Plant Cell Physiol. 39(1): 124–130 (1998)

Short Communication

Soybean *BRU*1 Encodes a Functional Xyloglucan Endotransglycosylase That is Highly Expressed in Inner Epicotyl Tissues during Brassinosteroid-Promoted Elongation

Man-Ho Oh [1], William G. Romanow [2], Rachel C. Smith [1, 5], Eli Zamski [1, 6], Jenneth Sasse [3] and Steven D. Clouse [1, 7]

[1] *Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695, U.S.A.*
[2] *PharMingen Co., 10975 Torreyana Road, San Diego, CA 92121, U.S.A.*
[3] *Forestry School, University of Melbourne, Parkville 3052 Australia*

Brassinosteroids promote soybean epicotyl elongation and regulate expression of *BRU*1, a gene with homology to xyloglucan endotransglycosylases (XETs). Recombinant BRU1 protein possesses XET activity and in situ hybridization reveals highest *BRU*1 transcript accumulation in inner epicotyl tissue, particularly the phloem and paratracheary parenchyma cells. These results suggest a role for *BRU*1 in vascular development in addition to cell elongation.

Key words: Brassinosteroid — *BRU*1 — Elongation — Soybean — XET — Xyloglucan endotransglycosylase.

Brassinosteroids (BRs) are widely distributed plant natural products with structural similarity to animal steroid hormones. BRs have been shown to promote cell elongation, regulate gene expression and enhance vascular differentiation in a number of plant systems (see Clouse 1996 for a recent review). Mutations that result in BR insensitivity (Clouse et al. 1996, Kauschmann et al. 1996) or lesions in genes encoding BR biosynthetic enzymes (Li et al. 1996, Szekeres et al. 1996) result in a similar phenotype that includes severely dwarfed stature, abnormal leaf development, reduced fertility, loss of apical dominance, and deetiolation of dark-grown plants. Genetic and physiological analyses of these mutants have provided convincing evidence that BRs are essential for normal plant growth and development.

We have shown that BRs promote elongation in apical epicotyl segments of soybean and have cloned a gene, *BRU*1, that is regulated specifically by BR during early stages of elongation in this tissue (Clouse et al. 1992, Zurek and Clouse 1994). Correlative evidence concerning spatial and temporal expression of the *BRU*1 gene, and the relationship of *BRU*1 expression to BR-mediated changes in cell wall mechanical properties, suggest that this gene product may play a role in BR-modulated cell elongation in soybean (Zurek et al. 1994). Sequence analysis of *BRU*1 reveals a marked degree of sequence identity to numerous xyloglucan endotransglycosylases (XETs) and XET-like sequences from *Arabidopsis, Azuki* bean, soybean, tomato, nasturtium, wheat and maize (Arrowsmith and de Silva 1995, Medford et al. 1991, Okazawa et al. 1993, Saab and Sachs 1995, de Silva et al. 1993, Xu et al. 1995, 1996).

Xyloglucans are major constituents of the hemicellulosic component of the primary cell wall in non-*Poaceous* monocotyledenous and dicotyledonous plants and are thought to be tethers that attach adjacent cellulose microfibrils (Carpita and Gibeaut 1993). For cell expansion to occur without loss of wall integrity, slippage or breakage of these tethers is required followed by reformation of the bonds. The discovery of XET (Fry et al. 1992, Nishitani and Tominaga 1992), an enzyme that cleaves xyloglucan specifically and attaches the cut ends to new xyloglucan acceptors, provided a candidate for a wall-loosening enzyme involved in expansion growth or an enzyme involved in wall strengthening after expansion has occurred. Both roles have been proposed, but no definitive evidence for either of these roles in muro is currently available (Cosgrove 1993, Nishitani 1997). It has also been proposed (Fry et al. 1992) that in addition to elongation, XETs may be involved in developmental processes requiring cell wall modification, such as abscission, fruit ripening and the formation of perforation plates and sieve plates in developing xylem and phloem cells. While the exact role of XETs remains uncertain, their widespread distribution (Nishitani 1997) and presence as differentially regulated multi-gene families (Xu et al. 1996) indicates that these enzymes are likely to play an important role in some critical process involving cell walls.

Tominaga et al. (1994) showed that in squash hypocotyls, the effect of IAA on elongation and cell wall mecha-

Abbreviations: BR, brassinosteroid; XET, xyloglucan endotransglycosylase.

[5] Present address: SAC, W. Mains Rd, Edinburgh EH9 3JG Scotland.
[6] Department of Agricultural Botany, Hebrew University, PO Box 12, Rehovot 76100 Israel (E.Z.).
[7] Corresponding author, e-mail: steve_clouse@ncsu.edu

nical properties was limited to the epidermis. BR, on the other hand, stimulated elongation and wall relaxation in both epidermal and inner tissues, but with a more pronounced effect on inner tissues. They therefore proposed that BR had inner stem tissue as its primary target. It was of considerable interest then, to examine the spatial distribution of *BRU*1 transcripts to determine if there was preferential expression in inner tissues of soybean epicotyls.

Moreover, while the sequence similarity of *BRU*1 to other proven XETs suggested a biological function, no direct evidence that *BRU*1 encoded an active XET was available. It was possible that *BRU*1 encoded a wall metabolizing enzyme related to XET but with a different wall polymer as substrate. In this paper we show that the BRU1 gene product in fact has XET activity and also provide studies on spatial regulation of expression of an XET (and a BR-regulated gene) by in situ hybridization.

To construct an expression cassesette, the Baculovirus vector pAcSG1 (PharMingen Co., San Diego, CA, U.S.A.) was digested with *Eco*RI and *Kpn*I and ligated to a full-length *BRU*1 cDNA (Zurek and Clouse 1994) cut with the same enzymes. SF9 insect cells (*Spodoptera frugiperda*) were grown in TNM-FH medium (Hink 1970) and maintained in exponential growth by splitting cultures 1 : 3 every 48 h. For cotransfection, 0.5 μg of linearized BaculoGold DNA (PharMingen) and 2.0 μg purified recombinant vector DNA were added to SF9 cells followed by incubation at 27°C for 5 days. For production of recombinant protein, the supernatant from the cotransfection plates was used to generate high titer viral infection stock which was incubated with *Trichoplusia ni* cells in BaculoGold Protein Free Insect Cell Medium (PharMingen) at a multiplicity of infection of 5 to 10. For XET assays of recombinant BRU1, cell lysates were prepared from 30 ml of infected insect cell cultures by centrifugation at 2,500 × *g* for 5 min followed by resuspension of the cell pellet in 1 ml of lysis buffer (50 mM Tris, pH 7.5; 650 mM NaCl; 5% Triton-X 100; 50 mM NaF; 50 mM sodium phosphate, pH 7.5; 50 mM sodium pyrophosphate, pH 7.5) per 4×10^6 cells. Cells were lysed on ice for 45 min and the lysate was cleared by centrifugation at 45,000 × *g* for 30 min.

High molecular weight xyloglucan was purified from *Tropaeolum majus* (nasturtium) seeds by the method of Edwards et al. (1985). A portion of the nasturtium xyloglucan was digested with *Trichoderma viride* cellulase and subjected to Bio-Gel P2 (Bio-Rad, Richmond, CA, U.S.A.) chromatography to generate xyloglucan oligosaccharides (XG7, XG8, XG9) as described by McDougall and Fry (1990). Oligosaccharides were tritiated by incubating with NaB^3H_4 as previously described (Hetherington and Fry 1993). To determine XET activity, 10 μl of extract (clarified cell lysate or soybean epicotyl segment homogenate) was added to 30 μl of reaction mixture containing 0.3% (w/v) nasturtium xyloglucan and 9.4 kBq [^{3}H-reducing termini] xyloglucan oligosaccharols in 67 mM MES, pH 6.0. Reactions conditions were those described in Fry et al. (1992).

For XET assay or in situ hybridization, soybean seeds (*Glycine max* L., cv. Williams 82, purchased from Wilkens Seed Grains, Pontiac, IL, U.S.A.) were sown in flats containing 50% vermiculite/50% perlite in a greenhouse with minimum day/night temperatures of 24/18°C. Seedlings were harvested between 10 and 14 days and apical epicotyl segments were treated with the brassinosteroid, brassinolide (2α,3α,22(R),23(R)-tetrahydroxy-24(S)-methyl-B-homo-7-oxa-5α-cholestan-6-one), or buffer exactly as described (Clouse et al. 1992). For in situ hybridization, 1 to 2 mm long epicotyl segments were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 6 h, dehydrated in a graded ethanol series and embedded in a 'hard grade' L.R. White resin (London Resin Co., U.K.). One μm thick sections were prepared using an 'Ultratome Nova' microtome (LKB, Sweden) and mounted on silylated microscope slides. Sections were rehydrated in PBS (100 mM sodium phosphate, 150 mM NaCl, pH 7.4) and incubated in 0.02 M HCl for 10 min, followed by washing in PBST (PBS + 0.1% Tween-20 [v/v]). Slides were then washed in PBS and incubated in Proteinase K (100 μg ml^{-1}) at 37°C for 30 min. Finally, slides were washed in PBS with glycine (2 mg ml^{-1}), incubated in cold 20% acetic acid (v/v) for 15 s, rinsed in PBS, and air dried prior to hybridization.

RNA hybridization probes labeled with biotin were synthesized as previously described using as the template a truncated form of p*BRU*1 consisting of 820 bp of 3′ sequence (Zurek and Clouse 1994). Fluorescein labeled probes were synthesized using the Fluorscript in vitro Transcription Kit (Ambion, Austin, TX, U.S.A.). Unincorporated nucleotides were removed by Sephadex G-50 chromatography or LiCl precipitation. Slides were hybridized overnight a 55°C with 4 μg probe in 500 μl of hybridization buffer (50% formamide, 37 mM PIPES, pH 7.2, 750 mM NaCl, 5 mM EDTA, 5 × Denhardt's solution (1 × = 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinyl pyrollidine), 0.1 mg ml^{-1} sheared salmon sperm DNA). After hybridization, slides were gently washed with 4 × SSC (1 × = 0.15 M NaCl, 0.015 M sodium citrate) at room temperature for 10 min, followed by 2 × 30 min rinses with 2 × SSC at room temperature. The slides were washed again in 1 × SSC at 55°C for 45 min. Slides were incubated for 45 min in 1.0% BSA in PBS (v/v) followed by a 1 h incubation in streptavidin-gold (50 μg ml^{-1} in PBST) and washed 2 × 5 min with PBST. After additional 2 × 5 min washes in PBS the slides were flooded with 1% gelatin (Bio-Rad EIA grade) for 15 min, washed with water and enhanced with silver (Amersham). When fluorescein labeled RNA probes were used, the protocol of the 'RNA Color Kit For Non-Radioactive In Situ Hybridization' (Amersham) was followed.

Figure 1 shows that cell lysates of *Trichoplusia ni* ex-

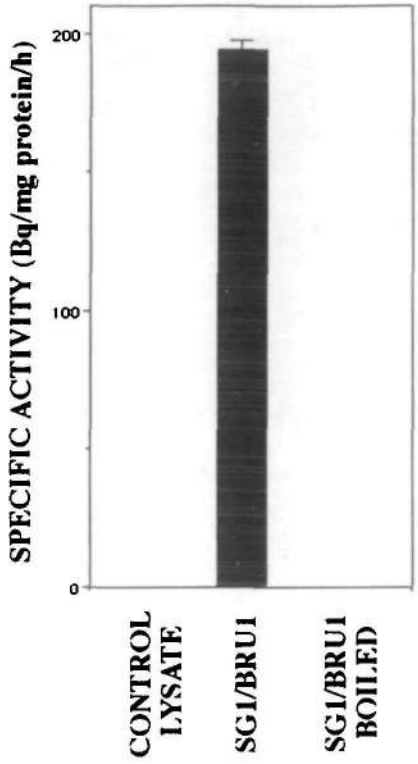

Fig. 1 Xyloglucan endotransglycosylase activity of recombinant BRU1 protein. A full length *BRU*1 cDNA was cloned into a baculovirus expression vector to generate SG1/BRU1. Plasmid constructs were cotransfected with linear baculovirus DNA into *Trichoplusia ni* insect cells. Cell lysate supernatants were examined for XET activity using ^{3}H-xyloglucan oligomers and unlabeled nasturtium xyloglucan as described by Fry et al. (1992). Specific activity is expressed as Bq of tritiated, high molecular weight xyloglucan formed per mg soluble protein per hour. Each data point represents the average of six independent assays ±SE.

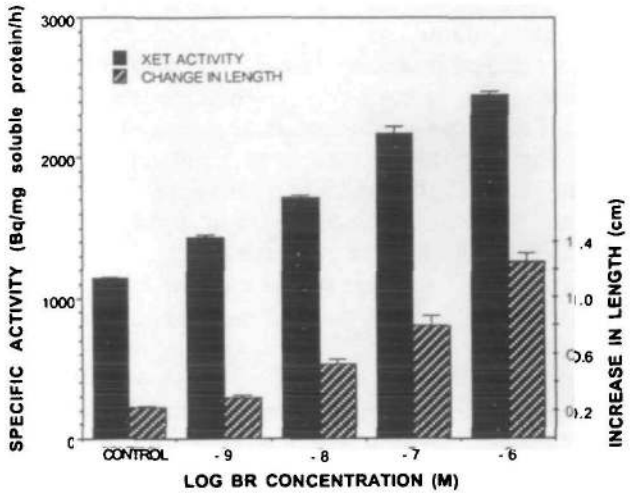

Fig. 2 Effect of brassinosteroid on xyloglucan endotransglycosylase activity in elongating soybean epicotyls. Light-grown soybean seedlings were harvested at day 10 and the apical 1.5 cm portions of the epicotyls were treated with various concentrations of the brassinosteroid (BR), brassinolide, for 17 h to induce elongation (Clouse et al. 1992). Epicotyls were then measured for elongation, homogenized and assayed for XET activity as described (Fry et al. 1992). Specific activity is expressed as Bq of tritiated, high molecular weight xyloglucan formed per mg soluble protein per hour. Each enzyme assay point represents the average of six independent assays ±SE. Each elongation point is the average of 10 independent measurements ±SE.

pressing SGI/BRU1 contain XET activity of 190 Bq (mg protein)$^{-1}$ h^{-1}. Neither control lysates of *T. ni* cells transfected with non-recombinant vector or boiled recombinant SG1/BRU1 showed XET activity. Based on sequence homology to known XETs and the ability of recombinant BRU1 protein to catalyze xyloglucan transglycosylation as measured by the standard XET assay (Fry et al. 1992), it is now apparent that *BRU*1 encodes a BR-regulated XET in soybean stems. It follows from this, and what is known about BR-promoted stem elongation (Clouse et al. 1992), that XET levels should increase with BR treatment of soybean epicotyls. Figure 2 shows that extractable XET activity increases linearly with increasing BR concentrations and is positively correlated with increasing epicotyl length.

Previous correlative evidence, including expression levels in elongating tissue, correspondence of increased *BRU*1 transcript levels with increased plastic extensibility of cell walls and structure-function analyses, suggested that *BRU*1 encoded a wall-modifying enzyme involved in BR-modulated stem elongation. The identification of *BRU*1 as an XET strengthens the argument for a role of BRU1 in wall alterations during elongation. *BRU*1 can now be added to the growing list of cloned genes with proven XET activity such as *NXG*1, a nasturtium cotyledon gene (de Silva et al. 1993), *Azuki* bean *EXT* (Okazawa et al. 1993), tomato fruit pericarp *EXT* (Arrowsmith and de Silva 1995) *Arabidopsis TCH*4 (Xu et al. 1995), tomato *tXET*-2 (Arrowsmith and de Silva 1995) and barley *EXT*11 (Smith et al. 1996). *BRU*1 is the first XET to be expressed in the baculovirus/insect cell system which provides the advantage of eukaryotic post-translational modifications, such as glycosylation. Direct comparison of the effectiveness of the *E. coli* vs. the insect cell systems in producing recombinant XET protein is not possible since TCH4 and tXET-2 were purified before assaying, whereas BRU1 assays were performed on crude cell lysates.

With respect to the mechanism of soybean epicotyl elongation, it is probable that BR promotes elongation in part by increasing the abundance of the *BRU*1 message which is translated into an XET that acts either alone or with other factors to alter cell wall properties. The correlative evidence cited above and our present finding that increasing BR concentrations cause extractable XET activity

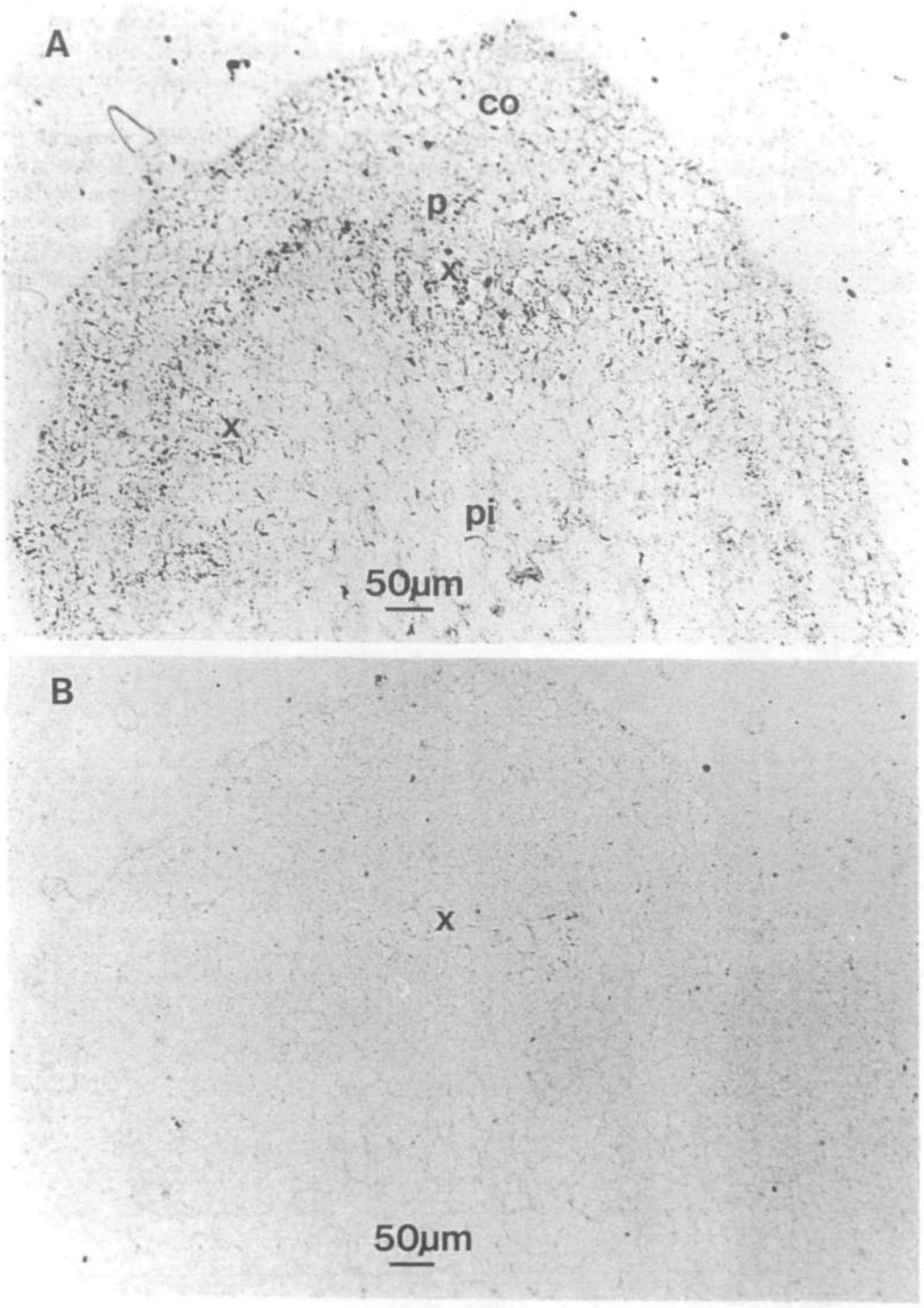

Fig. 3 In situ hybridization of *BRU*1 in elongating soybean epicotyls. Light-grown soybean seedlings were harvested at day 14 and the apical 1.5 cm portions of the epicotyls were treated with 10^{-7} M brassinolide for 17 h to induce elongation. One micron cross sections were hybridized with antisense (panel A) and sense (panel B) biotin-labeled RNA probes followed by streptavidin-gold treatment and silver enhancement. Sections treated with the antisense probe show labeling throughout the entire section, but the strongest reaction appeared in the inner tissues, particularly the xylem parenchyma cells and the phloem. CO, cortex; P, phloem; Pi, pith; X, xylem.

from soybean epicotyls to increase at the same linear rate as overall elongation, are in agreement with this hypothesis. Studies on the *TCH*4 gene in *Arabidopsis* provide further evidence that XETs are involved in BR-promoted elongation. The *TCH*4 gene has been shown to encode an active XET and application of 1.0 μM BR results in an increase in *TCH*4 transcripts within 30 minutes, with a maximum at two hours (Xu et al. 1995). A *TCH*4 promoter:β-glucuronidase fusion consisting of 958 bp of 5′ flanking region and 48 bp of the 5′ untranslated leader transformed into *Arabidopsis*, resulted in BR-regulated β-glucuronidase expression in elongating tissue (Xu et al. 1995). Moreover, BR deficient and insensitive mutants in *Arabidopsis*, which exhibit an extremely dwarfed phenotype due to decreased cell elongation (Clouse 1996), show much reduced *TCH*4 expression (Kauschmann et al. 1996), suggesting that XETs are an important component of the cell elongation machinery.

Previous studies of XET transcript levels have relied on gel blot hybridizations and RNase protection assays to assess organ-specific, but not tissue specific, expression. To more precisely define the spatial pattern of *BRU*1 expression in elongating soybean epicotyls, we synthesized labeled *BRU*1 RNA transcripts for in situ hybridization of 1

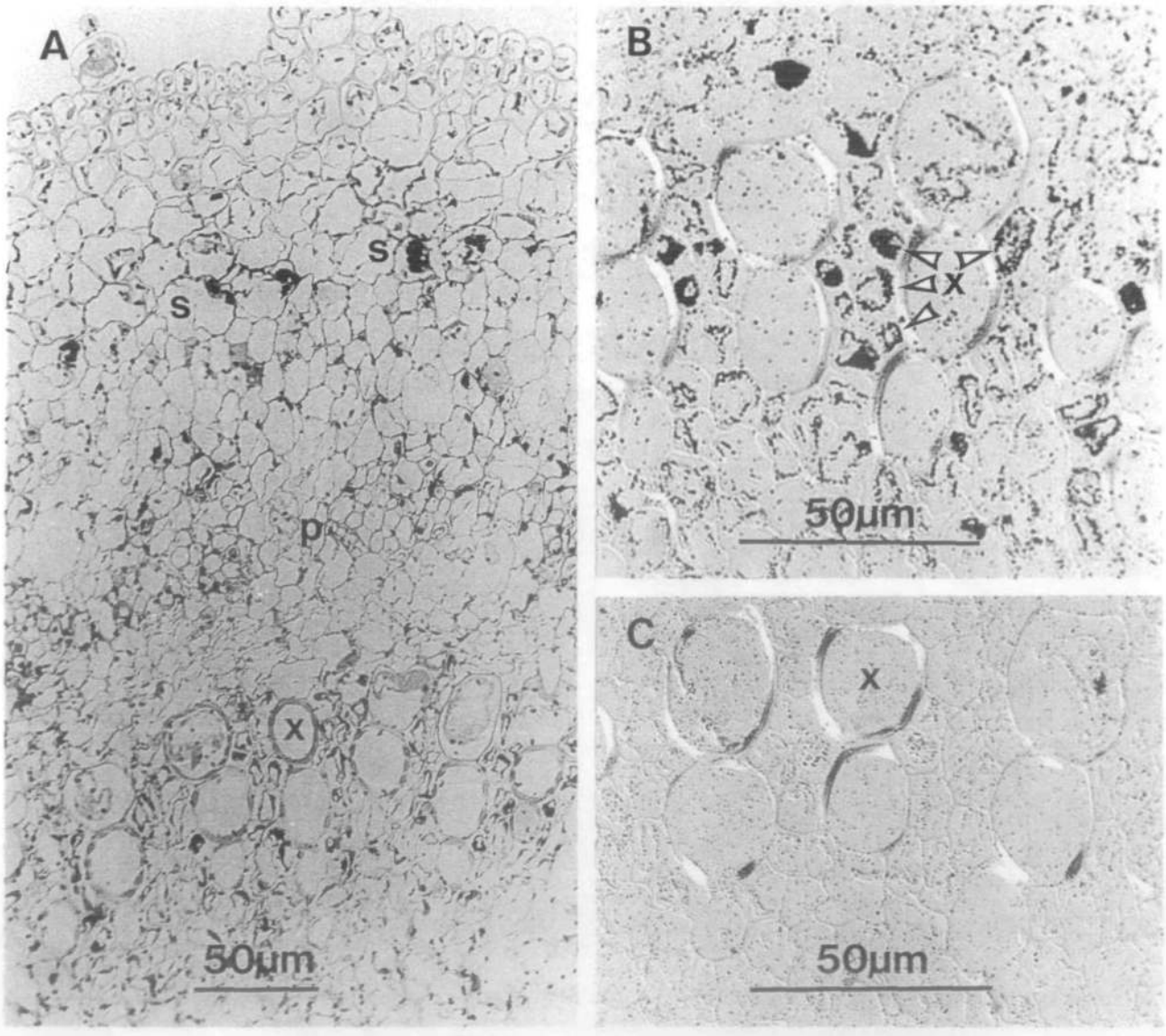

Fig. 4 *BRU*1 is expressed at high levels in vascular tissue of elongating soybean epicotyls. Panel A shows a one micron cross section as described in Fig. 3, hybridized with a fluorescein-labeled *BRU*1 antisense probe. Strong expression in the phloem (P), xylem (X) and starch sheath cells (S) is observed. Panels B (antisense probe) and C (sense probe) are higher magnifications of Fig. 3A and 3B respectively. Note the strong labeling in the cytoplasm of the parenchyma cells around the vessels (arrow heads in panel B).

μm epicotyl cross-sections. Figure 3a shows that the biotinylated antisense *BRU*1 probe hybridized to a corresponding message throughout the BR-treated, apical epicotyl cross-section, but the signal was particularly strong in vascular tissue. Higher magnification (Fig. 4a, b) confirmed the very high expression of *BRU*1 in phloem cells and in parenchyma cells surrounding the xylem elements, and also in the starch sheath layer (the innermost cortex layer with large starch grains). A duplicate section hybridized with a biotinylated sense *BRU*1 probe showed no signal (Fig. 3b; Fig. 4c). A similar experiment performed on apical epicotyl sections that were not treated with BR showed a similar pattern of expression with the antisense probe, but at a much reduced level of intensity (data not shown).

These results are consistent with the hypothesis of Tominaga et al. (1994) that inner tissue may be the primary target of BR action. However, we previously found that BR could promote hypocotyl elongation in an auxin-insensitive tomato mutant (Zurek et al. 1994), indicating that the effect of BR on outer tissue may be sufficient to promote elongation without auxin activity, although perhaps with a slower response.

The in situ hybridization also showed marked accumulation of *BRU*1 transcripts in vascular tissue. The role of auxins and cytokinins in xylem differentiation are well known (Fukuda 1996), but several lines of evidence have also implicated BRs in this process. The promotive effect of BR on xylogenesis has been demonstrated in both major in vitro model systems of tracheary element formation, *Helianthus tuberosus* explants and *Zinnia elegans* mesophyll cells (Clouse and Zurek 1991, Iwasaki and Shibaoka 1991); BRs have been isolated from differentiating xylem tissue (Kim et al. 1990); a BR-deficient *Arabidopsis* mutant exhibits abnormal vascular development (Szekeres et al. 1996); and *TCH*4 promoter-driven expression of β-glucuronidase is strong in the vascular tissue of roots and shoots in transgenic *Arabidopsis* plants (Xu et al. 1995). Our results show a clear relationship between BR treatment and enhanced expression of XET message in vascular tissue, thereby strengthening the argument that BRs, and XETs, are involved in vascular development.

The complex molecular architecture of the plant cell wall suggests that the enzymology and hormonal regulation of wall loosening and cell elongation is also likely to be complex. While the correlative evidence linking XET with elongation has become quite extensive, there are still troubling exceptions and it is not yet possible to conclude that XET has wall loosening activity. It may be that other enzymes, such as expansins (Cosgrove 1993), mediate hormone-promoted wall loosening and XETs may function to incorporate new xyloglucan into the growing wall to maintain structural integrity. Our identification of the *BRU*1 gene as an XET will allow examination of the specific role of this enzyme in BR-promoted growth in a well-defined system. We are currently generating transgenic soybeans expressing antisense *BRU*1 to determine the effect of loss of function of this XET on BR-modulated cell wall mechanical properties in soybean epicotyls. Furthermore, our findings that BR strongly enhances XET expression in vascular tissue should give impetus to the study of BR effects on vascular development.

This work was supported in part by grants from the United States Department of Agriculture National Research Initiative Competitive Grants Program, the U.S. National Science Foundation and the North Carolina Agricultural Research Service. M-H.O. was supported in part by a postdoctoral fellowship from the Korea Science and Engineering Foundation. We thank Dr. T.C. McMorris, University of California, San Diego, for supplying the brassinolide used in these experiments. We also thank Drs. P.E. Taylor, G.I. McFadden, and R.B. Knox, University of Melbourne; and Dr. N. Allen, North Carolina State University, for contributions to the in situ hybridization experiments.

References

Arrowsmith, D.A. and de Silva, J. (1995) Characterization of two tomato fruit-expressed cDNAs encoding xyloglucan endotransglycosylase. *Plant Mol. Biol.* 28: 391–403.

Carpita, N.C. and Gibeaut, D.M. (1993) Structural models of the primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* 3: 1–30.

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

Clouse, S.D., Langford, M. and McMorris, T.C. (1996) A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. *Plant Physiol.* 111: 671–678.

Clouse, S.D. and Zurek, D. (1991) Molecular analysis of brassinolide action in plant growth and development. *In* Brassinosteroids; Chemistry, Bioactivity and Applications, ACS Symposium Series 474. Edited by Cutler, H.G., Yokota, T. and Adam, G. pp. 122–140. American Chemical Society, Washington D.C.

Clouse, S.D., Zurek, D.M., McMorris, T.C. and Baker, M.E. (1992) Effect of brassinolide on gene expression in elongating soybean epicotyls. *Plant Physiol.* 100: 1377–1383.

Cosgrove, D.J. (1993) How do plant cell walls extend? *Plant Physiol.* 102: 1–6.

de Silva, J., Jarman, C.D., Arrowsmith, D.A., Stronach, M.S., Chengappa, S., Sidebottom, C. and Reid, J.S. (1993) Molecular characterization of a xyloglucan-specific *endo*-(1,4)-β-D-glucanase (xyloglucan *endo*-transglycosylase) from nasturtium seeds. *Plant J.* 3: 701–711.

Edwards, M., Dea, I.C., Bulpin, P.V. and Reid, J.S. (1985) Xyloglucan (amyloid) mobilisation in the cotyledons of *Tropaeolum majus* L. seeds following germination. *Planta* 163: 133–140.

Fry, S.C., Smith, R.C., Renwick, K.F., Martin, D.J., Hodge, S.K. and Matthews, K.J. (1992) Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. *Biochem. J.* 282: 821–828.

Fukuda, H. (1996) Xylogenesis: initiation, progression, and cell death. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 299–325.

Hetherington, P.R. and Fry, S.C. (1993) Xyloglucan endotransglycosylase activity in carrot cell suspensions during cell elongation and somatic embryogenesis. *Plant Physiol.* 103: 987–992.

Hink, W.F. (1970) Established insect cell line from the cabbage looper, *Tichoplusia ni*. *Nature* 226: 466–467.

Iwasaki, T. and Shibaoka, H. (1991) Brassinosteroids as regulators of tracheary-element differentiation in isolated *Zinnia* mesophyll cells. *Plant Cell Physiol.* 32: 1007–1014.

Kauschmann, A., Jessop, A., Koncz, C., Szekeres, M., Willmitzer, L. and Altmann, T. (1996) Genetic evidence for an essential role of brassinosteroids in plant development. *Plant J.* 9: 701–713.

Kim, S.K., Abe, H., Little, C.H. and Pharis, R.P. (1990) Identification of two brassinosteroids from the cambial region of Scots pine (*Pinus silvestris*) by gas chromatography-mass spectrophotometry, after detection using a dwarf rice lamina inclination bioassay. *Plant Physiol.* 94: 1709–1713.

Li, J., Nagpal, P., Vitart, V., McMorris, T.C. and Chory, J. (1996) A role for brassinosteroids in light-dependent development of *Arabidopsis*. *Science* 272: 398–401.

McDougall, G.J. and Fry, S.C. (1990) Xyloglucan oligosaccharides promote growth and activate cellulase: evidence for a role of cellulase in cell elongation. *Plant Physiol.* 93: 1042–1048.

Medford, J.I., Elmer, J.S. and Klee, H.J. (1991) Molecular cloning and characterization of genes expressed in shoot apical meristems. *Plant Cell* 3: 359–370.

Nishitani, K. (1997) The role of endoxyloglucan transferase in the organization of plant cell walls. *Int. Rev. Cytology* 173: 157–206.

Nishitani, K. and Tominaga, R. (1992) Endo-xyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule to another xyloglucan molecule. *J. Biol. Chem.* 267: 21058–21064.

Okazawa, K., Sato, Y., Nakagawa, T., Asada, K., Kato, I., Tomita, E. and Nishitani, K. (1993) Molecular cloning and cDNA sequencing of endoxyloglucan transferase, a novel class of glycosyltransferase that mediates molecular grafting between matrix polysaccharides in plant cell walls. *J. Biol. Chem.* 268: 25364–25368.

Saab, I.N. and Sachs, M.M. (1995) Complete cDNA and genomic sequence encoding a flooding-responsive gene from maize (*Zea mays* L.) homologous to xyloglucan endotransglycosylase. *Plant Physiol.* 108: 439–440.

Smith, R.C., Matthews, P.R., Schünmann, P.H. and Chandler, P.M. (1996) The regulation of leaf elongation and xyloglucan endotransglycosylase by gibberellin in 'Himalaya' barley (*Hordeum vulgare* L.). *J. Exp. Bot.* 47: 1395–1404.

Szekeres, M., Nemeth, K., Koncz-Kalman, Z., Mathur, J., Kauschmann, A., Altmann, T., Redei, G.P., Nagy, F., Schell, J. and Koncz, C. (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. *Cell* 85: 171–182.

Tominaga, R., Sakurai, N. and Kuraishi, S. (1994) Brassinolide-induced elongation of inner tissues of segments of squash (*Cucurbita maxima* Duch.) hypocotyls. *Plant Cell Physiol.* 35: 1103–1106.

Xu, W., Campbell, P., Vargheese, A.K. and Braam, J. (1996) The *Arabidopsis* XET-related gene family: environmental and hormonal regulation of expression. *Plant J.* 9: 879–889.

Xu, W., Purugganan, M.M., Polisensky, D.H., Antosiewicz, D.M., Fry, S.C. and Braam, J. (1995) *Arabidopsis TCH4*, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. *Plant Cell* 7: 1555–1567.

Zurek, D.M. and Clouse, S.D. (1994) Molecular cloning and characterization of a brassinosteroid-regulated gene from elongating soybean epicotyls. *Plant Physiol.* 104: 161–170.

Zurek, D.M., Rayle, D.L., McMorris, T.M. and Clouse, S.D. (1994) Investigation of gene expression, growth kinetics and wall extensibility during brassinosteroid-regulated stem elongation. *Plant Physiol.* 104: 505–513.

(Received August 7, 1997; Accepted October 17, 1997)