Differential Expression of Phenylalanine Ammonia-Lyase in Different Tissues of Sugarcane (*Saccharum officinarum* L.) during Development

Maryam Kolahi,[a,*] Parisa Jonoubi,[a] Ahmad Majd,[a] Mohamad Reza Tabandeh,[b] and Mahmoud Hashemitabar [c]

The phenylpropanoid pathway serves as a rich source of metabolites in plants. It is required for the biosynthesis of lignin and acts as a starting point for the production of many other important compounds involved in growth and development. Phenylalanine ammonia-lyase (PAL) catalyzes the first step of the phenylpropanoid pathway. PAL gene expression changes during the growth and development of plants as it regulates the synthesis of lignin and other phenylpropanoid compounds. The gene expression of sugarcane (*Saccharum officinarum* L.) PAL (SoPAL) was analyzed using quantitative real-time PCR (qPCR) and the comparative ΔΔCt method in different tissues during different developmental stages. The results showed that SoPAL was expressed in all tissues and developmental stages. SoPAL mRNA levels were increased from germination to tillering stages, except in the sheath, and from tillering to grand growth stages in the leaf and stem. PAL expression decreased from the grand growth to maturation stages in all tissues except the sheath. The highest expression of SoPAL occurred in the stem during the grand growth stage, while its lowest expression occurred in the leaf during germination ($p<0.05$). In conclusion, PAL, as a rate-limiting enzyme of the phenylpropanoid pathway, displays critical roles in the development of sugarcane, particularly in lignified tissues.

Keywords: Development; Lignified; Phenylalanine ammonia-lyase; Saccharum officinarum L.*; Tissue expression*

Contact information: a: Department of Plant Biology, Faculty of Biology Science, Kharazmi University, Tehran, Iran; b: Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; c: Cellular and Molecular Research Center, Faculty of Medicine, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran;
** Corresponding author: mkolahi@scu.ac.ir*

INTRODUCTION

Enzymes catalyze many biochemical reactions that regulate plant growth and development through biosynthesis of required metabolites. Enzymes also display key roles in the modification of natural plant products that help plant resistance to different pathogens (Ferrer *et al*. 2008; Jayalakshmi *et al*. 2011).

The phenylpropanoid pathway serves as a rich source of metabolites in plants, is required for the biosynthesis of lignin, and serves as a starting point for the production of many other important compounds involved in growth and development (Hyun *et al.* 2011).

Phenylalanine ammonia-lyase (EC 4.3.1.5) catalyzes the deamination of L-phenylalanine to yield *trans*-cinnamic acid and NH_4, which is the first step of the

phenylpropanoid pathway (Huang *et al.* 2010). Secondary metabolites produced from the phenylpropanoid pathway include the cell wall structural polymer, lignin, flavonoids, UV protectants, wound protectants, isoflavonoid and furano coumarin phytoalexin antibiotics (Ozeki *et al.* 2000; Ludwikow and Sadowski 2008; Hyun *et al.* 2011).

Derivatives from the phenylpropanoid pathway are ubiquitous in plants and affect plant development by regulation of structural integrity and cell signaling (An *et al*. 2011). Furthermore, phenylpropanoids act as chemical modulators in plant communication with other organisms (Ferrer *et al.* 2008).

The levels of PAL activity or its gene expression may be related to the developmental stages of the plant. Activity and expression are related to PAL actions in the synthesis of various phenylpropanoid products that are concentrated in plants during different stages of growth. Moreover, PAL activity may also be related to various environmental signals such as wounding, infection, and light associated adaptation or defense (Liang *et al.* 1989; Raju *et al.* 2008; Cao *et al.* 2011).

There have been several reports of increased PAL activity in plants under various stresses, such as irradiation, fungal infections, and wounding (Briidenfeldt and Mohr 1988; Dubery and Franchoan 1994; Lafuente *et al.* 2003; Panina *et al*. 2005; Yang *et al.* 2011; Koc and Ustun 2012). Induction of PAL gene expression or increasing its enzyme activity by stress-related hormones and herbicides has been extensively reported (Jiang and Joyce 2003; Lee *et al*. 2003; Panina *et al.* 2005; Sakurai *et al.* 2007; Ferrer *et al.* 2008; Moura *et al.* 2010; Jayalakshmi *et al*. 2011; Jeong *et al*. 2012; Koc and Ustun 2012).

Previous researchers have recognized the contribution and control of PAL expression during the development of Arabidopsis, bamboo, olive, kenaf, and melon (Diallinas and Kanellis 1994; Ortega-García *et al.* 2009; Huang *et al.* 2010; Gao *et al.* 2012; Jeong *et al.* 2012).

Saccharum officinarum is widespread and economically very important. It is the main source of industrialized cane sugar production and is used as a cheap source for ethanol in some countries (Sticklen 2008). Sugarcane supplies more than 70% of the world's sugar. Bagasse is also the main by-product of the sugarcane industry, representing by weight about 30% of the sugarcane agricultural production. It is also the main by-product of the alcohol industry (Selman-Housein *et al*. 1999). Biofuels provide a potential route to avoiding the global political instability and environmental issues that arise from confidence on petroleum. The most important resource for biofuel in the future will be based on the use of lignocellulose products and by-products from grasses. Sugarcane industry also has the high potential to generate biofuel (Sticklen *el al.* 2008). Sugarcane lignification is also very important with regards to the use of this plant in the pulp and paper industry (Selman-Housein *et al*. 1999).

To date, there is no report about the developmental regulation of PAL gene expression in sugarcane. In the present work, the gene expression of PAL in the leaf, sheath, stem, and root of sugarcane (*Saccharum officinarum* L.): cv. CP69-1062, during different developmental stages was investigated.

EXPERIMENTAL

Plant Materials

Samples of commercial sugarcane (cultivar CP69-1062) were prepared from leaf, sheath, stem, and root during the germination (1 to 3 month), tillering (3 to 7 month), grand growth (7 to 11 month), and maturation (11 to 15 month) stages, respectively. Plant materials were grown on a farm of the Sugarcane Research Center, Sugarcane and By-products Development Co. in a region situated in the province of Khozestan (Ahvaz, Iran). The plants were harvested in four replicates. Samples that were taken from each plant included the lamina and sheath from the last fully expanded leaf; the stem from the 7^{th} and 8^{th} internodes; and the roots. All parts were immediately dissected into small pieces, placed in 50-mL polypropylene tubes, snap-frozen in liquid nitrogen, and transported to the laboratory for further analysis.

RNA Preparation and Reverse Transcription

Total RNA was extracted from frozen samples using the Trizol RNA isolation reagent (Life Technologies, USA) and stored at -80 °C. RNA was quantified at a wavelength of 260 nm with a nanodrop spectrophotometer (Nano Drop 2000™, Thermo Scientific, USA). The integrity of RNA was verified by OD 260/280 nm between 1.8 and 2.0. For genomic DNA removal, an in-solution DNase digestion was carried out by treating 1µg of RNA with 2 units of DNase I (Fermentas Inc, Vilnius, Lithuania).

Reverse Transcription–Polymerase Chain Reaction

Reverse transcription was performed using an Ampli Sencec DNA synthesis kit (AmpliSens Enterovirus-Eph, Russia) as recommended by the manufacturer. To detect the expression of the SoPAL gene in different tissues, PCR was performed using a PCR kit (Vivantis Technologies, Selangor DE, Malaysia). Thermal conditions for amplification of PAL were 35 cycles consisting of denaturing at 94 ºC for 1 min, annealing at 58 ºC for 30 sec, extension at 72 ºC for 45 sec, an initial denaturing step at 95 ºC for 5 min, and a final extension step at 72 ºC for 5 min. A reaction without cDNA was used as a negative control. PCR products were visualized using 1% agarose gel electrophoresis.

Quantitative Real-Time PCR with SYBR Green I®

To evaluate the levels of PAL gene expression in different tissues (root, stem, leaf, and sheath) of the sugarcane, quantitative real-time PCR (qRT-PCR) was performed using the ABI Step One plus real-time PCR detection system (ABI plus; Applied Biosystems, USA): and the qPCR™ Green master kit for SYBR Green I® (Applied Biosystems, USA). The relative expression levels of PAL transcripts were normalized to RNA loading for each sample using GAPDH mRNA as described previously (Schmittgen and Livak 2008).

Sequences of sense and antisense primers (BIONEER, Seoul, South Korea) for SoPAL and SoGAPDH were designed using Primer Express 3 software (ABI, USA) and were as follows: SoPAL: 5’-tcttgtgcgaggtgatgaac-3’ and 3’-aagcacgccaagaaggtgaa-5’, and for ScGAPDH: 5’-agttcaacggcacagtcaag-3’ and 3’-tactcagcaccagcatcacc-5’.

Primers were designed based on the sequence of the conservative domain (GenBank accession No. for PAL and GAPDH respectively HQ24271301, KC416026).

Real-time PCR reactions were performed in a final volume of 15 µL containing forward and reverse primers (100nM): cDNA (3µL): DNase free water (4 µL) and 2x

Green star qPCR Master Mix (7.5µL). The reactions were performed using the following program: predenaturation (95 ºC for 5 min) followed by 40 cycles at 95 ºC for 30 s and 60 ºC for 45 s. Reactions were performed in duplicate. Reactions without cDNA were performed in parallel as negative controls. To analyze the qRT-PCR results based on the ΔΔCt method, Step One software 2.1 was used. Relative quantification was performed according to the comparative $2^{-\Delta\Delta Ct}$ method described previously (Schmittgen and Livak 2008). Validation of the assay to check that the primer for the housekeeping gene (GAPDH) and PAL had similar amplification efficiencies was performed as described previously (Rieu and Powers 2009).

Statistical Analyses

Data analyses were performed using the SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to test differences between various means, followed by a post-hoc Tukey test. All experimental data are presented as the mean ± SD. The level of significance for all tests was set at $p< 0.05$.

RESULTS

The pattern of SoPAL expression was analyzed in various sugarcane tissues during the different developmental stages. At first, to ensure SoPAL expression in all of samples, conventional PCR was done. After confirmation of SoPAL expression in different tissues, we studied its developmental changes at transcriptional level using quantitative real-time PCR. It was observed that the PAL gene was expressed in all tissues including the leaf, sheath, stem, and root from the seedling to maturation stages. The highest expression of SoPAL was observed in the stem and the root, while the lowest level of its mRNA was detected in the leaf ($p <0.05$).

Our results demonstrated that fully expanded leaves had the higher PAL transcript level than young, expanding leaves ($p < 0.05$). Although the lowest PAL mRNA level was determined in leaves during the germination stage ($p < 0.05$), no developmental changes of transcript accumulation were seen in other stages (Fig. 1-A). The expression level of SoPAL in the sheath gradually decreased from germination to the grand growth stages; and it exhibited a remarkable increase during the maturation stage (Fig. 1-B). As shown in Fig. 1-B, RNA extracted from the sheath during germination and maturation stages contained higher levels of PAL transcripts than RNA from the sheath on tillering and ground growth stages ($p < 0.05$).

The expression level of SoPAL in the stem was identical to that of the leaf and sheath in all stages. Accumulation patterns of PAL transcripts in the stem started at the tillering stage, reaching an accumulation peak during ground growth and maturation stages (Fig. 1-C).

During the formation of sugarcane roots, the lowest level of PAL mRNA was determined in the germination stage ($p < 0.05$). In the tillering stage, the expression of *PAL* gene in the roots markedly increased in comparison to that of the germination stage and was significantly reduced in the ground growth and maturation stages ($p < 0.05$) (Fig. 1-D).

A comparison was made of the SoPAL expression in different tissues during each developmental stage. The results indicated that in the germination stage, SoPAL mRNA levels in the root and sheath were significantly higher than in the leaves ($p < 0.05$). The

maximal levels of SoPAL transcript were detected in the sheath, while its minimal RNA level was found in the leaf during germination ($p < 0.05$) (Figs. 1-A, B D).

Analysis of SoPAL expression patterns through the tillering stage showed maximal levels of PAL expression in both the root and stem, while minimal RNA level was found in the sheath (Figs. 1- B and D).

During the grand growth stage, SoPAL expression levels in the stem were significantly higher than that in other tissues. The lowest expression levels of PAL were found in the sheath ($p < 0.05$) (Figs. 1-B and C).

At the maturation stage, the highest transcript intensity of PAL was observed in the stem and was higher than that of the root. The lowest SoPAL expression levels in the maturation and ripening stage were detected in the leaf ($p < 0.05$) (Figs. 1-A and C) ($P < 0.05$).

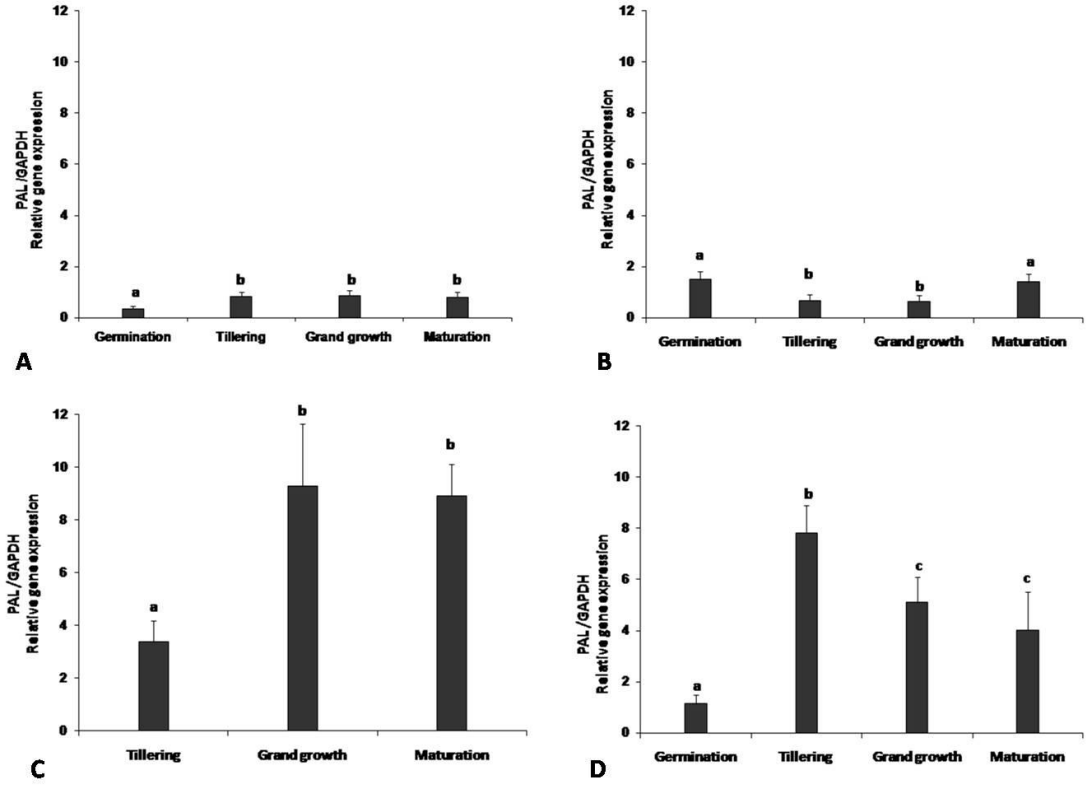

Fig. 1. Relative quantities of SoPAL mRNA in organs during different developmental stages of sugarcane (n=5 in each stage). A: Leaf, B: Sheath, C: Stem, D: Root. Data were presented as the mean ± SD. Letter above each bar demonstrates significant differences among groups at P<0.05.

DISCUSSION

It is commonly believed that various environmental factors, including temperature, moisture, light, fungi, heavy metals, ultraviolet light, and ultrasound have significant roles in the growth and metabolism of plants (Berim *et al.* 2008). The majority of higher plants respond to these stimuli by producing secondary metabolites via the phenylpropanoid pathway, which is present in various tissues of the plant, such as the seeds, roots, woody stems, fruits, flowers, and leaves (Shadlea *et al.* 2003; Ozeki *et al.*

2000). The gene expression pattern of the phenylpropanoid pathway in various tissues of the plant during development can influence the rate of secondary metabolite production and tissue development (Vogt 2010). Quantification of expression of these genes in economical plants like sugarcane will help to establish more efficiently the maturation stages of these plants. This identification can contribute to the development of these plants with improved by products quality.

Here, we studied for the first time the gene expression pattern of PAL, a key rate-limiting enzyme of the phenylpropanoid pathway, during the different stages of development in sugarcane. PAL transcripts accumulated differentially in the four major organs (leaves, stems, roots, and sheaths) of sugarcane; with evidence of developmental regulation of PAL gene expression that differs from one organ to another**.**

The expression level of SoPAL in all growth stages was higher in root and stem in relation to leaf and sheath. It was observed that the expression level of SoPAL was the lowest at the beginning of leaf and stem growth and it increased gradually and reached the maximum at tillering stage and then remained relatively constant. The mRNA accumulation of SoPAL showed similar developmental changes during the beginning of root growth. However, at the final stages of development, SoPAL abundance was reduced. It was also found that there was a different pattern of SoPAL transcript accumulation during the development of sheath. In this tissue, the expression level of *PAL* was the highest during the germination stage; however it decreased to a constant level in the tillering and ground growth stages and subsequently it increased again during the maturation stage.

Accumulation of *PAL* transcript in different tissues of sugarcane was consistent with other studies showing that *PAL* mRNA was typically high in roots and stems and low in leaves of other plants, such as *A. thaliana*, *Hordeum vulgare*, and *Solanum tuberosum* (Wanner *et al*. 1995; Kervinen *et al*. 1997, Joos 1992).

A study of the PAL gene expression patterns in plant tissues is a useful approach to detect the trend of lignification and the rate of production of other phenylpropanoid metabolites which are required for survival and growth of the plant cells.

The levels of PAL mRNA in different tissues of plants directly depend on the age and the rate of lignification (Diallinas and Kanellis 1994). During germination, when the stem is absent in sugarcane, a high level of the PAL mRNA was observed in the sheath. In monocotyledonous plants, aerial pseudo stems are created through the long, stiff sheath and leaf bases that are rolled around one other (Aeber 1918). Higher expression of the PAL gene during this growth stage in the sheath in relation to other tissues may be critical for the expansion of lignification and development of the stem.

During the germination and tillering stages of growth, roots are well established and partially converted to lignified tissues, a process that requires high levels of PAL gene expression. We found that the SoPAL transcripts were increased in the roots and stems during these stages. Although the roots of monocotyledonous plants do not go through a secondary growth stage, however, an abscission zone of the root hair is formed and the external part of the root is suberificated (Esau 1977). During the tillering stage, when the transformation of soft to woody roots is taking place, lignin formation accelerates in roots. The high level of PAL gene expression in a seven-month old plant root seems logical to protect the plant against drastic changes that occur in the root structure and composition (Esau 1977).

It has been shown that increasing the strength and length of the leaves depend on transformation of soft tissues to lignified tissues. In addition, different studies

demonstrate that the production of phenylpropanoid compounds that neutralize the reaction oxygen species increase consistent with extensive respiration by the growing leaves. Also, secondary metabolite accumulation in leaf epidermal cells can regulate the development of the whole plant and individual organs (Giovanni*et al*. 2009). Taking these findings, it was hypothesized that increasing the expression of the PAL gene in growing leaves, roots, and stems may be due to an increase in the rate of lignin or other metabolites formation to modulate these structural and physiological changes (Babar Ali *et al*. 2006).

During the tillering stage, when the transformation of soft to woody roots takes place, PAL gene expression accelerates in roots and protects the plant. The high level of PAL gene expression in a seven-month-old plant root seems logical to protect the plant against drastic changes that occur in the root structure and composition (Esau 1977). The acceleration of PAL gene expression in the leaves at the tillering stage may be due to an increasing conversion of soft tissues to lignified tissues in the leaves and their subsequent growth in length, up to 1 m (James 2004). At this stage, PAL gene expression in the sheath is significantly reduced, while it becomes intensified in the stem and new shoots.

During the grand growth period, the expression of PAL genes in the roots is reduced, and the enzymes reach their equilibrium state with previous developmental stages when the rate of expression of the *PAL* gene in the leaves and sheaths remains unchanged, but the trend in conversion of these parts to lignified tissues continues (James 2004). *PAL* gene expression in the stem accelerates, which could be the result of an increase in the growth of vascular bundles and conversion of the stem into lignified tissues. At this stage, while the plant is increasingly growing in length, the plant strengthens and health becomes more critical, especially in the stem.

During the maturation stage, there is a decrease in PAL mRNA levels in the root, which may be a consequence of the development of lignified tissues. Another important change during the maturation stage was increasing the level of PAL mRNA in the sheath. This change is an indication of the transformation of soft tissue in the stem, particularly during vascular bundle lignification. The number of concentric and peripheral vascular bundles which are distributed within the stems increases consistent with stem growth. Previous studies have shown that fluctuation in enzyme activities and gene expressions alter many metabolites linked to stem development and subsequent sucrose accumulation (Moore 1995; Lingle 1999; Casu *et al*. 2004, 2007). Accumulation of PAL transcript in the bundle sheath may indirectly affect storage of sugar in the stem, because sucrose concentration starts to increase in the internodes as elongation ceases and PAL accelerate stem elongation by increasing the number of lignified vascular bundles.

Increasing PAL gene expression in the stem during the developmental stages of sugarcane is very noticeable when compared to other tissues of the plant, particularly the root. This change is an indication of the transformation of soft tissue in the stem, particularly during vascular bundle lignification. The concentric and peripheral vascular bundles are distributed within the stem, and the peripheral vascular bundles are normally greater in number and larger in size compared to the concentric ones (Esau 1977). Comparing stem vascular bundles to those of the root, which are fewer in number and form a tight circular distribution, it is concluded that the higher expression of the PAL gene in the stem in relation to the root is due to an increased number of vascular bundles in the stem.

PAL is an indication of higher production of other secondary metabolites for resistance to pathogenesis and environmental stress (Huang *et al*. 2010). It has been

found that flavonoids and other phenolic compounds inhibit pathogens ranging from bacteria to fungi and insects (Hassan and Mathesius 2012). It is also known that PAL is considered as one of the key endogenous signals involved in the production of these compounds. The PAL could be markedly induced by many environmental stressors and activation of phenylpropanoid metabolism may play a role in the development of protective barriers in damaged cells. These observations lead to the hypothesis that increasing the PAL gene expression in different tissues of sugarcane especially in roots and stems, which had the highest exposure to many stressors, may play an important role in plant defense during the development.

Investigation with transgenic plants and mutants indicates that the specific down regulation of the expression of each gene involved in the lignin synthetic pathway induces changes not only in lignin content and composition but also in secondary cell wall formation in plant growth and development (Toham *et al.* 2007; Shadle *et al.* 2007). Knowledge of enzymes involved in the phenylpropanoid pathway suggests that the amount of lignin in sugarcane could be changed by genetically engineering the plant. In addition to understanding regulation of gene expression, the results could be applied by genetically engineering the plant in order to produce other phenylpropanoid compounds.

CONCLUSIONS

In conclusion, the data revealed that SoPAL gene is expressed in the four major organs (leaves, stems, roots, and sheaths) of sugarcane and its level changes during the development. These findings are novel, in that they offer support for the hypothesis that PAL changes during the normal physiology of sugarcane and it could be considered as a potential target gene to be used in genetic engineering for creation of transgenic plants with improved sucrose production or stress tolerance. The present work only provides data on developmental regulation of SoPAL expression under normal conditions. Further studies are necessary to understanding the potential role of PAL in phenylpropanoids and sucrose accumulation and also in response to different environmental stressors.

ACKNOWLEDGMENTS

This work was funded by grants from Ahvaz Jundishapour University of Medical Sciences (Project No: CMRC-35), Shahid Chamran University of Ahvaz Research Council (Grant No: 636410, 1391.4.6), and the Sugarcane Research Center, Sugarcane & By-products Development Co.

REFERENCES CITED

Aeber, A. (1918).“The phyllode theory of the monocotyledonous leaf, with special reference to anatomical evidence,”*Ann. Bot.* 32(4), 465-491.

An, C., and Mou, Z. (2011).“Salicylic acid and its function in plant immunity,”*J. Integr. Plant Biol.* 53(6), 412-428.

Babar Ali, M., Singh, N., Shohael, A., Eun, J., and Paek, K. (2006). "Phenolics metabolism and lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress," *Plant Sci.* 171(1), 147-154.

Berim, A., Ebel, R., Schneider, B., and Petersen, M. (2008). "UDP-glucose: (6- methoxy) podophyllotoxin 7-O-glucosyltransferase from suspension cultures of *Linum nodiflorum*," *Phytochemistry.* 69(2), 374-381.

Briidenfeldt, R., and Mohr, M. (1988). "Time courses for phytochrome-induced enzyme levels in phenylpropanoid metabolism (phenylalanine ammonia-lyase, naringenin-chalcone synthase) compared with time courses for phytochrome-mediated end-product accumulation (anthocyanin, quercetin)," *Planta*. 176(3), 383-390.

Cao, F. Y., Yoshioka, K., and Desveaux, D. (2011). "The roles of ABA in plant-pathogen interactions," *J. Plant Res.* 124(4), 489-499.

Casu, R. E., Jarmey, J. M., Bonnett, G. D., and Manners, M. J. (2007). "Identification of transcripts associated with cell wall metabolism and development in the stem of sugarcane by Afymetrix GeneChip sugarcane genome array expression profiling," *Funct. Integr. Genomics.* 7(2), 153-168.

Casu, R., Dimmock, C. M., Chapman, S. C., Grof, C. P. L., McIntyre, C. L., Bonnett, G. D., and Manners, J. M. (2004). "Identification of differentially expressed transcripts from maturing stem of sugarcane by in silico analysis of stem expressed sequence tags and gene expression profiling," *Plant Mol. Biol.* 54(4), 503-517.

Diallinas, G., and Kanellis, A. K. (1994). "A phenylalanine ammonia-lyase gene from melon fruit: cDNA cloning, sequence and expression in response to development and wounding," *Plant Mol. Biol.* 26(1), 473-479.

Dubery, I., and Franchoan, S. (1994). "Phenylalanine ammonia-lyase from cotton (*Gossypium hirsutum*) hypocotyls: Properties of the enzyme induced by a *Verticillium dahliae* phytotoxin," *Biochimica et Biophysica Acta* 1207(1), 24-30.

Esau, K. (1977). *Anatomy of Seed Plants*, Wiley, New York.

Ferrer, J. L., Austin, M. B., Stewart, C. Jr., and Noel, J. P. (2008). "Structure and function of enzymes involved in the biosynthesis of phenylpropanoids," *Plant Physiol. Bioch.* 46(3), 356-370.

Gao, Z. M., Wang, X. C., Peng, Z. H., Zheng, B., and Liu, Q. (2012). "Characterization and primary functional analysis of phenylalanine ammonia-lyase gene from *Phyllostachys edulis*," *Plant Cell Rep.* 31(7), 1345-1356.

Giovanni, A., Giovanni, S., Biricolti, S., and Massimiliano, T. (2009). "Mesophyll distribution of 'antioxidant' flavonoid glycosides in *Ligustrum vulgare* leaves under contrasting sunlight irradiance," *Ann. Bot.* 104(5), 9-19.

Hassan, S., and Mathesius, U. (2012). "The role of flavonoids in root–rhizosphere signalling: Opportunities and challenges for improving plant–microbe interactions," *J. Exp. Bot.* 63(9), 1-16.

Huang, J. G., Lai, M., Fan, Z., Shi, B., Zhou, K., Yu, Y. H., and Chen, Z. (2010). "Functional analysis of the *Arabidopsis* PAL gene family in plant growth, development, and response to environmental stress," *Plant Physiol.* 153(4), 1526-1538.

Hyun, M. W. Y., Kim, Y. H., and Kim, J. (2011)."Fungal and plant phenylalanine ammonia-lyase," *Mycobiology* 39(4), 257-265.

James, G. (2004). *Sugarcane*, Blackwell Science, Oxford.

Jayalakshmi, S. K., Raju, S., Usharani, S., Kurucheve, V., Benagi, V. I., and Sreeramulu, K. (2011). "Differential expression of defense related enzymes and protease inhibitors

in two different genotypes of chickpea by *Trichoderma harzianum* L1," *Aust. J. Crop. Sci.* 5(7), 885-894.

Jeong, M. J., Choi, B. S., Dong, W. B., Shin, S. C., Un Park, S., Lim, H., Kim, J., Kim, J. B., Cho, B., and Bae, H. (2012). "Differential expression of kenaf phenylalanine ammonia-lyase (PAL) ortholog during developmental stages and in response to abiotic stresses," *Plant Omics.* 5(4), 392-399.

Jiang, Y., and Joyce, D. C. (2003)."ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit," *Plant Growth Regul.* 39(2), 171-174.

Koc, E., and Ustun, A. S. (2012)."Phenylalanine ammonia lyase activity in stem of pepper (*Capsicum annuum* L.) infected by *Phytophthora capsici* L.,"*Gazi University Journal of Science* 25(2), 307-312.

Lafuente, M. T., Zacarias, L., Martínez-Téllez, M. A., Sanchez-Ballesta, M. T., and Granell, A. (2003)."Phenylalanine ammonia-lyase and ethylene in relation to chilling injury as affected by fruit age in citrus,"*Posth. Biol. Tech.* 29(3), 308-318.

Lee, B. K., Park, M. R., Srinivas, B., Chun, J. C., Kwon, I., Chung, I. M., Yoo, N. H., Choi, K. G., and Yun, S. J. (2003)."Induction of phenylalanine ammonia-lyase gene expression by paraquat and stress-related hormones in *Rehmannia glutinosa*," *Mol. Cells.* 1(1), 34-35.

Liang, X., Dron, M., Carole, L., Cramer, C. L., Dixon, R. A., and Lamb, C. J. (1989). "Differential regulation of phenylalanine ammonia-lyase genes during plant development and by environmental cues," *J. Biol. Chem.* 26(4), 14486-14492.

Lingle, S. E. (1999). "Sugar metabolism during growth and development in sugarcane internodes," *Crop Sci.* 39, 480-487.

Ludwikow, A., and Sadowski, J.(2008). "Gene networks in plant ozone stress response and tolerance," *J. Integr. Plant Biol.* 50(10), 1256-1267.

Moore, P. H. (1995). "Temporal and spatial regulation of sucrose accumulation in the sugarcane stem," *Aust. J. Plant Physiol.* 22(4), 661-679.

Moura, J. C., Bonine, C. A., De Oliveira Fernandes Viana, J., Dornelas, M. C., and Mazzafera, P. (2010). "Abiotic and biotic stresses and changes in the lignin content and composition in plants," *J. Integr. Plant Biol.* 52(4), 360-376.

Ortega-García, F., Blanco, S., Peinado, M. A., and Perag, J. (2009). "Phenylalanine ammonia-lyase and phenolic compounds in leaves and fruits of *Olea europaea* L. cv. Picual during ripening," *J. Sci. Food Agr.* 89(3), 398-406.

Ozeki, Y., Ito, Y., Sasaki, N., Oyanagi, M., Akimoto, H., Chikagawa, Y., and Takeda, J. (2000). "Phenylalanine ammonia-lyase genes involved in anthocyanin synthesis and the regulation of its expression in suspension cultured carrot cells,"*J. Plant Res.* 113(3), 319-326.

Panina, Y. S., Gerasimova, N. G., Chalenko, G. I., Vasyukova, N. I., and Ozeretskovskaya, O. L. (2005). "Salicylic acid and phenylalanine ammonia-lyase in potato plants infected with the causal agent of late blight," *Russ. J. Plant Physiol.* 52(4), 573-577.

Raju, S., Jayalakshmi, S. K., and Sreeramulu, K. (2008). "Comparative study on the induction of defense related enzymes in two different cultivars of chickpea (*Cicer arietinum* L) genotypes by salicylic acid, spermine and *Fusarium oxysporum* F. sp. Ciceri," *Aust. J. Crop. Sci.* 2(3), 121-140.

Rieu, I., and Powers, S. J. (2009). "Real-time quantitative RT-PCR: Design, calculations, and statistics," *The Plant Cell.* 21(4), 1031-1033.

Sakurai, T., Plata, G., Rodriguez-Zapata, F., Seki, M., Salcedo, A., Toyoda, A., Ishiwata, A., Tohme, J., Sakaki, Y., Shinozaki, K., and Ishitani, M. (2007). "Sequencing analysis of 20,000 full-length cDNA clones from cassava reveals lineage specific expansions in gene families related to stress response," *BMC Plant Biol.* 7, 1-17.

Schmittgen, T. D., and Livak, K. J. (2008). "Analyzing real-time PCR data by the comparative CT method," *Nature Protocols* 3, 1101-1108.

Selman-Housein, G., Lopez, M. A., Hernandez, D., Civardi, L., Miranda, F., Rigau, J., and Puigdomenech, P. (1999). "Molecular cloning of cDNAs coding for three sugarcane enzymes involved in lignification," *Plant Sci.* 143, 163-171.

Shadlea, G. L., Wesleya, S. V., Kortha, K. L., Chen, F., Lamb, C., and Dixona, R. A. (2003). "Phenylpropanoid compounds and disease resistance in transgenic tobacco with altered expression of l-phenylalanineammonia-lyase," *Phytochemistry* 64(1), 153-161.

Sticklen, M .B. (2008). "Plant genetic engineering for biofuel production: Towards affordable cellulosic ethanol," *Nat. Rev. Genet.* 9(6), 433-443.

Vogt, T. (2010). "Phenylpropanoid biosynthesis," *Mol. Plant* 3(1), 2-20.

Wanner, L. A., Li, G., Ware, D., Somssich, I. E., and Davis, K. R. (1995). "The phenylalanine ammonia-lyase gene family in *Arabidopsis thaliana*," *Plant Mol. Biol.* 27, 327-338.

Yang, H. R., Tang, K., Liu, H. T., and Huang, W. D. (2011). "Effect of salicylic acid on jasmonic acid-related defense response of pea seedlings to wounding," *Sci. Hortic.* 128(3), 166-173.

Article submitted: May 23, 2013; Peer review completed: July 9, 2013; Revised version accepted: August 1, 2013; Published: August 8, 2013.