MYB transcription factor PdMYB118 directly interacts with bHLH transcription factor PdTT8 to regulate wound-induced anthocyanin biosynthesis in poplar

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Abstract

Background: R2R3-MYB transcription factors (TFs) play important roles in plant growth and development, and response to biotic and abiotic stresses. However, their regulatory mechanisms in wound-induced anthocyanin biosynthesis in woody plants are largely unknown.

Results: In this work, we report that expression of anthocyanin biosynthesis genes (ABGs) were activated by PdMYB118, a MYB TF encoding gene from Populus deltoids, and the activation of PdMYB118 was significantly enhanced by PdTT8, a bHLH protein, through its direct interaction with PdMYB118. PdMYB118 and some ABGs were evidently induced by wound induction and methyl jasmonate (MeJA) treatment. Overexpression of PdMYB118 promoted anthocyanin accumulation in transgenic poplar upon wound induction. Furthermore, a poplar JASMONATE ZIM-domain (JAZ) protein, PtrJAZ1, repressed the transcriptional function of PdMYB118/PdTT8 complex by binding to PdTT8, and wound stimulated the biosynthesis of jasmonic acid (JA) and the degradation of PtrJAZ1.

Conclusions: Based on these observations, we proposed that PtrJAZ1 degradation triggered the expression of ABGs, leading to increased biosynthesis of anthocyanins in the wounded leaves of transgenic poplar. Therefore, our findings not only illustrate the crucial role of PdMYB118 in wound-induced anthocyanin biosynthesis in poplar, but also provide a molecular basis for the genetic engineering of colorful tree species.

Keywords: Anthocyanin, JAZ1, PdMYB118, PdTT8, Poplar

Background

As the major pigments, anthocyanins not only provide colors to plant flowers and fruits [1], but also function in plant resistance to different biotic and abiotic stresses [2–12]. In plants, anthocyanins are biosynthesized via the specific branch of flavonoid pathway [1, 13, 14]. In poplar, the biosynthesis of anthocyanins is catalyzed by a series of enzymes, including chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavanone 3′-hydroxylase (F3′H), flavonoid 3′5′-hydroxylase (F3′5′H), dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS) [7, 8]. The expression of anthocyanin biosynthetic genes (ABGs) are regulated by the MBW complexes, which is composed of two kinds of transcriptional factors (TFs), R2R3-MYB and basic helix-loop-helix (bHLH), and WD40-repeat proteins [15–17]. In Arabidopsis, the MBW complexes are composed of the
to PdMYB118 is involved in wound induced anthocyanin biosynthesis in poplar

To explore the possible functions of PdMYB118 in anthocyanin biosynthesis in poplar, we previously generated transgenic Shanxin Yang plants [34]. Overexpression of PdMYB118 led to red leaf phenotype in adult transgenic plants grown in both green house and field, whereas young tissue culture plantlets showed green leaves as did the wide type (WT) (Additional file 1: Fig. S1a). When young plantlets were sub-cultured onto new MS medium, their leaves turned red and produced more anthocyanins after being cut off from their mother plants (Additional file 1: Fig. S1b-d). Therefore, wound may have prompted the biosynthesis of anthocyanin in transgenic plants. To confirm this speculation, wound induction was applied to both young leaves of tissue cultured transgenic plantlets and mature leaves of greenhouse grown transgenic plants. As we have hypothesized, both WT and transgenic leaves from tissue cultured plantlets were green, but transgenic leaf discs formed more red speckles 2 days after wound induction (Fig. 1a). Similar results were also observed in the mature leaf discs of greenhouse grown transgenic plants (Fig. 1b). Transgenic leaf discs produced more anthocyanins than did the WT control (Fig. 1c, d). These results indicate that PdMYB118 is involved in wound induced anthocyanin biosynthesis in poplar.

Wound induces the expression of ABGs

Based on the observation that wound promoted anthocyanin accumulation in PdMYB118 overexpressing transgenic plants, we postulated that wound signaling may also induce the expression of ABGs in wild type poplar. We found that PdMYB118 transcripts were gradually increased in the leaf discs during wound induction, and reached to the highest transcription level at 24 h (Fig. 2). Some ABGs, including PtrF3'H, PtrDFR2 and PtrANS1, showed similar expression pattern as PdMYB118. A responsive expression of PtrCHS1 to wound induction was also observed: it increased at 1 h, then gradually reduced within 12 h and restored to the high expression level at 24 h. PdTT8 was slightly induced in wounded leaves within 3 h, and obviously up-regulated at 24 h. PtrCHI1 and PtrF3'H were down-regulated within 3 to 12 h after wound induction and then reached to the normal expression level. The transcripts of PtrF3'S'H2 reached a peak level within 1 h after wound induction and rapidly decreased to lower levels at later time points. From these data, we identified four wound-inducible ABGs: PtrCHS1, PtrF3'H, PtrDFR2 and PtrANS1.

The transcriptional activation activity of PdMYB118 is enhanced by a bHLH TF PdTT8

To clarify the exact function of PdMYB118 in wound induced anthocyanin biosynthesis, we first transiently expressed it in the leaf protoplasts of wild type Shanxin Yang plants. We found that transient expression of PdMYB118 enhanced the transcription of most ABGs, including the wound-/JA-inducible ABGs: PtrCHS1, PtrF3'H, PtrDFR2 and PtrANS1 (Fig. 3a). In plants, anthocyanin-related MYB factors interact closely with bHLH TFs to control anthocyanin biosynthesis [15]. We cloned a poplar bHLH TF PdTT8, the homolog of MdbHLH3 in apple and TT8 in Arabidopsis, which interacted with different MYB TFs, and investigated its transcription activity. PdTT8 alone did not regulate the
expressions of ABGs, but could significantly enhance the transcriptional activation activity of PdMYB118. When PdTT8 was transiently co-expressed with PdMYB118 in the protoplasts, the transcription levels of all ABGs were remarkably higher than those expressing PdMYB118 alone (Fig. 3a). These results imply that PdTT8 could be an efficient enhancer of PdMYB118 to regulate the expression of ABGs.

The enhanced transcriptional activation function of PdMYB118 by PdTT8 suggested a possible interaction of these two proteins. Therefore, we performed BIFC assays to confirm this possibility. The N- and C-terminal fragment of yellow fluorescent protein (nYFP and cYFP) was fused with PdMYB118 and PdTT8, respectively. As expected, when PdMYB118-nYFP and cYFP-PdTT8 were co-transfected into poplar leaf mesophyll protoplasts, the nuclei of protoplasts showed strong YFP fluorescence; whereas no signal was detected in the control (Fig. 3b). These results demonstrate that PdTT8 could directly interact with PdMYB118 to enhance its transcriptional activation activity.

Wound prompts JA biosynthesis and JAZ1 degradation
JA biosynthesis is catalyzed by a series of biosynthetic enzymes step by step [26]. It is widely considered that JA is rapidly synthesized in plant leaves suffering wound stimulus [23, 24]. To detect the JA changes in response to wound, we examined the expressions of JA biosynthesis genes PtraOC2–3, PtraOPR3–1, PtraACX1–2 and PtraARI1–1. All these genes were rapidly expressed to their peak levels within 1 h after wound induction and then reduced gradually as the treatment elongated (Fig. 4a). The responsive expression of JA biosynthesis genes may result in JA accumulation. We then measured JA and JA-Ile contents at two time points: 1 h (short time) and 24 h (long time) after mechanical damage. JA and JA-Ile contents were about 10 ng g⁻¹ and 0.58 ng g⁻¹ fresh weight tissues (FW) in undamaged leaves,
respectively. However, after 1 h of wound induction, the contents of JA and JA-Ile increased by about 16 and 200 folds (Fig. 4b). After 24 h, JA and JA-Ile contents reduced to about 1.9 and 0.53 ng g$^{-1}$ FW. The changes in JA and JA-Ile contents in wounded poplar leaves were tightly correlated with the expression changes of JA biosynthesis genes.

It has been reported that JAZ genes are obviously induced by mechanical wounding [23]. We analyzed the expression of a poplar JAZ gene PtrJAZ1 during wound induction, and found that PtrJAZ1 transcripts were quickly up-regulated within 1 h after wound induction, rapidly decreased within 3 h, and dropped to the lowest level after 24 h (Fig. 4c). The change in PtrJAZ1 protein content in response to wound induction was then tested by Western blotting. Consistent with its gene expression variation in wounded leaves, PtrJAZ1 protein accumulation reached to a higher level within 3 h, began to decline after 8 h, and reduced to a lower level after 12 h (Fig. 4d). These results indicate that wound induced anthocyanin biosynthesis in poplar may be mediated by JA signaling.

JA regulates the expressions of ABGs in poplar
We further examined whether JA can regulate the expression of ABGs. Poplar leaves were sprayed with MeJA solution for qRT-PCR analyses. As expected, the expression levels of wound inducible ABGs (PtrCHS1, PtrF3'H, PtrDFR2 and PtrANS1) were up-regulated more than 3 to 20 folds by MeJA treatment (Fig. 5). JA signaling is
regulated by JAZ proteins, which inhibit the expression of JA response genes by binding to other transcription factor [35]. We transiently expressed \textit{PtrJAZ1} in the leaf protoplasts of \textit{PdMYB118} transgenic plants. The up-regulated expression of most \textit{ABGs} by \textit{PdMYB118} was inhibited by the expression of \textit{PtrJAZ1} (Fig. 6a, b). These results indicate that JA induced expression of \textit{ABGs} in poplar is negatively regulated by \textit{PtrJAZ1}.

Fig. 3 \textit{PdTT8} enhances the transcriptional activation activity of \textit{PdMYB118}. a Transient expression of \textit{PdMYB118} or \textit{PdTT8} in poplar mesophyll protoplasts. The constructs of \textit{pGreenII62-SK-PdMYB118}, \textit{pGreenII62-SK-PdTT8} or \textit{pGreenII62-SK-PdMYB118 + pGreenII62-SK-PdTT8} were transfected into the poplar leaf protoplasts, respectively. The empty vector was used as a negative control. RNA was extracted from the transfected protoplasts for qRT-PCR analyses of \textit{ABGs}, \textit{PdTT8} and \textit{PdMYB118}. \textit{PtrCHS1}, \textit{PtrCHI1}, \textit{PtrF3H}, \textit{PtrF3'H}, \textit{PtrDFR2} and \textit{PtrANS1} are the \textit{ABG} genes respectively encoding chalcone synthase, chalcone isomerase, flavanone 3-hydroxylase, flavanone 3′-hydroxylase, flavonoid 3′,5′-hydroxylase, dihydroflavonol 4-reductase and anthocyanidin synthase in \textit{Populus}. C, protoplasts transfected with \textit{pGreenII62-SK} (control); M, protoplasts transfected with \textit{pGreenII62-SK-PdMYB118}; T, protoplasts transfected with \textit{pGreenII62-SK-PdTT8}; M + T, protoplasts transfected with \textit{pGreenII62-SK-PdMYB118} and \textit{pGreenII62-SK-PdTT8}. b BiFC assays to detect the interaction of \textit{PdTT8} and \textit{PdMYB118}. \textit{PdMYB118} or \textit{PdTT8} was respectively fused with N-terminal and C-terminal fragments of YFP. Construct pairs indicated on the left were co-expressed in the leaf protoplasts of WT poplar plants. Gene expression level in the control sample was set to 1. Values are means and standard deviations of three biological replicates (\(n = 3\)). *** significant difference in comparison to C and T at \(P < 0.001\), respectively (Student’s t-test).
PtrJAZ1 represses the transcriptional activation activity of PdMYB118/PdTT8 complex by binding to PdTT8

The suppressed transcriptional activation activity of PdMYB118 by PtrJAZ1 could be regulated by PdTT8. Therefore, we transiently expressed PdTT8 alone or together with PtrJAZ1 in the leaf protoplasts of PdMYB118 transgenic plants (Additional file 2: Fig. S2a, b). We observed that transient expression of PdTT8 in the leaf protoplasts of PdMYB118 transgenic plants further increased the expression levels of ABGs (Fig. 7a). However, when PtrJAZ1 and PdTT8 were co-expressed in the leaf protoplasts of PdMYB118 transgenic plants, the

Fig. 4 JA biosynthesis gene expression, JA content and JAZ1 degradation analyses. The mature leaves of poplar plants grown in greenhouse were treated using leaf-disc method for various time intervals and then used for RNA extraction. a qRT-PCR analyses of JA biosynthesis genes in the wounded leaves. PtrAOC2-3, a putative allene oxide cyclase gene in Populus; PtrOPR3-1, a putative OPDA reductase 3 gene in Populus; PtrACX1-2, a putative acyl-CoA oxidase gene in Populus; PtrJAR1-1, a putative JASMONATE RESISTANT gene (a JA-Ile biosynthesis gene) in Populus. b JA and JA-Ile content in the wounded leaves of poplar plants grown in greenhouse. The leaves treated for various time intervals were used to be tested. c qRT-PCR analyses of PtrJAZ1 expression in the wounded leaves. d Western blotting analyses of JAZ1 protein in the wounded leaves. Total proteins were extracted from the leaf-discs treated for various time intervals. An amount of 30 μg proteins were separated by 10% SDS-PAGE and hybridized with Arabidopsis JAZ1 antibodies (JAZ1) or plant actin antibodies (Actin), respectively. Each gene expression in the untreated leaves (0 h) was set to 1. Three independent replicates of measurements were performed for each time point, and the values are means and standard deviations (n = 3). * and ***, significant differences at P < 0.05 and P < 0.001 (Student’s t-test). The original uncropped blot image was shown in additional file 6: Fig. S6
expression levels of ABGs were repressed to the levels of control samples (Fig. 7a). These results suggest that PtrJAZ1 suppressed the transcription activity of PdMYB118/PdTT8 complex. Then, we performed BiFC assays to examine whether PtrJAZ1 interacts with PdMYB118 or PdTT8. PtrJAZ1-nYFP was transiently co-expressed with cYFP-PdTT8 or cYFP-PdMYB118 in the leaf mesophyll protoplasts of wide type poplar. Co-expression of PtrJAZ1-nYFP with cYFP-PdTT8 produced strong YFP fluorescence in the nuclei of protoplasts, whereas no fluorescence signal was detected in the protoplasts co-expressing PtrJAZ1-nYFP and cYFP-PdMYB118 (Fig. 7b; Additional file 3: Fig. S3). This result is consistent with the finding in apple that MdJAZs interact with MdbHLH3 (the analogue of TT8) but not with the PA biosynthesis associated MdMYB9/11 [36].

We further confirmed the inhibition of PtrJAZ1 on the transcriptional activation activity of PdMYB118/PdTT8
complex by dual-luciferase assays. Three wound inducible ABGs (*PtrCHS1*, *PtrDFR2* and *PtrANS1*) were selected. The transcriptional activation activity of PdMYB118 on the promoters of *PtrCHS1*, *PtrDFR2* and *PtrANS1* was higher than that of PdTT8, showing a higher ratio of LUC/REN (Fig. 7c-e). The highest ratio values were
observed in the proplasts co-expressing PdMYB118 and PdTT8. And the transcription activity of PdMYB118/PdTT8 complex decreased obviously when PtrJAZ1 was co-transformed with PdMYB118 and PdTT8 (Fig. 7c-e). These results suggest that by binding to PdTT8, PtrJAZ1 could restrain the transcriptional activity of PdMYB118/PdTT8 complex.

**Degradation of JAZ1 proteins promotes anthocyanin biosynthesis in the wounded leaves of transgenic plants**

Based on the observations that expression of ABGs was inhibited by PtrJAZ1 and JAZ1 proteins were degraded upon wound induction, we speculated that the increased anthocyanin biosynthesis in the wounded transgenic leaves may be a result of JAZ1 degradation. By Western blotting analysis using Arabidopsis JAZ1 antibodies, we found that JAZ1 accumulation in the wounded leaves of transgenic plants were distinctly lower than that in the no-treated control leaves (Fig. 8a). Therefore, the decreased JAZ1 protein level may have released the transcriptional activation activity of the MYB118/TT8 complex and increased the expression of wound-inducible ABGs. We further analyzed the expression of ABGs in the leaves of transgenic plants overexpressing PdMYB118. In the untreated leaves of transgenic plants (lines L2 and L7), wound-JA-inducible ABGs (PtrCHS1,PtrDFR2,PtrF3H and P트AN31) were up-regulated in transgenic plants. Upon wound induction, their expression levels increased in the leaves of both WT and transgenic plants, with a more significant increase in the wounded leaves of transgenic plants (Fig. 8b). Similarly, the expression level of PdMYB118 increased in the wounded leaves of both WT and transgenic plants, but the increase was significantly higher in transgenic plants (Additional file 4: Fig. S4a). However, the expression level of PdTT8 was only slightly changed in the leaves of both WT and transgenic leaves (Additional file 4: Fig. S4b). These results indicate that the increased anthocyanin accumulation in the wounded leaves of transgenic plants could be due to the enhanced expression of ABGs resulted from the wound-induced degradation of JAZ1 proteins.

**Discussion**

Although wound induced anthocyanin accumulation has been widely observed in plants, the molecular mechanism that how wound regulates anthocyanin biosynthesis in poplar still remains unknown [28, 37]. Recently, we reported that PdMYB118 regulated the biosynthesis of anthocyanin in poplar [34]. Overexpression of PdMYB118 obviously activated the expression of anthocyanin biosynthesis genes but not the two proanthocyanin synthesis genes, leading to red leafed phenotype in transgenic plants. Interestingly, during the process of shoot propagation, we observed that the leaves of tissue cultured plantlets or greenhouse grown young transgenic plants remained in green or less red color, but once the shoots were cut off, the leaves of new cut shoots turned into red color (Additional file 1: Fig. S1a-d). The stability of this phenomenon (wound-induced anthocyanin biosynthesis) was further confirmed using green leaves of both tissue cultured plantlets and greenhouse grown plants (Fig. 1a-d).

Previous studies have showed that JA regulates WD-repeat/bHLH/MYB (WBM) complex-mediated anthocyanin accumulation via the interaction of JAZ proteins with bHLH and MYB transcription factors [29]. PAspecific R2R3-MYB factors like MYB134 in poplar and MdMYB9/11 in apple dramatically responded to wound treatment, whereas the anthocyanin biosynthesis R2R3-MYB factor MdMYB1 in apple did not [8, 36]. MYB/bHLH complex, such as PAP1/TT8 in Arabidopsis, AN4/AN1 in Petunia hybrid, VvMYBA1/VvMYC1 in grape, and MdMYB10/MdBHLH3 in apple, are also involved in anthocyanin synthesis [13, 38–40]. We found that expression of most ABGs was responsive to wound treatment (Fig. 2). The poplar bHLH TF PdTT8 physically interacted with PdMYB118 to form the PdMYB118/PdTT8 complex, and more efficiently activated the expressions of ABGs (Fig. 3a, b). Therefore, PdTT8 may function as an enhancer to promote the transcriptional activation activity of PdMYB118.

Wound can rapidly activate the biosynthesis of JA, which functions as an important plant defense signal in response to various stresses [23, 24]. In plants, the biosynthetic pathway of JA is initiated from the triunsaturated fatty acids α-linolenic acid (18:3) [26]. After two oxidation steps, α-linolenic acid is converted to an unstable allene oxide, which is subsequently catalyzed to 12-oxophytodienoic acid (OPDA) by an allene oxide cyclase (AOC) in chloroplasts. Then the second half of JA biosynthesis is finished in peroxisomes, where OPDA is reduced to OPC-8 by OPDA reductase 3 (OPR3) [41]. After β-oxidation, JA is transported to cytosol, where it is conjugated to amino acids by JA-amino acid synthetase (JAR1) to form jasmonoyl-isoleucine (JA-Ile), a major biologically active jasmonate among a growing number of jasmonate derivatives. We found that wound treatment rapidly up-regulated the expression of JA biosynthesis genes within 1 h (Fig. 4a), leading to the rapid output of JA and JA-Ile in the wounded leaves (Fig. 4b). These results indicate that JA biosynthesis responds rapidly to wound induction in poplar. Increased JA biosynthesis by wound treatment could activate the JA signaling. Indeed, similar to the expression pattern of JA biosynthesis gene, P트JAZ1 was also up-regulated within 1 h after the initiation of wound induction, and then gradually decreased (Fig. 4c). This result is consistent with previous reports that most JAZ genes were strongly induced within 1 h after wound induction and declined
at later time points [23, 24]. JA signaling also promotes the degradation of JAZ transcriptional repressors through the activity of the E3 ubiquitin-ligase SCF COI1 JAZs [27]. We found that the content of poplar JAZ proteins was also obviously decreased after 8 h of wound treatment (Fig. 4d). Therefore, in poplar, wound induction could rapidly induce JA biosynthesis by activating its biosynthesis genes and promote the degradation of JAZ protein.

JA regulates several anthocyanin biosynthetic genes involved in anthocyanin accumulation [42, 43]. In poplar, MeJA treatment could induce the expression of some ABGs such as PtrCHS1, PtrF3H, PtrDFR2 and PtrANS1 (Fig. 5). Interestingly, expressions of these ABGs were also induced by wound, indicating that wound-induced anthocyanin biosynthesis may be mediated by JA signaling. It has been reported that JA regulates WD-repeat/bHLH/ MYB complex-mediated anthocyanin accumulation via the interaction of JAZ proteins with bHLH and MYB factors [29]. We found that PtrJAZ1 specifically bound to PdTT8 and attenuated the transcriptional activation activity of PdMYB118/PdTT8 complex on the promoter of JA/ wound-inducible ABGs (Fig. 7a-e). JA regulated expression of ABGs in poplar is similar to that in Arabidopsis [29], but different from that in apple, where MdJAZ2 inhibits the recruitment of MdbHLH3 to the promoters of MdMYB9 and MdMYB11 which regulate the biosynthesis of anthocyanin and proanthocyanin [24]. Therefore, JA induced JAZ protein degradation could abolish the
interaction of JAZ proteins with bHLH and MYB factors, and then activate the biosynthesis of anthocyanin [29]. In agreement with this speculation, when PtJAZ1 protein was degraded in the wounded leaves of transgenic plants, JA−/wound-inducible ABGs were highly induced to promote anthocyanin biosynthesis (Fig. 8a, b).

**Conclusions**

Taken together, our study illustrates the biological function of PdMYB118 in wound-induced anthocyanin synthesis in poplar. Although more detailed studies are still needed to completely understand the mechanism of PdMYB118 in anthocyanin synthesis, our data presented here imply a possible model of wound-induced anthocyanin synthesis in poplar: upon wound induction, JA biosynthetic genes are rapidly activated for JA biosynthesis, subsequently triggered the degradation of PtrJAZ1; Then, the transcriptional activation activity of PdMYB118/PdTT8 complex is restored to promote the expression of anthocyanin biosynthesis genes; Finally, anthocyanins were biosynthesized, leading to red leafed phenotype in transgenic plants (Additional file 5: Fig. S5a, b).

**Methods**

**Plant materials and growth conditions**

Wild type Shanxin Yang (WT) and transgenic Shanxin Yang (*P. davidiana × P. bolleana*) overexpressing PdMYB118 used in our previous report were propagated on MS medium, transferred to soil, and grown in greenhouse [34, 44]. Shanxin Yang was provided by Prof. Guifeng Liu (Northeast Forestry University, China). *Populus deltoids* and its red leaf mutant were purchased from Qingyuan HiTech Ltd. (Yantai, China). Plants were grown in greenhouse at 25 °C (day)/18 °C (night) in a 12 h light/12 h dark photoperiod.

**Wound treatments**

For wound induction, the leaves of wild type and transgenic poplar grown on MS medium or in green house were cut into 1.5 cm dices and transferred onto MS medium. At various time points after wound induction, leaf discs were harvested, frozen in liquid nitrogen, and stored at −80 °C for RNA, protein, JA and anthocyanin extraction.

**MeJA treatment**

To analyze the induction of PdMYB118 and ABGs by JA, 100 μM MeJA (Sigma, Shanghai, China) solution was sprayed onto the mature leaves of WT poplar plants grown in greenhouse. After 3 h, leaves were collected and used for RNA extraction. Leaves sprayed with water were used as a negative control.

**Quantitative real-time RT-PCR**

Total RNA was extracted from leaves and leaf protoplasts with RNAiso Reagent (Takara, Shanghai, China) and qPCR was performed as described previously [45]. The relative expression of each target gene was normalized using *PtrEF1β*. Gene specific primers used in this study were listed in additional file 8: Table S1. Three independent replicates of measurements were performed for each sample.

**Anatomical observations**

For histological observation of anthocyanin speckles, leaf dices of wild type and transgenic plants were examined with a light microscope after wound treatment for 48 h. Images were captured under the SMZ800 microscope.

**Anthocyanin content determination**

After wound induction for 48 h, anthocyanin content in the leaf discs of WT and transgenic plants grown in greenhouse was measured as described previously [46]. To detect the quantity of anthocyanin, A_{530} per gram fresh weight (FW) was used. Three replicates were carried out for each measurement, and the variability was indicated with the standard deviation (SD).

**Western blotting**

Western blotting analyses were performed as described previously [47]. Briefly, a total amount of 30 μg proteins were extracted from the wounded leaf discs at various time points and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then total protein was electrotransferred onto polyvinyllidene difluoride membranes. Immunoblotting was performed using JAZ1 antibodies against the *Arabidopsis* JAZ1 protein (Agrisera, http://www.agrisera.com/), then incubated with the secondary antibody goat anti-rabbit IgG-horseradish peroxidase (HRP) (Abmart, China). To detect the Actin, the primary antibody (mouse monoclonal Actin antibody) and the secondary antibody (goat anti-mouse IgG-HRP) were used to perform the immune reaction (Abmart, Shanghai, China). After incubation in the chemiluminescence detection solution Lumiglo (KPL, USA), membranes were imaged with a chemiluminescence image system Tanon 5500 (Tanon, Shanghai, China). Proteins were quantified with a BCA Protein Assay kit (Thermo, Shanghai, China).

**JA content assays**

For JA and JA-Ile content assays, the wounded leaves of wild type poplar plants at 0 h, 1 h and 24 h time points were ground into fine powder in liquid nitrogen, respectively. JA extraction was performed as described previously [48]. Three replicates were carried out for
each assay, and the variability was indicated with the standard deviation (SD).

Transient expression of TFs in poplar mesophyll protoplasts
To analyze the transcription function of PdMYB118, PdTT8 and PdMYB118/PdTT8 complex, CaMV 35S promoter driven transcriptional factor effectors were produced by inserting PdMYB118 or PdTT8 into pGreenII62-SK [49]. Leaf protoplasts were isolated from the leaves of Shanxin Yang as described previously [34]. The resultant constructs pGreenII62-SK-PdMYB118, pGreenII62-SK-PdTT8 and pGreenII62-SK-PdMYB118 + PdTT8 were transferred into protoplasts, respectively. The empty vector was used as a negative control. After kept in dark for 16 h, the transfected protoplasts were collected for RNA extraction and qRT-PCR analyses of ABGs as described above.

To detect the effects of PdTT8 or PtrJAZ1 on ABGs expression in the leaves of transgenic plants overexpressing PdMYB118, effectors pGreenII62-SK-PdTT8, pGreenII62-SK-PtrJAZ1 and pGreenII62-SK-PdMYB118 + pGreenII62-SK-PtrJAZ1 were transformed into the mesophyll protoplasts of transgenic plants, respectively. The transfected protoplasts were used for qRT-PCR analyses of ABGs. Three replicates were carried out for each assay, and the variability was indicated with the standard deviation (SD).

BiFC assays
Full-length coding sequences of PtrJAZ1, PdMYB118 and PdTT8 were individually cloned and subsequently recombined into YFP BiFC vectors so that they were fused with the N- or C-terminal of YFP (nYFP or cYFP) to generate pSAT4-nYFP-PdMYB118/PtrJAZ1 and pSAT4-cYFP-PdTT8/PdMYB118 plasmids. Primers used for gene clone are given in Supporting Information Table S1. To detect the interaction of PtrJAZ1 with PdTT8 or PdMYB118, and the interaction of PdMYB118 with PdTT8, the relative constructs were co-transfected into the mesophyll protoplasts of wild type plants. After incubated at 23 °C for 16 h, the protoplasts were analyzed using a confocal microscope at 514 nm wavelength (Zeiss LSM 510 META). Three replicates were carried out for each assay, and the variability was indicated with the standard deviation (SD).

Transient transcription dual-luciferase assays
For dual-luciferase assays, the LUC reporter constructs were generated by cloning the promoter of PtrCHS1, PtrDRF2 or PtrANS1 into pGreenII0800-LUC [47]. The resultant pGreenII62-SK-PdMYB118, pGreenII62-SK-PdTT8 or pGreenII62-SK-PtrJAZ1 was used as effector construct as described above. To detect the inhibition of PtrJAZ1 to the transcription activity of the PdMYB118/PdTT8 complex, effectors PdMYB118, PdTT8, PdMYB118 + PdTT8 or PdMYB118 + PtrJAZ1 were repetitively co-expressed with each reporter construct in poplar leaf protoplasts [45]. The LUC/REN ratio was used to represent the relative activity of the transcriptional factors. Three replicates were carried out for each assay, and the variability was indicated with the standard deviation (SD).

Statistical analysis
All data were obtained from three biological replicates each. For statistical analyses, Student’s t-test (two-tailed) or ANOVA (one-way) was used to generate every P value. The variability was indicated with the standard deviation (SD). *, ** and *** indicate p-values < 0.05, <0.01 and < 0.001, respectively.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12870-020-02389-1.

Additional file 1: Figure S1. Wound induced anthocyanin biosynthesis in the leaf of transgenic plants. Additional file 2: Figure S2. BiFC assays to test the interaction of PtrJAZ1 with PdMYB118. Additional file 3: Figure S3. Expression of PtrJAZ1 and PdTT8 in the protoplasts isolated from the leaves of transgenic plants overexpressing PdMYB118. Additional file 4: Figure S4. qRT-PCR analyses of PdMYB118 and PdTT8 in the wounded leaves of WT and transgenic plants overexpressing PdMYB118. Additional file 5: Figure S5. A proposed model of wound induced anthocyanin biosynthesis in poplar. Additional file 6: Figure S6. Western blotting analyses of JAZ1 protein in the wounded leaves. Additional file 7: Figure S7. Western blotting analyses of JAZ1 protein in the wounded leaves of transgenic poplar plants. Additional file 8: Table S1. Primer sequences used in this study.

Abbreviations
TF: Transcription factors; ABG: Anthocyanin biosynthesis gene; MeJA: Methyl jasmonate; JAZ: JASMONATE ZIM-domain; CHS: Chalcone synthase; CHI: Chalcone isomerase; F3’H: Flavanone 3’-hydroxylase; F3’5’H: Flavonoid 3’5’-hydroxylase; DFR: Dihydroflavonol 4-reductase; ANS: Anthocyanidin synthase; bHLH: Basic helix-loop-helix; WT: Wild type

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Authors’ contributions
HW, XW, CY, CW and YL performed the experiments and analyzed data. HW and HZ wrote the manuscript and arranged all data. All authors contributed revision of the manuscript. All authors read and approved the manuscript.

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