Expression of gibberellin 3 β -hydroxylase gene in a gravi-response mutant, weeping Japanese flowering cherry

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Abstract Expressions of the gibberellin biosynthesis gene were investigated in a normal upright type and a gravi-response mutant, a weeping type of Japanese flowering cherry (*Prunus spachiana*), that is unable to support its own weight and elongates downward. A segment of the gibberellin 3 β -hydroxylase cDNA of *Prunus spachiana* (*Ps3ox*), which is responsible for active gibberellin synthesis, was amplified by using real-time RT-PCR. The content of *Ps3ox* mRNA in the weeping type was much greater than that in the upright type, while the endogenous gibberellin level was much higher in the elongating zone of the weeping type. These results suggest that the amount and distribution of synthesized gibberellin regulate secondary xylem formation, and the unbalanced distribution of gibberellin affects the gravi-response of the *Prunus* tree.

Key words: Japanese flowering cherry, weeping type, gibberellin, 3 β -hydroxylase gene

Introduction

Woody plants develop secondary xylem inside the cambium to support a large body and to extend their lives. It is generally believed that cambial activity and differentiation are under the control of several factors, including plant hormones. One of the plant hormones, gibberellin, plays a major role in the regulation of shoot elongation and the production of xylem and phloem by the vascular cambium in woody plants. It has been reported that treatment of trees with gibberellin improves development of xylem and cambial activity (Aloni *et al.* 1987; Bradley and Crane 1957; Wang *et al.* 1995), and the elongation of secondary xylem fibers was correlated with endogenous levels of gibberellin A₁ (GA₁) (Ridoutt *et al.* 1996). Tension wood is formed in secondary xylem reacting to the stimulation of gravity and causes negative gravitropism in branches of the normal upright type of Japanese cherry. On the other hand, the branches of the weeping type of Japanese cherry are unable to support their own weight and elongate downward because tension wood is not formed in its branches. Because gibberellin applied to apical buds of the weeping type promoted the development of secondary xylem and inhibited bending (Baba *et al.* 19995; Nakamura 1996; Nakamura *et al.* 1994a, b, 1996), we investigated whether the inadequate development of xylem in the weeping type is resulted

from shortage of gibberellin. In this study we examined gibberellin biosynthesis, including the gene expression and endogenous level of gibberellin in both the upright and weeping types of Japanese flowering cherry. GA₁ was analyzed in this research because we had already confirmed that the main active gibberellin was GA₁ by its endogenous level and bioactivity (Kobayashi *et al.*, 1996).

Materials and Methods

Plant material

The current year's branches of five-year-old grafts of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenorosea*, a weeping type, and *Prunus spachiana* Kitamura f. *ascendens* Kitamura, an upright type, of Japanese cherry were used for plant material. These grafts were grown in the agricultural field of Japan Women's University.

RNA extraction and cDNA cloning

Total RNA was extracted from the buds using a method described by Gévaudant *et al.* (1999). For first-strand cDNA synthesis, reverse transcription PCR (RT-PCR) was performed using the SMART RACE cDNA Amplification kit according to the manufacturer's protocol (BD Biosciences Clontech, CA, USA) with approximately 5 μ g of total RNA.

Cloning of gibberellin 3 β -hydroxylase full-length cDNA

A pair of degenerate primers was designed based on the corresponding sequences of other plants (Kang *et al.* 2002) and synthesized to isolate the gibberellin 3 β -hydroxylase cDNA. The following primers were used for degenerate PCR: forward (5'-ATGTGGHVIGARGG ITTYAC-3') and reverse (5'-GTRTGIGSIGCIARICCC AT-3'), where H was not G, R was A or G, S was C or G, V was not T, Y was C or T and I was inosine. First-strand

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Gibberellin biosynthesis in Japanese weeping cherry

cDNA synthesized by RT-PCR was used as the template. The PCR reaction was initiated by heating to 94°C for 5 min, followed by 35 cycles of 95°C for 1 min, 55°C for 90 s and 72°C for 30 s. This reaction was completed by

a 10-min incubation at 72°C. The products were purified by 1.5% (w/v) agarose gel electrophoresis and cloned into pGEM-T easy vector (Promega, Madison, WI, USA). A DYEnamic ET Terminator Cycle Sequencing

Prs Ps3ox	1	MPAR-LSDAFKSHPLNLQQLKHP-DFSSQLQELPDSYAWSQ-ND-DQYPPSPGSGFAGQDSV	61
At GA4	1	MPAM-LTDVFRGHPIHLPHSHIPDFTSLRELPSYKWTPKDD-LLFSAAPSPATGE-NIPLIDL	62
At GA4H	1	MSST-LSDVFRSHPIHPLSNPPDF--KSLPDSYTWTPKDD-LLFSA--S--ASDE-TLPLIDL	55
Ps LE	1	MPS--LSEAYRAHPVHVNHKHP-DFNSLQELPESYNWTHLDD-HTLIDSNNIMKESTTTVEIDL	61
Le Le3OH-1	1	MPSR-ISDSCRPH--H-SQKHF-DLNSIKELPESHAWTS-SN-DHYTQENSC-NFE--SIEVIDL	55
Ls Ls3h1	1	MHTR-VADAFKAHPM-VNQKHL-DLKSMKELPESHAW--LSQ-DG-SPSYGSSSSEQ--VEVINL	56
Nt Nty	1	MPSR-ISDSFRAH---S-QKHL-DLNSIKELPESHAWTS-SN-D-YPSENSC-NFE--SIEVIDL	53
Ptt GA3ox1	1	MPSRSLADAFRSHPVHLHQKHL-DFSSLQELPDSHKWTQLDDIEQQHPSVESFITE--SVEVIDL	62
St Ga3b-1	1	MPSR-ISDSCRPH--H-SQKHF-DLNSIKELPESHAWTS-SN-DHYTQENSC-NFE--SIEVIDL	55
Prs Ps3ox	62	SD-PNA--LK-L--TCHACTRWGVFCVTNHGIPSKLLDDIESAGRSLESLPVQQKLKAARSPDGI	120
At GA4	63	DH-PDA--TN-Q--IGHACTRWGAFQISNHGVPLGLLQDIEFLTGSLEGLPVQRKLKSARSETGV	121
At GA4H	56	SD-IHV--AT-L--VGHACTRWGAFQITNHGVPSRLDDIEFLTGSLEGLPVQRKLKAARSENGV	114
Ps LE	62	ND-PNA--SK-L--IGHACKTWGVVQVMNHGIPSLLEDIQWLQOTLPSLPHQKHKATRSPDGV	120
Le Le3OH-1	56	DNNNNNNNNNILDHIGHACKKWGAFCIINHSEIKLLQDIEVAGKTLPSLPMQQKLKAARSPDGV	120
Ls Ls3h1	57	KD-PNA--MK-L--VGHACKTWGVFCVTNHGVPNTNLEEMEAAGRKLKALPTQQKLKAARAPDGV	115
Nt Nty	54	DNYINNINNINVLHIGGACKKWGAFCIINHSEIKLLQDIELAGKSLPSLPMQQKLKAARSPDGV	118
Ptt GA3ox1	63	LD-PNV--LQ-N--IGHACKTWGVFCVTNHGIPISLLESVEGVSRSLPSLPVQQKLKAARSPDGV	121
St Ga3b-1	56	DNNNNNNNNNILDHIGHACKKWGAFCIINHSEIKLLQDIEVAGKTLPSLPMQQKLKAARSPDGV	120
Prs Ps3ox	121	SGYGFARISFFQKLMWSEGFTIVGSPLDHFRQLWPDYDNKFCNIEEYEEKVMKRLAGRLMWLML	185
At GA4	122	SGYGVARIASFENKQMWSEGFTITGSPLNDFRKLWPDYDNKFCNIEEYEEKVMKRLAGRLMWLML	186
At GA4H	115	SGYGVARIASFENKQMWSEGFTIVGSPLDHFRKLWPDYDNKFCNIEEYEEKVMKRLAGRLMWLML	179
Ps LE	121	SGYGIARISFFQKLMWYEGFTIVGSPLDHFRQLWPDYDNKFCNIEEYEEKVMKRLAGRLMWLML	185
Le Le3OH-1	121	TGYGAARISFFQKLMWSEGFTIVGSPLEHARQIWPQDYDNKFCNIEEYEEKVMKRLAGRLMWLML	185
Ls Ls3h1	116	SGYGVARIASFENKQMWSEGFTIVGSPLEHARQIWPQDYDNKFCNIEEYEEKVMKRLAGRLMWLML	180
Nt Nty	119	TGYGVARIASFENKQMWSEGFTIVGSPLEHARQIWPQDYDNKFCNIEEYEEKVMKRLAGRLMWLML	183
Ptt GA3ox1	122	SGYGVARIASFENKQMWSEGFTIVGSPLEHARQIWPQDYDNKFCNIEEYEEKVMKRLAGRLMWLML	186
St Ga3b-1	121	TGYGAARISFFQKLMWYEGFTIVGSPLEHARQIWPQDYDNKFCNIEEYEEKVMKRLAGRLMWLML	185
Forward			
Prs Ps3ox	186	GSGLISMEDVKNW-GPKGDFRDASAALQLNSYPACPDPRAMGLAAHTDSTLLTILYQNNTSGLO	249
At GA4	187	NSLGVSEEDIEWA-SLSSDLNWAQAALQLNHYPVCPEPDRAMGLAAHTDSTLLTILYQNNTAGLO	250
At GA4H	180	GSGLVEEKDIQNA-GPNSDFGQTQAALQLNHYPVCPEPDRAMGLAAHTDSTLLTILYQNNTAGLO	243
Ps LE	186	DSLGITKEDIKNA-GSKAQFEKACAAALQLNSYPACPDPRAMGLAAHTDSTLLTILYQNNTSGLO	249
Le Le3OH-1	186	GSGLITKDDVKNWAVGPKGETKEGCAALQLNSYPACPDPRAMGLAAHTDSTLLTILYQNNTSGLO	250
Ls Ls3h1	181	GSGLVTTEDVKNW-GSQG---SCPALQLNSYPACPDPRAMGLAAHTDSTLLTILYQNNTSGLO	240
Nt Nty	184	GSGLITKEEVKNWAVCPKGESKGGSAALQLNSYPACPDPRAMGLAAHTDSTLLTILYQNNTSGLO	248
Ptt GA3ox1	187	GSGLITKDDLNAW-GPKGESKGGSAALQLNSYPACPDPRAMGLAAHTDSTLLTILYQNNTSGLO	250
St Ga3b-1	186	GSGLITKDDVKNWAVGPKGETKEGCAALQLNSYPACPDPRAMGLAAHTDSTLLTILYQNNTSGLO	250
Reverse			
Prs Ps3ox	250	VLREGTGWVTVPPFPGSLVNVNVDLHILSNGLYPSVLHRAVNVNRSRHLSTAYLYGPPASVQIS	314
At GA4	251	VFRDDLGWVTVPPFPGSLVNVNVDLHILSNGLYPSVLHRAVNVNRSRHLSTAYLYGPPASVQIS	315
At GA4H	244	VFRDDVGVVTPAPPVPSGLVNVNVDLHILSNGLYPSVLHRAVNVNRSRHLSTAYLYGPPASVQIS	308
Ps LE	250	VNREGSGWVTVPPFPGSLVNVNVDLHILSNGLYPSVLHRAVNVNRSRHLSTAYLYGPPASVQIS	314
Le Le3OH-1	251	VYQEGNGWVTVPPFPGSLVNVNVDLHILSNGLYPSVLHRAVNVNRSRHLSTAYLYGPPASVQIS	315
Ls Ls3h1	241	AHREGAGWVTVPPFPGSLVNVNVDLHILSNGLYPSVLHRAVNVNRSRHLSTAYLYGPPASVQIS	305
Nt Nty	249	VFKEGSGWVTVPPFPGSLVNVNVDLHILSNGLYPSVLHRAVNVNRSRHLSTAYLYGPPASVQIS	313
Ptt GA3ox1	251	VLKEGIGWVTVPPFPGSLVNVNVDLHILSNGLYPSVLHRAVNVNRSRHLSTAYLYGPPASVQIS	315
St Ga3b-1	251	VYQEGNGWVTVPPFPGSLVNVNVDLHILSNGLYPSVLHRAVNVNRSRHLSTAYLYGPPASVQIS	315
Prs Ps3ox	315	PLSKLLGPSQPLYPRIPTWNEYLGTAKKHFNKALSSVRLCAPLNALVDVSD-H-NSVKVG	372
At GA4	316	PVPKLVPVESPLYQSVTWKBYLRKATHFNKALSMIRNHREE-----	358
At GA4H	309	PLPKLVDPLOSPLYPSITWKOYLATKATHFNKALSSVRLCAPLNALVDVSD-H-NSVKVG	347
Ps LE	315	PHAKLIGPTKPLYPRIPTWNEYLGTAKKHFNKALSSVRLCAPLNALVDVSD-H-NSVKVG	374
Le Le3OH-1	316	PLSKLVDRHHPPLYRAVTWSBYLGTAKKHFNKALSSVRLCAPLNALVDVSD-H-NSVKVG	373
Ls Ls3h1	306	PLSKLTDHVHPPLYRAVTWSBYLGTAKKHFNKALSSVRLCAPLNALVDVSD-H-NSVKVG	363
Nt Nty	314	PLAKLVDRHHPPLYRAVTWSBYLGTAKKHFNKALSSVRLCAPLNALVDVSD-H-NSVKVG	371
Ptt GA3ox1	316	PIQKLVGNHHPPLYRAVTWSBYLGTAKKHFNKALSSVRLCAPLNALVDVSD-H-NSVKVG	373
St Ga3b-1	316	PLSKLVDRHHPPLYRAVTWSBYLGTAKKHFNKALSSVRLCAPLNALVDVSD-H-NSVKVG	373

Fig. 1. Alignment of the deduced amino acid sequences for *Ps3ox* cDNA with other gibberellin 3 β -hydroxylases from different plants. Identical residues among the nine sequences are displayed in black boxes. Gaps (-) were introduced to optimize the alignment. The primers for the degenerate RT-PCR performed to obtain the 290-bp cDNA fragment are as indicated below the sequences. Asterisks (*) indicate the Fe binding motif for 2-oxoglutarate-dependent dioxygenases, and the number signs (#) indicate the 2-oxoglutarate binding motif. Accession numbers for each sequence are Ps3ox (AB188145), GA4 (L37126), GA4H (AF070937), LE (AF001219), Le3OH-1 (AB010991), Ls3h1 (AB012205), Nty (AB032198), GA3ox1 (AY433958) and GA3b-1 (AY039111). Prs, *Prunus spachiana*; At, *Arabidopsis thaliana*; Ps, *Pisum sativum*; Le, *Lycopersicon esculentum*; Ls, *Lactuca sativa*; Nt, *Nicotiana tabacum*; Ptt, *Populus tremula* x *P. tremuloides*; St, *Solanum tuberosum*.

Kit (Amersham Biosciences, Bucks, UK) and an ABI Prism 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) were used to determine the nucleotide sequence, and a full-length cDNA was obtained by 5'- and 3'-RACE using a SMART RACE cDNA Amplification kit with gene-specific primers. A homology search was performed with the BLAST program of the DNA Databank of Japan.

Quantitative real-time RT-PCR

For quantitative RT-PCR experiments, first-strand cDNA synthesis and real-time RT-PCR were performed using a SYBR[®] RT-PCR Kit according to the manufacturer's protocol (Code: RR045A, Takara, Tokyo, Japan) in a Smart Cycler II (Cepheid, Sunnyvale, CA, USA) and analyzed with Smart Cycler software (Ver. 2.0). The PCR reaction mixture included SYBR Green I. The following primers were used to amplify a 142-bp pair fragment: forward (5'-CATGGGTTTGGCAGCAC ATACA-3') and reverse (5'-ACCGACGTTGACCACG AGAG-3'). Melt-curve analysis showed a single sharp peak for all samples. Sample normalization was carried out by quantification of actin cDNA (142 bp) amplified with forward (5'-AGGGTACATGTTTCACCACCA-3') and reverse (5'-CCATCGGGAAGCTCATAGTT-3') primers. The nucleotide sequence of the actin cDNA amplified from the same plant material was determined by ourselves (accession no. AB188144). Thermal cycling conditions were 95°C for 10 s, followed by 45 cycles of 95°C for 10 s, 55°C for 10 s and 72°C for 10 s. Each reaction was carried out in triplicate.

Analysis of endogenous GA₁

To examine the distribution of endogenous GA₁ in branches from the current year, 100 branches each of the weeping and upright types were collected. For extraction of plant growth substances, branches of both the weeping and upright types were divided into eight parts: bud (B), elongating zone (E), older elongating and younger non-elongating zone (N1), middle part of the non-elongating zone (N2), basal part of the non-elongating zone (N3), leaves at N1 (NL1), leaves at N2 (NL2) and leaves at N3 (NL3), as shown in Fig. 3. All segments from each part were frozen immediately in liquid nitrogen. Then they were subjected to extraction with about 80% methanol as reported previously (Saotome *et al.*, 1993), and the resultant extracts were stored at -20°C for analysis of

endogenous GA₁. For analysis of GA₁, the extracts from each part of 1 g fresh weight equivalent were used. [17,17-³H]GA₁ which was purchased from Prof. Mander (Australian National University, Canberra) was added to the initial extract as the internal standards. After the solvent fractionation, these extracts were purified and submitted to analysis by gas chromatography-selected ion monitoring (GC-SIM) as described previously (Saotome *et al.*, 1993; Kobayashi *et al.*, 1996) except for the use of the ODS-h 3151 column in HPLC. The analysis was repeated three times.

Results

To obtain full-length cDNA for *Prunus* gibberellin 3β-hydroxylase, 290-bp cDNA fragments were obtained initially by degenerate RT-PCR using the RNA from the upright and weeping types of *Prunus* trees, and they were extended to both directions by a RACE method. The resulting two full-length cDNA clones were 100% identical in nucleotide sequences and named *Ps3ox* (accession no. AB188145). The full-length cDNA contained 1440-bp nucleotides, which included a 204-bp 5' untranslated region, 117-bp 3' untranslated region and a 1119-bp open reading frame encoding 372 amino acid residues. gibberellin 3β-hydroxylase is one of 2-oxoglutarate-dependent dioxygenases (Yamaguchi *et al.*, 2000). The deduced amino-acid sequence had the conserved regions typical for 2-oxoglutarate-dependent dioxygenases (Valengard *et al.*, 1998, Hedden and Phillips, 2000), including an Fe-binding motif consisting of two His residues and one Asp residue, as well as the 2-oxoglutarate-binding motif consisting of an Arg and a Ser (Fig. 1). The amino acid sequence of *Ps3ox* was aligned with those of gibberellin 3β-hydroxylase in *Arabidopsis*, *Nicotiana*, *Pisum*, *Lycopersicon*, *Lactuca*, *Solanum* and hybrid poplar. The *Ps3ox* protein showed from 62 to 76% amino acid identity with the gibberellin 3β-hydroxylase of other plants (Fig. 1).

To analyze the expression of *Ps3ox* in the buds during growth of the current year's branches, total RNA was extracted from leaf buds of four growth stages. The growth stages were Stage I, winter buds collected on Feb. 15, 2004; Stage II, closed bud with visible leaves of 7 to 10 mm length collected on Mar. 15, 2004; Stage III, opening leaf buds of 15 to 20 mm length collected in Mar. 25, 2004 and Stage IV, buds of the 300 to 400

Table 1 Details of branches used for endogenous gibberellin extraction. Mean values with standard errors are shown (n>8).

Type of branches	Direction of branch growth	Growth rate, mm day ⁻¹	Total length of branch, mm	Number of internodes	Width of branch, mm
Weeping	D	5.86±0.32 (3.0±0.12)*	274.4±7.8	13.2±0.4	2.71±0.07
Upright	U	4.70±0.21 (3.1±0.16)*	231.5±10.2	13.5±0.4	3.55±0.13

*: Number of elongating internodes

D: Downward, U: Upward

Fig. 2. Comparison of the relative *Ps3ox* mRNA content of different growth stages.

Ps3ox mRNA contents were normalized by actin mRNA contents. Moreover, the results are shown as relative values of the level of growth stage I in the upright type, which is defined as 1. Bars show means $\pm$ SE ($n=3$). *, significant difference at $p=0.05$ (t-test).

Growth stage I, winter bud; II, closed bud with visible leaves that was harvested on March 15, 2004; III, opening bud that was harvested on March 25, 2004; IV, bud of the elongating branch that was harvested June 20, 2004.

mm length branches collected on June 20, 2004. Fig. 2 shows a comparison of the relative concentration of *Ps3ox* mRNA in each growth stage of the upright and weeping types. The results are shown as relative values of the level of the winter bud in the upright type, which is defined as 1. The *Ps3ox* mRNA content in the winter bud was the same in both types, but the content in the

weeping type was much greater than that in the upright type after the opening of leaf buds (Fig. 2).

Because high expression of *Ps3ox* was shown in the weeping type, the levels of endogenous GA_1 of the current year's branches of about 250 mm length were measured. The distributions of endogenous GA_1 were obtained in each of the eight parts of the branch (Fig. 3) in both the weeping and upright types. The characteristics of the branches are shown in Table 1. The representative distribution of endogenous GA_1 per fresh weight is shown in Fig. 4. The endogenous GA_1 level of the current year's branches was much higher in buds and elongating zones than in other parts in both types. In the comparison of the two types, the endogenous GA_1 level was much higher in elongating zones of the weeping type, but the differences between the other parts of the weeping and upright types were not as distinct.

Discussion

It has been reported that treatment of a tree with gibberellin improves the development of xylem and cambial activity (Aloni *et al.* 1987; Bradley and Crane 1957; Wang *et al.* 1995, 1997), and the elongation of secondary xylem fibers has been correlated with endogenous levels of GA_1 (Ridoutt *et al.* 1996). Since gibberellin applied to apical buds of the weeping type promoted the development of secondary xylem and inhibited bending (Nakamura *et al.* 1994b), the active gibberellin biosynthesis in the current year's branches was investigated in detail in both types of cherry in this study. It is notable that the expression of *Ps3ox*, coding for the enzyme responsible for active gibberellin biosynthesis, was more than two times higher in the weeping type than that of the upright type during growth of the current year's branches (Fig. 2) while there

Fig. 3. Parts of upright (A) and weeping (B) types of branches used for endogenous GA_1 level analysis: B, bud; E, elongating zone; N1, older elongating and younger non-elongating zone; N2, middle non-elongating zone; N3, basal non-elongating zone; NL1, leaf at N1; NL2, leaf at N2; NL3, leaf at N3.

Fig. 4. Distribution of GA_1 in the eight parts of current year's branches of weeping and upright types.

Bars show means $\pm$ SE ($n > 3$). *, significant difference at $p = 0.05$ (t-test).

Details of the branches' sizes were shown in Table 1.

See Fig. 3 for abbreviation of each part.

were no differences in the cDNA sequences of the two types. When the endogenous GA_1 level in both types was compared, it was much higher in the elongating zone of the weeping type (Fig. 4). It was confirmed that the elongation speed and the amount of the branch elongation of the weeping type were higher than that of the upright type. On the other hand, the current year's branches in both types had the same number of internodes and similarly developed leaves. It was necessary to thicken the secondary xylem adequately so that the branch might support its own weight. However, the weeping branch could not support its own weight and grew downward because the branch elongated before developing sufficient secondary xylem and grew longer than the upright type. In addition, the thickening of the non-elongating zone just below the elongating zone was not sufficient. The lack of thickening might be caused by insufficient gibberellin for its differentiation and development of xylem cells. Alternatively, the sensitivity to gibberellin for the secondary xylem development in the weeping type may be lower than that in the upright type. As a result, exogenous gibberellins would promote the development of secondary xylem in the weeping type. In branches of the weeping type, most gibberellin was used in the elongating zone, and the shortage of gibberellin in the supporting tissue caused the lack of tension wood.

In this study it was suggested that the weeping habit might be caused by abnormality of the distribution of active gibberellin which was synthesized by the *Ps3ox*. Since *Ps3ox* cDNA of the weeping type did not differ from that of the normal upright type, there may be mutations in the intron or other parts of the regulatory elements of the gene. It is also possible that some

regulatory genes controlling *Ps3ox* expression may be altered. A similar example of gibberellin causing negative gravitropism was observed in weeping types of peach trees (*Prunus persica*) (Nakamura *et al.* 1997). Further, it was suggested that gibberellin participates in the secondary xylem development in several woody plants (Wareing 1958; Ridiou *et al.* 1996; Little and Pharis 1995; Eriksson *et al.* 2000). Moreover, several reports showed that auxin (Du *et al.* 2004; Olsen *et al.* 1995; Schrader *et al.* 2003; Sundberg *et al.* 1994; Tuominen *et al.* 1997; Uggla *et al.* 1998) or auxin with gibberellin (Wang *et al.* 1995; Wang *et al.* 1997) took part in the development of secondary xylem in woody plants. However, auxin did not affect the growth of *Prunus* trees (Nakamura *et al.* 1994a, b). The previous and present results suggest that secondary xylem formation of the *Prunus* tree is regulated mainly by the synthetic level of gibberellin and imbalanced gibberellin distribution affects the gravity response. Further studies using a more detailed genetic approach to analyze mutation alleles of the weeping type of cherry tree and the gravi-response of woody plants are required.

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