GENE EXPRESSION OF *DLNPGR1* IN DIFFERENT TISSUES, DURING SE AND SEED GERMINATION IN *DIMOCARPUS LONGAN* LOUR.

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Abstract

Two important structural units, tetratricopeptide repeat (TPR) motifs and calmodulin-binding domain, are known for their involvement in protein-protein interaction and binding to calmodulin (CaM), respectively. Although many TPR-containing proteins, CaM-binding proteins (CBPs) and their respective encoding genes have been studied, further detail studies are still needed for more understanding of those important proteins and their respective genes. In this study, expression of a gene encoding CBP with TPRs, *NPGR1*, was analysed in different tissues, during SE and germination in *Dimocarpus longan* Lour. By using RT- PCR analysis, the expression levels of *DlNPGR1* in 12 different tissues, in different developmental stages during longan somatic embryogenesis (SE), and during seed germination in different days after sowing. According to our results, among 12 different tissues, the relatively highest expression level was detected in young fruit, followed by floral bud and female flower while mature root, pollen and mature leave exhibited the lowest expression. During SE, the highest expression of *DlNPGR1* was detected in NEC followed by CE while the lowest expression level was detected in EC stage. During seed germination, the highest expression of was detected in day 0 then the expression was significantly lower in day 4 followed by day 8 where the expression level was the lowest. In conclusion, being a gene encoding CBP with TPRs, *DlNPGR1* might be involved in cell division.

Keywords: *No pollen germination related 1*, gene expression, *Dimocarpus longan*, somatic embryogenesis, TPR motifs, CBD domain

Introduction

A sub-tropical and tropical tree *Dimocarpus longan* Lour. is economically and medicinally important. Somatic embryogenesis (SE) of longan was used as a model system for better understanding of the early development of the woody trees (Lai & Chen, 1997; Lai et al., 1997; Lai et al., 2000; Lai et al., 2010; Lai & Lin, 2013).

Embryogenesis is a critical process in the development of higher plants. Somatic embryogenesis (SE) is an in vitro developmental process of plant. SE is a module system that can be used to study of plant embryogenesis (Zimmerman, 1993). It can be used as an alternative experimental system for zygotic embryo development since SE showed close similarities to the development stages to zygotic embryogenesis (Dodeman et al., 1997; Willemsen & Scheres, 2004). Although many studies have been conducted to understand molecular basis during SE, molecular mechanism at early SE is still largely unknown (Elhiti et al., 2013).

There are various calmodulin-binding proteins (CBPs) which are involved in numerous cellular activities such as in metabolism regulation, transport of ions, etc. (Snedden & Fromm, 2001) and pollen germination (Safadi et al., 2000; Golovkin & Reddy, 2003; Zhang et al., 2012; Shin et al., 2014). Tetratricopeptide repeat (TPR) motif has been reported that it is involved in protein-protein interactions including cell cycle control, transcription repression, stress response, protein kinase inhibition, mitochondrial and peroxisomal protein transport and neurogenesis (Lamb et al., 1955).

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Many TPR protein encoding genes (D'Andrea, & Regan, 2003; Rosado et al., 2006; Zeytuni & Zarivach, 2012; Cerveny et al., 2013) and CBP encoding genes (Safadi et al., 2000; Golovkin & Reddy, 2003; Zhang et al., 2012; Shin et al., 2014) have been studied until now. However, further detail studies are still needed for better understanding of the involvement of those important genes. In this work, expression of a gene encoding CBP with TPRs, *DlNPGR1*, was analyzed by using RT-PCR techniques in different tissues, during SE and germination in *D. longan* Lour. cultivar 'Honghezi'. This study was conducted in Fujian Agriculture and Forestry University, China from January, 2015 to January, 2017. This work was conducted to determine the expression profile of *DlNPGR1* in some tissues of longan.

Materials and Methods

Embryogenic Cultures, Different Tissues and Seedlings

By following the protocols reported of Lai and Chen (1997), and Lai et al. (2000), different developmental stages of *Dimocarpus longan* embryogenic cultures i.e., embryogenic callus (EC), incomplete compact proembryogenic cultures (ICpEC), globular embryos (GE), cotyledon embryos (CE) and non-embryogenic callus (NEC) were obtained. Then those tissues were stored at -80 °C or subcultured to use in further study.

A total of 12 different tissues or organs of longan (mature root, pollen, mature leaf, pulp, young stem, vegetative bud, ripe seed, inflorescence, ripe fruit, female flower, floral bud and young fruit) were used to detect the expression pattern of *DlNPGR1* using qPCR analysis. Germinated seeds of longan in different days after sowing (das) (0d, 4d, 8d, 12d, 16d, 20d) were also used to detect the expression pattern of *DlNPGR1* during germination.

Sequence Alignment of *DlNPGR1* and *DlNPG1*

The opening reading frame (ORF) sequence of *DlNPGR1* used in this study was retrieved from NCBI (GenBank accession no. KP402183.1) published by Thu, et al. (2020a). In order to determine the sequence identity of *DlNPGR1* with downloaded previously published *DlNPG1* (GenBank: KP402181.1) (Thu et al., 2020b) we used DNAMAN (v.6.0, Lynnon Corporation, Quebec).

Quantitative Real-time PCR (qRT-PCR) Analysis

In order to determine the expression levels of the *DlNPGR1* gene during development of longan SE and in 12 different tissues, qRT-PCR analysis was performed on the LightCycler 480 (Roche Applied Science, Switzerland) after following the previous methods (Lin & Lai, 2010, 2013). The gene specific primers (GSPs) for qRT-PCR analysis were designed based on previously cloned, analyzed and published sequence of *DlNPGR1* cDNA on NCBI (GenBank: KP402183.1). The GSPs were *DlNPGR1*-qF (5′-TGTCTGCCAGTGTATTGTCA-3′) and *DlNPGR1*-qR (5′-TGCTCAAGTTCTTCCAGTGA-3′). For normalization of the expression of *DlNPGR1*, *DlFSD1a, DlEF-1a* and *DleIF-4a* were used as reference genes.

We examined and verified annealing specificity of primers by melting curve analysis. Then PCR efficiency was determined by four-point standard curve of a fivefold dilution series (1:5, 1:25, 1:375 and 1:625) from pooled cDNA. Each reaction well contained a total reaction volume of 20 µL (7.4 µL of ddH$_2$O, 10 µL of 2 x SYBR Premix ExTaq II (Takara, Japan), 0.8 µL of each primer (100 nM) and 1 µL of cDNA template (1:5 dilution)). The PCR conditions were as follow – preincubation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, 60 °C for 30 s, and 72 °C for 10 s. The relative gene expression of *DlNPGR1* was evaluated using the method described by Lin & Lai (2010, 2013).

Analysis of Data

Data were analyzed using SPSS v.19 (SPSS Inc., Chicago, USA). Means were separated by Tukey HSD test at $p < 0.05$.

## Results

Sequence Identity

We determined the sequence identity between ORF sequence of *DlNPGR1* and that of *DlNPG1* (Min Kyaw Thu et al., 2020b) by using DNAMAN (v.6.0). The result showed that there was 53.26% identity between the two sequences (Figure 1).

Figure 1 Multiple alignment of *DlNPGR1* with *DlNPG1* using DNAMAN.

Expression of *DlNPGR1* in Different Tissues

      qRT-PCR analysis was used to determine the expression pattern of *DlNPGR1* gene in 12 different tissues of longan. Our results showed that the relatively highest expression level was detected in young fruit, followed by floral bud, female flower and so on (Figure 2). Mature root, pollen and mature leave exhibited the lowest expression.
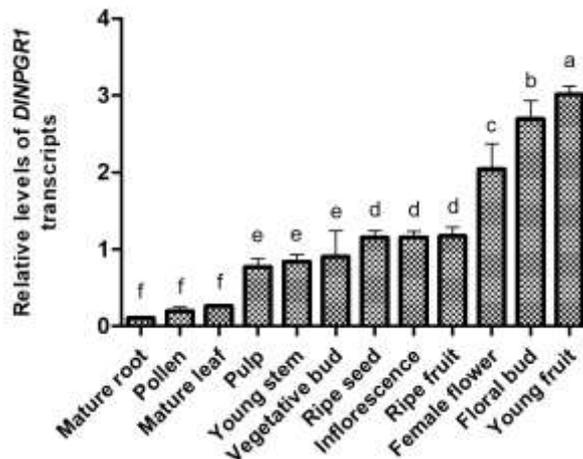
Figure 2 Expression levels of *DlNPGR1* in 12 different tissues of longan. Data are shown as means ± SD (n = 3). Different letters above each bar represent significant differences at $p < 0.05$ by Tukey HSD test.

Expression of *DlNPGR1* during Longan SE

      In order to know the transcriptional regulation of *DlNPGR1* in prominent stages during longan SE, we analysed the expression levels of the gene. The highest expression of *DlNPGR1* was detected in NEC followed by CE (Figure 3). Our results showed that the lowest expression level was detected in EC stage. Then the expression level was increased in ICpEC and GE.
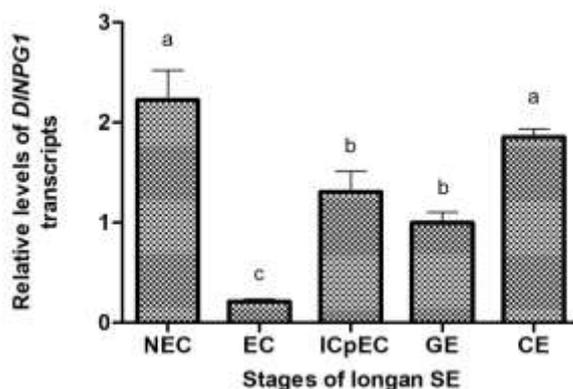
Figure 3 Expression pattern of *DlNPGR1* during longan somatic embryogenesis (SE). The tested stages were non-embryogenic callus (NEC), embryogenic callus (EC), incomplete compact proembryogenic cultures (ICpEC), globular embryo (GE), and cotyledon embryo (CE). Data are shown as means ± SD (n = 3). Different letters above each bar represent significant differences at $p < 0.05$ by Tukey HSD test.

Expression of *DlNPGR1* during Seed Germination

      We determined the expression pattern of the *DlNPGR1* during seed germination from day 0 to day 20 after sowing. The highest expression of *DlNPGR1* was detected in day 0 (Figure 4).

Then expression level was lower in day 4 followed by day 8 where the expression level was the lowest. After that, the expression level was significantly higher and higher until day 20 at which we stopped the study.
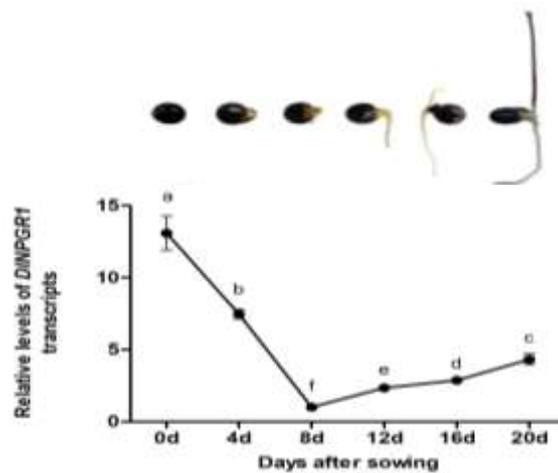
Figure 4 Expression levels of *DlNPGR1* during longan seed germination. Data are shown as means ± SD (n = 3). Different letters above each bar represent significant differences at $p < 0.05$ by Tukey HSD test.

Discussion

Expression Pattern of *DlNPGR1* in Different Tissues

The expression pattern of *DlNPGR1* might differ from that of *DlNPG1* since the similarity between them were low (53.26%). Although the high expression of MPCBP (Safadi et al., 2000), *AtNPG1* (Golovkin & Reddy, 2003) and *OsPCBP* (Zhang et al., 2006) was found in pollen, *DlNPGR1* was not highly expressed in pollen. Instead, the highest expression of this gene was found in young fruit. It can be clearly seen that *DlNPGR1* was actively expressed in developing young tissues such as in young fruit and floral bud.

It has been already reported that *DlNPGR1* gene contains eight TPRs and one CBD (Thu et al., 2020a). TPR-containing proteins are involved in many cellular processes such as cell cycle control, transcription repression, stress response, protein kinase inhibition, mitochondrial, peroxisomal protein transport etc. (Goebl & Yanagida, 1991). As a protein possessing eight TPRs and a CBD, DlNPGR1 might be involved in several cellular activities. *DlNPGR1* might be involved in developmental processes such as tip extension, cell proliferation and cell elongation in young and growing tissues.

Expression of *DlNPGR1* during Longan SE

The expression pattern of *DlNPGR1* suggests that it might be actively involved in NEC and CE followed by ICpEC and GE stages. As cytosolic free calcium is elevated during cell division (Trewavas et al., 1998), CaM and CaM-binding protein NPGR1 might be active at CE stage during longan SE. Moreover, being a TPR-containing protein, it might be involved in cell division and polarized growth.

Expression Pattern of *DlNPGR1* during Seed Germination

We can see that the highest expression of *DlNPGR1* was detected in das 0, then the expression level went down steeply until das 8. Then the expression level rose again after das 8 significantly but gradually until das 20. According to our results, there might be relatively higher

accumulation of the transcripts of *DlNPGR1* in mature seed in order to be used in subsequent germination. After using most of the *DlNPGR1* transcripts for starting germination, it might be necessary for the growth of young root and young shoot. That might be the reason we could detect higher expression after das 8. According to Lamb et al., (1955) and our finding suggests that as a TPR-containing CaM-binding protein DlNPGR1 might be involved in cell division.

Conclusion

In conclusion, *DlNPGR1*, TPR-containing CBP-encoding gene, might be involved in cell division and polarized growth during somatic embryogenesis and seed germination, and during the growth and development of young fruit and floral bud.

Acknowledgments

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