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Characterization of Gibberellin Biosynthetic Gene Cluster from *Fusarium proliferatum*

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Gibberellins (GAs) are a group of phytohormones that control many developmental processes in higher plants. We report the cloning and expression pattern of gibberellin biosynthesis genes from a new GA-producing fungus, *Fusarium proliferatum* (strain KGL0401). These genes sequences are deposited in the National Center for Biotechnology Information (NCBI) under accession numbers EF119831, EF119832, DQ313173, DQ313174, DQ313175, DQ313176, and DQ313177. The expression level of these genes was maximal at a 0.5 M : 0.17 M carbon : nitrogen ratio, and minimal at a 0.25 M : 0.47 M carbon : nitrogen ratio.

Key words: *Fusarium proliferatum* KGL0401, gibberellin, GA biosynthesis genes

Gibberellins (GAs) are a large family of isoprenoid plant hormones that influence many aspects of plant development, including seed germination, stem elongation, flowering, seed development, and fruit set [16, 26, 29]. GAs were first isolated from the rice pathogen *Gibberella fujikuroi* but are also produced by other fungal strains, some bacteria, and all plants [2, 16]. A total of 136 GAs have been identified [13, 31]. Owing to the importance of GAs as a plant growth regulator in the fields of agriculture and horticulture, the commercial production of GAs has increased immensely [23, 28, 32].

The biosynthetic pathway of GAs has been investigated for many years in *G. fujikuroi* and in higher plants [7, 9, 11, 17]. At least seven genes of the GA biosynthesis pathway are clustered in *G. fujikuroi,* and the order of these genes was shown to be *des, p450-4, p450-1, p450-2, ggs2, cps/ks*, and *p450-3*, which encode GA_4 desaturase, *ent*-kaurene oxidase, GA_{14} synthase, GA20-oxidase, geranylgeranyl-diphosphate synthase, copalyl diphosphate/*ent*-kaurene synthase, and C13-oxidase [7, 22, 23, 25–28]. Most genes of the isoprenoid pathway have been cloned from *G. fujikuroi*, including HMG-CoA reductase, farnesyl diphosphate synthase (FDP), and a general geranylgeranyl diphosphate synthase (GGDP) [16, 30]. Biosynthesis of the major metabolite GA_3 from GGDP requires 13 steps. GGDP is converted to *ent*-kaurene *via ent*-copalyl diphosphate in a 2-step cyclization reaction. The cyclization of GGDP is catalyzed through a bifunctional *cps/ks* enzyme. As a result, *ent*-kaurene is metabolized to GAs in *G. fujikuroi* by cytochrome P450 monooxygenases [8, 23, 24, 26].

In this report, we describe the isolation and functional characterization of the GA biosynthesis genes cluster in GA-producing *F. proliferatum* KGL0401. Real-time gene expression studies [6, 10, 21] of GA biosynthesis genes in regard to high (carbon : nitrogen = 0.5 M : 0.17 M), and low (carbon : nitrogen = 0.25 M : 0.47 M) GA production revealed significant differences in their regulation.

Materials and Methods

Fungal Strain and Culture Conditions

The strain *F. proliferatum* KGL0401 used in this study was previously isolated from *Physalis alkekengi* var. *francheti* plant roots and exhibited higher GA productivity than wild-type *Gibberella fujikuroi* (ATCC 12616) [19]. For DNA isolation, the fungal strain was grown in 40 ml of liquid Czapek's optimized medium (1% glucose, 1% peptone, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, pH 7.3 ± 0.2) for 7 days at 30°C on a rotary shaker set at 180 rpm. For semiquantitative RT-PCR and real-time PCR, *F. proliferatum* KGL0401 was grown in high GA production conditions (0.5 M sucrose, 0.17 M NH_4Cl, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, pH 7.3 ± 0.2) and low GA production conditions (0.25 M sucrose, 0.47 M NH_4Cl, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$,

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0.001% $FeSO_4 \cdot 7H_2O$, pH 7.3 ± 0.2). Mycelia were harvested after 7 days of cultivation and the RNA was isolated.

Bacterial Strains and Plasmid

The *E. coli* strain DH5α (Real Biotech Corp., Korea) was used for plasmid propagation. The PCR fragments were cloned into the pGEM-T Easy Vector System (Promega), according to the manufacturer's instructions.

DNA and RNA Isolation

Genomic DNA was isolated from lyophilized mycelium with hexadecyltrimethylammonium bromide (CTAB). The 2.5 g lyophilized mycelium was ground to a fine powder for 10 min in liquid nitrogen with a mortar and pestle, 25 ml of lysis buffer (20 mM Tris-HCl pH 8.0), 10 mM EDTA, 1% SDS) was added, and the mixture was incubated for 1 h at 50°C. Then 5 M NaCl was added to a final 0.7 M concentration and 10% CTAB buffer (10% CTAB, 100 mM EDTA, 500 mM Tris-HCl, pH 8.0) added to 1/10 volume. After mixing thoroughly and incubating for 1−2 h at 65°C, chloroform extraction was repeated two times. Nucleic acids were concentrated by precipitation in two volumes of absolute ethanol, dissolved in 0.1× TE buffer (0.1 mM EDTA, 1 mM Tris-HCl, pH 8.0), and treated with RNase A to obtain pure DNA. RNA was isolated using the RNeasy Mini Kit (Qiagen, USA).

Construction of a Genomic DNA Library

To construct pools of uncloned, adaptor-ligated genomic DNA fragments, the Universal Genome Walker Kit (BD Biosciences, USA) was used (according to the manufacturer's instructions). Full-length genomic DNA encoding GA biosynthesis genes were obtained by PCR screening of the genomic DNA library, using outer and nested gene-specific primers and adaptor 1,2 primers.

Cloning of the GA Biosynthesis Gene Cluster

Specific forward and reverse primers of GA biosynthesis genes were designed using nucleotide sequences from *G. fujikuroi*. For amplification of the *P450-4* and *P450-1* genes based on *F. proliferatum* KGL0401, P450-4F and P450-4R and P450-1F and P450-1F primers were used, respectively. The PCR fragment was cloned into the pGEM Easy vector and sequenced. For amplification of *F. proliferatum* (KGL0401) *P450-1* to *cps/ks* genes, and from *cps/ks* to *P450-3*, primers FP1F/FP1R and FP2F/FP2R were used, respectively. The approximately 9 and 5 kbp product fragments obtained were cloned and sequenced directly. The *des* and remaining *P450-3* genes were cloned using the Genome Walker Kit (BD Biosciences, USA). For amplification of *des* and *P450-3* genes, outer (P450-4outer) and nested (P450-4nested) gene-specific primers were used. For AP1, outer (P450-3outer) and nested (P450-3nested) gene-specific primers were used. The entire GA biosynthesis gene cluster was cloned using the Genome Walker Kit (BD Biosciences, USA).

PCR Conditions

The *F. proliferatum* KGL0401 genomic DNA and genomic DNA library were used as a template for amplification of GA biosynthesis genes. DNA amplification was performed in 50 µl mixtures, using 5 U of *Taq* DNA polymerase (Takara Ex Taq, Japan), 25 ng of genomic DNA/µl, 10× Ex Taq buffer, 2.5 mM concentrations of deoxynucleoside triphosphates, and 100 pmol concentrations of each primer. PCR was carried out at 95°C for 2 min followed by 35 cycles at 95°C for 1 min, 55°C to 60°C for 1 min, and at 72°C for 1 to 2 min. The annealing and elongation times were applied differently, depending on the annealing temperatures of each primer, as well as amplified fragments. The PCR product was purified by using a gel extraction kit (NucleoGen, Korea).

Sequence Analysis

DNA sequencing of recombinant plasmid clones was accomplished with an automatic sequencer (ABI 3730xl; Applied Biosystems, USA). DNA clones were sequenced using specific oligonucleotides. DNA and protein sequence alignments were conducted using DNAstar (USA). BLAST analyses were conducted through the NCBI server (http://www.ncbi.nlm.nih.gov/blast/).

cDNA Construction

cDNA construction was accomplished using SuperScript III Reverse Transcriptase (Invitrogen, UK), according to the manufacturer's protocol.

Confirmation of Introns by cDNA Screening

The cDNA was screened by PCR, using the gene-specific primers as described in Table 1. The PCR protocol was 5 min at 95°C, then 35 cycles of 30 s at 95°C, 1 min at 55°C to 60°C, and 2 min at 72°C, followed by a final extension for 5 min at 72°C.

Transcriptional Analysis of GA Biosynthesis Genes

An analysis of GA biosynthesis gene expression under high and low GA production media conditions was conducted by semiquantitative RT-PCR and real-time PCR of mRNA, and the rDNA gene was used as a positive control. Primers specific to GA biosynthesis genes and rDNA-specific primers amplified products ranging in size from 264 to 560 bp. Semiquantitative RT-PCR amplification was conducted in a 50 µl PCR mixture containing 60 pg of cDNA, 5 U of *Taq* DNA polymerase (Takara, Japan), 10× Ex Taq buffer, 2.5 mM concentrations of deoxynucleoside triphosphates, and 100 pmol concentrations of each primer. The PCR conditions were as follows: 5 min at 95°C, followed by 25 cycles of 30 s at 95°C, 30 s at 58°C, and 30 s at 72°C, and a final extension of 5 min at 72°C. The real-time PCR was performed using a LightCycler rapid thermal cycler system (Roche, Germany) according to the manufacturer's instructions. Reactions were accomplished in a 20 µl volume with 60 pg of cDNA, 0.5 µM primers, and 1 mM $MgCl_2$.

RESULTS

Cloning and Sequencing of GA Biosynthesis Genes from *F. proliferatum* KGL0401

GA-biosynthesis genes (*des, P450-4, P450-1, P450-2, ggs2, cps/ks*, and *P450-3*) were determined by genomic walking by PCR and cDNA screening. Downstream and upstream walking of the genomic libraries was performed using outer and nested gene-specific primers for GA biosynthesis genes. The assembly of sequences led to creation of the complete gene sequence. These sequences are registered in GenBank under accession numbers EF119831, EF119832, DQ313173, DQ313174, DQ313175,

Table 1. Sequence of PCR primers for GA biosynthesis genes.

Primer name	Sequence (5'–3')	Target gene and application
P450-3outer	TCCAGTAAAGCAGATCCGTAGACT	*p450-3* gene/genomic DNA walking
P450-3nested	TGAGGCCCTTGCACAAGTGCTCTA	
P450-4outer	CTCAAAGATTCGCTCAGAC	*p450-1* and *des* gene/genomic DNA walking
P450-4nested	GATGGGAAAGGAAGACCAGC	
P450-4F	CCAAACTCCTCGGACATCTTG	*p450-4* gene/genomic DNA screening
P450-4R	GTGTCAGTACGAACCCATAGC	
P450-1F	AGAACAGTCGTCCAAGCATCAGCA	*p450-1* gene/genomic DNA screening
P450-1R	GGCTACATATCTCGTGCTAGACAG	
FP1F	CCTCAACATGAATGCCAACCCAACTGCAAA	from *p450-1* gene to *cps/ks*/genomic DNA screening
FP1R	CCAAGAAAGGCTTCAAGGGAGTGAAGTAGT	
FP2F	TAGATCATAGCGACACTCCTGAAG	from *cps/ks* gene to *p450-3*/genomic DNA screening
FP2R	ATCAGCTCACCCGTCTTACTGATT	
CdesF	TTTATGCCTCATAAAGATAAT	*des*/cDNA screening
CdesR	CTACCAGAATGCAATGAACTT	
Cp450-4F	TTCATGAGTAAGTCCAACAGC	*p450-4*/cDNA screening
Cp450-4R	GCTCATTCATCTCTCAGTGA	
Cp450-1F	TTCATGGCGAACCATTCTTCT	*p450-1*/cDNA screening
Cp450-1R	GCTCAAATCGCAATCTCCTC	
Cp450-2F	TTCATGAGCATCTTCAATATG	*p450-2*/cDNA screening
Cp450-2R	GCTCAAATTGCTTCCAAATC	
Cggs2F	TTCATGGCTGAACAACAGATC	*ggs2*/cDNA screening
Cggs2R	GCTCACTTCCAAAACCAGCA	
Ccps/ksF	GAGATGCACATTCTAACCTAT	*cps/ks*/cDNA screening
Ccps/ksR	GCTCACTTCATGCTGCTTGA	
Cp450-3F	GCTCATCTCCTTCGCATCTC	*p450-3*/cDNA screening
Cp450-3R	TTCATGAAATACACAACATGC	
AP1	GTAATACGACTCACTATAGGGC	*p450-1* and *p450-3* gene/genomic DNA walking
AP2	ACTATAGGGCACGCGTGGT	

DQ313176, and DQ313177, respectively. The 1,029 bp *des* ORF (open reading frame) was not an intron. The 1,730 bp *P450-4* ORF was interrupted by three introns at positions 277–325, 616–667, and 1246–1296, with lengths of 49, 52, and 51 nucleotides, respectively. The 1,682 bp *P450-1* ORF was interrupted by two introns at positions 382–431 and 604–654, with lengths of 50 and 51 nucleotides, respectively. The 1,799 bp *P450-2* ORF was interrupted by three introns at positions 407–468, 572–622, and 692–750, with lengths of 62, 51, and 59 nucleotides, respectively. The 1,445 bp *ggs2* ORF was interrupted by four introns at positions 286–335, 481–530, 800–853,

Table 2. The gibberellin biosynthetic gene cluster in *Fusarium proliferatum* KGL0401.

Abbr.	*des*	*P450-4*	*P450-1*	*P450-2*	*ggs-2*	*cps/ks*	*P450-3*
Enzyme	GA_4 desaturase	*ent*-kaurene oxidase	GA_{14} synthase	GA20-oxidase	Geranylgeranyl-diphosphate synthase	Copalyldiphosphate/*ent*-kaurene synthase	C13-oxidase
Chr. DNA	1,029 bp	1,730 bp	1,682 bp	1,799 bp	1,445 bp	2,973 bp	1,458 bp
No. of intron	0	3	2	4	4	2	4
No. of aa	342	525	526	524	392	958	388
% Identities with GF[a]	100%	100%	99%	100%	89%	98%	100%
% Identities with FP[b]	96%	96%	95%	NS	93%	89%	NS
GenBank Accession No.	EF119831	EF119832	DQ313173	DQ313174	DQ313175	DQ313176	DQ313177

NS, no signification similarity was found.

[a]GF, *Gibberella fujikuroi.*

[b]FP, *Fusarium proliferatum.*

and 1013–1124, with lengths of 50, 50, 54, and 112 nucleotides, respectively [5]. The 2,973 bp *cps/ks* ORF was interrupted by two introns at positions 865–913 and 1319–1365, with lengths of 49 and 47 nucleotides, respectively. The 1,458 bp *P450-3* ORF was interrupted by four introns at positions 67–191, 649–706, 905–560, and 1058–1109, with lengths of 125, 58, 56, and 52 nucleotides, respectively. The intron position of GA biosynthesis genes was confirmed by PCR screening of the cDNA synthesized from the extracted RNA. The position of introns was comparable with *G. fujikuroi*. The amino acid sequence (deduced and analyzed using NCBI) showed that each cloned gene had a 100% identity with *des, P450-4, P450-2*, and *P450-3,* 99% identity with *P450-1*, 98% identity with *cps/ks*, and 89% identity with *ggs2* from *G. fujikuroi.* Cloned genes revealed 96% identity with *des* and *P450-4*, 95% identity with *P450-1*, 93% identity with *ggs2*, and 89% identity with *cps/ks* from *F. proliferatum* (Table 2). Complete sequences of the *P450-2* and *P450-3* genes of the *F. proliferatum* strain were cloned for the first time.

Expression Level of GA Biosynthesis Genes

Gibberellin biosynthesis is highly influenced by carbon and nitrogen sources [1] and is triggered after cessation of growth and exhaustion of nitrogen sources [3]. For studies on the gene regulation of GA biosynthesis, the *F. proliferatum* strain KGL0401 was grown in high and low GA-product conditions [20]. The levels of GA biosynthesis genes were analyzed in conditions with high and low mycelium levels.

Semi quantitative RT-PCR was used to detect GA biosynthesis genes. A positive PCR control (rDNA) in each condition displayed the same signal. All GA biosynthesis genes showed positive expression levels in the high mycelium condition, with the exception of the *P450-4* gene (Fig. 1). Melting curve analysis demonstrated proper products from the primer pairs (Table 3). The CP values for GA biosynthesis genes in high and low conditions show that a high expression of GA biosynthesis genes was achieved by real-time PCR, compared with the low condition. Thus, the expression level of GA biosynthesis genes (*P450-1*, *P450-2*, *cps/ks*, and *P450-3*) exhibited by the high mycelium condition were 11.31 3.24, 2.41, and 2.11 times stronger than the low condition using real-time PCR (Table 4).

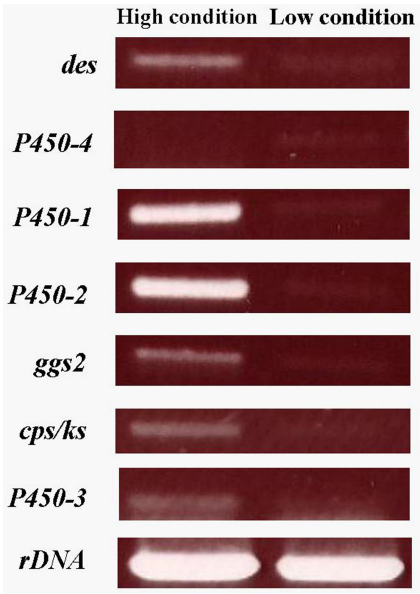

Fig. 1. Transcriptional analysis of GA biosynthesis genes using semiquantitative RT-PCR after cultivation under high conditions and low conditions.
The rDNA was used as a positive control. Semiquantitative RT-PCR amplification was done with 25 cycles using gene-specific primers (Table 3). The same sample volume of each PCR product was loaded onto the gel. *High condition (0.5 M sucrose, 0.17 M NH_4Cl, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, pH 7.3 ± 0.2). **Low condition (0.25 M sucrose, 0.47 M NH_4Cl, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, pH 7.3 ± 0.2).

DISCUSSION

The cloning and characterization of GA biosynthesis genes is very important in the fields of agriculture and horticulture.

Table 3. Primer sequences and amplification conditions for semiquantitative RT-PCR and real-time PCR.

Gene	Primer sequences (5'–3') forward and reverse	Anneal temp.	Product bp and Tm
des	CAGTCGTTCGCAAGGAAGTG TCCGACTCAAATGTCCCATG	65	550 bp 89.82(H) 89.47(L)
p450-4	CATCTGAGAGTGTCATGGATTGGT AGAGCCTGTTCATAACTGGCTT	65	500 bp 87.63(H) 87.32(L)
p450-1	AGTAGAAGAACTGCGTCTCTGG TGTTGATGGGCTGATACAAGCG	65	556 bp 89.59(H) 89.39(L)
p450-2	CGCAACGAAGCTTGGCTGAA GAGCGTAAGCTGGAAGATCACG	63	264 bp 90.95(H) 90.85(L)
ggs2	CATCGTGACCGACGATATCCTG AGCCAGAAGTTGGAGATTACGG	65	527 bp 88.04(H) 87.79(L)
cps/ks	CAAGCAATGGCTGTTTCCTGAG GATACTTCTGCAAGGGAACTCA	63	337 bp 88.66(H) 88.66(L)
p450-3	ATGAAATACACAACATGC ACGAGACCAGCATTTGATCTAG	63	502 bp 88.79(H) 88.77(L)
rDNA	ACCCGCTGAACTTAAGC TTCCACCCAAACACTCG	63	560 bp 88.02(H) 88.05(L)

The length of the specific amplification products and their Tm are indicated. The *F. proliferatum* KGL0401 were cultivated at high condition (H) and low condition (L). RNA was extracted from their mycelia.

Table 4. Expression levels of seven genes involved in the GA biosynthetic pathway.

Gene	Real-time PCR CP value[a]		Relative gene expression[d]	
	High condition[b]	Low condition[c]	High condition[b]	Low condition[c]
des	21.52	22.36	1.41	1.00
p450-4	22.51	22.92	1.04	1.00
p450-1	21.09	24.93	11.31	1.00
p450-2	23.69	25.73	3.24	1.00
ggs2	21.94	22.94	1.58	1.00
cps/ks	25.87	27.48	2.41	1.00
p450-3	27.19	28.61	2.11	1.00
rDNA	24.56	24.90		

[a]The CP value represents the number of cycles after which the real-time PCR indicated a positive result.
[b]Total RNA was prepared from *F. proliferatum* KGL0401 grown in high condition medium; 60 pg of cDNA was contained in each reaction.
[c]Total RNA was prepared from *F. proliferatum* KGL0401 grown in low condition medium; 60 pg of cDNA was contained in each reaction.
[d]The gene expression was calibrated by using the $2^{-\Delta\Delta CT}$ method [5, 12].

In this paper, the cloning of the GA biosynthesis gene cluster in *F. proliferatum* KGL0401 was investigated. In a previous study, *F. proliferatum* KGL0401 showed a 99% similarity to *F. proliferatum* (by analysis of an internal transcribed spacer) and GC-MS analysis of culture filtrates revealed that this strain had a higher GA productivity than wild type *G. fujikuroi* (ATCC 12616) [19]. This strain exhibited the highest plant growth promotion activity, two times stronger than wild-type *G. fujikuroi* (ATCC 12616) [19]. In this report, we identified a GA biosynthesis gene cluster containing seven closely linked genes (*des, P450-4, P450-1, P450-2, ggs2, cps/ks*, and *P450-3*) in *F. proliferatum* KGL0401. The *F. proliferatum* strain exhibited the highest level of phylogenetic relativity to *G. fujikuroi* [18]. Despite containing a complete GA biosynthesis gene cluster, *F. proliferatum* has not been reported to produce GAs as secondary metabolites, the loss of GA production possibly due to defective regulation of *ggs2* and *cps/ks* genes in *F. proliferatum* [14, 15]. In contrast, the orchid-associated *F. proliferatum* ET1 strain produces GAs [22]. In order to investigate the differences in GA production between *G. fujikuroi* and *F. proliferatum* strains (D-02945, KGL0401), we cloned and sequenced the GA biosynthesis genes from *F. proliferatum* KGL0401 and compared the sequence with *G. fujikuroi* m567 and *F. proliferatum* D-02945. Previously, several amino acid differences in *ggs2* and *cps/ks* between *G. fujikuroi* and *F. proliferatum* D-02945 were considered responsible for the loss of GA activity in *F. proliferatum* D-02945 [15]. The sequence analysis of amino acids in *ggs2* revealed 89% and 93% identity with *G. fujikuroi* and *F. proliferatum* D-02945, respectively. The positions of the introns and five catalytic domains [4] were also highly conserved in the species (data not shown). Sequence analysis of *cps/ks* gene amino acids revealed 98% and 89% identity with *G. fujikuroi* and *F. proliferatum* D-02945, respectively. The positions of the introns and the aspartate-rich domains [29] were highly conserved (data not shown). Interestingly, most of the amino acid sequences of GA biosynthesis genes from *F. proliferatum* KGL0401 showed a higher identity with *G. fujikuroi* than with *F. proliferatum*. This implies that GA biosynthesis genes are active in *F. proliferatum* KGL0401. Gene sequence comparisons between *F. proliferatum* D-02945 and *F. proliferatum* KGL0401 revealed significantly more differences in the intergenic regions (average 89% nucleotide identity) than in the coding regions (up to 93% amino acid identity). Thus, there is a possibility that putative unidentified GA-specific regulators are functional in *F. proliferatum* KGL0401 and responsible for the GA-producing ability of the strain.

Most of the GA biosynthesis genes of *F. proliferatum* KGL0401 were expressed in high GA conditions. The amounts of GA_1, GA_3, GA_4, and GA_7 were estimated at 144.5, 140.0, 354.1, and 146.3 ng/ml, whereas in low conditions, amounts are reported to be 46.8, 49.9, 0.4, and 68.4 ng/ml, respectively [19]. The amount of GA_4 was approximately 885 times higher in the high condition compared with the low condition. Of significant importance is the difference in *P450-1* gene expression between high and low conditions, about 11.31 times greater in high-GA production conditions. *P450-1* (GA_{14}-synthase) catalyzes four oxidation steps, from *ent*-kaurenoic acid to GA_{14}, *via ent*-7a-hydroxykaurenoic acid, GA_{12}-aldehyde, and GA_{14}-aldehyde [24]. *P450-2* gene expression was approximately 3.24 times greater in high-GA production conditions. *P450-2* is located in the GA biosynthesis gene cluster (between *P450-1* and *ggs2*) and is responsible for the oxidation of GA_{14} at C-20 and the final removal of the 20-carbon [24] for producing GA_4. These results support the hypothesis that high-level expression of the *p450-1* and *P450-2* genes, under high-GA conditions, up-regulates the yield of GA_4 (885 times) in *F. proliferatum* KGL0401. Almost all GA biosynthesis genes are more active in high-

GA conditions, with the exception of *p450-4*. The activities of these genes (with the exception of *P450-4*) seem to directly affect GA production in *F. proliferatum* KGL0401.

GA biosynthesis genes (*des*, *p450-4*, *p450-1*, *p450-2*, *ggs2*, *cps/ks*, and *p450-3*) from *F. proliferatum* KGL0401 were cloned and sequenced. The genome size of these genes were 1,029, 1,730, 1,682, 1,799, 1,445, 2,973, and 1,458 bp, and the number of amino acids they encode were 342, 525, 526, 524, 392, 958, and 388. The total number of introns in the genes were 0, 3, 2, 4, 4, 2, and 4, respectively. Most of the GA biosynthesis genes of *F. proliferatum* KGL0401 were expressed in high-GA conditions by semiquantitative RT-PCR and real-time PCR.

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