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Research Article

Contribution of taxane biosynthetic pathway gene expression to observed variability in paclitaxel accumulation in *Taxus* suspension cultures

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Variability in product accumulation is one of the major obstacles limiting the widespread commercialization of plant cell culture technology to supply natural product pharmaceuticals. Despite extensive process engineering efforts, which have led to increased yields, plant cells exhibit variability in productivity that is poorly understood. Elicitation of *Taxus* cultures with methyl jasmonate (MeJA) induces paclitaxel accumulation, but to varying extents in different cultures. In the current study, cultures with different aggregation profiles were established to create predictable differences in paclitaxel accumulation upon MeJA elicitation. Expression of known paclitaxel biosynthetic genes in MeJA-elicited cultures exhibiting both substantial (15-fold) and moderate (2-fold) differences in paclitaxel accumulation was analyzed using quantitative reverse transcriptase PCR. Each population exhibited the characteristic large increase in paclitaxel pathway gene expression following MeJA elicitation; however, differences in expression between populations were minor, and only observed for the cultures with the 15-fold variation in paclitaxel content. These data suggest that although upregulation of biosynthetic pathway gene expression contributes to observed increases in paclitaxel synthesis upon elicitation with MeJA, there are additional factors that need to be uncovered before paclitaxel productivity can be fully optimized.

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1 Introduction

Natural products represent many of the most effective drugs currently available for the treatment of a variety of diseases, and are particularly useful as anti-cancer and anti-infectious agents [1]. Paclitaxel (Taxol®), a taxane diterpenoid produced by *Taxus* genus is a potent chemotherapeutic widely used in the treatment of breast, ovarian, and non-small cell lung cancers as well as AIDS-related Kaposi's sarcoma [2]. Paclitaxel is also being investigated for use in the treatment of neurological disorders, additional cancers, and in post-surgery heart patients [3]. Large scale commercial synthesis of plant natural products via cell culture has proven to be challenging due to low yields and instability in productivities [4, 5]. Despite these limitations, plant cell culture has emerged as a sustainable source for paclitaxel supply, being commercially produced by Phyton Biotech Inc. and Samyang Genex Corp. using large scale fermentation. The successes of large scale industrial plant cell culture in the US and world-wide with paclitaxel [6–9] and several other plant-derived com-

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Abbreviations: cDNA, complementary DNA; **DW,** dry weight; **GAPDH,** glyceraldehyde 3-phosphate dehydrogenase; **MeJA,** methyl jasmonate; **qRT-PCR,** quantitative reverse transcriptase PCR; **REST,** relative expression software tool; **UPLC,** ultra-performance liquid chromatography

pounds such as ginseng [10], shikonin [11], and berberine [12], have clearly demonstrated both the feasibility of this technology, and broad applicability across a range of plant species and products.

The primary difficulties associated with establishment of plant cell culture bioprocesses are the low and variable yields in secondary metabolite accumulation [13]. To overcome low yields, a variety of traditional optimization strategies have been effective, including elicitation with MeJA [8, 14, 15]. By combining media and process optimization strategies with MeJA elicitation, paclitaxel accumulation of 110–120 mg/L have been reported in academic laboratories [8, 16] and up to 295 mg/L [17] and 900 mg/L [18] have been achieved in industrial environments. Along with high product yields, development of cell lines and processes with a high degree of biochemical and physiological stability is critical for the ultimate success of this technology platform [5, 19]. Variability in product accumulation has been observed in suspension cultures that are maintained over long periods of time [20, 21], including paclitaxel accumulation in *Taxus* cultures [22, 23]. Though production instabilities have been attributed to epigenetic and/or genetic causes [24, 25], few molecular studies have been performed [26, 27] to gain a mechanistic understanding of paclitaxel accumulation variability in *Taxus* cell cultures, and hence no rational engineering strategies have emerged to control or leverage favorable variability to optimize performance.

Understanding regulation of gene expression is critical to the design of targeted metabolic engineering approaches. To probe regulation of paclitaxel accumulation, expression patterns of known paclitaxel biosynthetic pathway genes can be quantified. Paclitaxel biosynthesis consists of 19 putative enzymatic steps [28] and has only been partially elucidated (see [27] for paclitaxel biosynthetic pathway). Regulation of paclitaxel/taxane biosynthetic pathway genes has been examined through RNA gel blot analysis and semi-qRT-PCR in MeJA-elicited *Taxus cuspidata* suspension cultures [27]. Similar studies using qRT-PCR have been reported for *T. chinensis* suspension cultures where the elicitation strategy focused on increasing Taxuyunnanine C, a 14β-hydroxy taxoid, generated through an early branch of the paclitaxel biosynthetic pathway [29]. For the majority of known pathway steps, mRNA abundance increased following elicitation, indicating substantial regulation of the pathway at the level of mRNA. However, these studies focused on comparison of cultures with and without elicitation. Variability in paclitaxel accumulation is often observed amongst cultures elicited with MeJA [23]. Therefore, to link variability fully to gene expression, comparison of multiple culture states with different biosynthetic capabilities is necessary.

Plant cells in suspension culture remain connected to each other after cell division, resulting in the formation of cellular aggregates of various sizes. In *Taxus* suspension cultures, aggregates ranging from less than 100 μm to well over 2 mm have been observed [30]. Cells within these aggregates are subject to different microenvironments, leading to cell-cell differences in morphology [31] and metabolism [32]. Recently, we have developed an approach to assess the effect of aggregate size as a process variable on paclitaxel accumulation, and have demonstrated that cultures with smaller aggregates accumulate significantly more paclitaxel upon elicitation with MeJA than cultures with larger aggregates [33]. Using this approach, it is possible to predictably and rapidly obtain cultures with different biosynthetic capabilities upon MeJA elicitation.

In this paper, we use this approach to quantify expression of paclitaxel biosynthetic pathway genes in cultures with varying levels of paclitaxel accumulation, to clarify the underlying causes of production variability that have been observed at the process scale. Using qRT-PCR, we first compared expression profiles in cultures elicited with MeJA that produce detectable levels of paclitaxel and unelicited cultures that do not produce detectable levels of paclitaxel, to elucidate the on/off state of paclitaxel accumulation. Next, we established cultures with small and large aggregate distributions [33] that exhibit variable paclitaxel accumulation patterns upon elicitation with MeJA, to enable comparison of gene expression between cultures in which taxane accumulation is positive but significantly different. Using this method we were able to examine cultures that had 15- and 2-fold differences in paclitaxel accumulation after MeJA elicitation, respectively. This is the first study to compare gene expression in cultures with variable levels of paclitaxel accumulation, providing valuable information regarding the regulation of paclitaxel synthesis.

2 Materials and methods

2.1 Cell culture maintenance, elicitation, and biomass measurements

The *T. cuspidata* P93AF cell line was provided by the United States Plant Soil and Nutrition Laboratory (Ithaca, NY, USA) and was used in all experiments. All chemicals were obtained from Sigma-

Aldrich Co. (St. Louis, MO, USA), unless otherwise noted. Cell cultures were maintained, as described previously [33]. For elicitation, 200 μM MeJA was added on day 7 post-transfer during the mid-exponential phase of growth, as described previously [34]. A Multisizer 3™ Coulter counter equipped with a 2000 μm aperture (Beckman Coulter, Brea, CA, USA) was used to measure culture aggregate size distributions, as described previously [30]. As the total aggregate volume was previously shown to correlate directly with biomass [30], this measurement was also used to determine dry weight (DW), and all DW data presented here were based on this correlation. For analysis, 2 × 2 mL samples of well-mixed culture broth from each flask were taken at each time point.

2.2 Initiation of cultures with different aggregate size distributions and sampling for growth, RNA, and metabolite analyses

Three 14-day old suspension flasks were combined into one culture and its aggregate size distribution and mean aggregate size were determined using the Multisizer 3™ Coulter counter. This culture was aseptically separated into two cultures (one "small aggregate culture" and one "large aggregate culture") using a filter corresponding to its mean aggregate size, based on correlations developed in previous work [30]. Three biological replicates each of small aggregate culture and large aggregate culture were initiated and maintained as 200 mL cultures in 500 mL shake flasks, as described previously [33]. Measurements for biomass were taken on the day of inoculation (day zero) and on the day of elicitation with MeJA (day seven). Prior to MeJA elicitation and 15 h post-elicitation, samples for total RNA extraction were collected. Cell suspensions (3–4 mL of well mixed cultures) were filtered through Miracloth® (Calbiochem, La Jolla, CA, USA) to yield ~500 mg fresh cell weight, thoroughly ground in liquid nitrogen and stored at –80°C in polypropylene tubes prior to RNA analysis (see below: section 2.4.1). Following MeJA elicitation, 1 mL samples of well mixed culture broth containing both cells and media were taken every 2–3 days with a cut pipette tip and stored at –80°C prior to metabolite analysis (see section 2.3).

2.3 Metabolite analysis

Taxanes were identified and quantified using a Waters (Milford, MA, USA) Alliance 2690 HPLC with a 996 photodiode detector [34] or a Waters Acquity ultra-performance liquid chromatography (UPLC) H-Class system. Separation on the HPLC was accomplished on a Taxsil (Varian, Inc., Torrance, CA, USA) column (particle size 5 μm, 250 mm × 4.6 mm). The UPLC separation was performed using Acquity (Waters, Milford, MA, USA) BEH C_{18} column (particle size 1.7 μm, 50 mm × 2.1 mm). Samples were prepared for taxane analysis, as described elsewhere [34], and analyzed on either the HPLC or UPLC system. Baccatin III and paclitaxel authentic standards were used to generate standard curves for quantification of taxane content.

2.4 Gene expression analysis

2.4.1 RNA isolation and reverse transcription

Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Genomic DNA was eliminated by treating each sample with Ambion's Turbo DNA-free DNase I (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The concentration of total RNA was estimated using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). RNA quality was further assessed by denaturing gel electrophoresis. One microgram of total RNA was subjected to reverse transcription using the Affinity Script complementary DNA (cDNA) synthesis kit (Agilent Technologies, Santa Clara, CA, USA) with oligo dTs as primers. Reactions were performed according to the manufacturer's protocol in a total volume of 40 μL. For use as templates for qRT-PCR, cDNA samples were diluted five-fold in sterile water.

2.4.2 Primer design

Primer pairs for qRT-PCR amplification were designed based on selected sequences using Primer3 software (http://frodo.wi.mit.edu/primer3/) with the following criteria: melting temperature of 63 ± 3°C, primer sequences of length between 20 and 25 bp, and GC content of 40–60%. Amplicon lengths were optimized to 100–250 bp to ensure maximal polymerization efficiency for qRT-PCR. Specificity of the primer pairs was evaluated by melting-curve analysis (Mx3000P real-time PCR instrument software, version 2.0) after 40 amplification cycles. Table 1 lists the primers used in this study.

2.4.3 Quantitative real time RT-PCR (qRT-PCR)

qRT-PCR reactions were performed in a 96-well plate with a Mx3000P real-time PCR system (Stratagene, La Jolla, CA, USA), using SYBR Green to monitor dsDNA synthesis. A 25-μL PCR reaction was prepared containing 12.5 μL of Brilliant 2× SYBR green master mix (Agilent Technologies,

Table 1. Sequences for forward and reverse primers of the paclitaxel biosynthetic pathway genes used in qRT-PCR.

Gene	Genbank accession	Primer pair (5′-forward-3′/5′-reverse-3′)	Product size (bp)
TASY	U48796	TGCAGCGCTGAAGATGAACG AGTGCCAGTGCTGCTGCTCA	181
T5αH	AY289209	GCAACAGCCACGCAGGATCT TTGGCGAAGTGGTGGTGTCA	136
DBBT	JF735996	CATGGCGGACAACGACCTTT CCCACAACAAATCCCCCACA	159
DBAT	AF193765	CCTGCAGCTCTCCACCCTTG CGCCCTGCAAAAGGGGAATA	162
PAM	AY582743	GAGGTCATGGAAGCGCTGGA CAATGTAGGCGAGCGGGATG	109
BAPT	AY082804	GCCTTCGCCCAAAACAATCC ACACCCTGCCCTGTGCACTC	228
DBTNBT	AF466397	GGAGTGCACAGGGGATGGTG GGCAATGCCCCCACATGTAA	190
GAPDH	L26922	TTCCCTGGGGTGAGGTTGGT GCCAAAGGAGCCAGGCAGTT	229
Actin	JF735995	GCGTGAAATTGTCCGCGATG CCGTTCTGCACCAATCGTGA	148

Santa Clara, CA, USA), 2 μL of template cDNA (or water for no template control), 100 nM of each primer, and 50 nM of diluted ROX dye (to compensate for non-PCR related variations in fluorescence, Agilent Technologies). The SYBR Green fluorescence data were collected with the following thermocycler conditions: initial denaturation at 95°C for 10 min followed by 40 cycles at 95°C for 30 s, 60°C for 1 min, and 72°C for 1 min. Melting-curve analysis was performed at the end of the amplification procedure to check the specificity of the PCR reaction. Mean PCR efficiency for each gene was calculated by LinRegPCR [35] by using 4–6 points and the best correlation coefficient. The reported fold induction is the geometric mean of the relative fold induction values for target genes normalized to each of the endogenous controls, actin (GenBank accession NO: JF735995) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (GenBank accession NO: L26922). The use of actin and GAPDH as internal reference genes was validated by ensuring that the relative expression of each gene remained constant in both small aggregate cultures and large aggregate cultures before and after elicitation with MeJA. The expression ratio results were tested for significance by a randomization test using the relative expression software tool (REST©) [36]. The statistical model used by this software is a pair-wise fixed reallocation randomization test. The software returns the probability of the alternative hypothesis (P(H1)), which is that the difference between sample and control groups is due only to chance. A p-value of less than 0.05 was considered significant.

3 Results and discussion

3.1 Analysis of paclitaxel/taxane biosynthetic pathway gene expression in unelicited and MeJA-elicited cultures

Cultures accumulated paclitaxel/taxanes only upon elicitation with MeJA (Fig. 1A, B), and therefore this experiment was designed to investigate gene expression in the on/off state of paclitaxel/taxane accumulation. Expression of seven known taxane biosynthetic pathway genes was quantified and compared between MeJA-elicited and unelicited cultures (Fig. 1C). Most genes were significantly upregulated in MeJA-elicited cultures relative to unelicited cultures (1.5–160-fold). These data concur with previous reports on different *Taxus* cell lines that show increased steady state mRNA levels of biosynthetic pathway genes upon MeJA-elicitation using Northern gel blot analysis [26, 27, 37, 38], RT-PCR [27], and qRT-PCR [29,39]. In this study, the fold change in T5αH expression upon elicitation with MeJA was lower than that quantified for most other pathway genes, which agrees with previous results [27, 40]. In a time course study using Northern gel blot analysis, T5αH expression was shown to be higher at 6 h relative to 18 h, after which transcripts were not detected [27].

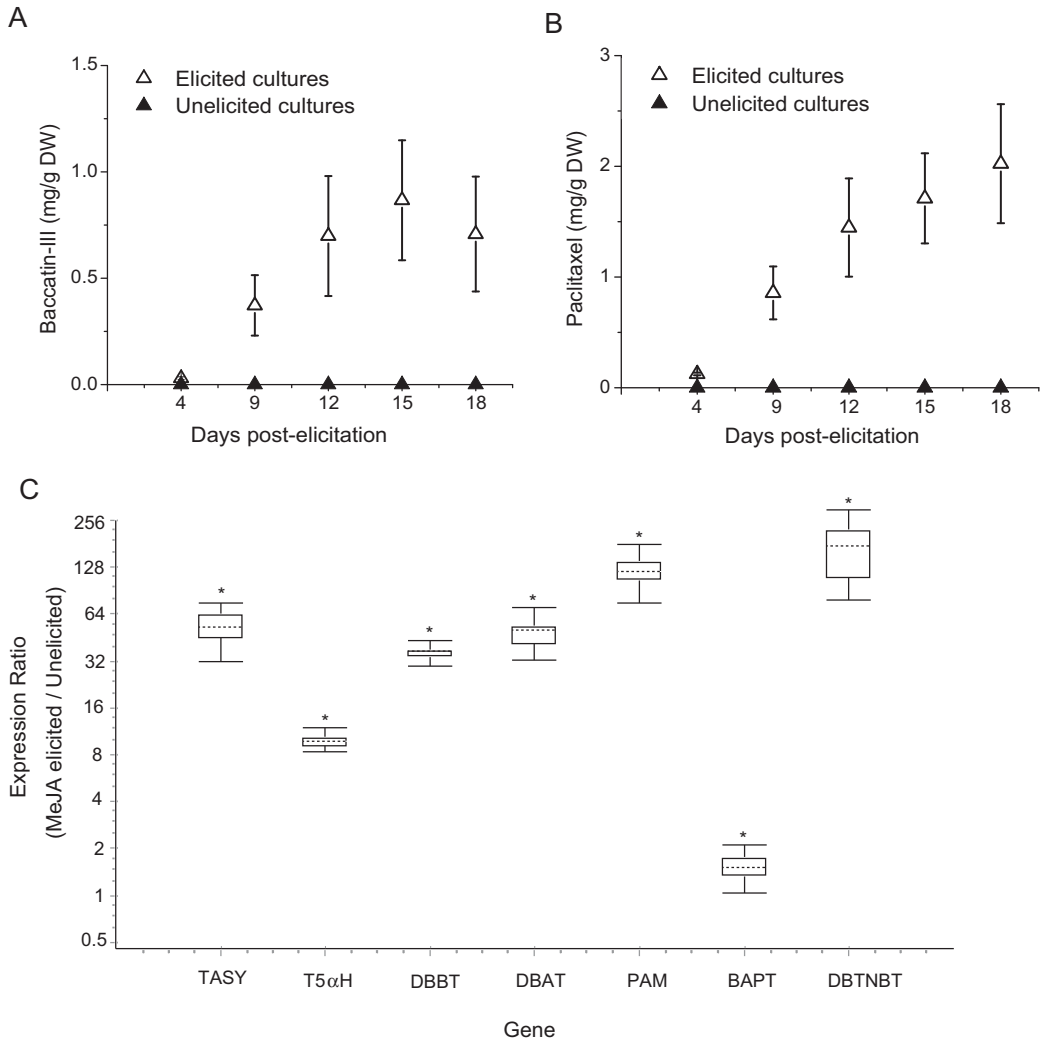

Figure 1. Effect of MeJA elicitation on taxane accumulation and expression of taxane biosynthetic pathway genes. (**A**) Baccatin III accumulation in MeJA-elicited and unelicited cultures. (**B**) Paclitaxel accumulation in MeJA-elicited and unelicited cultures. (**C**) Relative gene expression ratio of seven known paclitaxel biosynthetic pathway genes determined with qRT-PCR, calculated using REST 2009 software. Expression ratio represents the median fold change in MeJA-elicited samples relative to unelicited samples at 15 h post-elicitation, normalized by actin, and GAPDH reference gene expressions. The box represents the middle 50% of observations. The dotted line within the box represents the median expression ratio. Whiskers represent the minimum and maximum observations. $*p < 0.05$, as determined by randomization tests using REST software. Reported values are the average of three biological replicates.

Figure 1C shows results at 15 h post-elicitation, and therefore T5αH transcript abundance was significantly lower by this time point, resulting in a lower observed expression ratio. In addition, BAPT expression was relatively weakly induced with MeJA elicitation as compared to the other pathway genes, agreeing with previous data [27]. Thus, these data again support the hypothesis that BAPT may be a critical rate influencing step in the paclitaxel biosynthetic pathway and a primary target for metabolic engineering. Once the qRT-PCR technique was successfully established and induction of gene expression was confirmed with MeJA elicitation, we examined cultures that produced paclitaxel at different levels, enabling a more detailed understanding of metabolic differences amongst *Taxus* cultures with varying bulk paclitaxel/taxane production capabilities.

3.2 Analysis of cultures that exhibit high variability in paclitaxel/taxane accumulation

A comparison of gene expression in cultures that exhibit variations in paclitaxel/taxane accumulation after MeJA elicitation (i.e., higher-accumulating cultures vs. lower-accumulating cultures) has not previously been reported, but since variability in product synthesis is common amongst cultures, such a

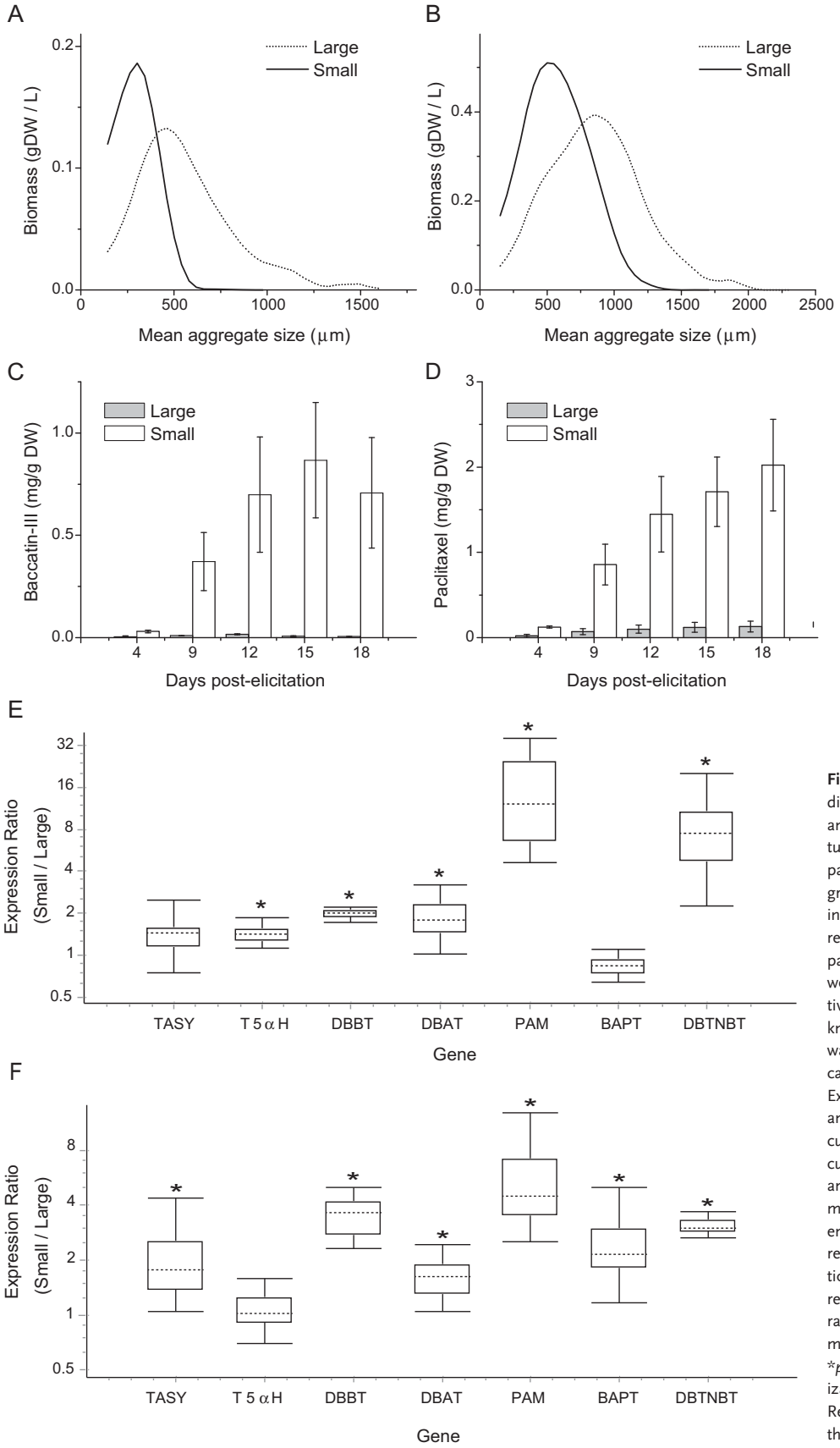

Figure 2. Profiles of aggregate size distributions, taxane accumulation and gene expression patterns in cultures exhibiting a large difference in paclitaxel accumulation. (**A, B**) Aggregate size distribution at culture initiation and prior to elicitation, respectively. (**C, D**) Baccatin III and paclitaxel accumulation over a three-week period, respectively. (**E, F**) Relative gene expression ratio of seven known paclitaxel biosynthetic pathway genes determined with qRT-PCR, calculated using REST 2009 software. Expression ratio represents the median fold change in small aggregate cultures relative to large aggregate cultures at 15 h post-elicitation (**E**) and just prior to elicitation (**F**), normalized to actin and GAPDH reference gene expressions. The box represents the middle 50% of observations. The dotted line within the box represents the median expression ratio. Whiskers represent the minimum and maximum observations. *$p < 0.05$, as determined by randomization tests using REST software. Reported values are the average of three biological replicates.

comparison is therefore of great practical interest. To obtain such differences in paclitaxel/taxane accumulation, we initiated cultures with different aggregate size profiles but the same initial biomass, and analyzed paclitaxel/taxane accumulation as well as pathway gene expression. Size distributions taken from small aggregate cultures and large aggregate cultures following inoculation on day 0 and prior to elicitation on day 7 indicated that once established, cultures maintained disparate aggregate size profiles (Fig. 2A, B). Growth is typically retarded upon elicitation with MeJA [8, 23] and small and large aggregate cultures maintain similar growth patterns post-elicitation [33]. Throughout the production period of the cell culture batch, small aggregate cultures accumulated higher levels of both baccatin III and paclitaxel in comparison to large aggregate cultures (Fig. 2C, D). The total baccatin III and paclitaxel levels were up to 120- and 15-fold higher, respectively, in small aggregate cultures as compared to large aggregate cultures, indicating substantial difference in metabolite accumulation.

Five of the seven genes investigated were upregulated in MeJA-elicited small aggregate cultures as compared to MeJA-elicited large aggregate cultures ($p<0.05$), but the level of upregulation varied amongst the genes (Fig. 2E). The relative transcript levels of early and middle pathway genes (T5αH, DBBT and DBAT) showed a minor (1.4-, 2-, and 1.8-fold, respectively) increase in small aggregate cultures relative to large aggregate cultures. A more substantial increase for the later pathway genes, PAM and DBTNBT (12- and 7-fold, respectively), was observed in small aggregate cultures. The relative expression levels of TASY and BAPT were not significantly different between the cultures. One possibility for suppressed secondary metabolism in large aggregate cultures is that MeJA elicitation is not uniform in larger aggregates, where transport limitations exist, and therefore MeJA may not effectively penetrate into the interior of all aggregates [41]. These effects may be amplified since MeJA is produced endogenously by cells in response to external stimuli [14], as jasmonic acid biosynthesis is regulated by a positive feedback loop [42, 43]. Overall, these data demonstrate some differences in pathway gene expression between cultures that accumulate substantially different paclitaxel levels (in this case 15-fold), but suggest there are additional factors that regulate paclitaxel accumulation. Interestingly, when the relative expression levels of the same pathway genes were compared in unelicited small and large aggregate cultures, higher mRNA levels for most pathway genes (six of the seven evaluated; $p<0.05$) were observed in the small aggregate cultures (Fig. 2F). Upregulation of greater than two-fold was observed for four genes, DBBT, PAM, BAPT, and DBTNBT, suggesting a difference in initial metabolic state prior to MeJA elicitation. However, no differences were observed in paclitaxel accumulation in unelicited cultures, as the levels of paclitaxel produced were below the limit of quantification of UPLC. These data indicate there are bottlenecks to product accumulation in unelicited cultures that are overcome through elicitation.

3.3 Analysis of cultures with smaller differences in paclitaxel/taxane accumulation but higher levels of production

A second experiment was conducted with the same *T. cuspidata* cell line, in which the aggregate sizes for both small aggregate cultures and large aggregate cultures were slightly larger (Fig. 3A, B). As in the previous experiment, a similar metabolite trend was observed, with small aggregate cultures accumulating higher levels of baccatin III and paclitaxel (Fig. 3C, D). However, there were two important differences: (i) the relative change in metabolite accumulation between small and large aggregate cultures maximally was only 4.6-fold for baccatin III and 1.7-fold for paclitaxel, compared to 120-fold for baccatin III and 15-fold for paclitaxel in the previous experiment; and (2) the large aggregate cultures in this experiment accumulated a relatively high level of paclitaxel (1.6 mg/g DW), which was 13-fold higher than large aggregate cultures and on the same order as small aggregate cultures from the previous experiment. Aggregate size is not the only factor influencing variability in paclitaxel accumulation amongst cultures, and it is not uncommon to observe varying differentials in paclitaxel accumulation [33]. Additional genetic and epigenetic factors not explicitly measured or controlled for likely contribute to the variability observed amongst *Taxus* cultures between experiments.

In this case, qRT-PCR analysis revealed no differences in the expression levels of pathway genes between small and large aggregate cultures after elicitation with MeJA (Fig. 3E). Both small and large aggregate cultures accumulated high levels of paclitaxel, indicating that pathway genes were upregulated by MeJA, but the differences in baccatin III and paclitaxel accumulation between small and large aggregate cultures could not be attributed to additional increases in mRNA abundance of pathway genes.

While this work collectively shows that some degree of variability in secondary metabolism can be linked to changes in biosynthetic pathway gene expression, there are clearly contributions from

other factors. Metabolite levels are not only determined by pathway gene expression levels, but also by post-transcriptional and/or post-translational regulation of enzyme activity [44]. For example, discrepancies in post-transcriptional and/or post-translational regulation were hypothesized for the

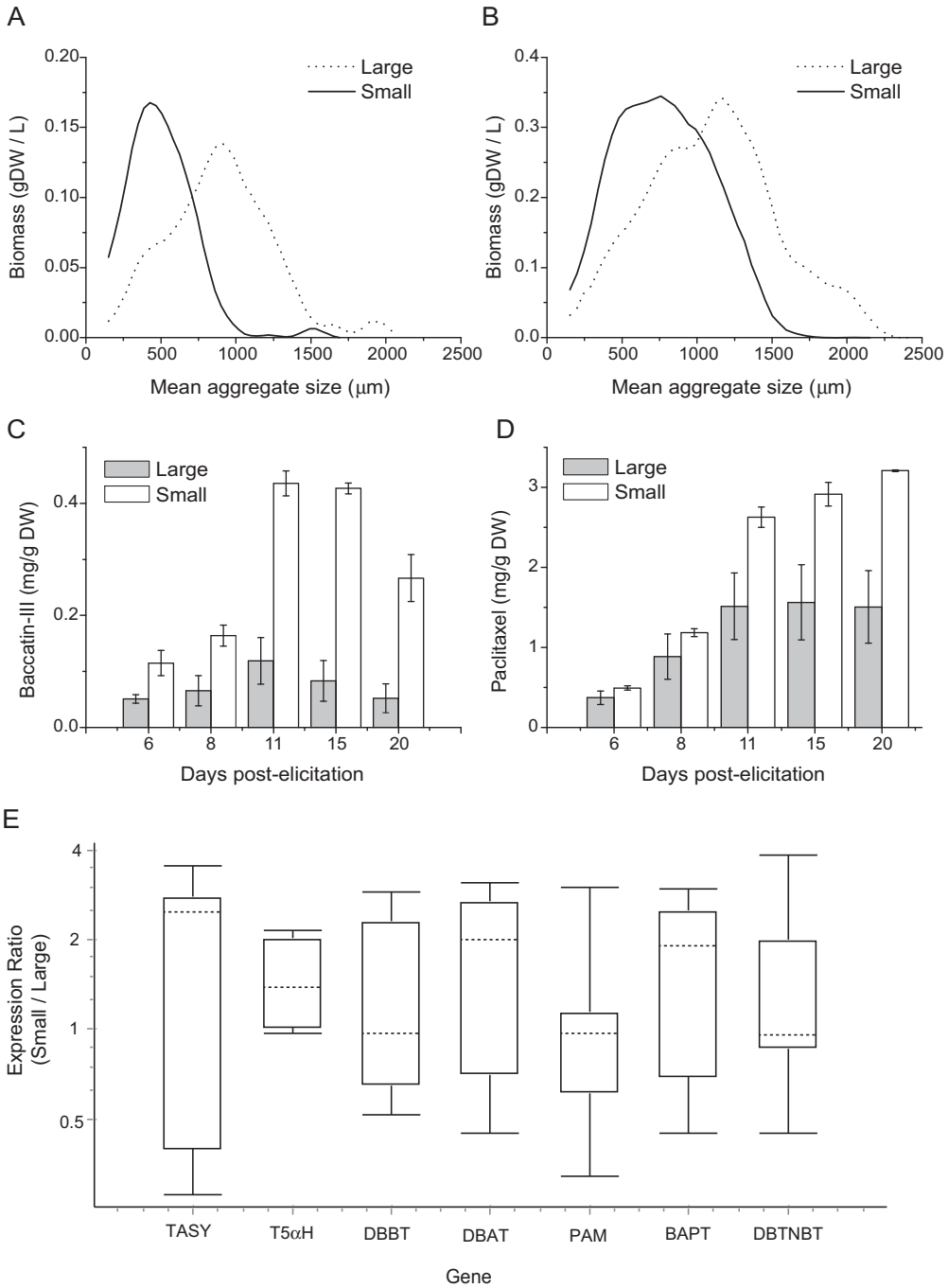

Figure 3. Profiles of aggregate size distributions, taxane accumulation, and gene expression patterns in cultures accumulating high levels of paclitaxel with a relatively small difference in paclitaxel accumulation. (**A, B**) Aggregate size distribution at culture initiation and prior to elicitation, respectively. (**C, D**) Baccatin III and paclitaxel accumulation over a three-week period, respectively. (**E**) Relative gene expression ratio of seven known paclitaxel biosynthetic pathway genes determined with qRT-PCR, calculated using REST 2009 software. Expression ratio represents the median fold change in small aggregate cultures relative to large aggregate cultures at 15 h post-elicitation, normalized using actin, and GAPDH reference gene expressions. The box represents the middle 50% of observations. The dotted line within the box represents the median expression ratio. Whiskers represent the minimum and maximum observations. Results are statistically similar for all genes ($p > 0.05$) as determined by randomization tests using REST software. Reported values are the average of three biological replicates.

lack of resveratrol production in *Vitis vinifera* cell cultures even after high induction of one of the key pathway genes [45]. Primary metabolism is known to provide critical substrates for secondary metabolic pathways and hence, precursor pool availability can also affect product accumulation. For example, phenylalanine addition to *T. cuspidata* cultures resulted in an increase in paclitaxel accumulation [46]. In addition to paclitaxel biosynthesis, post-biosynthetic events such as storage and degradation of paclitaxel and its precursors may be important [5, 47, 48]. The paclitaxel biosynthetic pathway originates in the plastid and involves hydroxylation in the endoplasmic reticulum and acylation in the cytosol [49]. Knowledge about subcellular trafficking and transport of paclitaxel and precursors, mechanisms for extracellular secretion and in vivo degradation will help suggest additional potential regulators of paclitaxel/taxane accumulation.

4 Concluding remarks

Significant variability is observed in paclitaxel accumulation amongst cultures of different *Taxus* species and over time within a single species. In this paper, we studied the relationship between paclitaxel biosynthetic pathway gene expression and the varying bulk levels of paclitaxel observed amongst cultures. Through qRT-PCR, we demonstrated induction of paclitaxel biosynthetic pathway genes through elicitation with MeJA, which is necessary for paclitaxel accumulation. By analyzing MeJA-elicited cultures with varying levels of paclitaxel accumulation, we observed additional upregulation of paclitaxel biosynthetic pathway gene expression between cultures with significant differences in paclitaxel accumulation capabilities (15-fold). In particular, increased expression of late pathway genes, such as PAM and DBTNBT, was observed when there were substantial differences in bulk paclitaxel accumulation levels. However, when paclitaxel accumulation levels are only moderately different (two-fold), no differences in pathway gene expression were measured, suggesting that other factors affect the bulk paclitaxel accumulation patterns. This is the first study to address the relationship between gene expression and variability in paclitaxel accumulation through generating cultures with different paclitaxel accumulation potentials upon elicitation with MeJA. A systems-wide genomics approach along with consideration of post-biosynthetic pathway events such as transport and degradation are necessary to fully understand the factors that regulate paclitaxel accumulation in cell culture.

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