Plant Physiol. (1970) 46, 497–499

Cytokinin Activity in Rose Petals and Its Relation to Senescence[1]

Received for publication September 29, 1969

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ABSTRACT

Cytokinin activity in young rose petals was higher than in old ones. The content of endogenous cytokinins in petals of a short-lived variety (Golden Wave) was lower than in a long-lived variety (Lovita). Application of the cytokinin, N^6-benzyladenine, increased longevity of the short-lived variety. This strengthens the view that cytokinins participate in the endogenous regulation of senescence in rose petals.

Cytokinins and other growth regulators have been shown to retard senescence of plants. Research has mainly dealt with the effect of exogenous application of cytokinins on senescence of various detached plant organs, including flowers (1, 2, 5, 10, 16); their effect upon the metabolism of protein and nucleic acid (6, 9); respiration; and photosynthesis (2, 3). Our knowledge about natural growth regulators in relation to senescence is still very meager. In order to claim that a particular hormone participates in the regulation of naturally occurring processes, it is essential to show its correlative change with the process being studied, as well as its influence via exogenous applications. We selected rose petals as a model for studying senescence because the complete development cycle of attached as well as detached petals is rather short. In our study we attempted to follow the changes in the level of phytohormones during maturation and senescence and to compare the hormonal activities in petals of a long-lived variety with those of a short-lived one.

MATERIALS AND METHODS

Rose petals of two varieties were used in this study: Golden Wave (Dr. Verhage), a short-lived variety (3–5 days of vase life), and Lovita, a long-lived variety (8–12 days of vase life). Flowers grown in a greenhouse were allowed to develop and age on the plant. They were cut at two stages: (*a*) young stage, tight bud, half the sepals are released, about half a day before commercial harvest; (*b*) old stage, initial senescent phase, flowers fully open, showing first signs of color fading. Generally, Golden Wave flowers required 7 days to pass from the first to the second stage, while 8.5 days elapsed in the case of Lovita. From this initial senescent stage to petal drop, an additional period elapsed of 3 and 7 days in the Golden Wave and Lovita varieties, respectively. Petals were harvested, immediately frozen in liquid air, and lyophilized. Four grams dry weight of lyophilized petals were covered with 80% methanol and homogenized in a Waring Blendor. The homogenate was shaken for 14 hr at 4 C and filtered. The residue was shaken with methanol for an additional 1 hr and filtered. The combined filtrate was evaporated to the water phase under reduced pressure, adjusted to pH 2.5 with HCl, and extracted twice with petroleum-ether and then four times with ethyl acetate. (In preliminary experiments no cytokinin activity was detected in the petroleum-ether and the ethyl acetate fractions.) The aqueous fraction was adjusted to pH 3.3, 15 ml of 10% (w/v) $AgNO_3$ was added, and the mixture was allowed to stand at 4 C for 12 hr. The precipitate was centrifuged, washed with cold $AgNO_3$ solution, and shaken with 0.2 N HCl for 3 hr at 45 C. The supernatant was concentrated under reduced pressure and assayed by the soybean callus growth bioassay. Three tests were carried out with Golden Wave and one with Lovita (Table I). In all the tests each treatment included 10 to 15 replicates.

To study the relationship between the varietal difference in flower longevity and natural occurring cytokinins, 18 g fresh weight of petals from young flowers of Lovita and Golden Wave were extracted and purified as above. The concentrate was made up to 3.5 ml with water, and 0.1 ml was applied to 4.5-cm wide strips of Whatman 3MM paper. Thirty paper strips were prepared from each extract and were developed by ascending chromatography with isopropanol-ammonium hydroxide-water (8:1:1, v/v) as a solvent. After drying, each paper strip was cut into 10 equal zones. Two corresponding zones from the paper strips served as one replicate, so that 15 replicates were used for each R_F zone. Soybean callus pieces (about 7–10 mg) were placed individually on agar in a test tube (23 × 100 mm) containing the two zone papers and 12 ml of Miller's medium (11). The tissue cultures were kept in darkness at 27 C and harvested after 4 to 5 weeks. Kinetin equivalents were calculated from standard curves obtained separately for each test.

The effect of external application of cytokinin on longevity of roses was studied with the short-lived variety, Golden Wave. Flowers were cut from a commercial greenhouse at the commercial harvest stage, transferred to the laboratory, and treated about 4 hr after harvest. N^6-Benzyladenine was applied in various concentrations as a 15 min dip of the flower bud. In another experiment BA[2] was applied to cut flowers aged in water at 5 C for 5 days. After treatment flowers were placed in tap water at room temperature. Four replicates (vases) of 3 flowers each (12 flowers) were used for each treatment. Longevity was determined as the number of days to petal drop.

RESULTS AND DISCUSSION

The results of four experiments (Table I) showed that cytokinin activity in petals declined considerably at the onset of senescence. The flowers aged on the plant until the initial senescent stage

[1] Contribution from the Volcani Institute of Agricultural Research, 1970 Series 1486-E.

[2] Abbreviation: BA: N^6-benzyladenine.

Table I. *Cytokinin Activity Assayed by the Soybean Callus Grow'h in Petals from Young and Old Rose Flowers*

Experiment	Variety	Young Petals		Old Petals	
		Callus	Kinetin equivalent[1]	Callus	Kinetin equivalent[1]
		mg fresh wt		*mg fresh wt*	
1	Golden Wave	235	0.045	101	0.022
2	Golden Wave	549	0.210	105	0.023
3	Golden Wave	622	0.302	154	0.036
4	Lovita	1548	0.518	515	0.028

[1] Kinetin equivalent in mg/100 g dry weight, calculated from standard curves obtained separately for each experiment.

Table II. *Cytokinin Activity Assayed by the Soybean Callus in Petals of Young Rose Flowers of Lovita and Growth Golden Wave Varieties*

Experiment	Golden Wave		Lovita	
	Callus	Kinetin equivalent[1]	Callus	Kinetin equivalent[1]
	mg fresh wt		*mg fresh wt*	
1	208	0.014	531	0.123
2	620	0.240	785	0.556
3	1068	0.044	1548	0.518

[1] Kinetin equivalent in mg/100 g dry weight, calculated from standard curves obtained separately for each experiment.

was reached. However, although the petals showed initial color fading at this stage, they were fully turgid and alive.

In other experiments it was found (Table II) that total cytokinins content in petals from young flowers of the long-lived variety Lovita is higher than in the short-lived variety Golden Wave. The chromatographic data presented in Fig. 1 shows that the difference in cytokinins between the two varieties was mainly restricted to one (R_F - 0.4) out of three activity zones.

In several experiments it was found that exogenous applications of the cytokinin BA directly to the flower bud delayed the senescence of both fresh and aged flowers of the short-lived variety Golden Wave (Table III). Application of BA as a base immersion or as a stem dip for up to 90 min was ineffective. The long-lived variety Lovita was less responsive to the BA treatment.

Since the work of Richmond and Lang (13) on the retardation of chlorophyll and protein breakdown in detached leaves, numerous reports have established the antisenescence effect of external application of cytokinins on plant organs. We know of only two cases (14, 15) in which endogenous changes in cytokinins were correlated with senescence. Sitton *et al.* (14) have correlated the level of cytokinins in root exudates of sunflowers and the onset of shoot senescence. They, however, did not present data on the effect of exogenous application of cytokinins on senescence of this plant. Thomas (15) determined cytokinin activity in Brussels sprouts, by the barley leaf section assay. Though this test seems reasonable for demonstrating antisenescence factors, it is not specific for cytokinins (4, 8). We have demonstrated here a decline in endogenous cytokinin activity in senescing rose petals and significant differences in the levels of cytokinins between a long- and a short-lived variety. Exogenous applications of cytokinin delayed the senescence of petals of the short-lived variety. This may strengthen the view that cytokinins participate in the endogenous regulation of

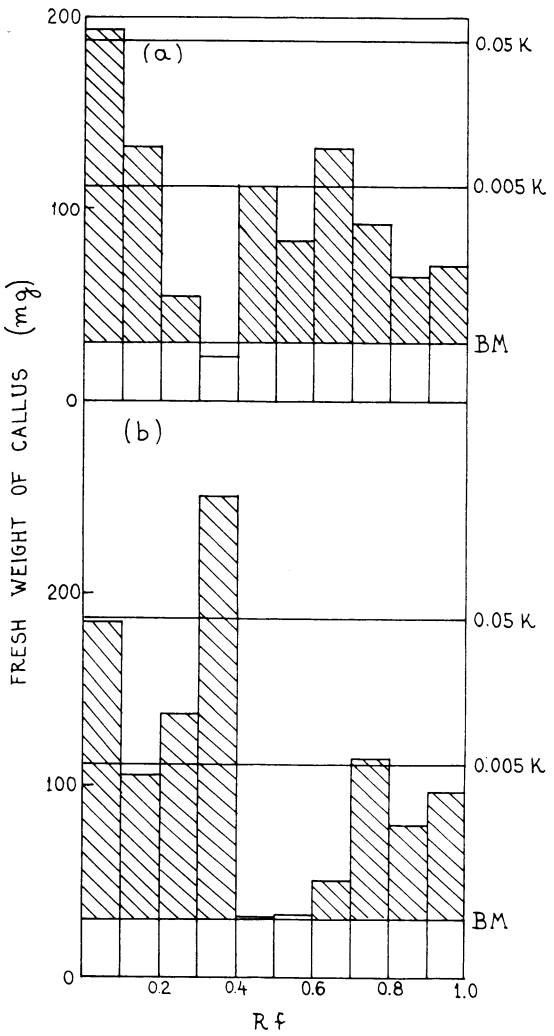

FIG. 1. Paper chromatograms of methanol extracts from rose petals of young flowers of Golden Wave (a) and Lovita (b) varieties. Extracts were bioassayed in the soybean callus test for cytokinins, after development in an isopropanol–ammonia–water solvent (8:1:1, v/v).

Table III. *Effect of 15-min Dip Treatment with the Cytokinin N^6-Benzyladenine on Longevity of Rose Flowers var. Golden Wave*

BA Concn	No. of Days to Petal Drop	
	Fresh flowers[1]	Aged flowers[1]
M		
0	7.1 c	4.3 c
10^{-5}	8.5 b	4.3 c
10^{-4}	8.2 b	4.8 b
5×10^{-4}	9.2 a	5.4 a

[1] Within each column, values not followed by the same letter are statistically different at the 5% level (Duncan's Multiple Range Test).

senescence in rose petals. Other phytohormones may also be involved in this regulation. Results on the endogenous levels of abscisic acid, gibberellin, and ethylene and the response to exogenous applications of these hormones will be reported in a forthcoming paper.

Cytokinin activity has been observed previously in various plant organs: seeds, fruits, stem tips, roots, tubers, and leaves (6, 7, 9, 12). To our knowledge, this is the first report on cytokinin activity in petals.

Acknowledgment—The technical assistance of Mrs. Leah Schlittner is gratefully acknowledged.

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