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Lysigenous aerenchyma formation: responsiveness to waterlogging in oil palm roots

S. NUANLAONG, S. WUTHISUTHIMATHAVEE, and P. SURANINPONG*

School of Agricultural Technology, Walailak University, Nakhon Si Thammarat 80160, Thailand

*Corresponding author: E-mail: potjamas@hotmail.com

Abstract

Oil palm (*Elaeis guineensis* Jacq.) responds to waterlogging stress by induction of lysigenous aerenchyma tissues, which facilitates the flow of oxygen through their root tissues for survival under waterlogged conditions. Thus, the morphological and genetic adaptation involved in lysigenous aerenchyma formation in the roots of the oil palm genotype Deli × Calabar under waterlogging stress was evaluated. This study found the highest number of dead cells after waterlogging stress for 2 d in the secondary root, while the percentage of root porosity was increased with increasing of time in both roots, especially at 1.0 - 2.0 cm from the root tip. This change in cell morphology implied the formation of lysigenous aerenchyma in oil palm roots under waterlogging stress. At the same time, most of the candidate genes involved in lysigenous aerenchyma formation revealed a higher mRNA expression after waterlogging stress for 3 d. Genes of ethylene synthesis group *ACS3*, *ACO*, and *ACO1* were highly up-regulated in both types of roots, while *XTH22*, *XTH23*, and *CEL12* in the cell wall modification group were more highly up-regulated in the primary roots than in the secondary roots. *CML11*, *CAMTA4*, *TCTP*, and *CPI1* in a signaling group were up-regulated in the primary roots, but they were down-regulated in the secondary roots. *NAC29*, *ERF1*, *ERF113*, and *HSFA2C* in a transcription factor group were strongly up-regulated in the oil palm roots. However, there have been no previous reports on the expression of *CAMTA4*, *bHLH79,* and *bHLH94* under waterlogging conditions. Our findings confirm gene expression during lysigenous aerenchyma development in oil palm roots under waterlogging. It can also be stated that primary roots are an important part of the adaptation mechanism of oil palm roots for survival under waterlogging stress. Furthermore, the molecular markers of all expressed genes will be developed and applied in our oil palm breeding project for selection of waterlogging tolerance.

Keywords: expression profile, ethylene synthesis, cell wall modification, transcription factor.

Introduction

Oil palm is a plant that requires relatively high amounts of water throughout the year. It needs an average annual rainfall of more than 2 000 mm, and consequently, a distribution of rain throughout the year of approximately 167 mm per month. Waterlogging is natural flooding or over-irrigation that brings water from underground levels to the surface. It usually occurs when rainfall or irrigation water is deposited in the soil surface or subsoil

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Abbreviations: ACC - 1-aminocyclopropane-1-carboxylic acid; *ACO* - 1-aminocyclopropane-1-carboxylic acid oxidase; *ACO1* - 1-aminocyclopropane-1-carboxylate oxidase 1; *ACS* - 1-aminocyclopropane-1-carboxylic acid synthase; *ACS3* - 1-aminocyclopropane -1-carboxylate synthase 3; *CAMTA4* - calmodulin-binding transcription activator 4; *CEL12* - endoglucanase 12; *CML11* - calmodulin 11; *CPI1* - cysteine proteinase inhibitor 1; *ERF1* - ethylene-responsive transcription factor 1; *ERF1B* - ethylene-responsive transcription factor 1B; *ERF91* - ethylene-responsive transcription factor 91; *ERF113* - ethylene-responsive transcription factor 113; *bHLH79* - transcription factor bHLH 79; *bHLH94* - transcription factor bHLH 94; *HSFA2C* - heat shock protein factor A-2c; *MYB1R1* - transcription factor MYB1R1; *NAC29* - NAC transcription factor 29; PCD - programmed cell death; *TCTP* - translationally controlled tumor protein; *XTH22* - xyloglucan endotransglucosylase/hydrolase protein 22; *XTH23* - xyloglucan endotransglucosylase/hydro-lase protein 23; *WRKY4* - transcription factor WRKY 4; TF - transcription factor.

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for a prolonged period. Waterlogging can also occur when the amount of water added through rainfall or irrigation is more than what can percolate into the soil within 1 or 2 d (Hardy *et al.* 2012). Because the roots of most plants are unable to respire when submerged in water, if waterlogging is prolonged, the roots may die (Corley and Tinker 2015). Oil palm seedlings develop aerenchyma tissue and/or pneumatophores as adaptation mechanisms under waterlogged conditions. Therefore, their gas exchange or their processes of micronutrient reduction and assimilation are not affected (Rivera-Mendes *et al.* 2016). However, oil palm seedlings have slower growth under waterlogging due to higher leaf respiration rates. Aerenchyma maintenance may potentially mimic the absorption and transport of macronutrients (Corley and Tinker 2015), resulting in oxygen uptate into submerged roots *via* internal aeration.

In general, plants have responsive mechanisms to unsuitable environments for their survival. Aerenchyma formation is a major physiological and morphological adaptation of plants to waterlogging or flooding conditions. It is known to enhance the internal diffusion of atmospheric and photosynthetic oxygen from the aerial parts to the flooded roots, allowing the roots to maintain aerobic respiration (Yamauchi *et al.* 2013). Aerenchyma formation increases the porosity of roots above the usual levels contributed by intercellular spaces (Colmer 2003). The increase in root porosity of tolerant genotypes in response to waterlogging stress could represent adaptation to anaerobic or hypoxic conditions (Hossain and Uddin 2011). Lysigenous aerenchyma is a developmental process that is triggered by the hormone ethylene. Its activity contributes to the opening of gas spaces within parenchymatic tissues due to programmed cell death (PCD) and cell wall modifications (Nishiuchi *et al.* 2012, Takahashi *et al.* 2014, Tavares *et al.* 2019). The formation of lysigenous aerenchyma has been studied extensively in the roots of various plant species, including barley (Settler and Waters 2003), rice (Colmer and Pedersen 2008), *Zea mays* (Rajhi *et al.* 2011), *Zea nicaraguensis* (Mano and Omori 2013), wheat (Herzog *et al.* 2016), and sugarcane (Grandis *et al.* 2019, Tavares *et al.* 2019). Therefore, lysigenous aerenchyma plays an important role in increasing waterlogging tolerance in dryland crops.

Waterlogging/hypoxia has been shown to stimulate the biosynthesis of ethylene and an increase in the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase, and ACC synthase has been observed in roots under hypoxia conditions (Drew *et al.* 2000). Ethylene rapidly accumulates in water-submerged roots and leads to the inducible formation of lysigenous aerenchyma in wheat (Yamauchi *et al.* 2014), maize (Rajhi *et al.* 2011), and rice (Yamauchi *et al.* 2015). Increasing direct and indirect evidence indicates that ethylene plays a regulatory role in the formation of lysigenous aerenchyma. The accumulation of ethylene inside the hypoxia root affects the activity of the PCD (Gunawardena *et al.* 2001, Mustroph *et al.* 2018, Sasidharan *et al.* 2018, Yamauchi *et al.* 2018). Recently, the production of ethylene-induced reactive oxygen species (ROS) that required aerenchyma formation in rice roots was discovered (Yamauchi *et al.* 2017). Due to H_2O_2 released as an oxidative burst, sequential propagation of PCD takes place. Either way, higher doses of ROS have been reported to cause cell death in higher plants (Ni *et al.* 2019, Yamauchi *et al.* 2019). ROS are important components in cell death signaling, and their regulated generation might be important in triggering physiological cell death to form lysigenous aerenchyma (Colmer *et al.* 2006, Sasidharan and Voesenek 2015). Moreover, Rajhi *et al.* (2011) reported that signaling pathways were based on the heteromeric G-protein, phospholipase C, inositol 1,4,5-triphosphate, and Ca^{2+} which are involved in the formation of lysigenous aerenchyma regulated by ethylene accumulation. The last step of lysigenous aerenchyma formation involves cell wall modification and degradation, with many glycosyl hydrolases enzymes including xylanases and cellulases (Rajhi *et al.* 2011, Leite *et al.* 2017). As the plant hormone ethylene is the main trigger that induced lysigenous aerenchyma, several families of transcription factors, including ethylene response factors (ERFs), NAC, bHLH, WRKY, and MYB, were reported as playing a role in aerenchyma formation (Rajhi *et al.* 2011). Members of these families are thought to regulate gene expression related to biotic and abiotic stresses (Rajhi *et al.* 2011, Safavi-Rizi *et al.* 2020). However, the role of certain genes involved in aerenchyma formation is still unknown in oil palm. Thus, gene expression during the development of lysigenous aerenchyma was investigated in oil palm roots under waterlogging stress.

Mature oil palms usually have four root sets that are formed in order of the primary root, secondary root, tertiary root, and quaternary root, respectively. Our previous study identified genes associated with waterlogging stress after 45 d in the roots of two oil palm cultivars (Nuanlaong *et al.* 2020). In addition, Rivera-Mendes *et al.* (2016) observed that pneumatophores began to appear after waterlogging stress for 15 d. To know where aerenchyma originated and how it forms in oil palm roots, both at the morphological and molecular levels, this study used oil palms at a nursery stage that has two root sets (primary roots and secondary roots). Hence, a study of cell death, root porosity and gene expression associated with lysigenous aerenchyma development in two types of oil palm roots within 15 d under waterlogging conditions was conducted for confirmation of our previous findings.

Materials and methods

Plants and waterlogging treatments: *Elaeis guineensis* Jacq. (cv. Deli × cv. Calabar) seedlings were grown in pot containing topsoil and sandy soil (4:1, v/v) in a greenhouse at a 12-h photoperiod, day/night temperatures of 34/26 °C, a relative humidity ranging from 81.8 to 85.4 %, and an irradiance 11.39 μmol m^{-2} s^{-1} in Nakhon Si Thammarat Province, Thailand. After cultivating for three months, the oil palm plants were divided into two groups: one group cultured under normal water supply used as the control and the second group submitted to waterlogging stress by placing the pot into a plastic tank, which imposed water

levels above the soil surface by approximately 10 cm. After the treatment for 0, 1, 2, 3, 7, and 15 d, primary and secondary roots were collected, cleaned, and frozen in liquid nitrogen for morphological studies and gene expression analysis.

Aerenchyma formation in oil palm roots under waterlogged condition: To determine an aerenchyma formation, a cell death and a root porosity were studied. The cell death was assessed by staining the roots with Evans blue according to the methods of Steffens and Sauter (2005) and Jiang *et al.* (2012), with slight modifications. The roots were washed and then immersed in 10-cm^3 centrifuge tubes containing 5 cm^3 of Evans blue solution (0.25 %, v/v) at room temperature for 30 min, and then they were washed several times to remove unbound Evans blue in distilled water and dried out with bibulous paper. To bleach the dye, the roots were transferred into 5 cm^3 of 50 % (v/v) methanol and 1 % (m/v) sodiumdodecyl sulphate (SDS) before being placed in a water bath at 50 °C for 30 min to release the trapped Evans blue from the cells. The mixture was centrifuged at 4 000 *g* for 15 min before measurement at 600 nm *via* spectrophotometer (*Thermo Scientific GENESYS 6*™, Wisconsin, USA). The amount of cell death was calculated using A_{600} g^{-1}(f.m.) (Xuewen *et al.* 2014).

Root porosity measurements were determined according to the method of Visser and Bögemann (2003). Transverse sections of the primary and secondary roots at 0.5 - 1.0 cm and 1.0 - 2.0 cm from the root tip were cut with a razor blade. The pieces of roots were submerged in water for 5 min in vacuum desiccators (5 kPa) to remove air bubbles. The treated roots were then stained with safranin-*o* before being photographed under a light microscope. The root area and air space were observed with *Image J* software (v. *1.50*). The root porosity percentage was calculated as (area of air space/total transverse-sectional area) × 100.

Expression analysis of genes involved in lysigenous aerenchyma formation: Genes associated with lysigenous aerenchyma formation were searched from transcriptome data of Nuanlaong (2018). The 27 genes found were validated *via* real-time qPCR in the primary roots and secondary roots under waterlogging stress for different times (Table 1 Suppl.).

The total RNA was extracted using the total RNA mini kit (*Geneaid*, Taipei, Taiwan) according to the manufacturer's instructions. The RNA quality and quantity were examined *via* spectrophotometer and 1.5 % (m/v) agarose gel electrophoresis. In addition, gene-specific primers received from Nuanlaong (2018) were used as templates for the primer design using the *Primer3Plus* software (Untergasser *et al.* 2007) and the *Oligo Calculator v. 3.27* program. Primer specificity was judged by melting-curve analysis and 1.5 % agarose gel electrophoresis of the amplification products (Table 2 Suppl.). For gene expression analysis, 1 µg of RNA was reverse transcribed using the *iScript*™ selected cDNA synthesis kit (*Bio-Rad*, Singapore). Real-time qPCR experiments were executed on a *7300 Real Time* PCR system (*Applied Biosystems*, Massachusetts, USA) using 5× *HOT FIREPol® EvaGreen® qPCR Mix Plus* (*ROX*) (*Solis BioDyne*, Tartu, Estonia) with specially designed primers. The relative expression of each gene was quantified with the comparative threshold cycle method, using the *18S rRNA* gene as the internal reference. The threshold cycle values (Ct value) of the genes and internal reference genes for the different samples were calculated by the $2^{-\Delta\Delta CT}$ method. The means ± SDs should always be calculated after the $2^{-\Delta\Delta CT}$ transformation in order to perform statistical analysis (Livak and Schmittgen 2001).

Statistical analysis: Cell death, root porosity, and gene expression data were analyzed by *ANOVA* and Duncan's multiple range tests at $P \leq 0.05$ through Statistical package for the social sciences (*SPSS v. 16.0*) software. The mean of each root was calculated from five representative sections, and the mean of each replicate was calculated from the three roots in each pot. For gene expression analysis, the treatment mean of three biological replicates was used.

Results

An average of Evans blue absorption showed the highest death of cells in the primary roots and in the secondary roots after waterlogging stress for 2 and 1 d, respectively. The secondary roots revealed a higher average of Evans blue uptake than did the primary roots, as shown in Fig. 1. The percentage of root porosity tended to increase followed by an increase in waterlogging stress time, except for the primary roots at 0.5 - 1.0 cm. Waterlogging for 15 d revealed the highest average percentage of root porosity in both types of roots. The average root porosity at 1.0 - 2.0 cm showed a higher percentage than at 0.5 - 1.0 cm from the root tip in both the primary and secondary roots. At 1.0 - 2.0 cm, the average root porosity for the primary roots (7.52 ± 3.65 %) was higher than for the secondary roots (5.30 ± 3.59 %) (Table 1 and Fig. 2).

For gene expression determination, 20 out of 27 genes could be amplified with cDNA fragments. The results of qPCR analysis revealed that more genes were up-regulated in the primary roots as compared to the secondary roots, and they were strongly expressed after waterlogging stress for 3 d. Three candidate genes in the ethylene synthesis groups (*ACS3*, *ACO,* and *ACO1)* were up-regulated in both the primary and the secondary roots under waterlogging stress for 3 d. In cell wall modification, the expression of *XTH22* and *XTH23* genes were up-regulated and highly expressed in the primary roots after waterlogging stress for 3 d with a significant difference ($P \leq 0.05$) between the times of waterlogging stress. However, the *XTH22* gene was down-regulated in the secondary roots. The *CEL12* gene was up-regulated only after waterlogging stress for 3 d in the primary roots, whereas in the secondary roots, it was up-regulated after waterlogging stress for 7 and 15 d. Similarly, the relative mRNA expression of four candidate genes of signaling transduction was up-regulated in the primary roots and down-regulated in the secondary roots. *CML11* was up-regulated after waterlogging for 2 d. *CPI1,*

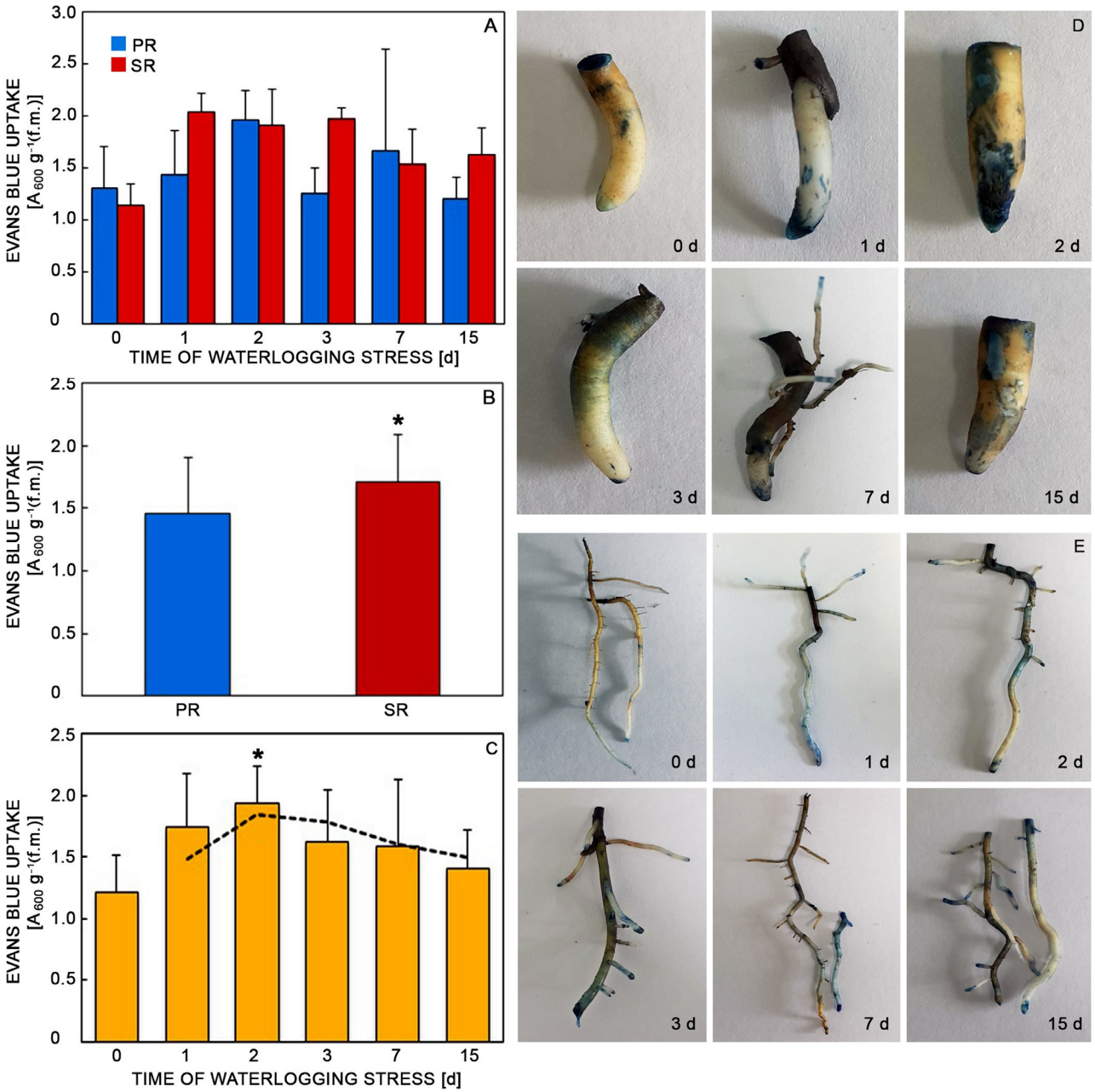

Fig. 1. Degree of Evans blue staining in waterlogged oil palm roots. *A* - Evans blue absorption in oil palm roots at various times; *B* - Evans blue absorption in primary roots (PR) and secondary roots (SR); *C* - Evans blue absorption in oil palm roots after waterlogging for 0, 1, 2, 3 , 7 and 15 d. *D* - Evans blue staining in primary roots stressed for various times; *E* - Evans blue staining in secondary roots stressed for various times. Means ± SDs, n = 5. * indicates significant differences at $P < 0.05$ compared between time of waterlogging stress (*A*), between root types (primary and secondary root, *B*) and between time of waterlogging (*C*).

CAMTA4, and *TCTP* were up-regulated after waterlogging stress for 3 d. In contrast, the expression of 10 candidate TF genes revealed both up and down-regulation in both roots under waterlogging stress. *NAC29* showed a higher mRNA expression in the primary roots and the secondary roots after waterlogging stress for 3 and 7 d, respectively, and was up-regulated at all times during waterlogging stress. *HSFA2C* and *WRKY4* were also up-regulated in the primary roots after waterlogging stress for 3 d. *ERF1*, *ERF91*, and *ERF113* showed a high relative mRNA expression in the primary roots after waterlogging stress for 3 d, but *ERF1B* had a high relative mRNA expression in the primary roots after waterlogging stress for 2 d and for 15 d in the secondary roots. *bHLH79* and *bHLH94* were down-regulated in the primary roots, but *bHLH94* was up-regulated in the secondary roots after waterlogging stress for 1, 3, and 7 d. In contrast, *MYB1R1* was mostly down-regulated under waterlogging stress in both types of oil palm roots. In addition, the relative mRNA expressions of *HSFA2C, MYB1R1*, and *WRKY4* were not significantly

Table 1. Percentage of root porosity in different types and positions of oil palm roots under waterlogging conditions for various times. (PR - primary roots, SR - secondary roots). Means ± SDs, $n = 5$. Means followed by different letters are significantly different at $P \leq 0.05$.
[1] - Average of values in the row. [2] - Average of values in the column.

Root types	Time of waterlogging stress [d]						Average[1] [%]
	0	1	2	3	7	15	
PR 0.5 - 1.0 cm	0.00±0.00	5.32±1.20	3.74±0.82	0.19±0.11	1.12±0.06	0.26±0.44	1.33±2.01^{d}
PR 1.0 - 2.0 cm	1.57±0.27	7.57±1.18	6.76±3.73	8.02±1.68	10.04±1.08	13.06±2.09	7.52±3.65^{a}
SR 0.5 - 1.0 cm	0.00±0.00	1.28±0.84	1.77±0.36	4.31±1.03	1.89±0.90	6.75±2.91	2.77±2.50^{c}
SR 1.0 - 2.0 cm	0.44±0.61	2.00±0.10	3.85±0.80	7.39±2.89	3.84±1.63	10.86±0.78	5.30±3.59^{b}
Average[2] [%]	0.50±0.73^{e}	4.04±2.78^{c}	4.03±2.50^{c}	4.98±3.56^{bc}	4.22±3.77^{c}	7.73±5.33^{a}	4.23±3.81

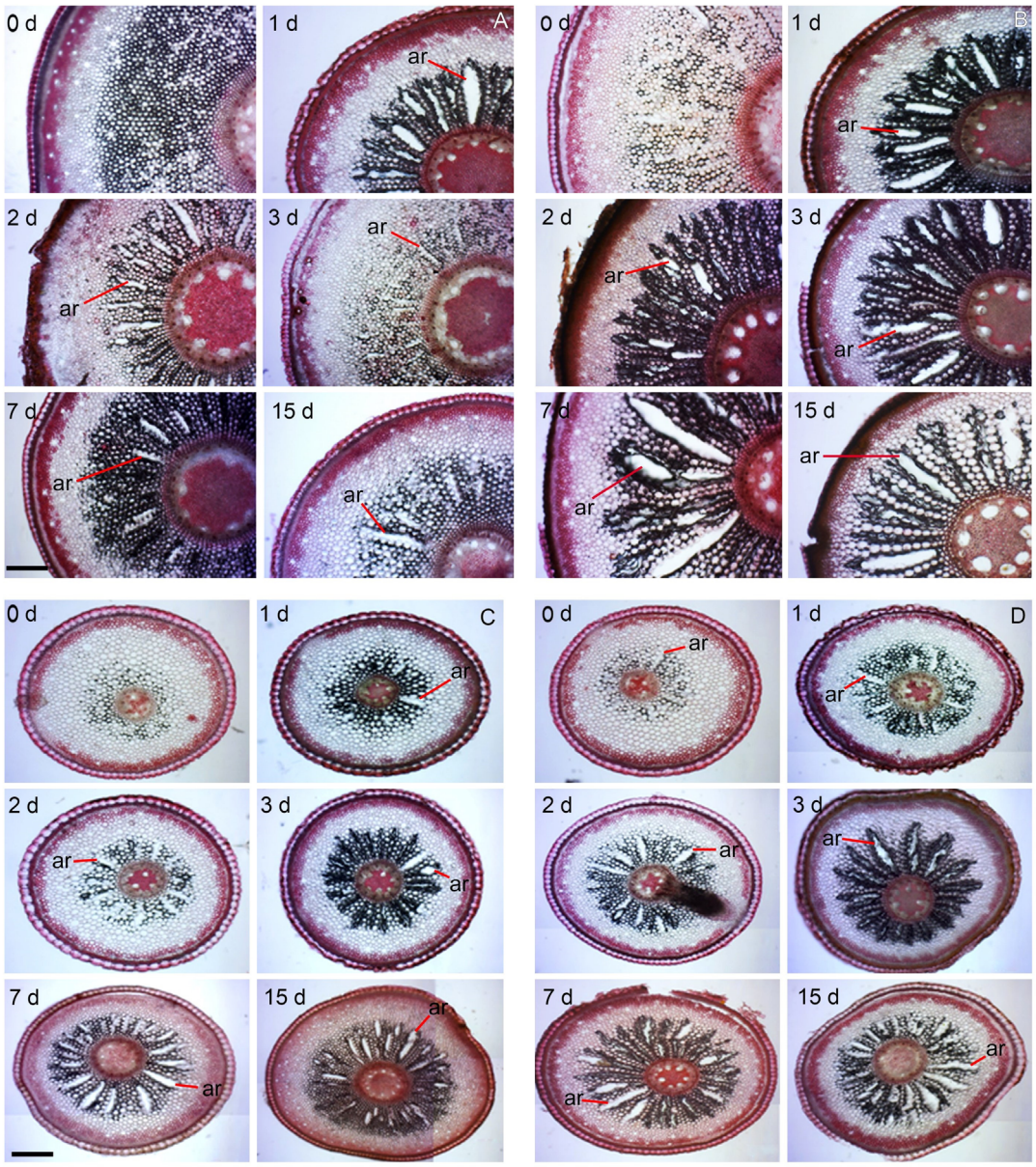

Fig. 2. Lysigenous aerenchyma formation in oil palm roots under waterlogging stress for various times. *A* - primary roots at 0.5 - 1.0 cm; *B* - primary roots at 1.0 - 2.0 cm; *C* - secondary roots at 0.5 - 1.0 cm; *D* - secondary roots at 1.0 - 2.0 cm (ar - lysigenous aerenchyma, *scale bar* = 200 μm).

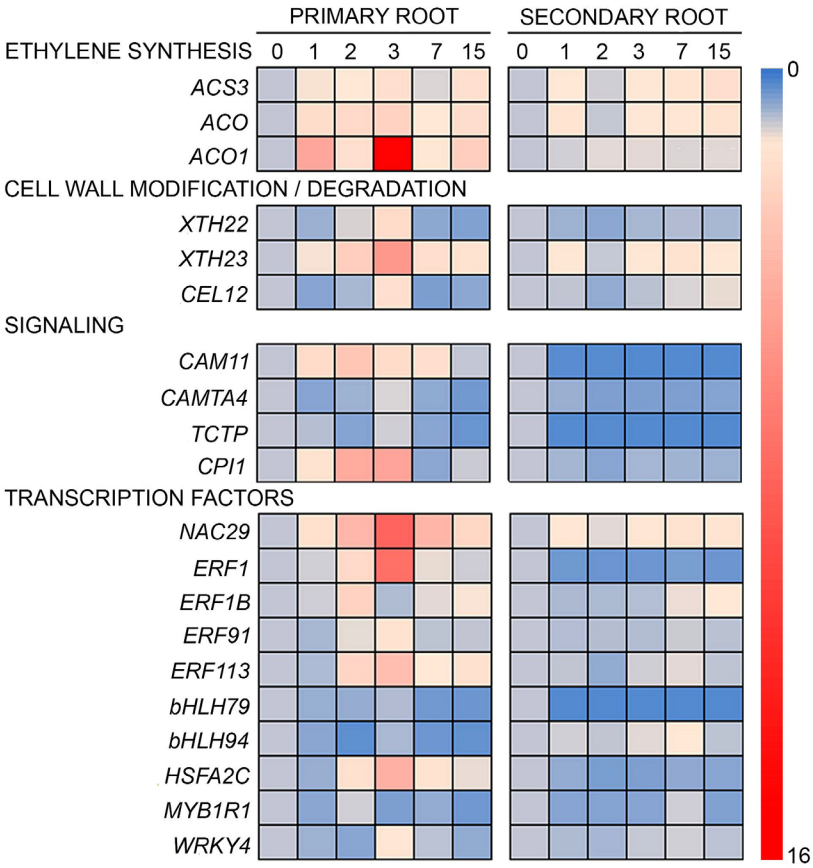

Fig. 3. Heat map of relative mRNA expression of candidate genes involved in lysigenous aerenchyma formation in oil palm roots under waterlogging stress for various times. The *red color* indicates up-regulated genes and the *blue color* indicates down-regulated genes.

different ($P \leq 0.05$) between the primary or secondary roots or between the times of waterlogging stress (Fig. 3).

Discussion

Aerenchyma formation, a consequence of PCD, is one of the morphological adaptations in plant species that makes them tolerant of waterlogging (Evans 2003). In our study, the highest Evans blue uptake in oil palm root appeared after waterlogging for 2 d, while the percentage of root porosity tended to increase with an increase in waterlogging time. This indicated that the death of cells was due to hypoxia-induction (Oh *et al.* 2014), in which root cortical cells are induced to die and form larger air spaces (Drew *et al.* 2000). As the secondary root is smaller than the primary root, the secondary root revealed the death of cells more quickly than did the primary root. However, the primary roots had a higher percentage of root porosity than the secondary roots. As the primary root is bigger and older than the secondary root, where O_2 from the air into the soil is effectively blocked, the death of root cells occurred followed by the degradation and total lysis of the cytoplasm. The constitutive formation of air spaces in primary roots was found to be higher than in the secondary roots. Furthermore, aerenchyma was formed in the basal region and not in the apical region (Nakazono *et al.* 2009). Thus, the root porosity was lower at 0.5 - 1.0 cm from the root tip than at 1.0 - 2.0 cm.

For gene expression analysis, a high relative gene expression of *ACS3*, *ACO*, and *ACO1* was similar to those of our previous study (Nuanlaong *et al.* 2020). This implied the beginning of aerenchyma formation in oil palm roots under waterlogging stress. After the start of ethylene biosynthesis, signaling transduction was activated. This experiment found the up-regulation of *CML11* at all times of waterlogging stress. Similarly, in maize roots, we found the up-regulation of Ca^{2+} signaling-related genes encoding calmodulin under waterlogged conditions (Rajhi *et al.* 2011). Furthermore, *CAMTA4*, *TCTP,* and *CPI1* were up-regulated in the primary roots after waterlogging stress for 3 d. *CAMTA4*, a member of the calmodulin-binding transcription activators (*CAMTAs*) family (Meer *et al.* 2019), is regulated in response to calcium signals and the positive regulation is a general stress response (Benn *et al.* 2014). In general, *CAMTAs* are reported in response to drought, cold, salinity, and hormones (*e.g.*, auxin, abscisic acid, and jasmonic acid) (Benn *et al.* 2014). As the expression of *CAMTA4* under waterlogging conditions has not been previously reported, the results from this study represent new findings. Moreover, *TCTP* could regulate PCD *via* a possible role of H_2O_2 during *TCTP* induction (Betsch *et al.* 2017). In a previous study, *TCTP* was increased 4 d after flooding treatment (Chen *et al.* 2014).

Nevertheless, CPI1 is one of the most important molecules involved in plant development and defense, especially in the regulation of stress responses. It is involved in the suppression of hypersensitive cell death activated by either a virulent pathogen or oxidative stress (Belenghi *et al*. 2003). Therefore, the expression of *CPI1* was found in this study.

Furthermore, the expression of three cell wall modification-related genes was highly up-regulated in the primary roots after waterlogging stress for 3 d. *XTHs* are not only involved in breaking down the cell walls, but they also allow rapid expansion and growth (Tsuchiya *et al*. 2015). The high expression of *XTH22* is the same result as that of our previous study (Nuanlaong *et al*. 2020). The deficiency of oxygen in waterlogged plants triggers the anaerobic stimulation of ethylene accumulation, which causes an increase in cellulase and xylanase activity leading to aerenchyma formation (Leite *et al.* 2017, Ni *et al.* 2019). Thus, the up-regulation of *CEL12* was found in the secondary roots after waterlogging stress for 7 and 1 d. This result was also confirmed by Rajhi *et al*. (2011), who found the up-regulation of the *CEL* gene in cortical cells of maize roots under waterlogging stress.

Moreover, the genes in the TF group proved to be the most important genes for the regulation of aerenchyma formation in oil palm roots under waterlogging condition. The ERF family is a large family of transcription factors and part of the AP2/ERF superfamily, which also contains the AP2 and RAV families (Riechmann *et al*. 2000). Currently, ERFs are reported as being controlled by miRNAs in the regulation of cell wall degradation during aerenchyma formation triggered by ethylene (Tavares *et al*. 2020). Furthermore, the ERF family is responsive to biotic stresses (Gu *et al*. 2000) and abiotic stresses (Dubouzet *et al*. 2003) in various plant species. *ERF1* was found to be an essential gene involved in the initial steps of pectin degradation during aerenchyma formation in sugarcane (Tavares *et al*. 2019), while *ERF91* exhibited a lack of oxygen in the first hours (24 and 48 h) followed by an increase after stress for 72 h (Pegoraro *et al*. 2013). *ERF113* was reported as a transcriptional activator involved in tolerance to abiotic stresses (Krishnaswamy *et al*. 2011), particularly to waterlogging stress. It also delays waterlogging-induced premature senescence by regulating stomatal closure and antioxidant enzyme activity (Liu *et al*. 2012). Therefore, in the case of oil palm roots under waterlogging stress or under the condition of hypoxia, *ERF1*, *ERF1B, ERF91,* and *ERF113* were expressed. Also, a high expression of *ERF1* and *ERF113* was similar to our previous study (Nuanlaong *et al*. 2020).

NAC and *MYB* are the other members of the *ERF* family (Tavares *et al*. 2019). *NAC29* was up-regulated at all times during the waterlogging treatments. The expression of *NAC29* appeared similar to our previous study (Nuanlaong *et al*. 2020), while *MYB1R1* was down-regulated in both types of oil palm roots. In general, *NAC* family genes play a role in abiotic stress response (Olsen *et al*. 2005). In *A. thaliana*, *ANAC102* was expressed in roots, shoots, and germinating seeds under low-oxygen stress (0.1 %) (Christianson *et al*. 2009). Additionally, in soybeans (PI408105A), *NAC2* is up-regulated in roots after waterlogging for 3 d and remains high after waterlogging for 7 d (Valliyodan *et al*. 2014), whereas *MYB1R1* is up-regulated in cotton leaves when responding to hypoxia conditions for 15 d (Zhang *et al*. 2017). However, our results resembled those of Shin *et al.* (2011), who reported that *StMYB1R-1* was enhanced in response to several environmental stresses but was unaffected by biotic stresses. Moreover, in sugarcane, the expressions of *NAC* and *MYB* in full nutrient and nutrient starvation show no significant difference (Tavares *et al*. 2019). These two genes are considered possible regulators of lignin and phenylpropanoid biosynthesis, a cell wall component (Nakano *et al.* 2015, Ferreira *et al.* 2016, Soler *et al*. 2016).

WRKY, another gene in the TF group, has been reported to have an important role in abiotic stress by interacting with hormone signaling pathways (Birhanu 2014, Aamir *et al*. 2017). In particular, transcriptions of *OsWRKY11* and *OsWRKY56* are involved in the submergence stress and cause aerenchyma development (Viana *et al*. 2018). Also, this study found a high relative mRNA expression of *WRKY4* in both roots.

In the HSF family, *HSFA2* is known to positively regulate plant tolerance to salt stress or osmotic stress, oxidative stress, heat stress, and anoxia (Zhuang *et al*. 2018). It is strongly induced by anoxia and heat stress in *A. thaliana*, which indicates that HSFs may play a role in survival under low oxygen conditions (Loreti *et al*. 2005). Hence, *HSFA2C* showed a high relative mRNA expression after waterlogging stress for 3 d.

The transcription factors of most of the bHLH-regulated metabolic processes are also related to the regulation of various abiotic stresses (Castilhos *et al*. 2014). In our study, *bHLH79* was down-regulated in both roots. *bHLH79* encoded the basic helix-loop-helix protein 79 and was involved in DNA-binding transcription factor activity. A previous study reported that TF *Glyma17g10290*, encoding a *bHLH79*-like protein, was found to be induced 2.8-fold in the soybean seedlings of drought-sensitive cultivar W82 under dehydration stress (Hua *et al*. 2018). In addition, *bHLH94* was down-regulated in the primary roots, but up-regulated in the secondary roots after waterlogging stress for 1, 3, and 7 d. In wheat, *OsbHLH094* interacts with jasmonic acid to mediate salt-stress sensitivity. In contrast, in sugarcane roots during osmotic stress, *bHLH94* is down-regulated (Pereira-Santana *et al*. 2017). As there was no previous report on the expression of *bHLH79* and *bHLH94* under waterlogging conditions, our findings are thus considered new information. Following our observation, the change in root morphology was in correlation with gene expression in this study and in our previous study (Nuanlaong *et al*. 2020). Although the oil palm genotype and the waterlogging time were different, most of the genes were expressed similarly. Thus, this study provided a better understanding of the mechanism of lysigenous aerenchyma formation on the anatomical and molecular levels of waterlogging in oil palm roots.

Conclusions

Under waterlogged conditions, the entry of oxygen from the atmosphere into the soil is impeded by restricted gas exchange that results in low oxygen content, whereas ethylene content in roots is elevated due to the restricted gas exchange. In oil palm roots, lysigenous aerenchyma was found to have originated in both primary and secondary roots, but it occurred more frequently in the primary roots. Also, primary roots at 1.0 - 2.0 cm developed more lysigenous aerenchyma than in the other positions. Hence, the change in morphology of primary root was an important adaptation mechanism for survival in flooding conditions in oil palms. This was confirmed by the higher gene expression involved in waterlogging stress in primary roots than in secondary roots.

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