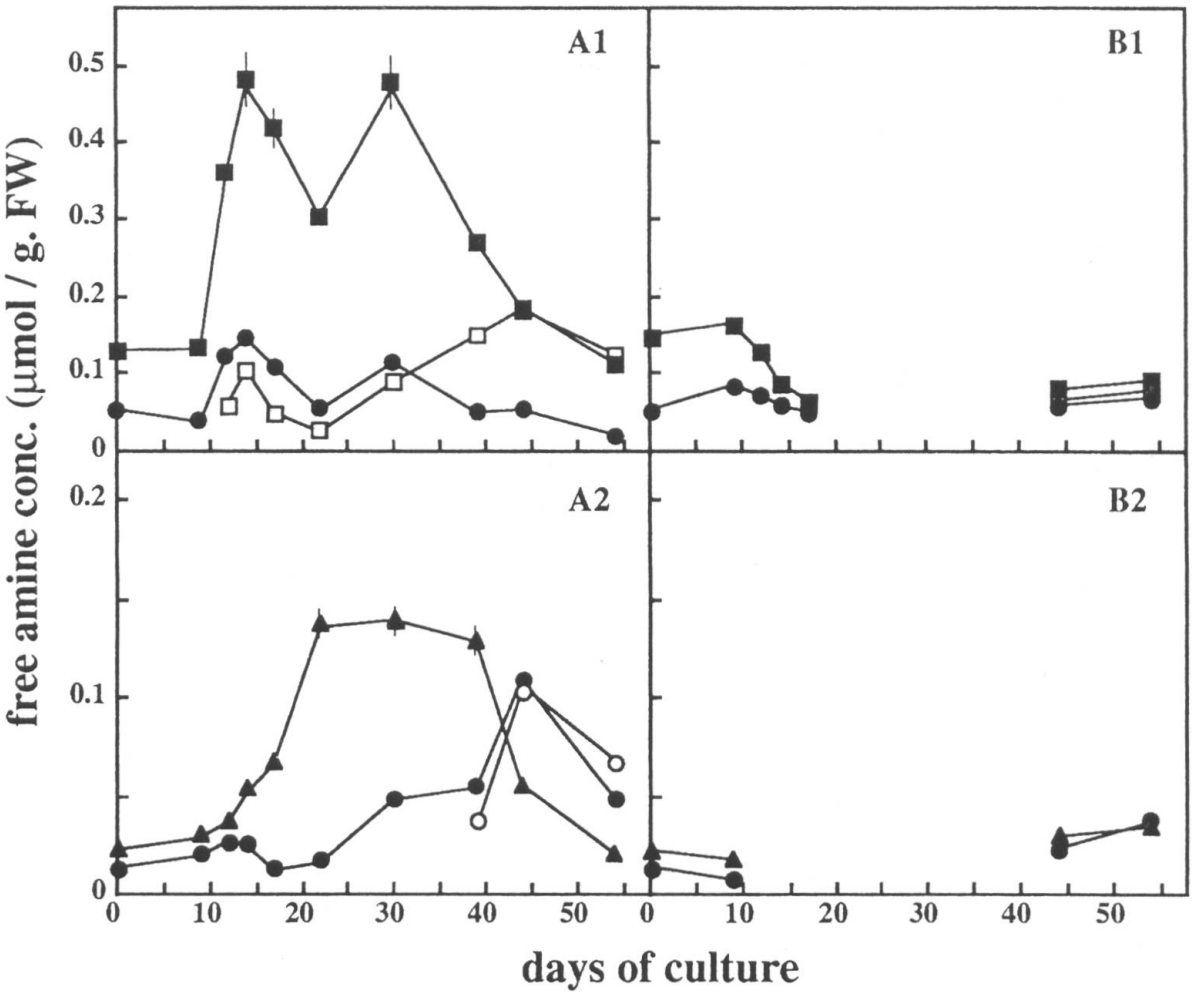


Fig. 2. – Free polyamine and aromatic amine levels in the shoot tips of chrysanthemum plants grown under short (A1, A2) and long (B1, B2) days and during flower development under short days (A1, A2). A1, B1: free polyamines; Put □, Spd ■, Spm ●; A2, B2: free aromatic amines; Phe ○, Tyr ▲, 3,4-Phe ●.Values expressed as µmol/g fresh weight. Means ± SD of 3 or 4 replicates each representing 10 to 20 plants. From ARIBAUD & MARTIN-TANGUY, 1994.

showing no function that can be ionized (aromatic amine conjugates). These compounds contain hydroxycinnamic acids (p-coumaric acid and caffeic acid) and amines linked by an amide bound. Until day 9 of culture the content and distribution of free amines (i.e., polyamines, spermidine and spermine and the aromatic amines, tyramine and 3,4-dimethoxyphenylethylamine) in shoot tips of plants cultivated under short days followed a similar pattern to that observed in shoot tips of plants cultivated under long days (Fig. 2). Under both conditions the levels were similar and remained low. However, under long days a marked decrease in polyamine and aromatic amine levels was observed until day 40; amine titers then increased slightly during the later stages of culture. Under short-day conditions considerable variation was observed in the concentrations of amines during floral induction and at different stages in flower development. Putrescine, spermidine, spermine changed more or less in parallel during the 50 days of culture (Fig. 2), showing an initial increase and reaching a peak on day 14 (before the visible appearance of the flower bud). After and until day 22 a decrease was observed, followed by an increase at day 30 for spermidine and spermine and at day 44 for

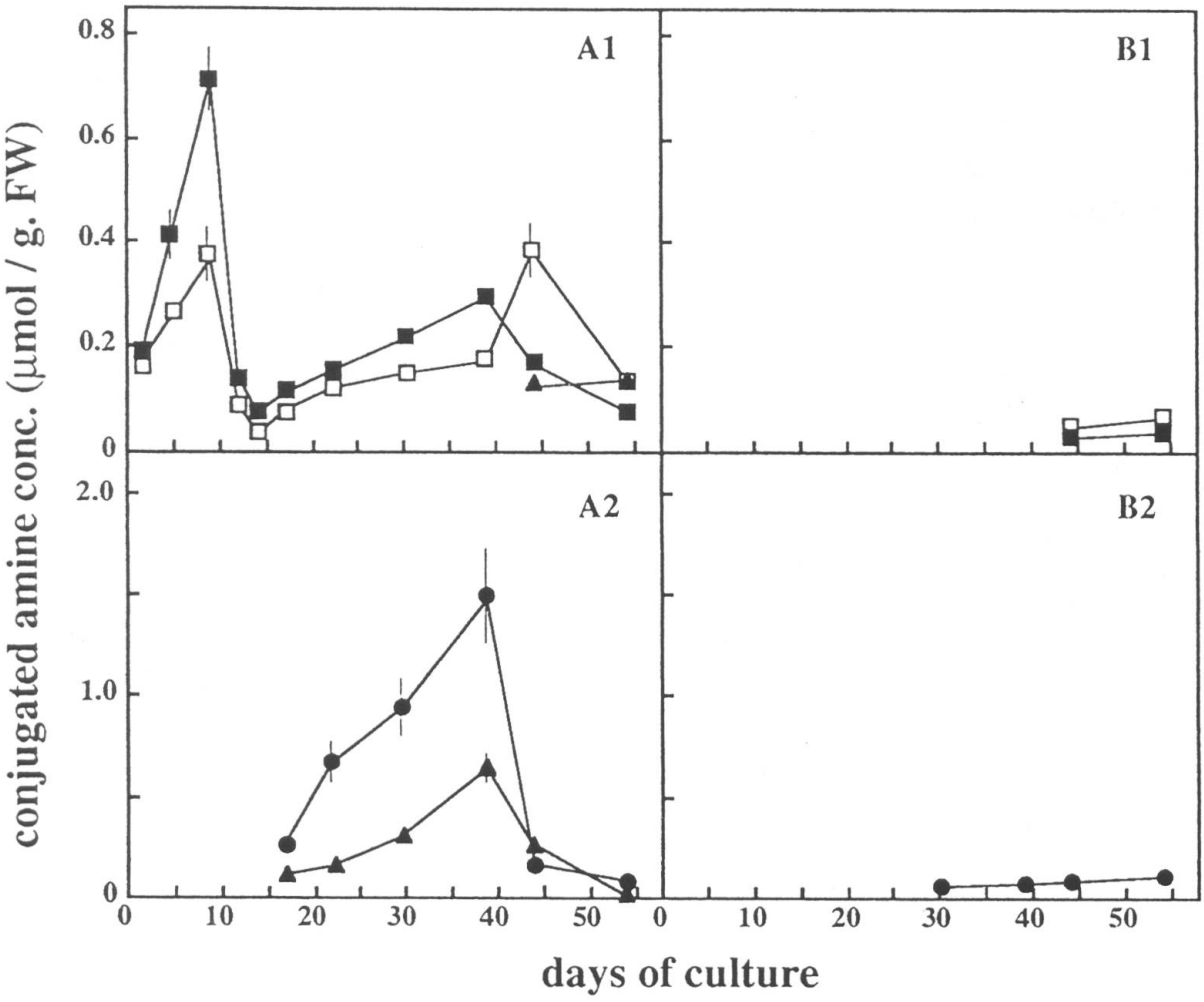


Fig. 3. – Conjugated polyamine and aromatic amine levels in the shoot tips of chrysanthemum plants grown under short (A1, A2) and long (B1, B2) days and during flower development under short days (A1, A2). A1, B1: conjugated polyamines ; Put □, Spd ■, Dap ▲; A2, B2: conjugated aromatic amines; Tyr ▲, 3,4-Phe ●. Values expressed as µmol/g fresh weight. Means ± SD of 3 or 4 replicates each representing 10 to 20 plants. From ARIBAUD & MARTIN-TANGUY, 1994.

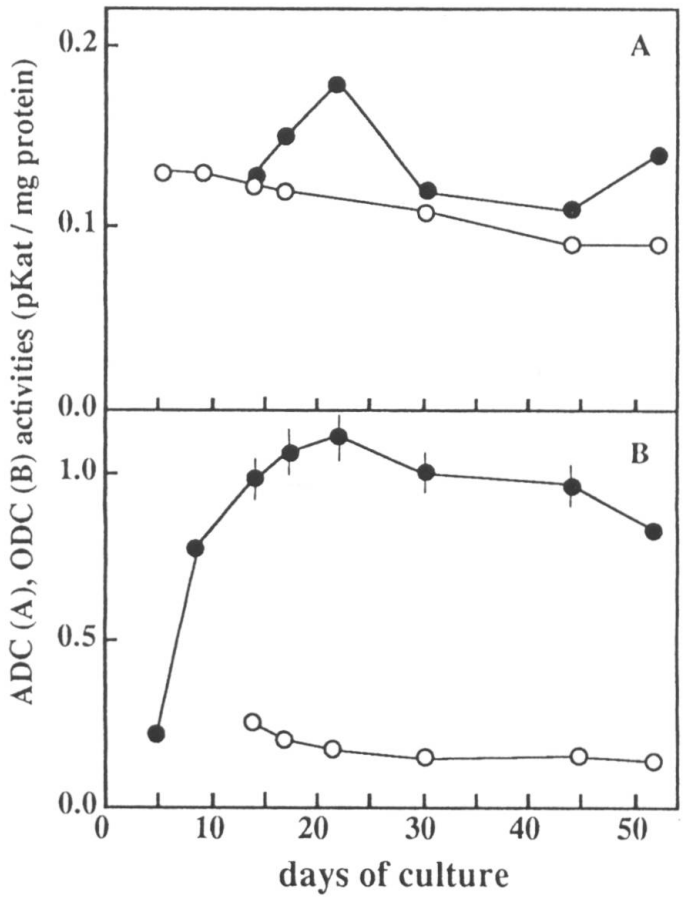


Fig. 4. – Changes in ADC (A) and ODC (B) activities (Pkat/mg protein) in the shoot tips of chrysanthemum plants grown under short (●) and long (○) days. Means ± SD of 3 or 4 replicates each representing 10 to 20 plants. From ARIBAUD & MARTIN-TANGUY, 1994.

putrescine. After day 30 a decrease of all substances was observed during the later stages of floral development. During the whole period of development, spermidine was the predominant polyamine, representing 50 to 60% of the total polyamine pool at day 14. During floral development tyramine accumulated substantially, reaching a maximum at day 22 and decreasing markedly thereafter. Phenylethylamine and 3,4-dimethoxyphenylethylamine behaved similarly, reaching a maximum at day 44, during the later stages of floral development. The levels of tyramine were higher than those of phenylethylamine and 3,4-dimethoxyphenylethylamine up to day 39.

Conjugated amine titers

Amine conjugates were not present at day 0 (Fig. 3). Under short days putrescine and spermidine conjugates increased rapidly in the shoot tips and reached a peak at day 9 before floral emergence of the shoot tip (Fig. 3). A decrease was then observed from days 9 to 14, followed by a slight increase. Peaks were observed at days 39 and 44 for spermidine and putrescine, respectively, followed by a decrease in the content of both compounds. Diaminopropane conjugates appeared at day 39, then increased up to day 44. Spermidine conjugates constituted 50% of the polyamine conjugate pool at day 9. Under long days putrescine and spermidine conjugates appeared at day 44, but remained at low levels during the later stages of culture (Fig. 3). Aromatic conjugates (tyra-

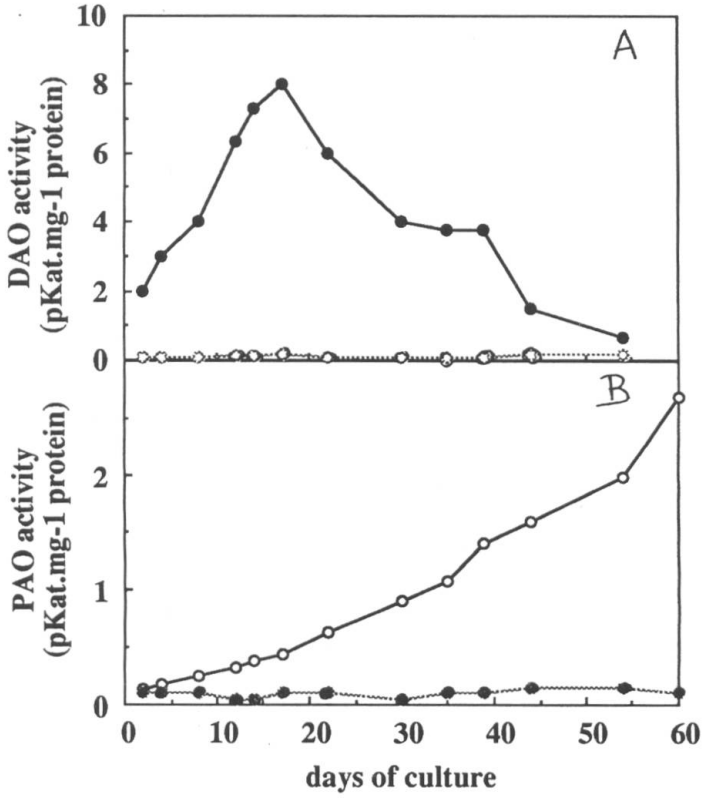


Fig. 5. – Changes in DAO (A) and PAO (B) activities (Pkat/mg protein) in the shoot tips of chrysanthemum plants grown under short (●) and long (○) days. Means ± SD of 3 or 4 replicates each representing 10 to 20 plants.

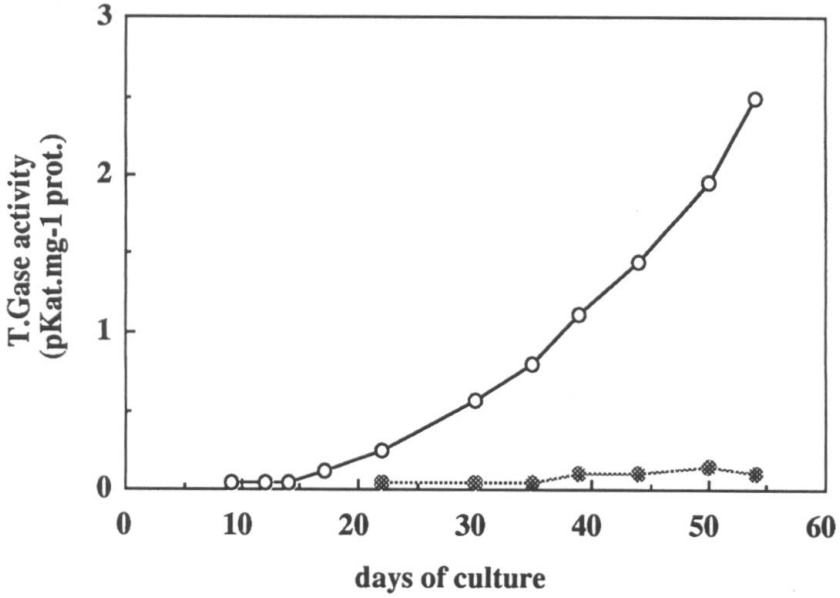


Figure 6. – Changes in TGase like activities (Pkat/mg protein) in the shoot tips of chrysanthemum plants grown under short (●) and long (○) days. Means ± SD of 3 or 4 replicates each representing 10 to 20 plants.

mine, 3,4-dimethoxyphenylethylamine) were not found in the shoot tips during floral initiation (Fig. 3) but increased gradually after day 16 to reach a maximum at day 39, and decreased thereafter. 3,4-Dimethoxyphenylethylamine conjugates were the predominant amine conjugates observed during the flower development, accounting for about 50% of the total by day 39. In plants cultivated under long day conditions, very low concentrations of 3,4-dimethoxyphenylamine appeared at day 39, and remained low as the plants aged (Fig. 3). Free aromatic amines and aromatic amine conjugates were located in male organs while conjugated polyamines were found in female organs. Amine conjugates appear to constitute biochemical markers for pollen and ovule fertility.

ADC and ODC activities

Under short days, the activity of ODC was much higher than that of ADC at all stages of development, reaching maximal activity between days 14 and 22. At day 14 ODC activity was approximately 7 times higher than that of ADC (Fig. 4). ADC activity was not found during floral initiation (Fig. 4). ADC and ODC activities remained low in shoot tips cultivated under long days (Fig. 4), with ODC detected only after 14 days of culture.

DAO and PAO activities

In plants cultivated under short days a substantial activation of DAO was observed with the maximum level at day 18 (Fig. 5). DAO activities remained low in shoot tips cultivated under long days (Fig. 5). Under short-day conditions PAO activities were low (Fig. 5). No change was observed. In contrast PAO activities appeared exponential in shoot tips cultivated under long days throughout the whole period of development (Fig. 5).

TGase activities

Under short-day conditions during floral induction and at different stages in flower development Tgase activities were low and only small changes were observed (Fig. 6). Shoot tips cultivated under long days exhibited an exponential activity of TGases.

Effects of polyamine biosynthesis inhibitors on development, polyamine titers, and related enzymes in Chrysanthemum cultivated under short-days

Treatment with DFMO at 2 mM inhibited flowering under a short day regime (Table 1), and produced plants having short internodes, wrinkled leaves and developed axillary buds (data not shown) (Table 1). Simultaneous treatment with DFMO plus putrescine at 2 mM did not produce these phenotypic changes (data not shown). Flowering was restored.

Free polyamines (spermidine and spermine) and conjugated polyamines (spermidine and spermine conjugates) were not detected after treatment with DFMO (Table 2), but agmatine accumulated in the shoot tips (Table 2). A combination of DFMO + putrescine increased free polyamines (spermidine and spermine) and polyamine conjugates (putrescine and spermidine); spermine conjugates also appeared. Free and conjugated polyamine titers were not lowered by DFMA at 2 mM.

No activation of ODC was detected after treatment with DFMO but ADC actively was promoted (0.30 pKat/mg protein) after 9 days of culture. No activation of DAO, PAO or TGase was observed after treatment with DFMO (data not shown). A combination of DFMO + putrescine promoted a transitory activation of DAO activities. In these treated plants DAO activities seemed to follow the same pattern as DAO in untreated plants.

Table 1. – Effects of DFMO and DFMA on growth and flower formation of chrysanthemum, when applied together with short day conditions from day 0.

Treatment	Emergence of floral buds (days)	Final height (cm)
–	17±2	35±4
2 mM DFMO	b	17±2
2 mM DFMA	17±2	35±5
2 mM DFMO	13+2	37+5
+ 2 mM putrescine		

Table 2. – Effects of DFMO and DFMA on free and conjugated polyamine titers in the shoot tips of chrysanthemum after 9 d culture under short days. Results are expressed un µmol g-1 fresh weight, as the means ± SD of 3 to 4 replicates, each representing 3 to 5 plants.

	Controls	+ 2 mM DFMO	+ 2mM DFMO + 2 mM put.	+ 2 mM DFMA
FREE:				
AGM.	nd	0.80±0.04	nd	nd
Put.	nd	nd	nd	nd
Spd.	0.18±0.04	nd	0.25±0.04	0.20±0.04
Spm.	0.06±0.04	nd	0.20±0.04	0.10±0.04
CONJUGATED				
Put.	0.42±0.04	nd	0.40±0.04	0.45±0.04
Spd.	0.85±0.04	nd	1.20±0.04	0.80±0.04
Spm.	nd	nd	0.60±0.04	nd

nd: not detected

Discussion

The relation between amines and flowering in a higher plant can be best studied in a strictly photoperiodically determinate plant. In the short-day plant, chrysanthemum, we observed that amides accumulated on floral initiation and well before floral evocation, i.e., before the first observable transformation of the meristem into a floral structure. Of these water-soluble polyamine conjugates, spermidine conjugates were predominant during floral initiation whereas no significant free amines accumulated during this period. Different associations of amides were observed during floral initiation as compared to fully reproductive tissues, where polyamine and aromatic amine conjugates were found. 3,4-Dimethoxyphenylethylamine conjugates (water-insoluble compounds) were the predominant amine conjugates observed during flower development, but these decreased rapidly after fertilization. Considerable changes in free amines were observed during flower development. In plants cultivated under long days amine conjugates appeared at very low levels during the last stages of culture.

Under short days, the activity of ODC was much higher than that of ADC at all stages of development. ADC activity was not found during floral initiation. ADC and ODC activities remained low in short tips cultivated under long days. Under short-day conditions DAO seemed to follow the same pattern as ODC. This indicates a direct correlation between biosynthesis and oxidation of putrescine which occur simultaneously in physiological stages of intense metabolism such as cell division and floral organ formation. DAO could be involved in the regulation of putrescine concentrations and/or

transport at the subcellular level, depending on the physiological stage of floral development. This result might suggest that putrescine catabolism is needed for floral initiation and/or floral development. In this light, we believe that the study of polyamine catabolism merits renewed interest and further experimental effort. It appears that PAO and TGase are active only in vegetative development.

The results presented indicate that ODC might regulate polyamine biosynthesis during the floral initiation and flower differentiation. When DFMO inhibited ODC, flowering was suppressed but a similar treatment with DFMA did not affect flowering. Simultaneous treatment with DFMO plus putrescine led to reversal of the effects of DFMO alone. The floral inhibition induced by DFMO was correlated with the expected changes in free and conjugated polyamines. Polyamine titers were lowered by treatment with DFMO but not by DFMA ; this effect of DFMO was reversed by putrescine. DFMO treatment led to a slight enhancement of ADC activity (accumulation of agmatine). These results suggest that ODC and polyamine conjugates are involved in regulating floral initiation in chrysanthemum. Considerable evidence now indicates that both ADC and ODC are active in plant tissues and that their relative contributions to putrescine and polyamine biosynthesis are dependent upon the type of tissue and the developmental process (GALSTON & FLORES, 1991). It thus appears that in several plants free polyamines derived through ADC may be involved in juvenile development, while polyamine conjugates derived through ODC may be required for floral initiation and sexual differentiation.

Recently we report that in the short-day plant, strawberry (*Fragaria ananassa* Duch.) ODC, free polyamines and conjugates may be involved in regulating floral induction and floral development (TARENGHI & MARTIN-TANGUY, 1995). We found that free polyamines, conjugated spermidine and bound amines (polyamines, aromatic amines) accumulated in the shoot tips of plants upon floral induction and well before the appearance of the first floral buds. Free and conjugated polyamines predominated in the last initiated leaves. Bound amines were located in apices. Different associations of free amines and conjugates were observed during floral development as compared to floral induction.

In *Xanthium strumarium,* a short-day plant, exposure of leaves to successive inductive nights resulted in a rise in polyamine conjugates, especially of spermine (GALSTON & al., 1990), per unit protein nitrogen. Spermine conjugates rose sharply after one inductive long night, remained high during the second cycle, then declined rapidly during the third and fourth inductive cycles. Spermidine and to a lesser extent, putrescine, followed the same trend. About eight days after the initial buds appeared, which also correlated well with the behaviour expected of a floral stimulus. The relationship between polyamine metabolism and flowering needs further investigation and analysis of vascular sap could provide some relevant information. It was proposed that during the early events of flowering in *Xanthium strumarium,* and *Sinapis alba* , a movement of polyamines occurred from young, expanding leaves to the buds and developing inflorescence (HAVELANGE & al., 1996). Such a mechanism would be consistent with the data obtained from studies on tobacco (MARTIN-TANGUY, 1985; CABANNE & al., 1981), chrysanthemum, strawberry and recent studies on polyamine translocability (BAGNI, 1988) and follows the general rules for florigen transport (VINCE-PRUE, 1975). But the fact that polyamine metabolism appears to be quite similar in both rooting and flowering induction (GASPAR & al., 1996) raises the question of its specificity in different developmental pathways.

If polyamines are causally involved in any type of morphogenesis it may be possible in the near future to alter development by manipulating the endogenous levels of polyamines in transgenic plants using tissue-specific or inducible promoters.

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