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Identification and expression pattern analysis of *YUCCA* and *ARF* gene families during somatic embryogenesis of *Lilium* spp.

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Abstract

Auxin is a key phytohormone in plant somatic embryogenesis (SE) and *YUCCA* and *AUXIN RESPONSE FACTORS* (*ARFs*) are two key genes involved in auxin biosynthesis and auxin signaling pathways, respectively. They have been reported in participating to the catalytic production of endogenous indole-3-acetic acid (IAA; a natural auxin) and regulating the transcription of auxin-responsive genes. To explore the structural characteristics of the *YUCCA* and *ARF* families of *Lilium* spp. L. and its expression pattern during SE induction and development processes, 6 *YUCCA* genes and 12 *ARF* genes were screened from the transcriptome database, and their nucleotides and encoded proteins were analyzed. At the same time, the expression patterns of *YUCCA* and *ARF* genes were analyzed by reverse transcription quantitative PCR, and endogenous IAA content was measured. Results show that the members of *Lilium indole-3-pyruvate monooxygenase YUCCA* (*LiYUCs*) are structurally conserved among *Lilium* spp., *Oryza sativa*, and *Arabidopsis thaliana*. The *LiARFs* are classified into six groups, most *LiARFs* have a closer affinity to the monocotyledon *Oryza sativa*. All the 12 *LiARFs* are involved in the SE induction and development, but their expression patterns differed. The *LiYUC2/4* and *LiARF5/7/21* had expression profiles corresponding with IAA content during the SE induction periods. The *LiYUC4/10* and *LiARF7/17/18/20/21/22* showed a similar downward trend with IAA content during the progress of the SE development. The results provide a basis for further research on the functions of *YUCCA* and *ARF* genes during somatic embryogenesis of *Lilium* spp.

Additional key words: indole-3-acetic acid biosynthesis and signaling pathways, *in vitro* culture, phylogenetic analysis.

Introduction

Auxin plays critical regulatory role in plant growth and development, especially in the plant morphogenesis including organogenesis and somatic embryogenesis (SE) (Su *et al.* 2009, Zhang *et al.* 2016, Wójcikowska and Gaj 2017). Exogenous auxins, including α -naphthalene acetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), picloram, *etc.*, has been utilized alone or in combination with other plant growth regulators to induce callus of *Lilium* spp. (Bakhshaei *et al.* 2016). But how exogenous auxin affects the embryogenic callus (EC) induction through changes in endogenous auxin has not been reported in *Lilium* spp.

Auxin activity can be achieved at different ways, including auxin biosynthesis and signaling pathways (Wang *et al.* 2017). There are many parallel synthesis

pathways of IAA in plants, whereas indole-3-pyruvic acid is currently considered as the main pathway (Mano and Nemoto 2012) and has been found in *Arabidopsis* and rice (Mashiguchi *et al.* 2011, Won *et al.* 2011, Abu-Zaitoon *et al.* 2012). *YUCCAs*, encoding flavin monooxygenase-like enzymes (FMOs), key enzymes in auxin biosynthesis, are highly conserved in plant (Liu *et al.* 2014). The homologous *YUCCA* genes have been cloned in various plants such as rice (Yamamoto *et al.* 2007, Yi *et al.* 2013), tomato (Kim *et al.* 2013), and petunia (Tobena-Santamaria *et al.* 2002). For example, in maize, the monocot-specific *YUCCA* gene *SPARSE INFLORESCENCE* (*SPIL*) is the key gene in the formation of axillary meristem and lateral organs (Gallavotti *et al.* 2008). The *ZmYUC1* is critical for maize endosperm development (Bernardi *et al.* 2012). *YUCCA* genes are required for SE induction and the

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Abbreviations: ABA - abscisic acid; ARFs - AUXIN RESPONSE FACTORS; CDS - coding sequence; CTD - C-terminal domain; 2,4-D - 2,4-dichlorophenoxyacetic acid; DBD - DNA binding domain; EC - embryogenic callus; FMO - flavin-containing monooxygenase; IAA - indole-3-acetic acid; NCBI - National Center for Biotechnology Information; SE - somatic embryogenesis; YUC - indole-3-pyruvate monooxygenase *YUCCA*.

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development in *Arabidopsis* (Bai *et al.* 2013). Bai *et al.* (2013) also reported that ethylene down-regulates auxin biosynthesis in *Arabidopsis* by inhibiting the expression of *YUCCA* genes. The quadruple mutations of *YUCs* in *yuc1yuc4yuc10yuc11* disturb local auxin distribution, which causes abnormalities in embryonic development such as the absence of hypocotyls or root meristem. Previous studies indicated that *YUCCA*-mediated auxin local synthesis is essential for *Arabidopsis* SE and participates in the formation and local distribution of auxin (Wójcikowska *et al.* 2013).

AUXIN RESPONSE FACTORS (ARFs), transcription factors that influences the expression of auxin-regulated genes, can specifically bind to auxin response elements in promoters of auxin-responsive genes to activate or inhibit the expression of downstream genes (Ulmasov *et al.* 1999, Hagen and Guilfoyle 2002, Shen *et al.* 2015). *ARFs* have been reported to have important functions in growth and development, including embryogenesis, vegetative organ development, flower or fruit development, vascular bundle formation, and so on (Carey and Krogan, 2017, Peng *et al.* 2017, Ren *et al.* 2017, Liu *et al.* 2018). Until now, the analysis of *ARF* gene family has been carried out in various plants such as *Arabidopsis* (Remington *et al.* 2004), rice (Wang *et al.* 2007), alfalfa (Shen *et al.* 2015), tomato (Kumar *et al.* 2011), and other plants. In addition, the function of *ARF* genes in plant regeneration also has been reported. Lee and Seo (2017) proved that high temperature promotion of callus formation requires the *BIN2-ARF7/19-LBD* in *Arabidopsis*. Roodbarkelari *et al.* (2015) reported that the ability of *AGO10* to maintain shoot meristem cells during *Arabidopsis* embryogenesis is connected with down-regulating *ARF2*-mediated auxin response. Lin *et al.* (2015) presumed that the expression of *ARF10*, *ARF16*, and *ARF17* negatively regulating auxin signals during the SE processes of *Dimocarpus longan* indicate the importance of *ARF* genes in plant regeneration.

Lilium spp., monocotyledonous bulbous plants belonging to *Liliaceae* family, have large flowers, bright colors, and strong adaptability, and thus they are utilized as important fresh cut flowers and potted flowers. More and more scientists have carried out research on their physiology and molecular biology (Bakhshaie *et al.* 2016). China is one of the world's major genetic resources and distribution countries of *Lilium* spp. From the 115 species of *Lilium*, 55 species and 18 cultivars are originated in China. Thus it has great theoretical and also economic significance to carry out research on somatic embryogenesis (SE) of *Lilium* spp.

The role of *ARFs* and *YUCCAs* in SE of *Lilium* spp. are still unclear. Therefore, the aim of this work was to analyze *ARF* and *YUCCA* families in *Lilium* by using bioinformatic methods. Based on the information from *Lilium* spp. transcriptome database, 12 *ARF* genes and 6 *YUCCA* genes containing intact coding sequence (CDS) regions we detected in the *Lilium* cultivar ConcaD'Or and their characteristic sequences annotated. Reverse transcription quantitative PCR method was conducted to analyze their expression patterns during the SE induction and developmental stages.

Materials and methods

Plants and cultivation: Seedling roots of the *Lilium* spp. L. Oriental × Trumpet hybrid called ConcaD'Or were used as explants. Roots approximately 1.0 cm in length, including the root tips, were inoculated on a Murashige and Skoog medium supplemented with 1.0 mg·dm⁻³ picloram and cultured in the dark and a temperature of 25 ± 2 °C to induce embryogenic calli (Zhou *et al.* 2013). Root explants were sampled after 0 d (control), 20 d (embryogenic callus induction1, ECI1), 40 d (ECI2), and 55 d (ECI3) on culture medium to represent different stages of ECI (Chen *et al.* 2019).

Then, embryogenic callus was inoculated on a Murashige and Skoog medium supplemented with 0.5 mg dm⁻³ abscisic acid (ABA) to observe its growth and development. Callus, globular embryo, shield-shaped embryo, cotyledonary shaped embryo, and mature embryos were sampled at 0 d (callus), 10 d (A1), 20 d (A2), 30 d (A3), and 40 d (A4), respectively (Fig. 1). Each development stage explants were sampled 3 times, each sample contained more than 10 explants. Materials were immediately frozen in liquid nitrogen and stored at -80 °C until total RNA was extracted.

Determination of IAA content: Root explants induced at 0, 20, 40, and 55 d, and embryos cultured at 0, 10, 20, 30, and 40 d were sampled and frozen in liquid nitrogen to detect the content of IAA. The sample preparation was conducted according to Pan *et al.* (2010). The IAA measurement was carried out high performance liquid chromatography mass spectrometry method as previously described (Liu *et al.* 2013). The biological samples were repeated more than three times.

Gene identification of the *ARF* and *YUCCA* family: The CDS sequences of the *LiARFs* and *LiYUCs* were derived from the transcriptome data of *Lilium* (SRP098955), *Arabidopsis thaliana*, and *Oryza sativa* sequence references were derived from the NCBI database. The conserved domain of the *LiARFs* and *LiYUCs* were analyzed using *SMART* (<http://smart.embl-heidelberg.de/>) and NCBI database. The molecular mass of the *ARF* and *YUCCA* proteins, the isoelectric point of the proteins as well as their affinity/hydrophobicity were predicted using the *ExPASy* (<http://web.expasy.org/protparam/>) tool. The *NetPhos 3.0* serve (<http://www.cbs.dtu.dk/services/NetPhos/>) was used to predict the protein phosphorylation sites. To exhibit the structural divergence of *LiARF* and *LiYUC* proteins, the conserved motifs were performed with multiple expectation maximization for motif elicitation (*MEME* v. 4.11.2) online program (Bailey *et al.* 2015).

Evolutionary tree construction: Homology comparisons of the multi-sequence amino acid were performed using the *ClustalW* program with the default parameters, and phylogenetic trees were constructed by using the *MEGA-X* software with the neighbor-joining method.

Reverse transcription quantitative PCR: Total RNA

was extracted from the samples using a *RNeasy Plant Mini* kit (*Omega*, Norcross, GA, USA) according to the instructions. First-strand cDNA synthesis was performed using the *Primer-Script™* RT reagent kit (*TaKaRa*, Dalian, China).

SYBR® Premix Ex TaqII (*TaKaRa*) was used to conduct the reverse transcription quantitative PCR reactions. The PCR reaction system consisted of 2 mm³ of cDNA (1:10), 10 mm³ of 2 × *SYBR Premix Dimer Eraser*, and 1 mm³ of each primer (Table 1 Suppl.), synthesized by *Shanghai Shenggong Bioengineering Technology Service Co.*. Results were normalized using the internal reference genes *EIF* (*EUKARYOTIC INITIATION FACTOR 1α*; acc. No. KP861874) and *EF1* (*ELONGATION FACTOR 1-α*; acc. No. KJ543461). PCR reactions were performed in a light cycler 480II (*Roche*, Penzberg, Germany) under the following conditions: an initial incubation at 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s, 60 °C for 34 s, and cooling at 40° C for 30 s. The gene-expressions were calculated over three biological replicates by *Genorm* software.

Statistical analysis was conducted by *Excel 2010* and *SPSS 17.0* software. In order to assess the statistical significance of the differences among sampling stages, a one-way analysis of variance followed by the Duncan's multiple range test ($P \leq 0.05$ and 0.01) was employed. Fold change were compared to the control period during the induction stages or callus period during the development stages.

Results

A total of 12 *LiARF* genes were identified by *BLASTP* analysis, each containing a plant-specific PRONE domain.

For example, the DNA binding domain (DBD) motif was found in *LiARF5/7/9/11/12/15/17/19/20/21*, the C-terminal domain (CTD) motif was found in *LiARF5/7/9/11/12*, the ARF motif was found in *LiARF5/7/9/11/12/15/17/19/21* (Fig. 1 Suppl.). The amino acid length ranges from 250 to 1 009, the molecular mass ranges from 27 142.88 to 112 309.86 and the isoelectric point ranges from 4.95 to 9.74. The specific characteristics are shown in Table 2 Suppl.

A total of 6 *LiYUC* genes were identified by *BLASTP* analysis, each containing flavin adenine dinucleotide-binding motif, GC motif, ATG-containing motif1, ATG-containing motif2, FMO-identifying sequence, and NADPH-binding motif (Fig. 2 Suppl.). The amino acid length ranges from 251 to 429, the molecular mass ranges from 27 854.23 to 48 347.92 and the isoelectric point ranges from 5.93 to 8.97. The specific characteristics are shown in Table 2 Suppl. The gene structure analysis of *LiARFs* and *LiYUCs* according to the phylogenetic relationship are shown in Fig. 3 Suppl.

To investigate the evolutionary relationship between *LiARFs* and those in other species such as *Arabidopsis thaliana* and *Oryza sativa*, a neighbor-joining tree was generated based on alignments of the amino acid sequences of 12 *LiARFs*, 22 *AtARFs* and 23 *OsARFs*. The results showed that all the *ARFs* could be grouped into six major clusters (I, II, III, IV, V, and VI) based on their phylogenetic relationships (Fig. 2A). Twelve *LiARFs* were distributed among the six clusters. For example, *LiARF16/22* were classified into *OsARF16*, *LiARF17/19/20/21* were classified into *OsARF8/10/13/18/20* and *AtARF5/10/16/17*, *LiARF12* was classified into *OsARF6/12/17/25*, *LiARF5* was classified into *OsARF5/11/19* and *AtARF6/7/8/19*, *LiARF15* was classified into *OsARF2/3/14/15* and *AtARF3/4*, *LiARF7/9/11* was classified into *OsARF1/7/4/24/9/23* and

Fig. 1. Different stages of somatic embryogenesis of *Lilium* spp. cv. Concea D'Or callus: embryogenic callus (A1); callus transferred to a Murashige and Skoog medium supplemented with 0.5 mg·dm⁻³ abscisic and grown for 10 d (A2), 20 d (A3), and 40 d (A4). Bars are 0.2 cm.

Fig. 2. Phylogenetic analysis of AUXIN RESPONSE FACTOR (ARF) and YUCCA proteins in *Lilium* spp., *Arabidopsis thaliana*, and *Oryza sativa* constructed by using the MEGA-X software with the neighbor-joining method.

Fig. 3. Indole-3-acetic acid (IAA) content during somatic embryo (SE) induction (A) and SE development (B) stages of *Lilium* spp. Root explants were sampled after 0 d (control), 20 d (embryogenic callus induction1, EC11), 40 d (EC12), and 55 d (EC13) on a culture medium. Then, the embryogenic callus was inoculated on a Murashige and Skoog medium supplemented with 0.5 mg dm⁻³ abscisic acid and calli, globular embryos, shield-shaped embryos, cotyledonary shaped embryos, and mature embryos were sampled at 0 d (callus), 10 d (A1), 20 d (A2), 30 d (A3), and 40 d (A4), respectively. Means $\pm$ SEs, $n = 3$, ** - significant differences between treatments at $P \leq 0.01$.

AtARF1/9/11/18/13/15/20/22/12/14/21/23.

To investigate the *LiYUCs* evolutionary relationship between *Lilium* spp. and *Arabidopsis thaliana* and *Oryza sativa*, a neighbor-joining tree was generated based on alignments of the amino acid sequences of 6 *LiYUCs*, 11 *AtYUCs*, and 10 *OsYUCs*. Results showed that all the *LiYUCs* could be grouped into four major clusters (I, II, III, and IV) based on their phylogenetic relationships (Fig. 2B). Six *LiYUCs* were distributed among the four clusters. For example, cluster I included *LiYUC4*, *AtYUC1*, *AtYUC4*, *OsaYUC1 4-a*, *OsaYUC1 4-b*, *OsaYUC1 4-c*; cluster II included *LiYUC10*, *AtYUC10*, *AtYUC11*, *OsaYUC10 11-a*, *OsaYUC10 11-b*, *OsaYUC10 11-c*; cluster III included *LiYUC2*, *LiYUC6*, *AtYUC2*, *AtYUC6*, *OsaYUC2 6-a*, *OsaYUC2 6-b*; cluster IV included *LiYUC5*, *LiYUC9*, *AtYUC3*, *AtYUC5*, *AtYUC7*, *AtYUC8*, *AtYUC9*, *OsaYUC3 5 7 8 9-a*, *OsaYUC3 5 7 8 9-b*.

Root explants induced at 0, 20, 40, and 55 d, and somatic embryos cultured at 0, 10, 20, 30, and 40 d were sampled to detect their IAA content. Results showed that the content of IAA increased during the embryogenic callus induction (Fig. 3A). During the somatic embryo development, ABA was added to the medium, and the content of IAA decreased during A1 period, increased during A2 period, and decreased during A3 and A4 periods (Fig. 3B).

The expression patterns of *LiARFs* were significantly different at different ECI stages (Fig. 4A). *LiARF5*, *LiARF7*, and *LiARF21* displayed a distinct up-regulation during the embryogenic callus induction stages. *LiARF17/20/22* showed an increased expression at the EC12 stage and decreased expression at EC13 stage. *LiARF11* showed a decreased expression at the EC11 stage and then its

expression increased at EC12 and EC13 stages. *LiARF12/18* showed a decreased expression at the EC11 stage and then its expression increased to a maximum at EC12 stage.

The expression patterns of *LiYUCs* also changed at different ECI stages. *LiYUC2* and *LiYUC4* showed a significantly increased expressions at the EC13 stage, while *LiYUC5*, *LiYUC6*, *LiYUC9*, and *LiYUC10* showed high expressions at 0 d (control) and the expressions decreased significantly at EC11, EC12, and EC13 stages (Fig. 5A). The content of IAA increased with the induction time of embryogenic callus which is consistent with the expression pattern of *LiYUC2* and *LiYUC4*.

The development of SE underwent different ontogenetic stages, i.e., globular, shield-shaped, and cotyledonary shaped, on a medium supplemented with 0.5 mg dm⁻³ ABA (Fig. 1). The expressions of *LiARF7/16/20* showed a trend of increasing first and then decreasing. The expressions of *LiARF11/12/15/17/18/21/22* displayed up-regulation at first, then their expressions firstly decreased and then increased at A4 period. The expression of *LiARF9* changed greatly and was the highest at A4 period, the expression of *LiARF5* decreased first and then increased. Except *LiARF5/9/11* which peaked at A4 period, the remaining *LiARFs* peaked at A2 period, indicating that these two periods were the turning points of the transcription levels after being treated with ABA.

The expression analysis of *LiYUCs* in the development stage of SE was detected. Results showed that the expressions of *LiYUC4* and *LiYUC10* increased significantly at globular embryo stage, then decreased at the shield-shaped embryo and cotyledonary embryo stages (Fig. 5B). *LiYUC2*, *LiYUC5*, *LiYUC6*, and *LiYUC9* showed an increased expressions with the development of somatic

Fig. 4. The expression pattern of *Lilium* spp. *AUXIN RESPONSE FACTORS* (*LiARFs*) during somatic embryo (SE) induction (A) and SE development (B) stages conducted by reverse transcription quantitative PCR method. Root explants were sampled after 0 d (control), 20 d (embryogenic callus induction1, EC11), 40 d (EC12), and 55 d (EC13) on a culture medium. Then, the embryogenic callus was inoculated on a Murashige and Skoog medium supplemented with 0.5 mg dm⁻³ abscisic acid and calli, globular embryos, shield-shaped embryos, cotyledonary shaped embryos, and mature embryos were sampled at 0 d (callus), 10 d (A1), 20 d (A2), 30 d (A3), and 40 d (A4), respectively. Changes were compared to the control period during the induction stages or to the callus period during the development stages. Means $\pm$ SEs, $n = 3$, *, ** - significant differences between treatments at $P \leq 0.05$ and 0.01, respectively.

embryos. The content of endogenous IAA decreased with the development of SEs which was consistent with the expression trend of *LiYUC4* and *LiYUC10*, indicating that they may play a positive role in the development of somatic embryos.

Discussion

The importance of auxin in determining cell fates in regenerating callus, tissues, or organs by activating specific developmental programs has been shown (Ikeuchi

Fig. 5. The expression pattern of *Lilium* spp. *indole-3-pyruvate monooxygenase YUCCA* during the somatic embryo (SE) induction (A) and SE development (B) stages conducted by reverse transcription quantitative PCR method. Root explants were sampled after 0 d (control), 20 d (embryogenic callus induction1, ECI1), 40 d (ECI2), and 55 d (ECI3) on culture medium. Then, the embryogenic callus was inoculated on a Murashige and Skoog medium supplemented with 0.5 mg dm⁻³ abscisic and calli, globular embryos, shield-shaped embryos, cotyledonary shaped embryos, and mature embryos were sampled at 0 d (callus), 10 d (A1), 20 d (A2), 30 d (A3), and 40 d (A4), respectively. Changes were compared to the control period during the induction stages or to the callus period during the development stages. Means $\pm$ SEs, $n = 3$, *, ** - significant differences between treatments at $P \leq 0.05$ and 0.01, respectively.

et al. 2019). The present study revealed the main structural features of the *YUCCA* and *ARF* gene families based on the transcriptome database of SE induction in *Lilium* spp. (Chen *et al.* 2019). The full length of CDS for 6 members in *YUCCA* family and 12 members in *ARF* family were successfully isolated. The *LiYUCs* and *LiARFs* were named based on their phylogenetic relationships with *Arabidopsis* homologs.

The *YUCCA* genes were highly conserved among *Lilium* spp., *Oryza sativa*, and *Arabidopsis thaliana* according to the analysis of results. *YUCCA* proteins can be grouped into 4 groups: *YUC1-4*, *YUC2-6*, *YUC3-5-7-8-9*, and *YUC10-11* (Poulet and Kriechbaumer 2017). In the

present study, *LiYUC4* was classified into the *AtYUC1-4/OsaYUC1-4* group and *LiYUC10* was classified into the *AtYUC10-11/OsaYUC10-11* group, which were referred to their function in embryogenesis (Stone *et al.* 2008, Bai *et al.* 2013). *LiYUC2* and *LiYUC6* were classified into the *AtYUC2-6/OsaYUC2-6* group, which were the main *YUCs* expressed in shoots, *LiYUC5* and *LiYUC9*, the main *YUCs* expressed in roots (Chen *et al.* 2014), were classified into the *AtYUC3-5-7-8-9/OsaYUC 3-5-7-8-9* group. In addition, the protein domain analysis of *LiYUCs* showed that they also contain the conserved motifs (flavin adenine dinucleotide binding motif, GC motif, ATG-containing motif, FMO-identifying sequence, and NADPH-binding

motif).

The function of *YUCCA* genes in somatic embryogenesis has been reported. Many plant embryonic regulators such as *LEAFY COTYLEDON 1 (LEC1)* and *LEC2* appear to promote somatic embryogenesis by modulating auxin biosynthesis and signaling (Ikeuchi *et al.* 2019). For example, *LEC1* can induce auxin biosynthesis via induction of *YUC10*, *LEC2* can activate *YUC2* and *YUC4* in *Arabidopsis* (Stone *et al.* 2008). In addition, Bai *et al.* (2013) reported that *yuc1yuc4yuc10yuc11* quadruple mutations showed abnormalities in embryonic development including absence of hypocotyls or root meristem. In our research, the expressions of *LiYUC2*, *LiYUC4*, and *LiYUC10* were also significantly changed during SE induction and development, which was consistent with the above conclusions. In addition, expressions of *LiYUC6*, *LiYUC5*, and *LiYUC9* were also changed which may play a role in the SE induction and development.

Different from the *YUCCA* genes, ARF protein has been reported to have three characteristic regions, including DBD, ARF, and CTD domains (Guilfoyle and Hagen 2007). The present study of LiARF protein showed that most LiARFs have the characteristic region, except LiARF16 and LiARF22 which do not have the DBD motif, ARF motif, and CTD motif. Phylogenetic analysis indicated that the most LiARFs were classified into OsARF group, indicating the closer relationship with OsARFs than AtARFs.

In general, ARFs are classified as activators or repressors during the gene transcription. *AtARF5/6/7/8/19* are reported as activators and *AtARF1/2/3/9* as repressors of the target genes transcription (Tiwari *et al.* 2001). In addition, *ARF7/19* also play key roles in callus induction in *Arabidopsis* root explants (Fan *et al.* 2012). In our research, the expressions of *LiARF5/7/21* were up-regulated during the induction stage and *LiARF7/11/12/15/16/17/19/20/21/22* showed a trend of increasing during the first SE development stage, indicating that the *LiARFs* play significant roles. The *ARF* functions in somatic embryogenesis has been reported in many plants such as *Arabidopsis*, *Dimocarpus longan*, and *Gossypium hirsutum* (Lin *et al.* 2015, Wójcikowska and Gaj 2017, Sun *et al.* 2019). However, the *ARF* functions in somatic embryogenesis of *Lilium* spp. still need more research to be conducted.

The induction and regeneration of plant SEs is a complex process. In this study, the embryogenic callus induction on root explants requires the addition of picloram, a herbicide with auxin-like properties similar to 2,4-D (Kefford and Caso 1966). The presence of picloram can induce the biosynthesis of IAA in our research, which is consistent with other reports (Vondráková *et al.* 2011, Pescador *et al.* 2012, Ayil-Gutiérrez *et al.* 2013, Song *et al.* 2020). The relationship between IAA biosynthesis and development of carrot callus has been reported (Michalczyk *et al.* 1992). In carrot, most of the initial cell clusters developed into somatic embryos after initial 2 weeks of culturing in medium without 2,4-D, and at least a 10-fold decreasing IAA content was detected (Michalczyk *et al.* 1992). Similar results were found in *Arabidopsis* where

correct *WUSCHEL* expression was induced by removal of exogenous auxin (Su *et al.* 2009). In *Lilium* spp., the removal of auxin from the medium and addition of ABA can promote the development of embryogenic callus into somatic embryos and IAA content decreased during this processes (Fig. 3B).

Absciscic acid is one of the commonly used hormones for inducing embryogenic callus. For example, exogenous ABA can promote the formation of embryogenic callus and SE maturation in *Glycine max*, *Cocos nucifera*, and *Vitis vinifera* (Fernando *et al.* 2000, Tian *et al.* 2000, Prado *et al.* 2014). Especially in some woody plants, ABA can assist the proembryo formation (Zavattieri *et al.* 2010, Jain *et al.* 2013, Prado *et al.* 2014, Zhang *et al.* 2014, Zhou *et al.* 2017). In our study, the addition of ABA also promote the SE development of *Lilium* spp., which provide reference on SE development in bulbous plants.

Taken together, we identified 6 *YUCCA* genes and 12 *ARF* genes in *Lilium* spp. In addition, the IAA content and the expressions of *LiYUCs* and *LiARFs* were detected to search the key genes during SE induction and development. According to the results, *LiYUC2/4* and *LiARF5/7/21* had the similar expression profile with IAA content pattern during the SE induction periods. *LiYUC4/10* and *LiARF7/17/18/20/21/22* expressions showed similar downward trend as the IAA content during the SE development stages. These genes seems to be engaged in the *Lilium* spp. somatic embryogenesis. Results of this study provided important information to reveal the functions of IAA in somatic embryogenesis of *Lilium* spp.

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