The Amino Acids Pool in the Style of Self-Incompatible Strains of *Petunia* after Self- and Cross-Pollination

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Summary. Shifts in the composition of amino acid patterns in the styles of *Petunia hybrida* were investigated during incompatibility reactions after self pollination.

High organ specificity (leaves, unpollinated styles, pollen) was observed, along with a high content of free tryptophane and proline in the pollen.

In unpollinated styles slow protein synthesis takes place during the first 24 hour period after their removal from the plant. After cross pollination a slight decrease in net protein content is seen which is due to the presence of penetrating pollen tubes. An increase noted in the concentration of amide containing groups can be linked to protein and amino acid degradation. In the first 12 - 18 hrs. after self pollination protein synthesis stops abruptly, to be resumed again slowly after 20 hrs. This change does not correspond to an alteration in free amino acid concentration.

In pollinated styles the amount of free amino acids from protein degradation is much higher than the actual increase of amino acid concentration, especially after selfing. This must mean that the amino acids resulting from protein hydrolysis are used up as an energy supplying substrate.

There is evidence for the existence of two different amino acid pools in the style: a storage pool to serve as source of oxygen for respiration, and another small pool which provides amino acids for protein synthesis. In a style penetrated by growing pollen tubes evidence of compartmenting and metabolic channeling has been obtained, thus variation in the free amino acid pool is the physiological expression of pollen tube growth. The striking increase of the tryptophane concentration, especially after outbreeding, seems to be linked to the metabolism of growth hormones of the indole group.

The specific influence of incompatible pollen tubes on the amino acid pools is discussed in connection with recent theories on the physiological mechanism of gametophytic incompatibility and the formation of allosteric molecules as genetic regulators of inhibitor synthesis.

I. Introduction

During the passage of the pollen tubes through the transmitting tissue of the style important biochemical transformations take place. Preceding the process of fertilization the pollen tube and the cells of the female tissues undergo definite physiological changes. There are 3 metabolic relations between the conducting tissue of the style and the pollen tube viz. (1) dissolution of the pectic material of the stigma by the pollen tube (v. d. PLUIJM and LINSKENS, 1966), (2) chemotropic guidance of the pollen tube and (3) absorption of metabolic substances by the pollen tube. There is a great lack of knowledge regarding the enzymatic relation between pollen tubes and stylar tissues (for literature see: LINSKENS, 1964, 1966a).

Physiological condition in connection with the incompatibility reaction (LINSKENS, 1965a) which result in an inhibition of the pollen tube growth is of special interest (STRAUB, 1946; LINSKENS, 1955). All experimental results available till to date suggest the possible formation of protein-like substances which can be interpreted as an immune-like reaction (LEWIS, 1965; LINSKENS, 1965a, b).

After an experimental confirmation that there is a change in the protein pattern in the incompatibility reaction (LINSKENS, 1953, 1955, 1965b; TUPÝ, 1963a) we looked for the net synthesis of proteins and the changes in the composition of the pool of free amino acids. These data would give us a detailed information about the metabolic relations between the pollen tube and the style in general and more so at the time of building up of the physiological incompatibility barrier and their biochemical background.

Detailed analysis of the amino acids by paper chromatography in the styles of *Nicotiana alata* have already been reported (TUPÝ, 1961a). From this it can be seen that the growing pollen tubes *in situ* have a characteristic influence on the amino acid spectrum of the style. It could be concluded that pollen tubes normally use amino acids from the amino acid pool of the stylar tissue as a substrate for energy transport. Quantitative differences were found in styles after incompatible and compatible pollination; but no specific disturbance of the amino acid metabolism in incompatibility reaction was found in the self or cross pollinated *Nicotiana*. In *Petunia* also, amino acid analyses (LINSKENS, 1955) of these two types of styles did not give any conclusive results.

The inhibition of the pollen tube growth after incompatible pollination may be caused by reactions which result or depend on modification of the protein pattern. This can also be demonstrated in shifts of the composition of the amino acid pattern.

II. Material and methods

The styles and pollen from *Petunia hybrida media vulgaris* of the clones W 166 k (self incompatibility alleles S_1 S_2) and T 2 U (self incompatibility alleles S_3 S_3) were used for analysis. These were grown in temperature-controlled glass houses with supplemented artificial light (Philips HPW 500, intensity ca. 10.000 lux), which are in use since many years (STRAUB, 1946; LINSKENS, 1953, 1955, 1958, 1959, 1960, 1961). Unpollinated styles were taken from flowers on the day of anthesis. The pollen used for pollination was mature and not older than one day. Pollination of the entire bulk of the material was done within about 10 minutes by a group of technicians. The pollinated flowers were removed from the plants and the cut end dipped in tap water and put in the dark at 25 °C. The styles were removed at various times synchronously and immediately extracted.

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Protein determination

Extraction: For each analysis 100 styles were taken and grinded with 5 ml cooled acetone p. a. with little quartz sand in a mortar. The suspension was centrifuged after 30 minutes (5 min, 3000 x g). The residue was washed once more with 5 ml cold acetone and centrifuged. The dried sediment was then twice extracted with 2 ml tris buffer (pH 8.6, μ 0.1) for 30 and 10 min respectively. This was followed by another extraction with citrate buffer (pH 6.5, μ 0.1) for about 30 minutes. The 3 buffer extracts combined together contained the total protein. This was further checked regularly by disc electrophoresis.

Dialysis: The combined extract was dialysed under pressure in Necol collodion shells (BDH 301—261). These were freshly prepared in conical test tubes and after drying (15 min) the shells were filled with dist. water and were ready within 10 minutes. After filling in the extracts, the shells were hung in a buffer (tris 0.01 m, pH 8.6) at a pressure of 0.35 atmos. The extract was concentrated to 1 ml in about 15 minutes. This procedure was repeated with another 3 ml of the buffer. The dialysed and concentrated extract was quantitatively analysed. All extractions and dialysis was done at a temperature of 4 °C.

Determination: The total protein determination was done by Biuret method (HEINEN, 1963).

Analysis of free amino acids

Extraction: 150 styles and 150, 75, or 15 mg pollen resp. were separately grinded with quartz sand and 2 ml 80% ethanol and stored for 30 minutes in the cold room (0 °C). The suspension was centrifuged (5 min, 3000 × g) and the loose sediment was once more extracted for 10 min with 1 ml alcohol and centrifuged. The combined extracts were shaken for 5 min with 6 ml chloroform (Vortex vibrator). Phase separation took place during night in the cold room. The supernatant water phase was pipetted and filtered through a Seitz candle. In the clear extract, 0.15 ml of 1N HCl was added and made to a volume of 1.5 ml with dist. water. All extractions were carried out in the cold room (0 °C).

Separation: For quantitative determination of free amino acids the Micro Column Amino Acid Analyser (Technicon Instruments Co., Ltd. Chertsy, Surrey, England) was used (fig. 1). It consists of a 133 cm column filled with Chromobeads, which following HAMILTON (1963) are comparable with Dowex 50 × 8 resins (— 400 mesh). The trial (1 ml) after application was eluated by a gradient of citrate buffer from pH 2.875 to 5.0. The effluent is mixed with a cellosolve-ninhydrin-solution and the absorption measured at 570 mμ (8 mm and 15 mm layers resp.) and at 440 mμ (15 mm layer). Recordings were done by a three-point-recorder. As an internal standard, 0.1 mol norleucin was added to each sample. From the recorded curves, the absolute amount in μmol of the various amino acids could be calculated by standard chromatograms (FERRARI and MACDUFF, 1959; TAYLOR and MARSH, 1959; CATRAVAS, 1964).

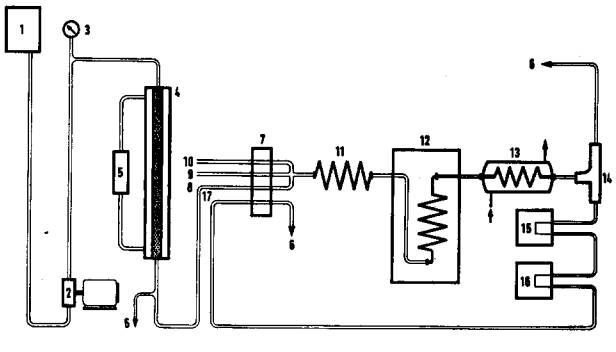

Fig. 1. Circulation scheme of automatic amino acid analyzer. — 1. Cascade chambers of AutoGrad (for producing eluent gradient from pH 2.85 to pH 5.00); 2. Positive displacement Pump, capacity 0.5 ml/min ca. 200 psi, pumping the eluent at a constant rate through the resin column regardless of resistant pressure; 3. Manometer; 4. Column, ⌀ 6 mm × 1400 mm, packed with CHROMOBEADS, 60 °C; 5. Water bath with re-circulation pump; 6. Flowing off to the fraction collector; 7. Proportioning pump; the combined streams are mixed and flow into heating bath (12); 8. Eflux from column, 0.42 ml/min (sample line); 9. Nitrogen gas, 0.60 ml/min: it is used for segmentation instead of air to allow lower concentration of hydringantin and so a less background color; 10. Ninhydrine solution 1.06 ml/min; 11. Mixing spiral; 12. Heating spiral, 95 °C, retention time about 12 min; 13. Cooling device; 14. Exflux of gas: on the upper arm nitrogen bubbles off, the lower arm feeds the nitrogen-free solid stream to the colorimeters; 15. Colorimeter, lightpath 15 mm, 440 μm, measures proline and hydroxyproline; 16. Colorimeters, lightpath 8 and 15 mm, 570 μm, measure the reacted ninhydrine-amino acids. Outputs of the colorimeters feed a multipoint recorder, simultaneously recording the reading of separate colorimeter in a different color; 17. Suck up through colorimeter.

Acknowledgements

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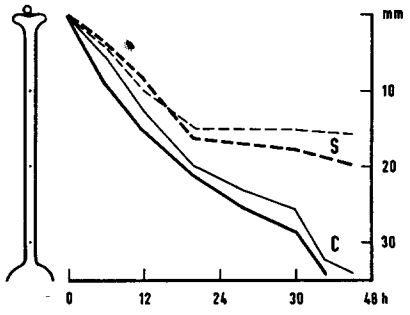

Fig. 2. Pollen tube growth in the pistil of *Petunia* at 25 °C after self-pollination [S] (— — — in isolated flowers, ——— in flowers on the plant) and cross-pollination [C] (- - - - in isolated flowers, ——— in flowers on the plant).

III. Results

The passage of the pollen tube through the transmitting tissue of the style is given in Fig. 2. It shows that at the climax of the vegetative period (May) the incompatible pollen tubes are inhibited half way to the ovule, while the compatible ones grow more or less with constant rate to reach the egg cell.

The results of the amino acid analysis are given in Tab. 1 and Fig. 4—6. They are all from fresh material and one style has a mean weight of 7,3 mg. For effective pollination of one style about 0.2 mg pollen was used. The variation of the results caused by the analysis method is ± 3 per cent. A maximum deviation of 10% was noticed for the seasonal variation of the plant material.

1. Starting material

In Tab. 1 the content of the free amino acids for unpollinated styles, and the pollen of both the species is compared with the amino acid content of fresh leaves of *Petunia*. Whilst the amino acid content of the pollen of both the species is almost identical, the styles have a much lower content. The already abundantly

Representation of results: Each point on the graphs represent an average of 3—7 repetitions. Tab. 1 and the graphs 4—6 represent mean values. In Tab. 1 the amount for each amino acid is given for one fresh style and 1 mg fresh pollen resp. always used for pollination, in 10^{-9} gram molecules. In Tab. 1 absolute values are shown for unpollinated styles and pollen. In the graphs the abscissae for the individual curve is omitted to be able to represent the time dependent changes simultaneously. Because the first measurement of the curves is identical with the values of Tab. 1 it is possible to verify the absolute values for each curve, because the scale of the abscissae, which is mentioned below, is identical for all curves.

Table 1. *Content of free amino acids in 10^{-9} gmol.*

Substance	Abbreviation	1 pistil S_1S_2 unpollin. fresh (= 7.3 mg)	Pollen S_1S_2 1 mg air dry	Pollen S_3S_3 1 mg air dry	Leaves S_1S_2 1 mg fresh
Cysteinic acid	CA	±	0.0	0.0	0.09
Aspartic acid	AspA	7.3	9.0	6.9	0.19
Threonine + Asparagine + Glutamine	Thr + $AsNH_2$ + $GlNH_2$	3.4	7.8	7.3	0.09
Serine	Ser	10.0	7.0	6.0	0.07
Glutamic acid	GluA	13.8	17.6	16.5	0.50
Proline	Pro	0.8	112.0	108.0	0.04
Glycine	Gly	1.8	2.6	2.2	0.014
Alanine	Ala	2.4	12.5	12.0	0.072
α-NH_2-isobutyric acid	α-NH_2isobA	0.3	0.7	0.6	0.0
Valine	Val	1.2	1.9	1.7	0,02
Cystine	Cys	0.7	0.1	0.15	0.004
Methionine	Met	0.06	0.1	0.13	0.005
iso-Leucine	iLeu	1.0	0.8	0.5	0.018
Leucine	Leu	0.7	0.3	0.3	0.013
Tyrosine	Thy	0.3	0.5	0.35	0.006
Phenylalanine	PhAl	0.3	0.4	0.17	0.019
Ethanolamine	$AeNH_2$	5.9	2.2	2.4	0.15
γ-Aminobutyric acid	γNH_2bA	0.3	0.5	0.4	0.0
Ornithine	Orn	0.2	0.17	0.2	0.003
Lysine	Lys	0.4	0.7	0.8	0.017
Histidine	His	0.4	3.2	2.7	0.006
Arginine	Arg	0.2	2.3	4.3	0.0
Penicillinamine	PenA	+	0.0	0.0	0.0
Tryptophane	Try	19.0	33.0	31.5	0.0

reported high proline content in pollen (BATHURST, 1954; BRITIKOV et al., 1964) should be mentioned. The important difference in the amino acid content between the styles and leaf tissue is surprising. The specialisation in function is represented in a higher total amino acid content, in the stylar tissue and in proportions between individual acids.

2. The unpollinated style

As for biochemical analysis a great amount of identical material had to be used the flowers were gathered, brought to the laboratory, put in tap water and between pollination and analysis stored in a dark room at 25 °C. All observations are from excised material. It was therefore necessary to have a control to know the fluctuation of free amino acid in unpollinated styles of emasculated flowers. As seen from Fig. 3 in unpollinated style, a slow protein synthesis takes place especially in the first 24 hours after removal from the plant. On the second day protein content remains more or less constant. Ripe, unpollinated styles are therefore protein synthezising systems. When compared with the amino acid pool (Fig. 4) we observe, independent from presence of tubes, that as a consequence of removal of the styles of the flowers in the first half hour there is a slight decrease in some amino acids, though hardly measurable. In some others(glutamic acid, γ-aminobuturic acid, threonine, valine) a distinct depression takes place. This is the result of the apparent disturbance of style isolation from the flower. For the following 24 hours the amino acid spectrum remains almost constant, with the exception of threonine, asparagine and glutamine. These compounds could not be separated by the present analytical method. This group shows a minimum after about 6 hours of isolation which is followed by a strong increase. But also the content of alanine during first hours and that of aspartic acid and tryptophane later rises.

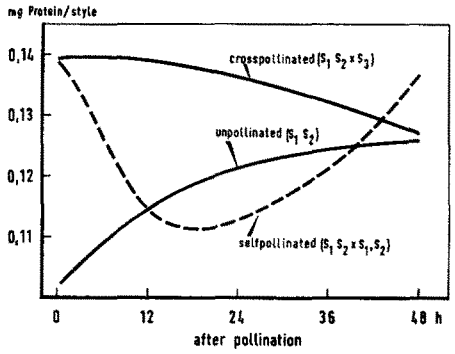

Fig. 3. Protein content (mg) in one style of *Petunia*, changing in time in unpollinated (u), cross-pollinated (C) and self-pollinated (S) condition.

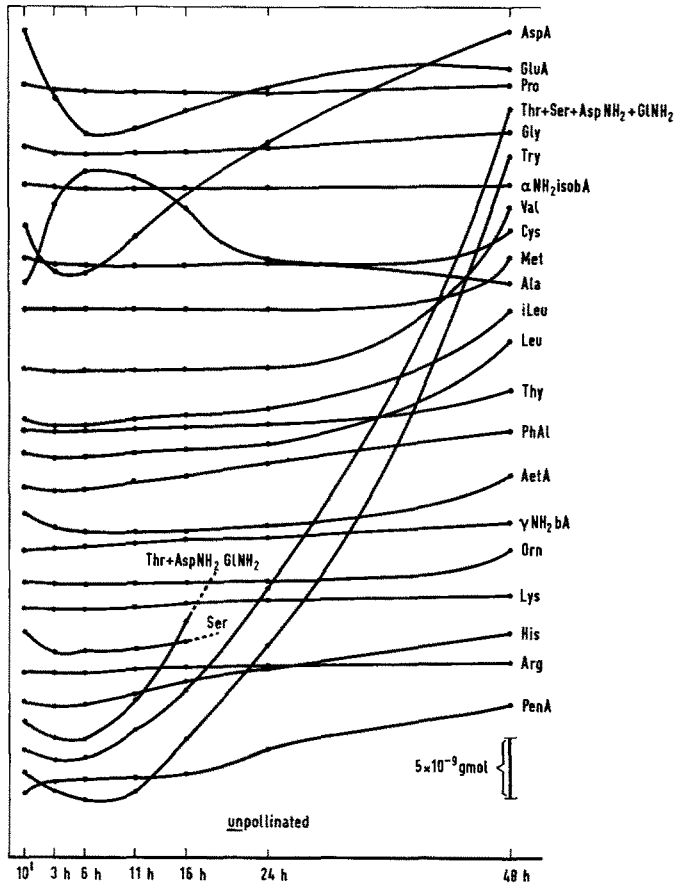

Fig. 4. Changing of amino acid amount in one unpollinated style (S_1S_2) in isolated, emasculated flower.

3. Pollinated styles

The amino acid spectrum for pollinated styles is represented in Fig. 5 and 6. A striking fluctuation is seen in the curves compared with the spectrum of unpollinated styles (Fig. 4), not identical for both types of pollination.

a) Styles after cross pollination. After cross pollination the net protein synthesis is immediately stopped and a slight decrease is seen due to the presence of growing pollen tubes (Fig. 3). This fact has little influence on the amino acid pool (Fig. 5) when compared with the unpollinated styles (Fig. 4). Increase of concentration can be observed for only a few acids (tryptophane, valine, leucine, iso-leucine and the amides threonine-serine group). Some amino acid concentrations remain constant for 48 hours (glycine, methionine, ornithine, lysine, arginine). The content of proline, an amino acid which is specially brought by the pollen tubes, shows an apparent depression in the first hours of growth.

The increase of the concentration of the amide containing group is linked apparently with the degradation of proteins and amino acids. Whilst this phenomenon in unpollinated style first appears after 11 hours, in the cross pollinated ones it starts to increase within 3 hours of pollination. In the same way changes in amount of tryptophane, valine, leucine and iso-leucine are shifted to earlier time. At the same time, when in unpollinated styles the amount of tryptophane as a concequence of flower isolation decreases, in cross pollinated styles a distinct increase is observed. Valine concentration increases immediately after pollen germination, while in unpollinated styles it remains constant. Characteristic metabolic changes as a reaction to compatible pollen tube growth are mostly evident in the curves of metabolically active amino acids (glutamic acid, aspartic acid, alanine) and of the amines (ethanolamine, penicillinamine).

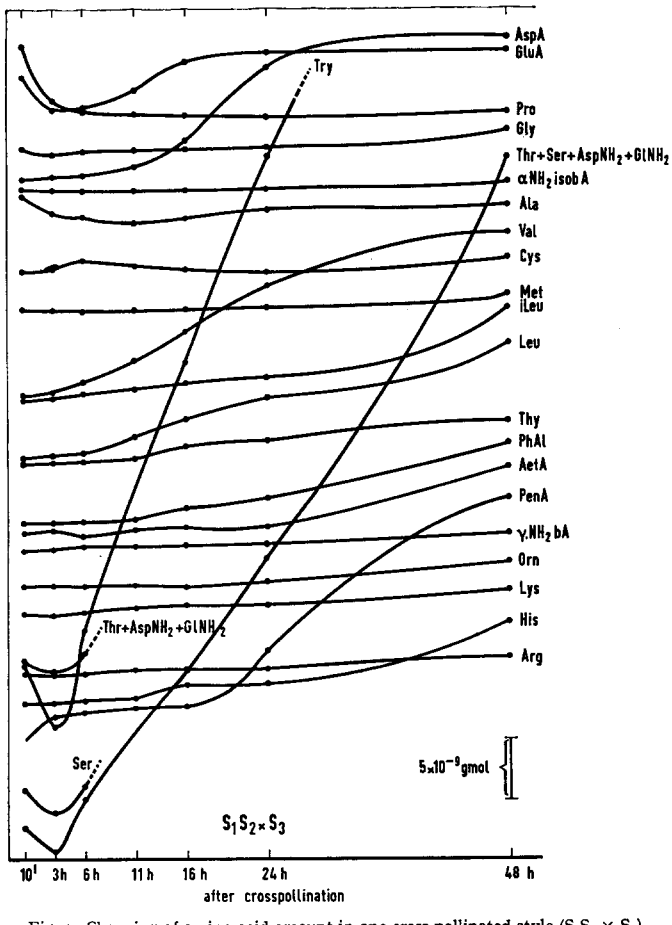

Fig. 5. Changing of amino acid amount in one cross-pollinated style ($S_1S_2 \times S_3$) in isolated flower.

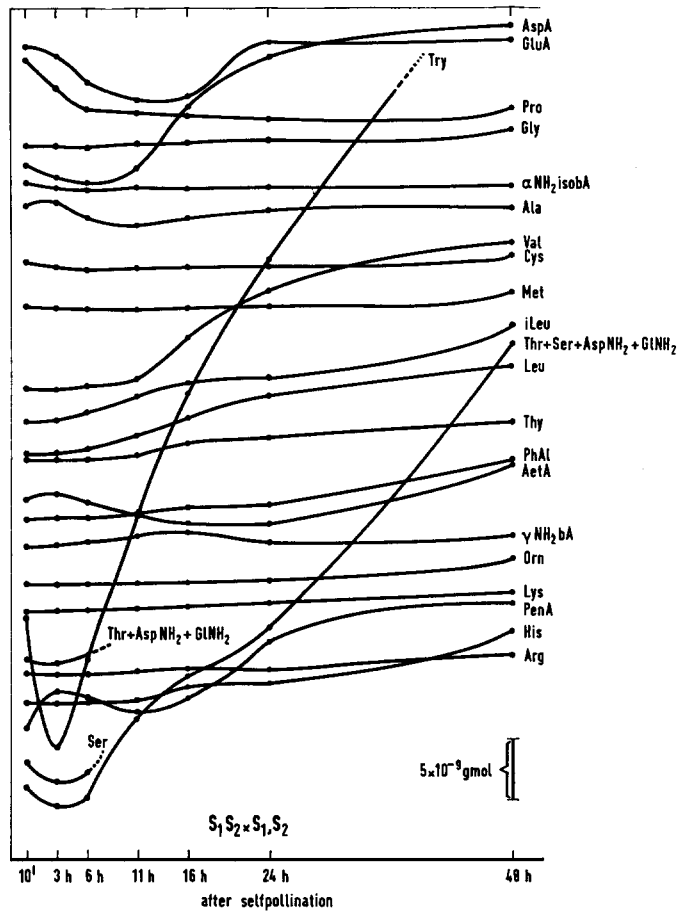

Fig. 6. Changing of amino acid amount in one self-pollinated style ($S_1S_2 \times S_1, S_2$) in isolated flower.

b) Styles after self pollination. After self pollination net protein synthesis in the styles is abruptly stopped in the first 12—18 hours of pollina-

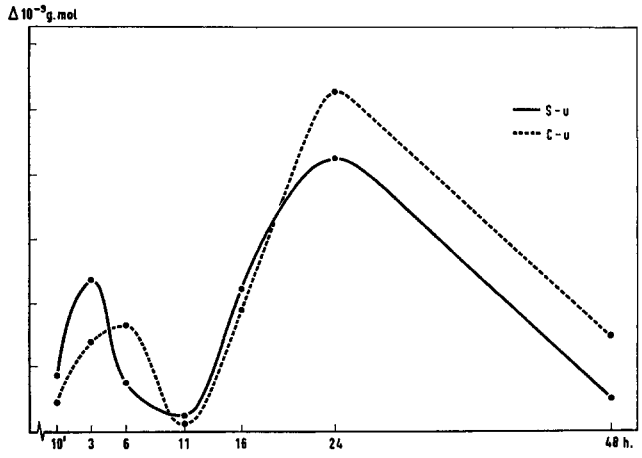

Fig. 7. Variation of the amount of total amino acids in one style. The amount of a self-pollinated (——— S—u) and of a cross-pollinated (- - - - C—u) is corrected by the amount of an unpollinated style, to demonstrate the difference between both the combination types.

tion and a strong breakdown of proteins takes place (Fig. 3). The amino acids, liberated in this manner do not accumulate (Fig. 6), quite comparable with the situation after cross pollination. There is no close relation between protein breakdown and concentration of amino acids. This is quite evident when the total amount of amino acids (Fig. 7) is taken into consideration. The amino acids, liberated after cross pollination obviously are consumed in energy delivering processes. This view is confirmed by the increase in concentration of the amides which is strongest during the period of quick protein breakdown.

After about 20 hours a shift back to protein net synthesis is observed. Even this tendency is not expressed in free amino acid concentration. In a similar way as in crossed styles an increase in concentration of most amino acids was measured. An exception will be observed for amides containing group, which are reduced after 20 hours. This is probably due to depression of the amino acid catabolism. Self as well as cross pollinated styles are different with regard to certain amino acids (aspartic and glutamic acid, proline, tryptophane, leucine, iso-leucine, tyrosine, alanine, ethanolamine, penicillinamine) as against control (unpollinated style) during the first 16—24 hours of pollen tubes passage through the style. This change of pattern is similar for both cross and self pollinated styles (proline, leucine, iso-leucine, tyrosine) or characteristic for self pollinated styles, as far as aspartic and glutamic acid, tryptophane, valine, alanine, ethanolamine and penicillinamine are concerned. This specific influence of the incompatibility reaction on the amino acid pool is represented in Fig. 8 which represents differential curves of self- and cross pollinated styles against unpollinated controls.

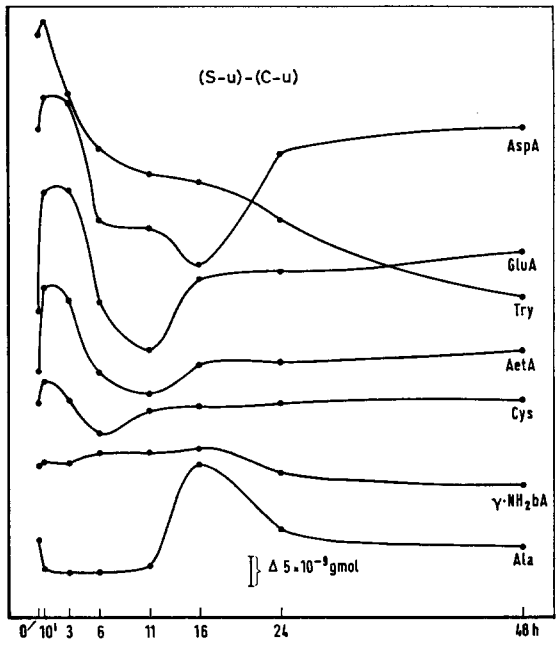

Fig. 8. The differential amount of some amino acids within the style in dependence from time of pollination. Curves represent the difference between a self-pollinated style (reduced with the amount of an unpollinated style) and a cross-pollinated one (reduced with the content of an un-pollinated one).

IV. Discussion of results

1. Differences in free amino acid content of pollen, styles and vegetative tissue of leaves

The high proline content in pollen confirms a known general fact. The relatively high amount of alanine is already observed in other species (BELLARTZ, 1956; TUPÝ, 1961a, 1963b). Surprising fact is the presence of high tryptophane content in *Petunia* pollen. This amino acid is rare and is found in very low amount in pollen (NIELSEN et al., 1955). It is especially the high content of these three amino acids which differentiates the spectrum of pollen from that of the styles and leaves.

The presence of γ-aminoisobutyric acid as well as of ethanolamine is reported for the first time in pollen.

Comparing with pollen and leaves, the high concentration of serine in the female tissue should be mentioned. Next to glutamic acid, serine is the most prominent amino acid in this organ at the pregametic phase. High concentration of serine is also characteristic for the pistil of *Nicotiana alata* (TUPÝ, 1961a). The presence of this amino acid seems to be linked with relatively high concentration of ethanolamine which is formed from serine by decarboxylation (STETTEN, 1942). The primary observed penicillinamine in styles of plants can be considered as a derivative of valine or cysteine (QUAGLIARIELLO, 1958).

The high specificity of the amino acid pool for distinct organs is not surprising, when the very distinct and different function of these organs is taken in consideration. This is specially true when referred to the well known organ specificity of proteins (VOM BERG, 1932) and its changing with developmental processes (LINSKENS, 1966b).

2. Change of the net protein synthesis as an effect of pollen tube growth in the pistil Amino acids as an energy substrata

It is a well known fact that in leaves and other plant organs after isolation there is partly protein degradation combined with increase in free amino acids and especially amides (KROTKOV, 1939; WEINSTEIN and PORTER, 1962). Similar derangement can be the result of shortening of light period (STEWARD et al., 1959) and especially darkening (MIETTINEN, 1955). One of the reasons is the lack of energy delivering carbohydrates which are partly replaced by amino acids delivered from protein hydrolysis. This metabolic consequence apparently has some influence also in protein and amino acid content in *Petunia* flowers, plucked and put in dark. After the initial depression of amino acid concentration which is a wound reaction and in particular of interruption of supply of organic material from the flower the difference between increasing consumption on one side and the formation resp. importation of amino acids on the other side is compensated by protein degradation. Later, a slow increase of the total amount of amino acids and amides is observed but this has no close relationship with change in protein content. In pollinated styles, especially after selfing, the amount of amino acids formed by protein degradation is much higher than the actual increase of the concentration of amino acids. That means that the amino acids resulting from protein hydrolysis are consumed as energy supplying substrate.

This relation between protein degradation and energy requirement of processes within the style can also be derived from time curves of respiration (LINSKENS, 1955). In pollinated styles respiratory intensity and sugar consumption is much higher than in unpollinated ones. Maximum utilization of energy is seen during the first 12 hours in incompatible pollinated styles. In this time period strongest protein degradation was observed. After 18 hours of pollination a striking reduction of respiratory intensity was found. This is the time when incompatible pollen tubes have stopped growth and protein synthesis recommenced. Pollen tube growth after compatible pollination showed continuous increase of respiratory intensity which corresponds with the continuous decrease of protein supply in the style.

The intensity of energy utilization of amino acids can also be derived from the increase in amide containing group. This follows from the function of amides formation in ammonia detoxication (MOTHES, 1958). In *Nicotiana alata* this relation between energy demand and asparagine accumulation could be demonstrated very clearly (TUPÝ, 1961a). The accumulation of asparagine takes place only in pistil from isolated flowers and is linked with decrease of sugars (TUPÝ, 1961b).

At first sight it seems surprising that in unpollinated styles with continuous net protein synthesis, an increase of the amount of amino acids and also amides took place. This could only be explained by the existence of two different amino acid pools. Following the hypothesis of STEWARD et al. (STEWARD and BIDWELL, 1961; BIDWELL et al., 1964) the storage pool derives its amino acids from protein. It is the source of carbon for respiration and CO_2 production. The other the little pool is the more active and delivers amino acids for protein synthesis. The main source of carbon for this pool is sugar.

The existance of two different amino acid pools in the style can also be confirmed by the rate of amino acid carbon and fructose carbon in styles of tobacco (TUPÝ, 1964). Labelled carbon from C^{14}-proline and especially C^{14}-glutamic acid is less incorporated in protein than from C^{14}-fructose. On the other hand, carbon from amino acids, participates in a higher rate in CO_2 production than carbon from sugar. It seems therefore possible that during protein synthesis in unpollinated styles the energy rich sugars were partly replaced by amino acids of the storage pool, which is provided by protein degradation and supply from other parts of the flower. In any case the increase of proteins and the free amino acids has to be explained by transport of nitrogen containing substances from other parts of the flower into the style.

After plucking flowers and placing them in dark, when both the sugar sources are interrupted (inflow and the photosynthesis of green parts of the flower) function of sugar as energy source in style will be taken over to some extent by the storage pool of free amino acids, which will be supplied by protein degradation. Under these conditions change in protein content represents the energy requirements of the growing pollen tubes, and the metabolic relation between tubes and transmitting tissue of the style.

Amino acids brought to the style by the pollen tubes can be considered exogenous in origin. The exchange of amino acids between tubes and the cells of the transmitting tissue shows that the participating cells are permeable to amino acids. The phenomenon of two sources of a compound contributing to two separate, independent metabolic pathways (protein synthesis, energy delivering) is termed metabolic channeling (DOUGALL, 1965). In the style penetrated by pollen tubes evidence for compartmentation has been obtained.

3. Characteristic shift in amino acid pools after incompatible pollination

First of all there is the variation in the pool of free amino acids which is the physiological expression of pollen tube growth. This concerns the variations in self-, cross- and eventually in unpollinated styles which differ in intensity and time pattern, e.g. increase of tryptophane, valine, leucine, iso-leucine, tyrosine and the group of threonine-serine-asparagine-glutamine, as well as the decrease of proline in pollen tubes growing in styles. In contrast to the situation in *Nicotiana* (TUPÝ, 1961a), the intensity of proline decrease does not differ in self- and cross-pollinated styles of *Petunia*.

The striking increase of tryptophane concentration, especially intensive after outbreeding seemed to be linked with the metabolism of growth hormones of indole group similar to *Nicotiana tabacum*. The increase of auxin concentration within the pollinated style was demonstrated by MUIR (1942) while that of tryptophane by LUND (1956) which compound possibly serves as precursor of IAA synthesis in the pollen tubes.

Specific changes induced by incompatible pollination, and which not only show quantitative deviations in analogy to cross-pollinated ones are seen in the concentration of aspartic and glutamic acid, tryptophane, alanine, cysteine and ethanolamine especially during the first 16 hours of pollination when inhibition reaction took place.

It is remarkable to note that glutamic acid, cysteine, alanine and tryptophane, the 4 out of the 8 amino acids, are the ones which participate in formation of specific protein-carbohydrate-complexes after pollination (LINSKENS, 1955). It was already found that the cross-, self- and unpollinated pistils of *Nicotiana alata* can be distinguished by their content in glutamic acid and alanine (TUPÝ, 1961a). As a whole both species of *Nicotiana* and *Petunia* differ in composition of amino acid pool in cross-, self- and unpollinated styles in another way. From this it follows the species specificity of the incompatibility reaction influence on the amino acid pool.

Binding away of specific amino acid like lysine, alanine and glutamic acid can give some explanation for abnormal tube wall formation. As reported by SHOCKMAN (1963) absence of the above mentioned single amino acids can result, in some instances, in thickened cell walls, in others a rapid autolysis and there are shadings in between. This seems to be dependent on the absence of a particular amino acid, or which nutrients still remain to influence the subsequent events. Combination of one of the current

theories of genetic-biochemical control mechanisms with the presented results on amino acid pool seems to be fruitful.

4. Amino acids participation in immune-reaction and regulation of pollen tube growth

Specific influence on the amino acid pool is of special interest for explanation of the physiological mechanism of gametophytic incompatibility. The findings are in agreement with former observations of formation of specific glycoproteids (LINSKENS, 1958, 1959). The dynamics of the amino acid pool and other little molecules demonstrate the metabolic dynamic of the specific processes leading to an inhibition reaction.

The dimer hypothesis of incompatibility (LEWIS, 1965) is derived from the theory of genetic complementation and supposes that S alleles produce specific peptides which polymerize in dimers. S dimer from pollen tubes can only combine with identical S dimers of the style and form by association of allosteric molecules — the tetramer. The so formed tetramer acts as a genetic regulator of the inhibitor synthesis. With regard to the participation of a new synthesis in formation of specific proteins after pollination, the tetramer is apparently formed not only by combination of protein subunits already present in pollen and styles. The production of inhibitory principals seems to be linked to the surface of the pollen tubes, because the inhibition reaction of tubes deriving from self-pollination, does not influence simultaneously present tubes in the same conducting tissue originating from cross-pollination. Obviously the tetramer is consumed, which like an effector inactivates the corresponding repressor on the surface of the pollen tubes. But also the fact, that the incompatibility reaction takes place after repeated selfing, this makes it necessary to assume a renovation of style S dimers. The regulations of dimer formation can be considered like activation and inactivation of the S dimer regulating repressor, where the effector is the S dimer as the end product. The dimer hypothesis does not explain, why the tetramers do not generate from identical S dimers already in unpollinated styles. It would happen especially in pistils of S allele homozygote plants.

Formation of the specific proteins is the basic concept of the theory which explains incompatibility reaction as an immune-like reaction (EAST, 1929). But definitive inhibition should be the result of binding of a sort of "antibodies" to the pollen antigen, which may be an enzyme. Production of "antibodies" is continued in pollinated styles, as soon as the concentration by binding to antigens diminishes continuously (LINSKENS, 1965a). This idea explains also the specific changes in the amino acid pool and the participation of carbon donors in formation of specific protein-like compounds in incompatible pollinated styles. Regulation of the "antibody" formation can happen by regulation of the functional activity of an enzyme, which is similar in configuration with the "antibody". This enzyme should produce an effector, which inactivates the repressor of the "antibody" coding messenger-RNA synthesis. The antigen renews the "antibody" production by binding to the enzyme (LINSKENS, 1965a). It seems to be easier, that the "antibody" itself acts as effector, which determines the activity of repressor for regulation of "antibody" synthesis.

All these hypotheses of gametophytic incompatibility reaction do not explain the specific variations in the amino acid pool and the formation of specific glycoproteid fractions after compatible pollination. Unilateral incompatibility between self-incompatible and self-compatible species, that is the impossibility of successful pollen tube growth of the latter species within the pistil of the former (LEWIS and CROWE, 1958) demonstrates that in the stylar tissue of incompatible species an inhibitory factor has to be supposed. This factor will be inactivated after compatible pollination and the specific protein changes may be the result of inactivation. A breakdown of inhibition seems to be more probable than induction of stimulation. It follows from the fact, that the length necessary for completing fertilization in nature is reacted by pollen tubes in presence of sugar and boric acid only (HRABĚTOVÁ and TUPÝ, 1964). For adaptation of the explanation to specific protein synthesis after compatible pollination and the unilateral interspecific incompatibility, more detailed experiments are necessary.

Zusammenfassung

Die Zusammenstellung des Pools freier Aminosäuren wird für Blätter, Pollen, unbestäubte, geselbstete und fremdbestäubte Griffel einer selbstinkompatiblen Rasse von *Petunia hybrida* quantitativ bestimmt.

1. Es ergab sich der Nachweis einer großen Organ-Spezifität des Aminosäuren-Musters mit einem hohen Gehalt des Pollens an freiem Tryptophan und Prolin. Außerdem wurden in *Petunia*-Pollen erstmalig α-Amino-iso-Buttersäure und Äthanolamin nachgewiesen.

2. Der unbestäubte Griffel zeigt in den ersten 24 Stunden nach der Anthese der Blüte eine deutliche Zunahme des Protein-Gehaltes. Nach Fremdbestäubung nimmt der Gesamtproteingehalt langsam ab; nach Selbstbestäubung nimmt der Proteingehalt in den ersten 12 Stunden nach Bestäubung plötzlich ab, um nach etwa 20 Stunden wieder langsam anzusteigen.

3. Die Änderung des Gehaltes an freien Aminosäuren entspricht nicht der Änderung des Proteingehaltes. Nach anfänglicher Depression kommt es in den bestäubten Griffeln zur Freisetzung großer Mengen freier Aminosäuren und Amide. Im bestäubten Griffel, besonders nach Selbstung, übersteigt die Menge der durch Protein-Hydrolyse freigesetzten Aminosäuren die wirkliche Zunahme der Konzentration derselben. Daraus kann geschlossen werden, daß ein Teil der freigesetzten Aminosäuren dem Atmungsstoffwechsel zugeführt wird. Die Intensität der energetischen Ausnutzung der Aminosäuren kommt auch in dem Konzentrationsanstieg der Amide zum Ausdruck.

4. Im Griffel muß die Existenz zweier getrennter Aminosäuren-Pools angenommen werden: ein kleinerer, aktiver Pool dient der Protein-Synthese, ein größerer Pool kann als Kohlenstoff-Quelle für die Atmung dienen.

5. Die Änderungen des Aminosäuren-Pools sind in erster Linie Ausdruck der physiologischen Aktivität des Pollenschlauchwachstums. Der Anstieg der Tryptophan-Konzentration, besonders nach Fremdbestäubung, hängt wahrscheinlich mit der Aktivierung des Wuchsstoffhaushaltes der Indol-Gruppe zusammen.

6. Selbst- und fremdbestäubte Griffel unterscheiden sich in der Änderung des Aminosäuren-Pools, die wahrscheinlich artspezifisch ist.

7. Die Ergebnisse werden im Zusammenhang mit der Theorie einer Wachstumshemmung des inkompatiblen Pollenschlauches durch die Synthese spezifischer Proteine diskutiert.

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