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Anther-specific Production of Antimicrobial Tuliposide B in Tulips

Kazuaki Shoji [1]*, Makoto Ubukata [2], Kazumi Momonoi [1], Toshiaki Tsuji [3] and Takashi Morimatsu [1]

[1] *Agricultural Experiment Station, Toyama Agricultural Research Center, Yoshioka, Toyama 939-8153*

[2] *Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589*

[3] *Vegetables and Ornamental Plants Experiment Station, Toyama Agricultural Research Center, Goromaru, Tonami 939-1327*

Summary

Strong antimicrobial activity was observed in water extracts of tulip anthers. Purification by column chromatography and structural analysis by various methods, such as liquid chromatography-mass spectrometry (LC-MS), ^{1}H-, and ^{13}C- nuclear magnetic resonance (NMR) revealed that the active compound was 6-tuliposide B (6-*O*-((*S*)-4', 5'-dihydroxy-2'-methylenebutyryl)-D-glucopyranose). The antimicrobial susceptibility test revealed that it showed a strong growth inhibition against Gram-positive, Gram-negative bacteria and certain fungicide tolerant strains except for a yeast. Among the 22 cultivars (*Tulipa gesneriana* L.) and 15 *Tulipa* species, antimicrobial activities of the anthers from cultivars were stronger than those of the wild species. These production abilities of 6-tuliposide B in anthers were not related to pollen fertility. During anther development, the production of 6-tuliposide B was confined for a short period of approximately 7 to 12 days before flowering. Hence, it appeared that the 6-tuliposide B in anthers was produced in a tissue- and stage-specific manner with higher 6-tuliposide B accumulation than that observed in other tissues that produce both 6-tuliposides A and B. These results suggest that a novel defense strategy evolved in tulips to protect pollens from bacterial pollution in the reproductive process by producing an anther-specific 6-tuliposide B.

Key Words: anther, antibiotics, phytoanticipin, tulip (*Tulipa gesneriana* L.), tuliposide.

Introduction

Higher plants, confronted with a large number of microorganisms, produce a variety of antibiotics for protection against these microbial pathogens. Phytoanticipins are antibiotics produced by plants during the course of normal plant development and are distinct from phytoalexins, which are synthesized in response to pathogen attack (Paxton, 1980, 1981; VanEtten et al., 1994).

Tulip cultivars synthesize antimicrobial substances in flowers, stems, leaves, and bulbs (Bergman and Beijersbergen, 1968; Bergman et al., 1967). These substances are identified as glycosides, 6-tuliposide A (6-*O*-(5'-hydroxy-2'-methylenebutyryl)-D-glucopyranose) and 6-tuliposide B (6-*O*-((*S*)-4', 5'-dihydroxy-2'-methylenebutyryl)-D-glucopyranose), and their lactonized aglycones, tulipalin A (1-meth-ylene-γ-butyrolactone) and tulipalin B (4(S)-hydroxy-1-methylene-γ-butyrolactone) (Christensen and Kristiansen, 1999; Tschesche et al., 1969). They are also widely distributed in the family, *Liliaceae* (Slob, 1973; Slob et al., 1975); 1-tuliposide A (1-*O*-(5'-hydroxy-2'-methylenebutyryl)-β-D-glucopyranoside) is also produced by some *Tulipa* species (Christensen and Kristiansen, 1999). In particular, tuliposide A and tulipalin A produced by *Tulipa* and *Alstroemeria* are known to cause allergic contact dermatitis (Santucci et al., 1985; Slob, 1973). On the other hand, tuliposide B is non-allergenic (Hausen et al., 1983; Slob et al., 1975). It is considered that a methylene group in the α position is responsible for the allergenic activity of tuliposide A and tulipalin A (Santucci et al., 1985). Another tuliposide, tuliposides D, has been isolated from *Alstroemeriaceae* (Christensen and Kristiansen, 1995b). Recently, tuliposide D and F were also found in *Tulipa turkestanica* and *Tulipa sylvestris* (Christensen, 1999). Hence, unique compounds have evolved in tulip and alstroemeria that play an important role in disease resistance (Bergman and Beijersbergen, 1968; Bergman et al., 1967; Hutchinson, 1974).

Much attention has been paid to the isolation of tuliposides and tulipalins (Beijersbergen and Lemmers, 1972; Bergman, 1966; Bergman et al., 1967), derma tologic studies on their allergenic properties (Christensen and Kristiansen, 1995a, b, 1999; Hausen et al., 1983; Santucci et al., 1985), chemical synthesis (Bestmann and Schmidt, 1987; Henin and Pete, 1983; Hutchinson, 1974; Tanaka and Yamashita, 1980), and antimicrobial

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* Corresponding author (E-mail: shoji@agri.pref.toyama.jp).

activities (Bergman, 1966; Bergman and Beijersbegen, 1968; Langenfeld, 1970; Schönbeck and Schroeder, 1972; Schroeder, 1972). However, few studies have been carried out on the biological properties of tuliposides and tulipalins, as to their tissue-specificity and relationship to the growth process of tulip plants.

In this paper, we report the production and accumulation of an anther-specific antimicrobial substance during the process of flower development. Accumulation of this antimicrobial substance may contribute to the success of the reproductive process because it coats the pollen which deters pathogens and serves as an excellent defense strategy.

Materials and Methods

Plant and microorganisms

Tulip cultivars and *Tulipa* species were grown in a field in Toyama Agricultural Research Center, and their bulbs were propagated each year. Anthers and other tissues used in the preparation of antimicrobial substances were stored at -20℃ prior to the experiment.

To analyze the antimicrobial activity, the following bacterial and fungal strains were used: *Escherichia coli* IFO3972, Gram(G)(-); *Salmonella enteritidis* IFO3313, G(-); *Pseudomonas aeruginosa* IFO13275, G(-); *Staphylococcus aureus* IFO13276, G(+); *Bacillus subtilis* IFO3007, G(+); *Candida albicans* IFO1594. The fungicide tolerant strains, *Pseudomonas glume* T12119 (oxolinic acid resistant) and *Pseudomonas avenae* T9020 (kasugamycin and oxsolinic acid resistant) that were isolated from rice plants grown in Toyama Agricultural Research Center were also used.

Extraction, purification, and identification of the antimicrobial substance

The antimicrobial substance was extracted from frozen anthers that were collected immediately after blooming without separating the pollens. The extraction was carried out with H_2O for 1 h on ice. The water-soluble extract was centrifuged at 27000 × g for 15 min at 4℃ and freeze-dried after filtering through a cellulose acetate membrane (0.2 μm).

Gel-filtration chromatography of the extracts was performed by using a fast protein liquid chromatography (FPLC) system (Amersham Bioscience Corp., USA) and a Superdex^{TM} Peptide HR 10/30 column (Amersham Bioscience Corp., USA) with a flow rate of 0.9 mL·min^{-1} of H_2O and detection at 280 nm. The eluent that was fractionated for 240 min at intervals of 1.5 min (1.35 mL) resulted in 160 fractions. Following the gel-filtration column chromatography, 10 μL of each fraction was applied on a paper disc (ϕ 8 mm) to carry out the antimicrobial bioassay. Further purification and quantitative analysis were carried out by high-performance liquid chromatography (HPLC, model Alliance 2695, Waters Corp., USA) as described by Christensen and Kristiansen (1999).

Identification of antimicrobial substance was performed by using a LC-MS system (model JMS-T100LC/Agilent-1100. ESI(+), JEOL Co., Ltd., Tokyo) under the following conditions; column: Mightysil ODS 2.0 mm ID × 50 mm (Kanto Chemical Co., Inc., Tokyo), flow rate: 0.2 mL·min^{-1}, eluate: H_2O for 5 min, and the NMR spectra were measured in CD_3OD by using a JEOL JNMLA400 spectrometer at room temperature, 2D NMR spectra including HMBC spectrum of the active substance and recorded on a Bruker AMX-500 spectrometer (Bruker Biospin Corp., Germany).

Bioassay for antimicrobial activity

Antimicrobial activity was analyzed by using an assay plate that was prepared as follows; 3 mL of H soft agar medium (10 g·L^{-1} bacto tryptone, 8 g·L^{-1} NaCl, and 8 g·L^{-1} bacto agar). This medium that was autoclaved and stored at 60℃ prior to use was mixed with 70 μL of *E. coli* bacterium grown to OD_{660} = 0.5 in liquid 2YT medium (16 g·L^{-1} bacto tryptone, 10 g·L^{-1} yeast extract, and 5 g·L^{-1} NaCl), and spread immediately on the H agar (12 g·L^{-1} agar) medium plate (ϕ 9 cm). For the assay, an anther that harbored the pollens or a paper disc that contained an anther extract was placed on the plate, and antimicrobial activity was determined by measuring the inhibitory zone (which was translucent compared to the area of the bacterial growth) after incubating at 30℃ for 24 h. To compare activity among cultivars and/or species, water extracts of the anthers were applied on paper discs, which were then incubated on assay plates as above. Extraction of the antimicrobial from the anthers was carried out as follows: 1 g of anther was extracted with 10 mL of ice water. The extraction was repeated twice and the two extracts were combined and freeze-dried. The crude extract was then dissolved in 1 mL of water, and 10 μL was applied onto a paper disc. Antimicrobial activity was measured by estimating the diameter of bacterial growth inhibition zone after incubation for 24 h at 30℃.

Inhibitory activity of antimicrobial substance

The minimum inhibitory concentration (MIC) of antimicrobial substance on the growth of microorganisms was determined by the standard dilution method of using bacterial and fungal strains cultured in SCD medium (Nihon Pharmaceutical Co., Ltd., Tokyo) at 30℃ for 24 h.

Detection of pollen fertility

Pollen fertility was determined by the fluorescein diacetate (FDA) method (Eady et al., 1995) under a fluorescence microscope (model AHBS3, Olympus Co., Ltd., Tokyo). Fertile pollen was distinguished by the yellow-green light fluorescence emitted upon UV irradiation.

Analysis of 6-tuliposide A

6-Tuliposide A in tissues of tulip cultivars was identified by the method of Christensen and Kristiansen (1999) from the result of retention time of HPLC analysis and the measurement of molecular weight of precursor and/or product ions by using LC-MS-MS system (model Q Trap/Agilent-1100. ESI(+), Applied Biosystems Japan Co., Ltd., Tokyo) under the following conditions; column: Mightysil ODS 2.0 mm ID × 50 mm (Kanto Chemical Co., Inc., Tokyo), flow rate: 0.2 mL·min^{-1}, eluate: H_2O for 10 min. 6-Tuliposide A was also purified from petals and quantified for antimicrobial substances as follows: 10 g each of the tissues from petals, pistils, anthers, leaves, stems, bulb scales, and roots were homogenized in 100 mL of water. After centrifugation at 27000 × g for 10 min, the supernatants were filtered through a cellulose acetate membrane (0.2 μm) and quantitatively analyzed by HPLC.

Results and Discussion

Antimicrobial substance from anthers

Tulip anthers showed strong antimicrobial activity on an assay plate as indicated by the translucent growth inhibition zones around them (Fig. 1). To identify the active component, 6.5 g of the frozen sample from 'Murasakizuisho' was water extracted, dehydrated and the resulting 0.83 g crude extract was separated by gel-filtration column chromatography. A 0.5 mL aliquot of the extract was dissolved in 2 mL water; the active fractions that were eluted at a retention time between 10-20 min were collected (Fig. 2) and assayed.

The LC-MS measurement of the active fraction showed 2 peaks at 1.4 min and 1.9 min. These peaks were interchangeable, and the fragment patterns of the MS spectra of the peaks were superimposable, m/z 317 $(M+Na)^+$, m/z 312 $(M+NH_4)^+$, m/z 295 $(M+H)^+$, m/z 277 $(M-H_2O)^+$, m/z 115 $(M-C_6H_{11}O_6)^+$, m/z 97 $(M-C_6H_{11}O_6-H_2O)^+$ (Fig. 3). The MS fragment patterns suggested that the two peaks belonged to α and β 6-*O*-

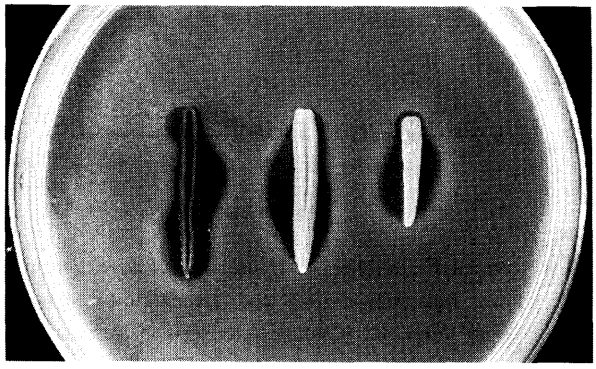

Fig. 1. Antimicrobial activity of anthers. Translucent parts surrounding the anthers indicate bacterial growth inhibition. Anthers from 'Halcro' (left), 'Dordogne', and 'Mirella' (right).

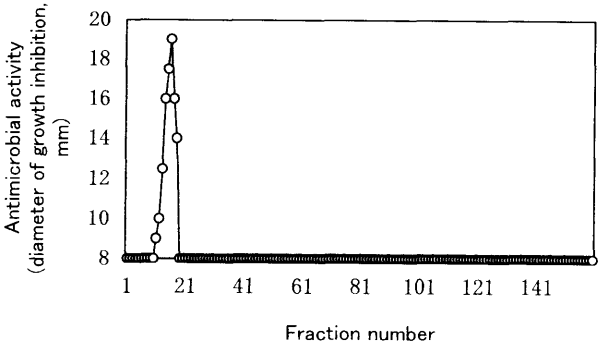

Fig. 2. Antimicrobial activity of fractions eluted from the gel-filtration chromatography column. The activity was indicated as the diameter of the bacterial growth inhibition zone by the paper disc method.

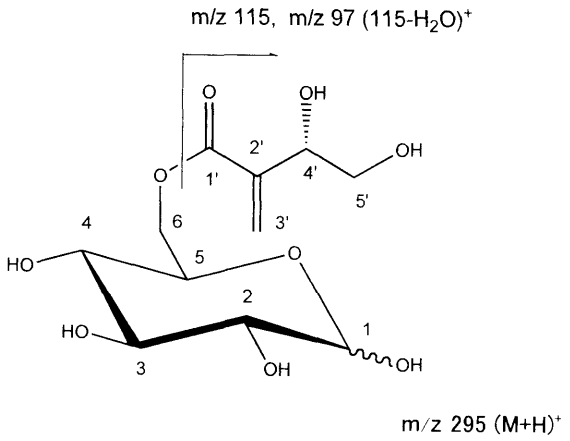

Fig. 3. Structure of 6-tuliposide B and its MS fragmentation. The pseudomolecular ion $(M+H)^+$ is m/z 295 and its fragment ions, m/z 115 $(M-C_6H_{11}O_6)^+$ and m/z 97 $(M-C_6H_{11}O_6-H_2O)^+$ indicate the presence of the aglycon of tuliposide B.

((*S*)-4', 5'-dihydroxy-2'-methylenebutyryl)-D-glucopyranose. ^{1}H-NMR spectrum of this mixture in CD_3OD showed the characteristic anomeric protons at 5.09 ppm (α, d, J = 3.6 Hz) and 4.5 ppm (β, d, J = 7.3 Hz) and protons of aglycon (C-3' proton, 5.99, and 6.35 ppm, each brs; C-4' proton, 4.56 ppm, m; C-5' proton, 3.44 and 3.72 ppm, overlapping). HMBC spectrum of the compound indicated the C-H long range coupling between C-6 methylene protons at 4.25 ppm (α anomer, dd, J = 6, 12 Hz) and 4.46 ppm (α anomer, dd, 2.8, 12 Hz), and 4.26 ppm (β anomer, dd, 6, 12 Hz) and 4.50 ppm (β anomer, dd, 2.8, 12 Hz) and between C-1' carbons at 167.4 and 167.3 ppm (α and β anomers). The entire ^{1}H- and ^{13}C-NMR spectral data of the mixture agreed with that of the reported value of tuliposide B (Christensen et al., 1999). Although sucrose was detected in this active fraction, 1-tuliposide B or other related compounds were not.

Anther-specificity of 6-tuliposide B

Tulip cultivars are known to produce both 6-tulipo-

sides A and B (Christensen and Kristiansen, 1999). To confirm the specificity of anthers with regard to tuliposide production, other tissues including petals, pistils, leaves, stems, bulb scales, and roots which were isolated immediately after blooming were extracted and assayed as above. The results showed that both 6-tuliposides A and 6-tuliposide B peaks were present in the petals as well as in other tissues. However, the analysis revealed that the anthers contained 6-tuliposide B; 6-tuliposide A was undetectable (Table 1). These results not only support the anther-specific production of 6-tuliposide B but also revealed the abundant accumulation of this compound in the anthers.

Susceptibility for tuliposide B

1-Tuliposide B is known to act against *B. subtilis* and *Pythium debaryanum* (Tschesche et al., 1969); however, the activity on bacteria or fungi of 6-tuliposide B has not been examined in detail. Therefore, the concentration of 6-tuliposide B which inhibited the growth of various bacterial and yeast strains (Table 2) showed that all strains with the exception of *C. albicans* were sensitive to 6-tuliposide B, regardless of their Gram nature and their fungicide-resistant traits. Experiments performed by Langenfeld (1970) suggested that tuliposide A and B chemically react with the SH-group of amino acids such as L-cysteine. These results on the inactivation of many enzymes in vivo caused a growth inhibition except in *C. albicans*, a yeast, which showed no sensitivity to 6-tuliposide B. In considering the working mechanism of tuliposides A and B, the growth of this strain, when treated with the antimicrobial, does not appear to be possible. However, a similar phenomenon was also observed in some other fungi (data not shown). This resistance may be the result of the difference in permeability of the cell wall or the ability of the yeast to degrade 6-tuliposide B. Currently, we are investigating these possibilities by using the pathogens that affect tulips and some other microorganisms.

Table 1. Tuliposide contents in tissues of 'Murasakizuisho'.

Tissue	Concentration (%)[z]	
	6-Tuliposide A	6-Tuliposide B
Petal	0.41	0.35
Pistil	0.25	0.42
Anther	n.d.[y]	2.31
Leaf	0.21	0.12
Stem	0.17	0.11
Bulb scale	0.25	0.11
Root	0.23	0.13

[z] Concentration are given in % fresh weight.
[y] Not detectable.

Table 2. Minimum inhibitory concentration (MIC) of 6-tuliposide B on the growth of microorganisms.

Microorganisms	MIC ($mg \cdot L^{-1}$)
Escherichia coli	15 ± 0.8[z]
Salmonella enteritidis	18 ± 0.5
Pseudomonas aeruginosa	14 ± 0.8
Pseudomonas glume[y]	15 ± 0.9
Pseudomonas avenae[x]	14 ± 0.8
Staphylococcus aureus	15 ± 0.6
Bacillus subtilis	20 ± 1.1
Candida albicans	–[w]

[z] Mean ± SE (n=3).
[y] Oxsolinic acid-resistant strain.
[x] Kasugamycin- and oxsolinic acid-resistant strain.
[w] No effect on the growth.

Developmental production of 6-tuliposide B

To understand the production and accumulation of 6-tuliposide B by anthers, antimicrobial activities were examined throughout the development of the flower

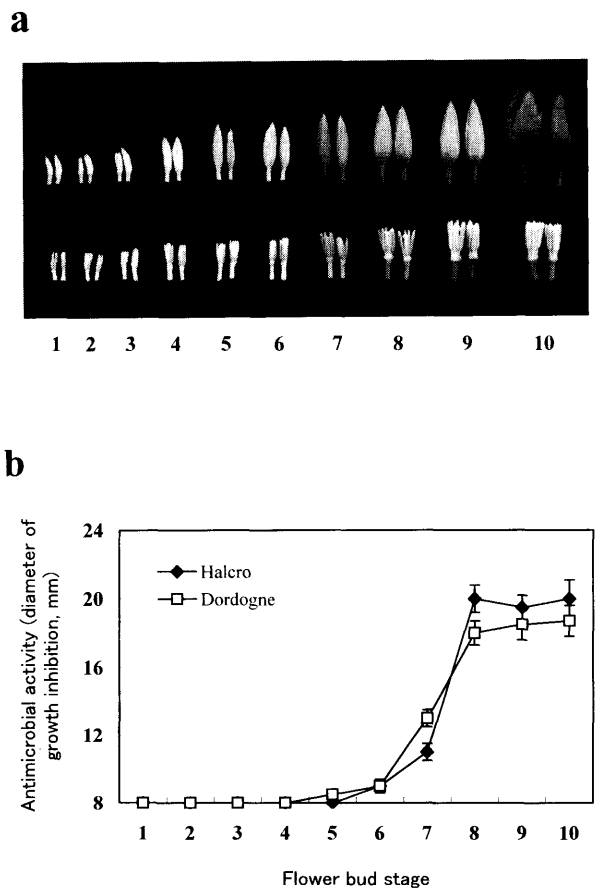

Fig. 4. Production of tuliposide B during floral development. a: Flower bud (upper row) and anther (lower row) in 'Halcro' from bulb planting to anthesis, b: Antimicrobial activity of anthers in the process of anther development in 'Halcro' and 'Dordogne'. 1, 148 days before flowering (DBF); 2, 77 DBF; 3, 45 DBF; 4, 25 DBF; 5, 14 DBF; 6, 12 DBF; 7, 10 DBF; 8, 7 DBF; 9, 4 DBF; 10, anthesis. Vertical bars represent SE of 5 replications.

Table 3. Antimicrobial activities and pollen fertilities in cultivars and Tulipa species.

Cultivar / Species	Antimicrobial activity (mm)	Pollen fertility (%)
Cultivars (classification group[z])		
Albino (T)	18.5 ± 1.2[y]	22.7
Bartigon (SL)	18.8 ± 1.1	29.3
Cashmir (SL)	20.3 ± 0.8	62.5
City of Vancouver (SL)	20.5 ± 1.4	7.1
Don Quichotte (T)	20.3 ± 0.8	45.7
Dordogne (SL)	19.2 ± 0.9	42.9
Halcro (SL)	20.8 ± 1.1	30.3
Hocus Pocus (SL)	20.3 ± 1.4	0
Kinkazan (SL)	18.5 ± 1.8	33.6
King Solomon (SL)	19.0 ± 0.3	58.5
Meissner Porzellan (T)	19.0 ± 1.3	83.3
Mirella (T)	20.5 ± 0.9	1.7
Murasakizuisho (T)	19.0 ± 1.2	98.0
Prince of Wales (SL)	19.8 ± 1.1	87.8
Red Advance (SL)	19.8 ± 1.8	98.5
Red Pitt (SL)	20.0 ± 1.5	81.3
Renown (SL)	19.5 ± 1.0	56.9
Rosabella (SL)	18.5 ± 1.1	52.4
Scotch Lassie (SL)	20.3 ± 0.4	95.7
Shanghai (SL)	19.3 ± 0.6	82.9
Winter Gold (SE)	17.0 ± 1.9	51.9
Yellow Emperor (SL)	18.0 ± 0.9	87.1
Tulipa species		
T. batalinii	13.5 ± 1.1	4.6
T. chrysanta	13.0 ± 1.3	93.2
T. clusiana	16.0 ± 0.9	77.8
T. hageri	14.3 ± 1.6	54.6
T. kolpakowskiana	12.0 ± 0.4	54.2
T. linifolia	19.3 ± 1.7	82.7
T. maximowizii	17.3 ± 2.1	91.7
T. orphanidea	13.8 ± 0.9	10.7
T. pulchella	15.0 ± 1.1	93.5
T. praestans	13.5 ± 1.7	92.2
T. sosnowskyi	16.5 ± 1.4	93.5
T. tarda	n.d[x]	97.4
T. urumiensis	10.3 ± 0.8	98.0
T. vvedenskyi	16.8 ± 1.7	98.1
T. wilsoniana	n.d	94.8

[z] Classification groups in cultivars indicate as follows: SL, Single late group; SE, Single early group; T, Triumph group.
[y] Mean ± SE (n=3).
[x] Not detectable.

bud. In tulips, flower buds start to form in the bulbs during the storage period in the summer after their harvest. The tapetal tissue, lining the anther locule, provides nutrients for the maturing pollens and deposits special chemicals, such as flavonol, anthocyanin, and carotenoid, onto the pollen surface so as to form a coating in tulips (Wiermann, 1979). We divided the anther developmental processes in 10 stages (Fig. 4a)

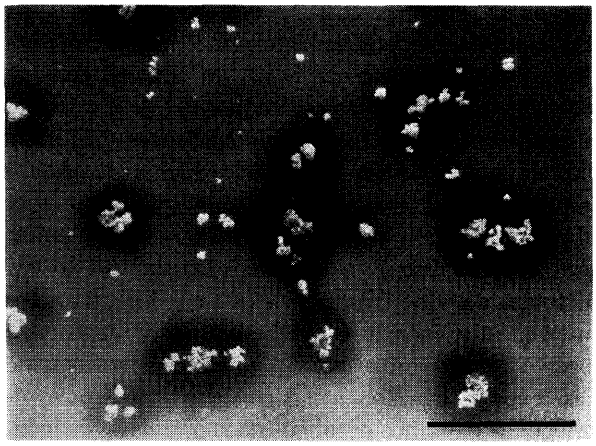

Fig. 5. Antimicrobial activity of mature pollens from 'Murasakizuisho' on the antimicrobial assay plate. Translucent parts surrounding the pollen clusters indicate bacterial growth inhibition by 6-tuliposide B coated on the surface of pollens. Bar indicates 1.0 cm.

based on the phases of tulip growth from planting of bulbs to anthesis. The antimicrobial activities that were examined in each stage (Fig. 4b) showed that the activity began approximately 12 days before anthesis (stage 6) and gradually increased to reach a maximum 7 days before anthesis (stage 8). This antimicrobial production ability was not related to pollen fertility because cultivars, such as 'Mirella' or 'Hocus Pocus', that produce mostly sterile pollen contain a relatively high content of 6-tuliposide B (Table 3). Thus, 6-tuliposide B in anthers was produced for a short period in a stage-specific manner. Similar to other substances such as flavonols or anthocyanins, the surfaces of pollens were coated with tuliposide B that protect the pollen from the bacterial contamination during pollination (Fig. 5). We also examined antimicrobial activity of anthers from alstroemeria which is known to produce 6-tuliposide A and tulipalin A, the causative agent for contact dermatitis; however, we could not detect any activity in the anthers (data not shown). Hence, the phenomenon of anther-specific production and accumulation of tuliposide is considered to be restricted to tulips.

Comparison of activities in cultivars and Tulipa species

More than 5600 cultivars are registered under tulips; 2600 cultivars are in cultivation (Zandbergen, 1996). To compare the productivity of anther-specific 6-tuliposide B, 22 cultivars and 15 *Tulipa* species were analyzed. The results obtained indicated that the activities varied; extracts of named cultivars tended to possess stronger activities than the species (Table 3). This phenomenon is considered to be the result of selecting for pathogenic tolerance in breeding processes, or the taxonomical differences of species between the *T. gesneriana* and the other species. Recently, the antimicrobial peptides, *Tu*-AMP1 and *Tu*-AMP2 that possess chitin binding properties were isolated from

tulip bulbs (Fujimura et al., 2004). These peptides may also contribute to pathogenic tolerance in addition to the tuliposides. On the other hand, no activity could be detected in some species. Slob (1973) investigated tuliposides A and B of flowers and leaves in liliflorae and reported that some tulip species such as *T. tarda*, *T. praestans*, and *T. greigii* contain tuliposide A but do not tuliposide B in any tissues. Our anther extracts obtained from *T. tarda* and *T. wilsoniana* also showed no antimicrobial activity. These species may lack the genes involved in tuliposide B synthesis. In *T. praestans*, we observed antimicrobial activity in anther extracts which was reported by Slob and Varekamp (1977) who described the existence of tuliposide B in tissues, including the flower of the same species. Therefore, the production of 6-tuliposide B in anthers of *T. praestans* is undoubted.

As the evolutionary production of 6-tuliposide B by tulip anther is intriguing, we are focusing on the entomophilous trait of the tulip flower. In several tulip cultivars, basal petal colors and/or anthers differ from the other parts; the colors are believed to attract insects more effectively. Recently, Kim et al. (1998, 1999) reported high insecticidal activity of tulipalin A, derived from 6-tuliposide A of thunberg spiraea (*Spiraea thunbergii*) leaves against *Thrips palmi* (Thysanoptera). On the other hand, the insecticidal activity of tulipalin B has not yet been reported. If tulipalin B has no insecticidal activity, and produce no allergic symptoms on animals, it may explain why anthers produce only the 6-tuliposide B. We postulate that tulips may have evolved a novel strategy for molecular mechanisms for the anther-specific production of 6-tuliposide B; it aids in pollination by preventing the contamination of pollen by pathogens and protecting the insects. Hence, we conclude that the production and accumulation of 6-tuliposide B in anthers and its coating of pollen are a novel defense strategy to aid the reproductive process of tulips.

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チューリップにおける抗菌性チューリッポシドBの葯特異的生成

荘司和明[1]・生方　信[2]・桃井千巳[1]・辻　俊明[3]・森松　敬[1]

[1]富山県農業技術センター農業試験場　939 8153　富山市吉岡

[2]北海道大学大学院農学研究科　060 0808　札幌市北区

[3]富山県農業技術センター野菜花き試験場　939 1327　砺波市五郎丸

摘　要

チューリップの葯における水抽出物に強い抗菌活性を見出した．カラムクロマトグラフィーによる精製とLC MSおよび^{1}H，^{13}C NMRによる構造解析の結果，活性本体は6 チューリッポシドB (6-*O*-((*S*)-4', 5'-dihydroxy-2'-methylenebutyryl)-D-glucopyranose)であることが明らかになった．抗菌性の感受性検定の結果，6-チューリッポシドBはグラム陽性およびグラム陰性とは無関係に細菌類に対して広く強い殺菌力を示し，農薬耐性を持つ細菌類に対しても同様の効果を示したが，酵母菌類には効果が見られなかった．チューリップ22品種と*Tulipa*属15種の葯を用いて抗菌活性を比較したところ，野生種に比べ栽培品種でより強い活性が示された．これらの活性は花粉稔性とは無関係であった．さらに，葯の発達過程を球根の移植期から開花期まで10段階に分けて抗菌活性を調べた結果，チューリッポシドBの生成は，開花12日前から始まり7日前には最大に達することが明らかになった．すなわち，葯以外の組織ではチューリッポシドAとBの両方が作られるのに対し，葯ではチューリッポシドBだけが組織特異的かつ生育時期特異的に生成され高濃度に蓄積していることが明らかになった．これらの結果から，チューリップは生殖過程においてチューリッポシドBを葯特異的に生産し，細菌類の汚染から花粉を守るという独特の防御機構を発達させていることが示唆された．