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Floral gradient in flowering tobacco in relation to free amino acids

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ABSTRACT

By employing TCLs (thin cell layers) culture, the floral gradient in flowering tobacco of different developmental stages was confirmed. The TCLs from early flowering tobacco regenerated more floral buds than those from the tobacco plants in full blooming or fruiting stages. Analysis of free amino acid levels revealed the acropetal gradient of Pro in flowering tobacco stem. L-Pro, L-Trp, D,L-Met and L-Arg were respectively added into the culture medium for testing their influence on floral bud formation from tobacco pedicel segments. Only L-Trp evidently enhanced the floral bud neoformation.

Key words: *tobacco, floral bud differentiation, floral gradient, free amino acid, thin cell layers culture, L- Trp.*

INTRODUCTION

In 1961, Chouard and Aghion[1] first discovered the floral gradient in flowering tobacco plants and this phenomenon was also observed in *Torenia fournieri*, Cichorium *intybus, Passiflora suberosa and Hieracium floribundum*[2]. It is hypothesized that this gradient reflected a gradient of floral substances. Based on this view point, some substances related to floral gradient were examined, e.g. DNA, phytohormones and polyamine[3-7]. Among them, phytohormones and polyamine were known to be involved in floral bud neoformation *in vitro,* such as spermidine could enhance

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Abbreviations used: TCLs-Thin Cell Layers, IAA-indole-3-acetic acid, IBA-indolebutyric acid, 6-BA-6-benzyl aminopurine, Pro-proline, Trp-tryptophan, Arg-arginine, Met-methionine, Asp-aspartic acid, Glu-glutamic acid, Gly-glycine, Ala-alanine, Val-valine, Ile-isoleucine, Leu-leucine, Tyr-tyrosine, Phe-Phenyl-alanine, His-histidine, Lys-lysine, Ser-serine, Thr-threonine, Cys-cysteine, Asn-asparagine, Gln-glutamine.

the floral bud differentiation in tobacco tissue culture[S]. In 1969, Vallee et al.[9] found the Pro acropetal gradient in flowering tobacco stem. However, there have been no further reports about Pro in tobacco floral bud differentiation. In addition, study on the influence of exogenous amino acids on floral bud differentiation has so far been very limited[10], although amino acids are essential in plant development. Therefore, in the present work, we tested the relation between floral gradient and free amino acids, and the effect of some exogenous amino acids on florM bud neo-formation *in vitro.*

MATERIALS AND METHODS

1. Plant materials

Flowering plants of day-neutral tobacco (*Nicotiana tabacum* L. cv. Ge Xing 1) grown in an artificially illuminated phytotron were used as the experimental material. The explants: 1. TCLs (about 5-8 mm long, 1-2 mm wide) composed of epidermis and several layers of parenchymatous cells [11] stripped from stem, floral branches and pedicels of tobacco plants in early flowering (only the first flower opened), full blooming or fruiting stages; 2. flower pedicel segments (about 1-2 mm in length); 3. leaf pieces (about 5 mm long, 5 mm wide) from vegetative tobacco plants. All the explants were sterilized with routine methods.

2. Culture methods

The basic medium was composed of inorganic salts and organic elements of modified Murashige and Skoog's medium (MS medium)[12], inositol 100 mg/L, sucrose 30 g/L and agar 5.2-6 g/L. According to the requirements of the experiments, phytohormones, plant growth regulators or amino acids were respectively added into the basic medium (for details, see Results). Before autoclaved, the medium was adjusted to pH 5.8 with 1N NaOH.

As a rule, 8-10 explants were inoculated for one petri dish (6 cm in diameter) and incubated in a culture box at 22°C (night) to 25℃ (day) under 15 h. illumination (about 4000 Lux) produced by cool-white fluorescence [13]. The results were recorded after culturing for about 4 weeks:

3. Extraction and determination of free amino acids

Free amino acids in fresh samples were extracted with 80% alcohol according to Wang and Fang(14). The contents of free amino acids were determined with high performance amino acid analyzer, the system 6300 series, Beckman. The standard sample of Trp was freshly made up for every determination.

RESULTS

1. Floral gradient in different developmental stages

The floral gradients in different developmental stages had been determined by the method of TCLs culture. The results were shown in Tab 1.

The results in Tab 1 showed that the floral gradients were obvious in 3 different developmental stages (see the percentages in Tab 1: F-TCLs/T-TCLs). The TCLs with the competence for floral bud formation were mainly taken from the apical region, i. e. from stem region I to floral pedicels. However, the TCLs from the early flowering plants formed more floral buds than those from other 2 stages. The floral buds formed from pedicel TCLs or fruit stalk TCLs were leafless (Fig 1), but

most of the floral buds formed from floral branches and stem region I TCLs were, in fact, floral shoots with one or more leaves. In addition, fewer vegetative buds were formed from pedicel and fruit stalk TCLs (see V/TCLs in Tab 1).

2. Levels of free amino acids in different regions of flowering tobacco

The assaying results of free amino acids listed in Tab 2 revealed that the contents of free amino acids in apical parts of flowering tobacco plants were higher than those in basal parts, and the acropetal gradient of Pro in flowering tobacco stem could obviously be visible.

The Pro level reached about 2 mg/g fresh tissue in the apical zone of stem or flower zone. However, the contents of the other free amino acids (Val, Ile, Tyr, Arg and so on) were extremely low, even not detectable.

Tab 1. De novo floral bud differentiation from TCLs as influenced by the origins of explants*

Origins of TCLs		Total of TCLs	F-TCLs (%) T-TCLs	F/F-TCLs	V/TCLs	D-TCLs
	Pedicel	30	100	10.2	0.6	0
Early	Floral branch	45	89	14.1	9.4	0
flowering	stem I	54	0	0	13.9	0
stage	II	43	0	0	5.4	0
	III	31	0	0	5.4	10
	IV	40	0	0	1.6	33
	Pedicel	74	77	2.5	0	0
Full	Florsl branch	77	22	7.9	1.7	0
blooming	Stem I	61	10	3.5	0.5	0
stage	II	47	0	0	0.4	1
	III	40	0	0	0.3	13
	IV	48	0	0	2.0	43
	Fruit stalk	68	99	3.5	0.4	0
Fruiting	Fruit branch	70	93	6.5	0.6	0
stage	Stem I	80	10	3	3.5	3
	II	70	0	0	2.7	7
	III	60	0	0	2.7	33
	IV	72	0	0	3.2	44

* All TCLs were cultured on the basic medium + IBA 2 mg/L + 6-BA 0.2 mg/L.
F- TCLs: TCLs with regenerated flower buds.
T- TCLs: Total of TCLs cultured.
D- TCLs: TCLs died in culture.
F /F- TCLs: Average of regenerated flower buds on one F-TCLs.
V /TCLs: Average of regenerated vegetative buds on one TCLs (TCLs = T-TCLs - D-TCLs).
Stem I — IV: representing the different regions of tobacco stem from apex to base at an interval of about 8 nodes each.

Tab 2. The contents of 15 free amino acids in different parts of flowering tobacco*

Type of amino acids	The contents of free amino acids(μg/g fresh weight)					
	Flowering zone			Stem		
	Fruit stalk	Pedicel	Floral branch	Apical zone	Middle zone	Basal zone
Pro	2155	1616	2039.5	2146	1433	407.1
Trp	128.59	321.88	80.67	84.40	81.90	47.38
Aeg	nd	17.02△	17.40	33.96	nd	nd
Met	14.47	12.69	11.11	15.25	14.32	5.21
Asp	204.01	135.50	197.80	134.9	138.8	107.4
Glu	552.10	275.50	285.30	277.3	260.9	177.7
Gly	17.11	13.78	22.40	14.30	14.65	6.09
Ala	57.40	63.90	41.28	29.77	27.85	15.49
Val	nd	nd	nd	nd	nd	nd
He	nd	nd	nd	nd	9.57△	nd
Leu	31.76	20.30△	28.88	36.12	37.79	19.88
Tyr	22.73	nd	nd	nd	nd	nd
Phe	20.80△	32.04	32.19	27.9	20.80△	12.27△
His	24.97	25.04	13.80	20.51	19.85	10.39
Lys	13.99	20.53	11.91	21.08	15.67	17.24

*Except for fruit stalk, the other samples were all taken from flowering tobacco plants with only a few flowers blossomed.
The figures were the average of the 2 determinations, the mean error was 11.73% and the standard deviation was 9.7%.
nd: not detectable; △: 1 determination, the other sample not detectable.
Ash, Ser, Thr, Gin and Cys had not been determined in the present work.

3. Influence of exogenous amino acids on floral differentiation from pedicel segments

According to the results in Tab 2 and the known roles of amino acids in plant metabolism, 4 amino acids (Pro, Trp, Arg and Met) were chosen to be added into the No.120 medium (the basic medium + IA A 2 mg /L + 6-BA 0.2 mg/L) for testing their effects on flower bud formation. Tab 3 showed the results of this experiment.

It was evident from Tab 3 that 3 amino acids L-Pro (at 100, 1000 mg/L), D,L-Met and L-Arg (at all concentrations used) inhibited the floral bud formation and L-Pro 1 g/L was distinctly poisonous to explants (Fig 2). But, L-Trp could obviously enhance the floral bud formation from pedicel segments. Further researches revealed that L-Trp combining with 6-BA also promoted floral bud differentiation (Fig 3-4). The frequency of the pedicel segments regenerating floral buds reached 68.6%(48/70) on the medium (the basic medium + L-Trp 4 mg /L + 6-BA 2 mg /L). However, when only L- Trp was added into the basic medium, no floral bud neofor-

mation could be observed (Fig 3)

Tab 3. The influence of exogenous amino acids on floral bud differentiation from tobacco pedicel segments*

Type	Concentration(mg /L)	TPS	FPS /TPS(%)	MFBS
CK	0	125	72.8	10
L-Pro	10	156	82.7	18
	100	177	29.9	5
	1000	188	0	0
L-Arg	5	150	39.3	5
	25	150	33.3	5
	125	150	41.3	7
	5	130	71.5	17
	25	120	48.3	7
	125	130	24.6	9
L-Trp**	16	200	96.0	25

* The medium used here was No. 120 medium.

** The medium used in this experiment was the basic medium + IAA 1 mg/L+6-BA 0.3 mg/L. TPS-Total of pedicel segments cultured; FPS Amount of pedicel segments with regenerated floral buds; MFBS The most floral buds formed from a segment.

4. Effect of L-Trp and L- Pro on organ differentiation from vegetative explants

The results outlined above showed that L-Trp and lower concentration of L-Pro enhanced floral bud differentiation from the tobacco pedicel segments. Therefore, further research on the effect of L-Trp and L-Pro was carried out with explants from vegetative tobacco plants.

The stem TCLs from the region II of flowering tobacco plant (Early flowering stage, cf. Tab 1) and leaf pieces from vegetative tobacco plants were cultured on a series of media containing L-Trp + 6-BA or L-Pro + IAA + 6-BA. In all cultures, the TCLs and leaf pieces differentiated into adventitious vegetative buds, but no floral buds, and the higher concentration of L-Pro obviously inhibited the vegetative bud formation (Fig 5-6). Apparently, L-Trp or L-Pro was unable to induce floral bud formation from vegetative explants.

DISCUSSION

Plant gradient (e.g. Gradients of signals or of cellular traits) may be resulted from the changes occurring in the apical meristems, and the changes were maintained in the mature cells they produced[15]. Thus, it is possible to investigate the gradients of substances related to flowering through the floral gradient. For example, ABA has been found to be closely related to floral gradient in Torenia fournieri

in this way[16]. The results here showed that the floral gradient in flowering tobacco plant was obvious in different stages, although the frequencies of floral bud regeneration from TCLs varied with the developmental stages. The determination of free amino acids confirmed the Pro gradient in flowering tobacco stem and showed higher contents of free amino acids (cf. Tab 2) (e.g. pro, Trp, Ala, etc.) in flowering zone and stem apical zone. But gradient of tryptobane and its quantity were not as obvious and large as gradient of prolive Kutacek reported that tobacco tissues *in vitro* could utilize L-Trp to synthesize IAA via indolylpyruvate pathway[17], so the enhancement of floral bud formation by L-Trp could logically be explained. The explants from tobacco pedicel, whose Trp level was the highest among the tissues analyzed in flowering tobacco, had stronger competence for floral bud formation than that from other parts of the plant. But L-Trp could not induce flower buds from vegetative explants. Hence, according to the model of multifactorial control suggested by Bernier[18], L-Trp may be just only one of the factors related to floral bud differentiation.

Pro was usually considered as non-poisonous to plants[19]. In the present work, L-Pro at low concentration (10 rag/L) had a promoting, although mild, effect on floral bud neoformation. This promotion was also reported in the work with root explants of Cichorium on floral bud differentiation *in vitro*[10]. However, it is very difficult to explain the poisonous effect of L-Pro at high concentration (1 g/L) *in vitro,* considering the large accumulation of Pro *in vivo* (see Tab 2) and the high concentration of L-Pro (100 m*M*, about 11.5 g /L) used in the culture of maize[20]. This problem remains to be investigated.

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Fig 1. Floral buds without any leaves directly regenerated from Thin Cell Layers (TCLs) of pedicel, about X 6

Fig 2. Flower formation from pedicel segments were inhibited with the concentration of L-Pro increasing (from left to right, L-Pro: 10, 100, 1000 mg /L). X 0.6

Fig 3. Any combination of 6-B A with L-Trp promoted flower bud formation from pedicel segments. But, single 6-BA or L-Trp (except for 6-BA 0.4 mg /L few segments regenerated few floral buds on this medium), no floral bud neoformation had been observed. X 0.5

Fig 4. Floral buds neoformation from pedicel segments cultured on the basic medium plus 6-BA 2 mg/L and L-Trp 4 mg/L for 24 d. X 1.1

Fig 5. Vegetative buds differentiated from stem TCLs cultured on the basic medium plus 6-BA 0.4 mg/L and L-Trp 32 mg/L for 22 d. X 1.1

Fig 6. Influence of different concentrations of L-Pro on vegetative bud differentiation of leaf pieces [the medium: the basic medium + I AA 2 mg /L + 6-B A 0.2 mg/L + L-Pro 0, 10, 100, or 1000 mg /L (from left to right)]. X 0.5

Florl gradient and free amino acids in flowering tobacco