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Acceleration of ripening of tomato pericarp discs by brassinosteroids

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Abstract

Brassinosteroids are now considered as the sixth group of hormones in plants. As brassinosteroids influence varied growth and development processes such as growth, germination of seeds, rhizogenesis, flowering, senescence and abscission, they are considered as plant hormones with pleiotropic effects. The effect of 28-homobrassinolide and 24-epibrassinolide on ripening of tomato pericarp discs was studied. Application of brassinosteroids to pericarp discs resulted in elevated levels of lycopene and lowered chlorophyll levels. In addition brassinosteroid-treated pericarp discs exhibited decreased ascorbic acid and increased carbohydrate contents. Fruit ripening as induced by brassinosteroids was associated with increase in ethylene production. The study revealed the ability of brassinosteroids in accelerating fruit-senescence.

1. Introduction

Brassinosteroids are a new type of phytohormones with significant growth promoting influence (Clouse and Sasse, 1998; Sasse, 1999). Based on the fact that brassinosteroids were found in all plants tested so far (9 monocots, 28 dicots, 5 gymnosperms, 1 pteridophyte and 1 alga), Sasse (1997) suggested that probably they are ubiquitous in the plant kingdom. The work with brassinosteroid biosynthetic mutants in *Arabidopsis thaliana* (Li et al., 1996) and *Pisum sativum* (Nomura et al., 1997) provided strong evidence that brassinosteroids are essential for plant growth and development. The growth inhibition of *Lepidium sativum* by brassinozole, the specific inhibitor of brassinolide synthesis was found reverted by the exogenous application of brassinolide indicating the necessity of brassinosteroids for plant growth (Asami et al., 2000). Sasse (1997) regarded brassinosteroids as plant hormones with pleiotropic effects as they influence varied developmental processes such as growth, seed germination, rhizogenesis, senescence and also confer resistance to plants against various abiotic stresses. Recently the ability of brassinosteroids to induce nodulation in groundnut (Vardhini and Rao, 1999), induction of gravitropic curvature in maize primary roots (Kim et al., 2000) and the ability of Ts303, a synthetic brassinolide analogue to increase fruit set in tomato (Kamuro and Takatsuto, 1999) were reported. In the present study the influence of brassinosteroids on the ripening of tomato pericarp discs is being investigated.

2. Results and discussion

Treatment of tomato pericarp discs with brassinosteroids resulted in substantial increase in lycopene content and considerable decrease in total chlorophyll content (Table 1). Fruit ripening involves chlorophyll degradation and carotenoid biosynthesis (Rhodes, 1980).

28-Homobrassinolide and 24-epibrassinolide application to tomato pericarp discs resulted in decrease in ascorbic acid content (Table 2). Gradual reduction in ascorbic acid content during tomato ripening was reported by Rick (1978). Tomato pericarp discs treated with brassinosteroids also exhibited elevated levels of reducing and total sugars (Table 3).The changes in the contents of ascorbic acid and carbohydrate levels are in tune with the observation made by Rick (1978) during the course of tomato fruit ripening.

The acceleration of ripening as induced by brassinosteroids was associated with increase in ethylene pro-

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Table 1
Effect of brassinosteroids on the lycopene and total chlorophyll contents of tomato pericarp discs

Compounds	4th Day		6th Day		8th Day	
	Lycopene content (A 503 g^{-1} fr. wt)[a]	Total chlorophylls (μg g^{-1} fr. wt)[a]	Lycopene content (A 503 g^{-1} fr. wt)[a]	Total chlorophylls (μg g^{-1} fr. wt)[a]	Lycopene content (A 503 g^{-1} fr. wt)[a]	Total chlorophylls (μg g^{-1} fr. wt)[a]
Control	0.469±0.31	180.71±0.24	0.602±0.22	8.197±0.02	0.721±0.12	1.024±0.04
0.5 μM HBR	0.641±0.12	15.498±0.33	0.799±0.08	7.585±0.08	0.826±0.11	–
1.0 μM HBR	0.726±0.08	13.940±0.29	0.864±0.16	6.560±0.09	0.915±0.02	–
3.0 μM HBR	0.899±0.11	13.187±0.32	0.939±0.14	5.495±0.19	1.226±0.08	–
0.5 μM EBR	0.629±0.01	15.182±0.20	0.727±0.18	6.442±0.01	0.814±0.22	–
1.0 μM EBR	0.704±0.22	13.463±0.15	0.801±0.08	5.242±0.13	0.900±0.21	–
3.0 μM EBR	0.854±0.32	12.421±0.11	0.916±0.21	4.350±0.11	1.104±0.18	–

HBR = 28-homobrassinolide.
EBR = 24-epibrassinolide.
[a] Mean±S.E.

Table 2
Effect of brassinosteroids on the ascorbic acid content of tomato pericarp discs

Compounds	4th Day (mg g^{-1} fr. wt)[a]	6th Day (mg g^{-1} fr. wt)[a]	8th Day (mg g^{-1} fr. wt)[a]
Control	0.196±0.032	0.126±0.010	0.642±0.011
0.5 μM HBR	0.186±0.022	0.101±0.011	0.587±0.012
1.0 μM HBR	0.180±0.032	0.094±0.011	0.487±0.011
3.0 μM HBR	0.180±0.021	0.088±0.021	0.381±0.013
0.5 μM EBR	0.193±0.010	0.114±0.011	0.525±0.012
1.0 μM EBR	0.187±0.012	0.110±0.011	0.452±0.010
3.0 μM EBR	0.186±0.032	0.090±0.032	0.393±0.010

HBR = 28-homobrassinolide.
EBR = 24-epibrassinolide.
[a] Mean±S.E.

duction (Table 4). Fruit ripening involves the transitory increase in ethylene production (Rhodes, 1956). Ethylene is necessary for manifestation of ripening in climacteric fruits (Yang, 1985). The studies conducted with transgenic and mutant tomato lines that inhibited ethylene biosynthesis or perception, revealed that the process of climacteric ripening represents a combination of ethylene regulation and developmental control (Giovannoni, 2001). Giovannoni (2001), who reviewed the work on fruit ripening suggested that both ethylene and additional developmental processes regulate the fruit ripening. Regulation and synergy of the multiple processes contributing to the ripening phenomenon remain unknown (Giovannoni, 2001). The present study revealed the ability of brassinosteroids to enhance ethylene production in ripening tomato discs. It is well established that brassinosteroids independently as well as in synergism with auxins induce ethylene production. (Kripach et al., 1999). It is also established that brassinosteroids stimulate ethylene biosynthesis between *S*-adenosylmethionine and 1-amino-cyclopropane 1-carboxylic acid (Schlagnhaufer et al., 1984). However, brassinosteroid induced growth responses are considered independent of ethylene. Brassinolide at low concentrations (0.1 μM) induced petiole elongation, upward leaf bending and the same effect was brought about by ethylene and ACC also, but at higher concentration of 200 ppm and 1 mM respectively and the growth response as influenced by brassinolide in *Arabidopsis* was found associated with the production of ACC and ethylene (Arteca and Arteca, 2001). However, based on the fact that brassinolide promoted exaggerated growth and elevated levels of ACC and ethylene even in the ethylene-insensitive mutant *etr1–3*, Arteca and Arteca (2001) suggested that the effect of brasssinosteroids on the growth is independent of ethylene. The possibility of other hormones in addition to ethylene in climacteric fruit ripening also exists (Giovannoni, 2001). The results of the present study indicate the acceleration of ripening process in tomato pericarps by brassinosteroids.

Recent studies employing brassinosteroid biosynthesis and insensitive mutants of tomato provided strong evidences to the importance of brassinosteroids in growth. The dwarf tomato mutant, dumpy (*dpy*) is an intermediate dwarf, displaying a curled leaf phenotype with dark ruggose leaves, suppression of axillary shoots and exogenous application of brassinolide completely rescued the phenotype to wild type (Clouse and Feldmann, 1999). Another brassinosteroid deficient tomato mutant is the *dwarf* (*d*) mutant, and it has been shown that the DWARF protein, the product of the dwarf gene(*D*) is responsible for the conversion of 6-deoxacastasterone to castasterone (Bishop et al., 1999). Tomato mutant *curl-3* (*cu-3*) has shown to be brassinosteroid insensitive considered equivalent to *brassinolide insensitive 1*(*brl 1*) mutant of *Arabidopsis* (Bishop and Yokota, 2001).

The results obtained in the present study with pigment levels, ascorbic acid, carbohydrate contents and ethylene clearly indicated the involvement of brassinosteroids in fruit ripening. Earlier, the involvement of brassinosteroids in the induction of senescence in detached cotyledons of cucumber seedlings (Zhao et al., 1990) and leaves of mung bean seedlings (He et al.,

Table 3
Effect of brassinosteroids on carbohydrate fractions of tomato pericarp discs

Compounds	4th Day		6th Day		8th Day	
	Reducing sugars (mg g^{-1} fr. wt)[a]	Total sugars (mg g^{-1} fr. wt)[a]	Reducing sugars (mg g^{-1} fr. wt)	Total sugars (mg g^{-1} fr. wt.)[a]	Reducing sugars (mg g^{-1} fr. wt.)[a]	Total sugars (mg g^{-1} fr. wt.)[a]
Control	7.36±0.37	11.69±0.22	9.39±0.29	12.56±0.19	12.59±0.21	16.01±0.10
0.5 μM HBR	9.17±0.28	13.27±0.31	10.12±0.06	14.07±0.37	14.22±0.50	18.28±0.16
1.0 μM HBR	9.73±0.10	14.17±0.08	10.21±0.12	15.40±0.23	15.24±0.41	18.76±0.35
3.0 μM HBR	9.92±0.11	15.07±0.08	10.42±0.11	16.61±0.15	17.28±0.44	20.31±0.18
0.5 μM EBR	8.86±0.28	12.28±0.13	9.62±0.17	14.09±0.09	13.53±0.61	18.12±0.33
1.0 μM EBR	9.31±0.34	13.31±0.16	9.94±0.13	15.34±0.12	15.43±0.32	19.00±0.30
3.0 μM EBR	9.89±0.12	15.02±0.07	10.15±0.07	16.07±0.07	17.26±0.46	20.14±0.08

HBR = 28-homobrassinolide.
EBR = 24-epibrassinolide.
[a] Mean±S.E.

1996) was reported. *Arabidopsis* mutants lacking bioactive brassinosteroids show delay in chloroplast senescence (Li et al., 1996).

3. Experimental

3.1. Chemicals and plant material

28-Homobrassinolide and 24-epibrassinolide were purchased from M/s. Beak Technologies Inc., Brampton, Ontario, Canada. Tomato (*Lycopersicon esculentum*. Mill. Var. Early Pusa Dwarf [P.E.D]) fruits were harvested from field grown plants.

3.2. Disc preparation and treatments

Uniformly shaped MG_2 stage fruits were used for disc preparation (Beaulieu et al., 1997). Fruits were washed with 1% (v/v) sodium hypochlorite solution and rinsed with several changes of sterile distilled water. Disc preparation was carried out with sterile technique using procedures described by Saltviet (1989). Pericarp discs were cut from equatorial regions with a cork borer and separated by hand into individual discs with epidermis included. Discs were rinsed in sterile water, carefully blotted off to remove adsorbed water and kept in petriplates of 5 cm diameter provided with Whatman No. 1 filter paper. Five pericarp discs were placed in each petriplate supplied with 2 ml of either test solution (viz., 0.5, 1.0 and 3.0 μM brassinosteroids) or distilled water (control). The petriplates were kept in a chamber whose temperature was maintained at 20±1 °C. On the 4th day, another dose of 1 ml solution was added. Lycopene contents, total chlorophylls, ascorbic acid and carbohydrate estimations were conducted on 4th, 6th and 8th day. On the 3rd day ethylene was estimated.

Table 4
Effect of brassinosteroids on ethylene produced by tomato pericarp discs

Compounds	3rd Day
	Amount of ethylene formed (nano moles of ethylene g^{-1} h^{-2})[a]
Control	107.08
0.5 μM HBR	122.83
1.0 μM HBR	134.83
3.0 μM HBR	144.20
0.5 μM EBR	121.75
1.0 μM EBR	132.08
3.0 μM EBR	138.91

HBR = 24-homobrassinolide.
EBR = 28-epibrassinolide.
[a] Mean of three replicates.

3.3. Lycopene

Lycopene content was determined by the procedure described by Beaulieu et al. (1997). Lycopene concentrations were studied in discs varying from green to red colour by measuring the absorbance at 503 nm of acetone extract of individual discs. This wavelength is best suited for tomato lycopene because the influence of carotenoids (473 nm) is negligible (Beerh and Siddappa, 1959). The results are expressed in absorbance units.

3.4. Total chlorophylls

Chlorophylls were extracted and estimated by the procedure described by Arnon (1949). Pericarp discs were homogenized with 80% (v/v) acetone and centrifuged. The acetone extract was used to calculate the total chlorophylls employing the formula given below.

$$\text{Total chlorophylls} = [\text{O.D. at } 645 \times 20.2 + \text{O.D. at } 663 \times 8.3] \times [(v/1000) \times w]$$

Where v volume of acetone extract
w weight of the pericarp discs.

3.5. Ascorbic acid

Ascorbic acid (reduced form) was estimated according to the method described by Raghuramulu et al. (1983). Discs were homogenized with 10 ml of 4% oxalic acid and centrifuged. The supernatant was titrated against 2,6-dichlorophenol indophenol dye. The amount of dye consumed indicated the amount of ascorbic acid present in the pericarp discs which was estimated by the following formula.

$$\frac{0.5}{v_1} \times \frac{v_2}{5} \times \frac{100}{w}$$

where v_1 titre value of working standard
v_2 titre value of sample
w weight of the sample.

3.6. Carbohydrates

Discs were homogenized with 80% (v/v) ethanol and the homogenate was heated and centrifuged. The supernatant was used for the estimation of total sugars (Yoshida et al., 1976) and reducing sugars (Nelson, 1944).

3.7. Ethylene

On the 3rd day the pericarp discs from the petriplates were transferred into glass tubes sealed with suba seal to allow it to be pierced by an hypodermic needle bearing a syringe. After 2 h of incubation 0.5 ml of accumulated gases was withdrawn from the sample container with a syringe and immediately injected into a gas chromatograph (Shimadzu GC-148) having Poropack 'n' column equipped with a flame ionization detector to detect the ethylene gas.

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