Trees (2006) 20: 286–291
DOI 10.1007/s00468-005-0041-3

ORIGINAL ARTICLE

A. B. Pereira-Netto · C. T. A. Cruz-Silva · S. Schaefer · J. A. Ramírez · L. R. Galagovsky

Brassinosteroid-stimulated branch elongation in the marubakaido apple rootstock

Received: 15 July 2005 / Accepted: 16 November 2005 / Published online: 16 December 2005
© Springer-Verlag 2005

Abstract Brassinosteroids (BRs) comprise a specific class of low-abundance plant steroids now recognized as a new class of phytohormones. In this paper, we demonstrate that a fluoro derivative of 28-homocastasterone (5F-HCTS) stimulates branch elongation in *in vitro*-grown shoots of *Malus prunifolia*, the marubakaido apple rootstock. In addition to that, we show that this BR-stimulated branch elongation is paralleled by an increase in ethylene release. However, either the presence of 1-amino-cyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene in higher plants, in the culture medium or an ethylene-enriched atmosphere resulted in inhibition of branch elongation, indicating that the stimulation of branch elongation observed for 5F-HCTS-treated shoots in this study was not, at least directly, related to the BR-induced enhancement in ethylene release rate. Besides its positive effect on the marubakaido shoot growth, i.e. branch elongation, the 5F-HCTS-driven enhancement of branch elongation found in this study is potentially useful to improve micropropagation techniques for other plant species as well, especially woody species, in which branch elongation is typically a constraint for efficient micropropagation.

Keywords 28-Homocastasterone · 5F-HCTS · Ethylene · *Malus prunifolia*

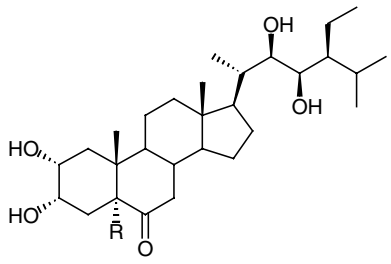
A. B. Pereira-Netto (✉) · C. T. A. Cruz-Silva · S. Schaefer
Department of Botany-SCB, Centro Politecnico-UFPR,
C.P. 19031, Curitiba, PR 81531-970, Brazil
e-mail: apereira@ufpr.br
Tel.: +55-41-33611631
Fax: +55-41-33662042

J. A. Ramírez · L. R. Galagovsky
Department of Organic Chemistry, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 1428 Buenos Aires, Argentina

Introduction

Brassinosteroids (BRs) comprise a specific class of low-abundance plant steroids which carry an oxygen moiety at C-3 and additional ones at one or more of the C-2, C-6, C-22 and C-23 carbon atoms (Bishop and Yokota 2001). Molecular genetic analysis of mutants defective in BRs biosynthesis or response revealed that BRs are essential for normal plant growth and development. These findings, together with the ubiquitous occurrence of BRs in plants (Fujioka 1999) and their highly effective elicitation of several responses (Mussig and Altmann 1999) have led to the recognition of BRs as a new class of plant hormones (Yokota 1997; Clouse and Sasse 1998). Among the several responses elicited by BRs are the stimulation of 1-amino-cyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene in higher plants, and ethylene biosynthesis (Lim et al. 2002).

Marubakaido (*Malus prunifolia* (Willd.) Borkh), the most widely used apple rootstock in countries like Brazil, displays simultaneous resistance against the woolly apple aphid (*Eriosoma lanigerum* (Hausman)) and the collar rot (*Phytophthora cactorum*) (Losso and Mondin 1991). The collar rot is considered to be the main root disease in apple trees, worldwide. However, the marubakaido rootstock is susceptible to several viruses, which usually kill the rootstock, making *in vitro* propagation (micropropagation) the most recommended propagation technique for virus-free propagules (Flores et al. 1999). In this paper, we demonstrate that a fluoro derivative (compound **2**, Fig. 1) of 28-homocastasterone (28-HCTS, compound **1**, Fig. 1) stimulates branch elongation in *in vitro*-grown shoots of the apple rootstock. We also demonstrate that this BR-stimulated branch elongation is paralleled by an enhancement of ethylene release from the shoots. In addition to that, we present evidence indicating that the stimulation of branch elongation observed for 5F-HCTS-treated shoots in this study was not, at least directly, related to the BR-induced enhancement in ethylene release rate.



1 - R= H 28-homocastasterone

2 - R= F 5α-fluoro-28-homocastasterone

Fig. 1 Chemical structure of 28-homocastasterone and 5-fluoro-28-castasterone

Materials and methods

Shoot microculture

Shoot segments measuring between 10 and 20 mm in length were taken from 30-day-old aseptically grown decapitated shoots of a clone of *Malus prunifolia* (Willd.) Borkh var. Marubakaido and used as explant sources in the experiments. Explants were grown on 40 ml of MS basal medium (Murashige and Skoog 1962) supplemented with 555 μM myo-inositol, 4.06 μM nicotinic acid, 2.43 μM pyridoxine–HCl, 26.64 μM glycine, 6.25 μM thiamine–HCl, 2.2 μM N^6-benzyladenine, 3% (w/v) sucrose and 0.6% (w/v) agar. The pH was adjusted to 5.7 prior to autoclaving.

Culture conditions

Cultures were maintained in a culture room using a completely randomised design. A photoperiod of 16/8 (light/dark) hours was provided by cool-white fluorescent tubes giving a photosynthetic photon flux density of 40 μmol m^{-2} s^{-1} at the culture level. Relative humidity was kept at 70±5%. Air temperature around the cultures was 26±1.0°C.

5F-28-Homocastasterone application

5F-HCTS (5fluoro-28-homocastasterone or (22R,23R)-2α,3α,22,23-tetrahydroxy-5α-fluorostigmastan-6-one) was synthesized from stigmasteryl acetate, as previously described (Ramirez et al. 2000). Microdrops (5 μl) of 95% (v/v) ethanol containing known amounts of 5F-HCTS were pipetted onto the uppermost leaf, which was at least 3 mm wide, of 15-day-old shoots originating from shoot segments obtained as described above. Only single applications were used and control shoots were treated with 5 μl microdrops of only 95% (v/v) ethanol. Single microdrops were used for each leaf.

Time course of ethylene release

Ten shoot segments, obtained as described above, were placed in each of eight 600 ml glass vessels containing 100 ml of culture medium (500 ml of headspace). Shoot segments from four glass vessels were treated with 0.5 μg of 5F-HCTS while shoot segments from the remaining four vessels were treated with 95% (v/v) ethanol. Every 96 h, gas samples (500 μl) were removed from the vessels for ethylene concentration measurements. After the gas samples were taken, the fresh weight of the shoots was measured. Afterwards, the vials were aerated for 3 min with sterile air at 252 ml min^{-1} and closed in such a way that allowed gas exchange between the vessel and the external atmosphere. Forty-eight hours prior to the gas sampling, the vessels were sealed with serum stoppers in order to allow ethylene accumulation. Ethylene concentration in the gas samples was measured with a Varian Star 3400 CX gas chromatograph (Varian, Inc., Palo Alto, CA) equipped with a Supelco porapak N column (Bellefonte, PA) and a flame-ionization detector. Gas chromatograph settings were 120°C column temperature, 40 ml min^{-1} N_2 carrier gas flow rate, and 4 kg cm^{-2} carrier gas pressure. Injection port and detector temperatures were 110 and 200°C, respectively. Ethylene peaks were calibrated against a 4.32 nmol ml^{-1} ethylene standard in nitrogen. Ethylene release rates were calculated on a fresh weight basis as nmol g^{-1} day^{-1}. Blank sample test tubes were analyzed for ethylene, and any background was subtracted from sample peaks.

ACC and ethylene treatments

A solution of 1-amino-cyclopropane-1-carboxylic acid (ACC) (Sigma Chem. Co., St. Louis, MO), the immediate precursor of ethylene in higher plants, was prepared immediately prior to the beginning of the treatment. The solution was filter sterilized through a 0.22 μm pore, polyvinylidene fluoride membrane (Millex, Millipore Co., MA) and added to the aforementioned culture media to achieve the desired concentration, after autoclaving. Shoot segments, obtained as described above and not treated with 5F-HCTS, were inoculated in the culture media.

For the ethylene treatments, 10 shoot segments per treatment, obtained as described above and not treated with 5F-HCTS were placed in each of the twenty 600 ml glass vessels (4 vessels per treatment × 5 treatments) containing 100 ml of culture medium (500 ml of headspace). Ethylene (White Martins-Praxair Inc., Rio de Janeiro, Brazil) was filter sterilized as described for ACC and injected into the vessels through serum stoppers to achieve the desired concentration. Every 5 days, the vessels were vented, as described under "Time course of ethylene release", resealed and ethylene was re-injected.

Statistical analyses

Unless otherwise stated, treatments consisted of eight replications (one replication = one culture vessel) with four explants per replication. Each experiment was repeated at least three times. The data were analyzed using the SAS-JMP 5 software package (SAS Institute, Inc., Cary, NC).

After a significant analysis of variance, the differences between means for treatments were analyzed by Student–Neuman–Keuls pairwise comparison test.

Results

Effect of 5F-HCTS on branch elongation

Progressive enhancement of elongation of main branches (originating directly from the initial shoot segment) and primary lateral branches (originating from the main branches) of *in vitro*-grown marubakaido apple rootstock shoots was associated with increased doses of 5F-HCTS (Fig. 1) until 0.5 µg per shoot (Fig. 2). This enhanced elongation was, however, statistically significant (P=0.05) only for the 0.5 µg of 5F-HCTS dose. The use of 5F-HCTS at doses over 0.5 µg per shoot led to reduced branch length, compared to the peak stimulation of branch elongation found at 0.5 µg per shoot. The primary lateral branches were more responsive than the main branches to BR treatment.

For the secondary lateral branches (branches originating from the primary lateral branches) no significant (P=0.05) change in elongation was found for BR-treated shoots at any of the doses tested. The variable data obtained for secondary lateral branches are very likely due to the small number of these branches formed during the culture cycle, which is typically only about one-tenth the number of main and primary lateral branches formed (Schaefer et al. 2002).

Time course of ethylene release

Ethylene release of *in vitro*-grown shoots of the marubakaido rootstock was within the range of rates reported for other plant species (Osborne 1989). An increase in rate of ethylene release occurred between Day 1 (the beginning of the culture cycle) and Day 8 for shoots treated with 5F-HCTS, and between Day 1 and Day 4 for shoots not treated with 5F-HCTS (Fig. 3). For either Day 4 or Day 8, shoots treated with 5F-HCTS typically presented higher rates of ethylene release than untreated shoots. After ethylene release peaked, a decrease in rate of release was observed until it stabilized around 0.04 nmol g^{-1} day^{-1} for shoots either treated or not with 5F-HCTS (Fig. 3).

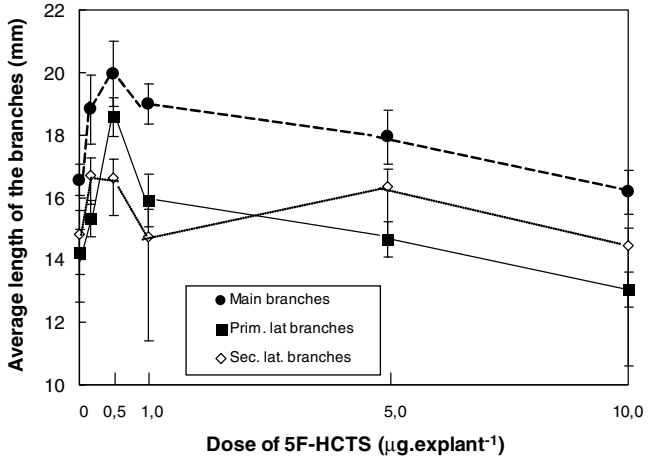


Fig. 2 Effect of 5F-HCTS on the average length of main (– – –), primary lateral (—) and secondary lateral branches (- - -). Vertical bars indicate ±standard error

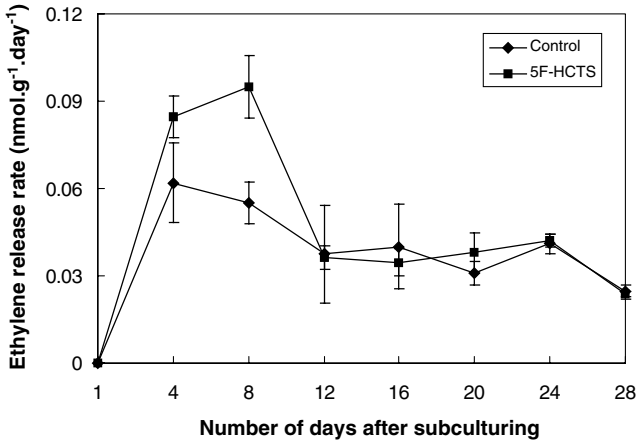


Fig. 3 Time course of ethylene release rate for shoots treated or not with 5F-HCTS. Vertical bars indicate ±standard error

Response to exogenous ACC and ethylene

Increasing concentrations of 1-amino-cyclopropane-1-carboxylic acid (ACC) in the culture medium resulted in decreased length of main, primary lateral and secondary lateral branches (Fig. 4). These decreases in length became significant (P=0.05) at 3.1525, 50 and 200 µM ACC, respectively, for secondary lateral, main and primary lateral branches, when compared to the control (0 µM ACC).

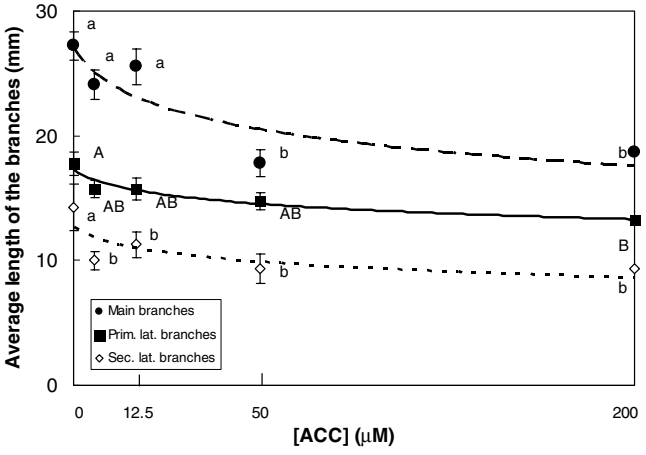


Fig. 4 Effect of ACC on the average length of main (– – –), primary lateral (—) and secondary lateral branches (- - -). R^2 is 0.7843, 0.9222 and 0.5673, respectively for the main, primary lateral and secondary lateral branches. Average lengths of main and secondary lateral branches followed by the same lower case letters, and primary lateral branches followed by upper case letters, do not differ at P=0.05. Vertical bars indicate ±standard error

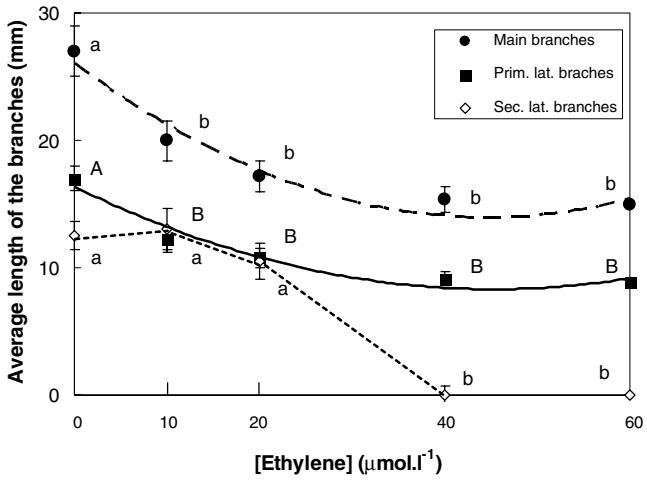


Fig. 5 Effect of exogenous ethylene on the average length of main (– – –), primary lateral (—) and secondary lateral branches (- - -). R^2 is 0.9542 and 0.9548, respectively for the main and primary lateral branches. Average lengths followed by the same lower case letters for main branches and secondary lateral branches, and by the same upper case letters, for the primary lateral branches, do not differ at P=0.05. Vertical bars indicate ±standard error

Similarly to what was found for shoots grown in ACC-containing media, a progressive decrease of elongation was found for main and primary lateral branches of shoots grown in atmospheres enriched with ethylene at 10–60 µmol l^{-1} (Fig. 5), a range that overlaps the range of ethylene concentrations found during the time course of the ethylene release experiment. These decreases were statistically significant (P=0.05) for all of the ethylene concentrations tested. However, for secondary lateral branches significant inhibition of elongation was only found for shoots grown at 40 and 60 µmol l^{-1} ethylene, indicating that secondary lateral branches are less sensitive to low ethylene concentrations, in terms of inhibition of elongation, when compared to main and primary lateral branches. In contrast, and differently from what was found for main and primary lateral branches, complete inhibition of secondary lateral branch elongation was found for shoots grown in atmospheres enriched with 40 and 60 µl l^{-1} ethylene.

Discussion

Exogenous application of BRs at nanomolar to micromolar concentrations to plants stimulates cell elongation and division, and tracheary element differentiation (Mandava 1988; Clouse and Sasse 1998; Sasse 1999), all-important to allow branch elongation. 28-Homocastasterone has been widely employed in field trials because of its greater synthetic accessibility compared to typically more active BRs, such as brassinolide (Mandava 1988; Fujioka and Sakurai 1997; Baron et al. 1998). In order to enlarge studies on the effects of BRs analogues on bioactivity, Ramirez et al. (2000) synthesized a new compound by introducing a fluoro group at the C-5 position of 28-homocastasterone. In the present work, we found that this fluoro derivative, 5F-HCTS, significantly (P=0.05) stimulated branch elongation in the marubakaido apple rootstock.

BRs have been demonstrated to stimulate ACC and ethylene biosynthesis in systems such as the primary roots of maize (*Zea mays*) in which exogenously applied brassinolide enhanced ethylene release and ACC oxidase activity in a dose-dependent manner (Lim et al. 2002). Furthermore, in etiolated mung bean hypocotyl segments, stimulation of ethylene biosynthesis, due to increased ACC synthase activity, has been found after brassinolide treatment (Arteca 1995). Indeed, in the past years, molecular genetic studies have been able to identify specific genes of the ethylene biosynthetic pathway activated by BRs, helping to understand how BRs affect ethylene biosynthesis at the gene expression level. Experiments carried out with etiolated plantlets of *cin5*, a loss-of-function *Arabidopsis* mutant defective in ACS5, a member of the ACC synthase gene family, have suggested that BR-induced stimulation of ethylene biosynthesis is at least in part dependent on accumulation of the ACS5 isoform (Woeste et al. 1999). Because BRs have been demonstrated to stimulate ethylene release in systems such as roots and etiolated hypocotyls segments, we decided to investigate whether or not the BR-induced stimulation of branch elongation found for the marubakaido rootstock was also paralleled by enhanced ethylene release rate. Indeed, we found in this study that ethylene release rate was also enhanced in BR-treated marubakaido shoots.

Ethylene has long been known to inhibit organ elongation, such as in hypocotyls of etiolated seedlings, in numerous plant species (Neljubow 1901; Ecker 1995). However, stimulation of elongation has been found for light-grown seedlings, as well. In *Arabidopsis*, for example, seedlings grown in the light in either an atmosphere enriched with 10 ppm ethylene or in the presence of 50 µM ACC in a low-nutrient culture medium presented longer hypocotyls, compared to controls grown in ethylene-free atmosphere or ACC-free culture medium (Smalle et al. 1997). In our system, enrichment of either the culture medium with ACC or the internal vessel atmosphere with ethylene resulted in inhibition of branch elongation, providing evidence that the stimulation of branch elongation observed for 5F-HCTS-treated shoots in this study was not, at least directly, related to the BR-induced enhancement in ethylene release rate.

Although BRs are known to stimulate elongation of young tissues (reviewed in Clouse 1996), these plant growth regulators have also been previously reported to inhibit branch elongation in various plant species such as rice (*Oryza sativa*) (Chon et al. 2000) and pea (*Pisum sativum*) (Kohout et al. 1991). These reductions in elongation have been attributed to enhanced ethylene release and/or enhanced cyanide, a byproduct of ethylene biosynthesis when ethylene is produced from ACC (Chon et al. 2000). Thus, a possible way that 5F-HCTS might inhibit elongation of main and primary lateral branches in marubakaido shoots treated with doses of 5F-HCTS over 0.5 µg per shoot might be through a stimulation of ethylene and/or cyanide biosynthesis, especially if ethylene and/or cyanide biosynthesis is BR dose-dependent. However, this hypothesis needs to be tested.

Cytokinins are known to stimulate branch elongation in several plant species. Transformed plants bearing the agrobacterial isopentenyltransferase (ipt) gene, an enhancer of endogenous levels of cytokinins such as zeatin, zeatin riboside and isopentenyladenosine, typically present longer branches compared to untransformed plants (Schwartzenberg et al. 1994). Like cytokinins, BRs have also been reported to stimulate branch elongation. Application of brassinolide and castasterone to the dumpy (dpy) mutant of tomato, a mutant presenting reduced axillary branching, rescued the dpy phenotype, as did C-23-hydroxylated, 6-deoxo intermediates of brassinolide biosynthesis. The brassinolide precursors campesterol, campestanol and 6-deoxocathasterone were not able to rescue the dpy phenotype, suggesting that dpy may be affected in the conversion of 6-deoxocathasterone to 6-deoxoteasterone (Koka et al. 2000). At the biochemical level, BRs have been demonstrated to change endogenous cytokinin concentrations in plant species. For example, when provided via a culture medium containing a growth-limiting level of auxin, 24-epibrassinolide increased the endogenous predominant cytokinins N-6-(Δ-2-isopentenyl) adenine and *trans*-zeatin in tobacco (*Nicotiana tabacum*) callus tissue (Gaudinova et al. 1995). Thus, BRs might stimulate branch elongation via a BR-driven enhancement of the endogenous levels of cytokinins. The presence of benzyladenine, a cytokinin previously thought not to occur naturally (van Staden and Crouch 1996), in the culture medium in concentrations over the concentration used in this work was shown not to lead to further increase in branch elongation in marubakaido shoots not treated with 5F-HCTS (Nunes et al. 1999). Consequently, the 5F-HCTS-driven stimulation of branch elongation found in this work, might be due to an eventual 5F-HCTS-induced enhancement of endogenous amounts of naturally occurring cytokinins other than benzyladenine. However, the possibility that 5F-HCTS might be able to stimulate branch elongation in our system by itself or synergistically with benzyladenine and/or other naturally occurring cytokinins cannot be ruled out at this point.

Besides its positive effect on the marubakaido shoot growth, i.e. shoot elongation, the 5F-HCTS-driven enhancement of branch elongation found in this study is potentially useful to improve micropropagation techniques for other plant species as well, especially woody species, in which branch elongation is typically a constraint for efficient micropropagation. Furthermore, this 5F-HCTS-induced increase of branch elongation might also be useful to help stimulate branching during the production phase in orchards.

Acknowledgements "Conselho Nacional de Pesquisa e Desenvolvimento"-Brazil is acknowledged for the fellowships granted to the senior author, S. Shaefer, S. A. Medeiro and C. T. A. C. Silva. Drs. F. Pedrosa, E. M. Souza and G. Klassen are acknowledged for their assistance with the gas chromatography

References

Arteca RN (1995) Brassinosteroids. In: Davies PJ (ed) Plant hormones: Physiology, biochemistry and molecular biology. Kluwer, Dordrecht, pp 206–213

Baron DL, Luo W, Janzen L, Pharis RP, Back TG (1998) Structure–activity studies of brassinolide b-ring analogues. Phytochemistry 49:1849–1858

Bishop GJ, Yokota T (2001) Plants steroid hormones, brassinosteroids: Current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. Plant Cell Physiol 42:114–120

Chon NM, Nishikawa-Koseki N, Hirata Y, Saka H, Abe H (2000) Effects of brassinolide on mesocotyl, coleoptile and leaf growth in rice seedlings. Plant Prod Sci 3:360–365

Clouse SD (1996) Molecular genetic studies confirm the role of brassinosteroids in plant growth and development. Plant J 10:1–8

Clouse SD, Sasse JM (1998) Brassinosteroids: Essential regulators of plant growth and development. Annu Rev Plant Physiol Plant Mol Biol 49:427–451

Ecker JR (1995) The ethylene signal-transduction pathway in plants. Science 268:667–675

Flores R, Lessa AO, Peters JA, Fortes GRL (1999) Efeito da sacarose e do benomyl na multiplicação *in vitro* da macieira. Pesqui Agropecu Bras 34:2363–2368

Fujioka S (1999) Natural occurrence of brassinosteroids in the plant kingdom. In: Sakurai A, Yokota T, Clouse SD (eds) Brassinosteroids: Steroidal plant hormones. Springer-Verlag, Tokyo, pp 21–45

Fujioka S, Sakurai A (1997) Biosynthesis and metabolism of brassinosteroids. Physiol Plant 100:710–715

Gaudinova A, Sussenbekova H, Vojtechova M, Kaminek M, Eder J, Kohout L (1995) Different effects of 2 brassinosteroids on growth, auxin and cytokinin content in tobacco callus-tissue. Plant Growth Regul 17:121–126

Kohout L, Strnad M, Kaminek M (1991) Types of brassinosteroids and their bioassay. In: Culter HG, Yokota T, Adam G (eds) Brassinosteroids, chemistry, bioactivity, and applications. ACS Symposium Series 474. American Chemical Society, Washington, DC, pp 56–73

Koka CV, Cerny RE, Gardner RG, Noguchi T, Fujioka S, Takatsuto S, Yoshida S, Clouse SD (2000) A putative role for the tomato genes *DUMPY* and *CURL-3* in brassinosteroid biosynthesis and response. Plant Physiol 122:85–98

Lim SH, Chang SC, Lee JS, Kim SK, Kim SY (2002) Brassinosteroids affect ethylene production in the primary roots of maize (*Zea mays* L.). J Plant Biol 45:148–153

Losso M, Mondin VP (1991) Sistema de produção para a cultura da macieira (Sistemas de Produção n° 19). DID/EMPASC, Florianópolis

Mandava NB (1988) Plant growth-promoting brassinosteroids. Annu Rev Plant Physiol Plant Mol Biol 39:23–52

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473–497

Mussig C, Altmann T (1999) Physiology and molecular mode of action of brassinosteroids. Plant Physiol Biochem 37:363–372

Neljubow DN (1901) Uber die horizontale nutation der stengel von *Pisum sativum* und einiger anderen. Beih Bot Zentralbl 10:128–139

Nunes JCO, Barpp A, Silva FC, Pedrotti EL (1999) Micropropagation of rootstocks "marubakaido" (*Malus prunifolia*) through meristem culture. Rev Bras Frutic 21:191–195

Osborne DJ (1989) The control role of ethylene in plant growth and development. In: Clijsters H, De Proft M, Marcelle R, Van Poucke MM (eds) Biochemical and physiological aspects of ethylene production in lower and higher plants. Kluwer, Dordrecht, pp 1–11

Ramirez JA, Gros EG, Galagovsky LR (2000) Effects on bioactivity due to C-5 heteroatom substituents on synthetic 28-homobrassinosteroid analogs. Tetrahedron 56:6171–6180

Sasse J (1999) Physiological actions of brassinosteroids. In: Sakurai A, Yokota T, Clouse SD (eds) Brassinosteroids: Steroidal plant hormones. Springer-Verlag, Tokyo, pp 137–161

Schaefer S, Medeiro SA, Ramirez JA, Galagovsky LR, Pereira-Netto AB (2002) Brassinosteroid-driven enhancement of the *in vitro* multiplication rate for the marubakaido apple rootstock [*Malus prunifolia* (Willd.) Borkh]. Plant Cell Rep 20:1093–1097

Schwartzenberg K, Doumas P, Jouanin L, Pilate G (1994) Enhancement of the endogenous cytokinin concentration in poplar by transformation with *Agrobacterium* T-DNA gene ipt. Tree Physiol 14:27–35

Smalle J, Haegman M, Kurepa J, Van Montagu M, van Der Straeten D (1997) Ethylene can stimulate *Arabidopsis* hypocotyl elongation in the light. Proc Natl Acad Sci USA 94:2756–2761

van Staden J, Crouch NR (1996) Benzyladenine and derivatives—Their significance and interconversion in plants. Plant Growth Regul 19:153–175

Woeste KE, Vogel JP, Kieber JJ (1999) Factors regulating ethylene biosynthesis in etiolated *Arabidopsis thaliana* seedlings. Physiol Plant 105:478–484

Yokota T (1997) The structure, biosynthesis and function of brassinosteroids. Trends Plant Sci 2:137–143