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SHORT COMMUNICATION

INDUCTION OF FEMALE ORGANS IN MALE FLOWERS OF *VITIS* SPECIES BY ZEATIN AND DIHYDROZEATIN*

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Abstract—Sixteen nucleic acid derivatives were scrutinized for induction of female organs in staminate plants of *Vitis* species. The results indicated that two naturally occurring cytokinins, zeatin and its dihydro derivative, markedly effected induction in addition to the synthetic compounds reported previously. N^6-Isopentenyladenine(6-(3-methyl-2-butenylamino)purine) was feebly active in only the limited species. The implications of the present results for the female organ induction are discussed.

INTRODUCTION

THE INDUCTION of female organs in staminate plants of *Vitis* by the application of a synthetic cytokinin, 6-benzylamino-9-(2-tetrahydropyranyl)-purine, has been reported.[1-3] In a further search for synthetic cytokinins which exhibit such sex conversion activity, 6-benzylamino-9-β-D-ribofuranosylpurine and 6-benzylaminopurine have been shown to possess this activity, while 6-benzylamino-9-β-D-glucopyranosylpurine, 6-benzylamino-9-β-D-ribopyranosylpurine, 6-allylaminopurine, 6-allylamino-9-β-D-ribofuranosylpurine have shown no activity. The compounds described above, however, are synthetic cytokinins and cannot be regarded as natural plant constituents, because they have not been shown to occur in living tissue. It is, therefore, of special interest to find out whether the naturally occurring cytokinin possesses induction activity or not. This paper reports the finding that the naturally occurring cytokinin, zeatin [6-(4-hydroxy-3-methyl-2-butenylamino)purine][4,5] and the dihydro derivative,[6] an enantiomeric pair, markedly effect the induction of female organs in staminate plants of several *Vitis* species

RESULTS AND DISCUSSION

Table 1 shows the induction activity of nucleic acid derivatives tested by the above assay. The clusters applied with zeatin, (±)-dihydrozeatin and 6-benzylamino-9-β-D-ribofuranosylpurine produced typical hermaphroditic flowers showing pistils with mucous on stigmas, upright stamens with functional pollen grains, and normal fruit set. Percentage of the sex-converted flowers to the total flowers treated in *V. thunbergii* and *V. coignetiae* are

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TABLE 1. FEMALE ORGAN INDUCTION ACTIVITY OF NUCLEIC ACID DERIVATIVES

Nucleic acid derivatives	Activity
6-Benzylamino-9-β-D-glucopyranosylpurine	−
6-Benzylamino-9-β-D-ribopyranosylpurine	−
6-Benzylamino-9-β-D-ribofuranosylpurine	+++
6-Benzylamino-9-(2-tetrahydropyranyl)purine	+++
6-(*o*-Chlorobenzylamino)purine	−
6-(*m*-Chlorobenzylamino)purine	−
6-(*p*-Chlorobenzylamino)purine	−
6-Allylaminopurine	−
6-Allylamino-9-β-D-ribofuranosylpurine	−
6-Isopentenylaminopurine	±
Zeatin	++
(±)-Dihydrozeatin	++
Kinetin	?
Inosine	±
5-Iododeoxyuridine	±
Adenine	−
D-Ribose	−

The activity of 6-benzylamino-9-β-D-ribofuranosylpurine was arbitrarily denoted by +++. The sign ++ refers to lower activity in respect of the percentage of the sex conversion occurred. The sign ± indicates the imperfect induction observed occasionally.

shown in Table 2. Imperfect induction, with only tiny stigmas, was occasionally observed in the clusters treated with inosine, 5-iododeoxyuridine and N^6-isopentenyladenine. No induction of female organs was observed with the other compounds.

It is worth noting that the structural modification of the ribofuranosyl moiety of the active cytokinin molecule, even the conversion to ribopyranosyl, causes inactivation as shown in Table 1. High activity of 6-benzylamino-9-(2-tetrahydropyranyl)purine may be explained by the instability of the glycosidic linkage.

In the previous paper,[3] synthetic cytokinins such as 6-benzylamino-9-(2-tetrahydropyranyl)purine, 6-benzylaminopurine and 6-benzylamino-9-β-D-ribofuranosylpurine were

TABLE 2. PERCENTAGE OF THE SEX CONVERTED FLOWERS TO THE TOTAL MALE FLOWERS EXERCISED

Chemical	No. of clusters	Pollen fertility (%)	Per cent* ♀	⚥	♂	Total flowers
In *V. thunbergii:*						
Control		99·1	0	0	100·0	
Zeatin	4	98·3	54·1	13·7	32·2	1124
Dihydrozeatin	2	98·0	10·3	61·2	28·5	263
6-BAR†	10	98·8	76·8	13·2	10·0	3130
In *V. coignetiae:*						
Control		99·1	0	0	100·0	
Zeatin	6	99·0	24·8	36·6	38·6	144
Dihydrozeatin	5	99·5	7·8	41·8	50·4	136
6-BAR†	21	99·6	21·0	42·2	36·8	622

* ♀ indicates the percentage of the flowers with complete female organs; ⚥ shows that of the flowers with incomplete female organs; ♂ represents that of the flowers with only male organs.

† 6-BAR stands for 6-benzylamino-9-β-D-ribofuranosylpurine.

shown to exhibit positive activity in *V. coignatiae*, *V. thunbergii*, *V. lincosemii*, *V. aestivalis*, *V. vulpina* and *V. labrusca*. These cytokinetic compounds, however, have not so far been shown to occur in living tissue. Zeatin and its dihydro derivative, found in the immature fruit of several plants,[4,6] and N^6-isopentenyladenine, found in serine and tyrosine transfer RNAs,[7,8] are the naturally occurring cytokinetic compounds. The occurrence of these compounds in *Vitis* species is not known at the present stage. The cytokinin activity (tobacco callus assay and chlorophyll retention test), however, has been detected in the sap of hermaphroditic grapevines collected in early spring.[9,10] Although the exact structure and function of such cytokinetic compounds in the grapevines remain to be investigated, the present finding that naturally occurring cytokinin induces female organs in male flowers appears to be significant for the elucidation of the mechanism of the induction. Because the results suggest that the morphogenesis of female organs in *Vitis* may be controlled by an endogenous zeatin like compound in a certain concentration during a critical stage of inflorescence development.

Sex ratio in progenies of self-pollinating sex-converted males was observed to be male/female = 3/1. This is consistent with the ratio expected on the basis of Oberle's hypothesis. Thus, the cytokinin in a certain concentration seems to function as a modifier of the expression of the major genes.

MATERIALS AND METHODS

Chemicals. 6-Benzylamino-9-β-D-glucopyranosylpurine, 6-benzylamino-9-β-D-ribopyranosylpurine, 6-benzylamino-9-β-D-ribofuranosylpurine were synthesized in one of the author's laboratory. The synthetic method was essentially similar to that previously described.[11–13] Details of the synthesis will be reported elsewhere. Zeatin was prepared according to the method described by Shaw *et al.*[14,15] N^6-Isopentenyladenine was generously donated by Dr. T. Fujii of Kanazawa University, and (±)-dihydrozeatin was a gift from Dr. K. Koshimidzu. 6-Allylaminopurine and the 9-β-D-ribofuranoside were obtained from commercial sources.

Assay of the female organ induction activity. Vitis species used in this experiment included *V. thunbergii* Makino var. *typica*, *V. coignetiae* Pulliat, *V. lincecomii* Buckel, *V. labrusca* L., *V. vulpina* L., *V. rupestris* Scheele. Each species shows segregation for male and female individuals under normal populations.

The chemicals were applied to clusters by dipping for approximately 20 sec in aqueous solutions ranging in concentration from 250 ppm to 1000 ppm. Airol-OP at 0·1 per cent was added to the solutions as a wetting agent. Applications were made 1–40 days before anthesis. Morphological changes of the clusters treated with chemicals were observed periodically during development. Matured berries were harvested and the seeds obtained from treated clusters were planted to determine the germination rate and sex ratio in progenies of self-pollinating sex-converted males.

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