Phytochemistry, Vol. 20, No. 9, pp. 2131–2134, 1981.
Printed in Great Britain.

0031-9422/81/092131-04 $02.00/0
Pergamon Press Ltd.

ALIPHATIC AND AROMATIC AMINES DURING DEVELOPMENT OF *NICOTIANA TABACUM*

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(*Revised received* 6 *February* 1981)

Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; development; aliphatic polyamines; aromatic amines.

Abstract—Putrescine, spermidine, spermine, tyramine and phenethylamine have been analysed in the apical parts, leaves, stems, flowers and roots of the tobacco plant, *Nicotiana tabacum* cv *Xanthi* n.c. The content of the five compounds differs according to tissue and stage of development. The variations are higher for the aromatic amines than for the aliphatic ones: phenethylamine is the main amine of the green vegetative tissues, but tyramine is the main amine in the stem and it accumulates in the flowers.

INTRODUCTION

There are few papers in the literature about free amines in plants. Apart from Smith's reviews on aliphatic [1, 2] and aromatic amines [3], the other reported results concern only very young plants such as legumes [4–7] or barley [8]. Regarding the tobacco plant there are only sporadic data [1, 9–11].

It is now well known that aliphatic amines function in plants and in animals as cations in membrane permeability and in the stabilization or regulation of nucleic acids and cellular division. By contrast nothing is known about the role of aromatic amines in plants. The recent discovery that hydroxycinnamic acid amides accumulate in the reproductive organs of numerous species from different plant families [12] suggests an answer to such a question. Indeed, hydroxycinnamic acids may be conjugated with aliphatic or aromatic amines; for example, tobacco flowers contain caffeylspermidine as well as ferulytyramine. The possible role of such compounds in sexual differentiation is complex and still hypothetical [13]. However, it is now important to study simultaneously aliphatic and aromatic amines as potential precursors of these various hydroxycinnamic amides.

This paper is a first approach of a dynamic analysis of the five main free amines found in one cultivar of *Nicotiana tabacum*. Their distribution in the different organs of the plant and their variation in the different parts during development from a vegetative to a floral stage are described.

RESULTS AND DISCUSSION

The main free amines which have been identified in *N. tabacum* cv *Xanthi* n.c. are putrescine, spermidine, spermine, tyramine and phenethylamine. We have never found cadaverine in the green parts of *N. tabacum*, in contrast to the results of Hohlt [11]. Its occurrence in roots must still be proved. Agmatine and dopamine were not detected in our experiments. Tryptamine was not analysed by our method: its absence in tobacco tissues still remains to be established.

Apical parts

The apical parts of a plant represent a very heterogeneous material but also the most active in plant metabolism. They include the vegetative—or floral—bud plus 1–2 cm of the stem just below the apex and the young leaves which are bounded with that stem fragment, the longest one reaching 10–12 cm. The amount of every amine was measured at eight different stages of development between the 50th and 80th day of culture (Figs. 1 and 2). The three aliphatic amines increase in parallel during this period. Putrescine and spermine content at the 80th day is twice that observed at the 50th day. Spermidine increases more slowly but its initial amount is higher. Few analyses have been made with very young plants (30–40 days old). At these stages apical parts represent nearly the whole plant. The amine concentration is low in these young plants. Spermine content is always at a low level, below 100 nmol/g fr. wt, much lower than that of other aliphatic amines. In particular, it is 8–10 times lower than the spermidine content. This does not indicate that spermine is a less important amine. Putrescine and spermidine reach a concentration of 400 nmol/g fr. wt after floral induction. This is the highest level for these two main aliphatic amines in all the tissues of tobacco, except flowers. Most interesting is the change of the ratio spermidine/putrescine. This is near or greater than 2 in vegetative plants, and near 1 when plants reach their flowering stage. In Fig. 1, vertical dotted lines represent the greatest differences between four replicates of such an experiment. These variations of amine content are important between the 60th and 65th day of culture; we think they are characteristic of the great metabolic modifications which affect the plant at the stage of floral induction [13, 14].

The changes of the concentration of the two aromatic amines is more significant. Tyramine is low in vegetative plants until the 60th day. Then it increases quickly and within 30 days the amount is 4 times higher. The phenethylamine content is very different and unique among the five amines. After a rapid accumulation in young vegetative plants, phenethylamine reaches a maximum level (500–700 nmol/g fr. wt) between the 52nd

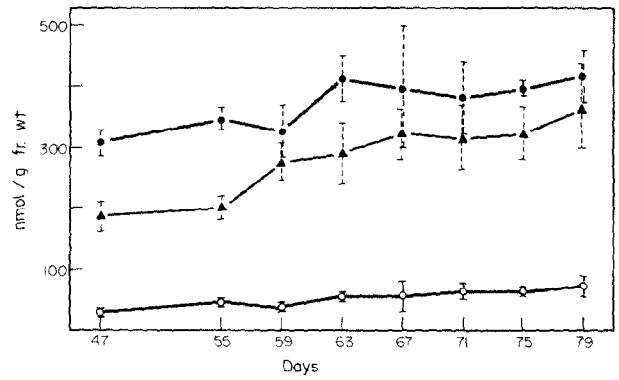

Fig. 1. Putrescine (▲), spermidine (●) and spermine (○) content in the apical parts of *Nicotiana tabacum* cv *Xanthi* during the development of the plant between the 47th and 79th day of culture. Vertical dotted lines represent the greatest differences for four experiments.

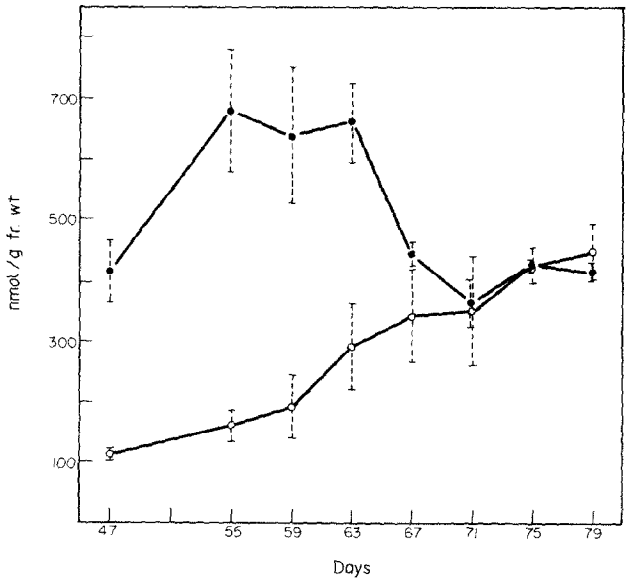

Fig. 2. Tyramine (○) and phenethylamine (●) content in the apical parts of *Nicotiana tabacum* cv *Xanthi* during the development of the plant between the 47th and 79th day of culture.

and 65th day. It is the main amine in the apical parts of tobacco during the whole vegetative phase. After the 65th day, the phenethylamine amount decreases, stabilizing at *ca* 400 nmol/g fr. wt. The opposite changes of tyramine and phenethylamine contents are clearly signified by the ratio phenethylamine/tyramine which decreases from 4 to 1 between the 45th and 80th day.

Leaves

The total content of the amines is clearly lower in the leaves than in the apical parts of *N. tabacum* cv *Xanthi*. Moreover, this content is very much the same for the leaves at the vegetative and floral stage (Table 1). On the contrary, the young leaves always have a relatively higher content of each amine than the older ones. Most noticeable is the constant predominance of spermidine among the aliphatic amines and of phenethylamine among the aromatic ones over respectively putrescine and tyramine. Therefore, the ratios spermidine/putrescine and phenethylamine/tyramine are always at least between 2 and 4, but sometimes they are considerably higher.

Stems

The free amine content of the stem was analysed every week between the 56th and 77th day of culture. In the first sample (56th day) the stem was still very short, so we analysed the whole stem. In proportion as the plants grow we cut the stem into 10-cm-long pieces. The results are shown in Fig. 3.

Tyramine is the main amine in the stem: at every stage of development and at every stem level, tyramine has the highest amine concentration, namely 40–80% of the whole amine pool. The older the stem, the higher is the amount of tyramine. There is also a decreasing tyramine

Table 1. Free amine content in young or old leaves of *Nicotiana tabacum* cv *Xanthi* at three different stages of development

	56th day	63rd day		70th day	
nmol/g fr. wt		Young	Old	Young	Old
Putrescine	52	85	20	105	44
Spermidine	138	205	80	260	83
Spermine	14	15	10	15	10
Tyramine	10	115	46	110	22
Phenethylamine	148	315	145	300	58

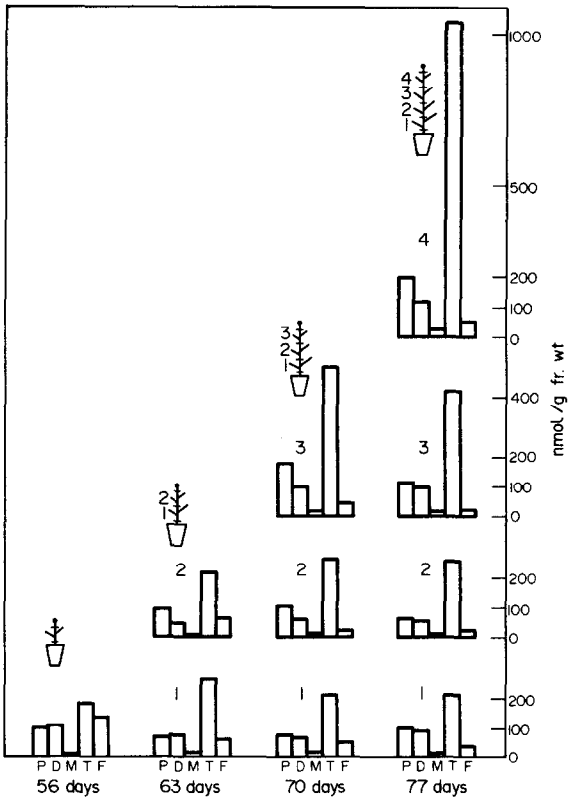

Fig. 3. Amine content in the stems of *Nicotiana tabacum* cv *Xanthi* at various levels (noted 1, 2, 3, 4) of the stem and at four different stages of development of the plant. P, putrescine; D, spermidine; M, spermine; T, tyramine; F, phenethylamine.

gradient along the stem from the top to the bottom. Phenethylamine presents an opposite situation: its amount decreases as the plant grows, so the relation phenethylamine/tyramine in the stem is always lower than 1 and may be as low as 0.02.

The aliphatic amine content and variation are less significant. Nevertheless, the amount of putrescine is always higher than spermidine, so the relation spermidine/putrescine is also lower than 1 in most cases. Spermine reaches a very low level, as in other plant organs.

Flowers

An illustration of free amine content in flowers, just before the anthers dehisce, is shown in Table 2. Tyramine accumulation in flowers is quite noticeable; it represents 80–90% of the total amine pool and a concentration 10 times higher than in any other organ of the plant. Stamens have a tyramine content twice that of the pistil. Therefore, such a specific accumulation in flowers must not be generalized to other species since the main aromatic amine in the reproductive organs is not the same in different *Nicotiana* species [16].

By contrast, phenethylamine is absent from the reproductive organs. It is found only in the perianth. If the aromatic amine content is quite characteristic in floral parts, the aliphatic amine content is not very different from that found in other organ tissues. It must be noted that the putrescine concentration is greater than that of spermidine in the stamens, i.e. where the tyramine accumulation is the greatest.

Roots

Some preliminary experiments have been made on washed roots after growth on sand or a mix of peat and gravel [15]. In roots, phenethylamine is found only in traces. Tyramine is the only aromatic amine present in a relatively important amount particularly during the flowering process. Putrescine is the predominant amine during the vegetative stage. Its amount is higher than that of spermidine before floral induction. Spermine level is low as in all the organs of the tobacco plant.

CONCLUSION

We have demonstrated the importance of the relation between the two main aliphatic amines on the one hand and between the two aromatic amines on the other hand.

Table 2. Free amine content in 98-day-old flowers of *Nicotiana tabacum* cv *Xanthi*

nmol/g fr. wt	Whole flowers	Stamens	Pistils
Putrescine	450	620	305
Spermidine	240	220	335
Spermine	60	30	95
Tyramine	5500	7500	3280
Phenethylamine	230	0	0

One can state that chlorophyllous tissues always have a spermidine content higher than that of the putrescine and a phenethylamine content higher than that of the tyramine. In other organs, such as stems, roots and flowers, these relations are reversed. The variations of aromatic amines are clearly more significant than aliphatic amine ones: phenethylamine is the main amine of vegetative chlorophyllous tissues; it is present only in traces in roots and flowers. On the contrary, tyramine is at a low level in young tissues; it becomes the main amine in stems and accumulates in large amounts in flowers.

EXPERIMENTAL

Plant material. Nicotiana tabacum cv *Xanthi* n.c. was utilized in all the expts. It is a herbaceous plant indifferent to photoperiodism but much more sensitive to temp: the flowering process is inhibited above 32°. Plants were grown in pots with a mix of peat and gravel. Every day, 200 ml of a nutrient soln [17] was supplied to each plant. About the 30th day of culture plants were set in controlled environment chambers (20° ± 0.5°; illumination 120–150 W/m^2; photoperiod 16 hr; r. h. 70–80%). Under such conditions *N. tabacum* cv *Xanthi* grows regularly; it reaches 1 m high and develops 35 leaves within 3 months of culture. About the 65th day floral induction is irreversibly established. The first inflorescence buds appear at *ca* the 90th day and develop during 30 days to produce 100–200 flowers.

Extraction and analysis of amines. Samples were always taken at the same time at the beginning of the photoperiod. Aliphatic and aromatic amines were extracted according to ref. [18]. Plant tissues were ground in a mortar with 0.1 M HCl; macromolecules were precipitated with 10% TCA. After filtration, TCA was extracted with Et_2O. The aq. phase was concd and kept in 0.1% HCl. The chromatographic analysis of the amines was performed by a semi-automated ion exchange method [19] using two buffers (pH 5.65) with increasing NaCl ionic strength. A good separation of the 10 usual amines was obtained in less than 2 hr with the second buffer. Contrary to ref. [19], in our chromatographic system, spermine is eluted from the column between spermidine and dopamine. The quantification of the amines was obtained by a fluorimetric method, using *o*-phthalaldehyde as reagent [20]. It is easy to detect 0.5 nmol of each amine with good accuracy.

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