Expression Analysis of *OsVTC1-1* Gene in Ascorbic Acid Biosynthesis Pathway during Rice Blast Fungus Infection

Kanyanat Loedkunchotiphat, Apinya Longya and Chatchawan Jantasuriyarat[1]*

Department of Genetics, Faculty of Science, Kasetsart University,

Bangkhen Campus, Ladyao, Chatuchak, Bangkok, 10900

Sureeporn Kate-Ngam

Department of Agronomy, Faculty of Agriculture, Ubon Ratchathani University,

Muang Si Khai, Warin Chamrap, Ubon Ratchathani 34190

Abstract

Rice (*Oryza sativa*) is an important staple food crop in the world especially in Asia. Nowadays, the rice yields in Thailand have lower productivity due to many factors such as fungal diseases. *Magnaporthe oryzae* also known as a rice blast fungus, is an important plant pathogen that causes lesions in all parts of the plant. Ascorbic acid (AsA) or vitamin C is a powerful antioxidant and plays an important role in the plant defensive response. Previous study showed that GDP-D-mannose pyrophosphorylase (*GMP* or *VTC1*) is a key gene in ascorbic acid synthesis pathway. In this experiment, we aim to study the expression level of *OsVTC1-1* gene during rice blast fungus infection. Two isolates of rice blast fungus that can and cannot infect Jao Hom Nin were used. Leave samples were collected at 0, 6, 12, 24 and 36 hours after inoculation. Then, the expression level of *OsVTC1-1* gene was examined using semi-quantitative RT-PCR. The results showed that *OsVTC1-1* gene was expressed at the highest level at 12 hours after inoculation and decreased to normal levels after 12 hours. The pattern of this gene expression showed the same in both resistance and susceptible reaction, which indicated that AsA may play a role in defense response upon fungal infection. *OsVTC1-1* gene was cloned to study gene structure and genetic relationship among plant species. This gene consists of 1,086 bp and encodes 362 amino acid residues. The phylogenetic tree revealed that *OsVTC1-1* was grouped into two groups, monocotyledon and dicotyledon. Our results suggested that *OsVTC1-1* is conserved throughout plant kingdom and may play an important role in rice blast fungus defense response.

Keywords: ascorbic acid; blast disease; L-galactose pathway; rice; vitamin C

[1]*Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart (CASTNAR, NRU-KU), Kasetsart University, Bangkhen Campus, Ladyao, Chatuchak, Bangkok, 10900*

**Corresponding author: fscicwj@ku.ac.th*

1. Introduction

Rice (*Oryza sativa* L.) is one of the world's most important food crops. Nearly all (90 %) world's rice was produced in Asia. It belongs to the family Poaceae or Gramineae, of the genus *Oryza* (Ohyanagi *et al*., 2015). Rice has long been Thailand's traditional food crop and the country's main export product. The United States Department of Agriculture (USDA) (2017) reported that Thai's rice production in 2017-2018 is forecast at 19.5 million metric tons, up 5 percent from the previous year (Prasertsri, 2017). There are many varieties of Thai rice, such as Khao Dawk Mali 105 or KDML105, Gor-Kor 15 or RD15 and Jao Hom Nin (JHN) (Titapiwatanakun, 2012). However, JHN is Thai commercial non-glutinous rice variety, which shows a broad-spectrum resistance to blast disease (Sreewongchai *et al*., 2009). Therefore, this variety may have some mechanisms to defense against infection by the blast fungal pathogen.

Rice blast caused by the fungus, *Magnaporthe oryzae*, is the most important diseases, affecting the yield loss in rice (Wang and Valent, 2009). This fungus can attack on all above ground part of the plant and show the white to gray lesions. The lesions may enlarge and combine to kill the entire leaves. The yield losses are increasingly severe in East Asia and other countries with temperate climate around the world (Herdt, 1991).

Although, plants have impenetrable barriers, such as cuticles or, producing toxin to protect them from pathogens, the most effective protection mechanism is the hypersensitive reaction (HR), which prevents the spread of infection by the rapid death of cells or programmed cell death (PCD). This mechanism leads to the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and peroxide (O_2^{-2}) (Dutta *et al*., 2015). These molecules are toxic to pathogens, therefore it can prevent the pathogen growth and enhance the expression of resistant genes (Nanda *et al*., 2010).

Ascorbic acid (AsA) or vitamin C, is a strong antioxidant, a cofactor for enzyme, regulates growth and development of both animal and plant (Zhang, 2013). Currently, the L-galactose pathway or Smirnoff-Wheeler pathway is the important pathway in AsA synthesis of cereal plants (Imai *et al*., 2012), which GDP-D-mannose pyrophosphorylase (GMP or VTC1) is the first key enzyme in this pathway. It catalyzes the conversion of D-mannose-1-phosphate to GDP-D-mannose, a precursor for AsA synthesis. Previous report showed that AsA plays a crucial role in against pathogenesis in plants. The mutation of *vtc-1* gene in *Arabidopsis* activated the expression of pathogenesis-related genes (PR) suggested that ascorbate might involve with the plant defensive response (Kiddle *et al*., 2003), but their roles in rice blast disease resistance have not been demonstrated.

In this experiment, we evaluated the expression level of *OsVTC1-1* gene in AsA synthesis pathway during rice blast fungus infection. The results obtain from this study will

help to unravel the role and the involvement of vitamin C in plant defense response. This knowledge can also be used to facilitate the rice blast resistance breeding program in the future.

2. Materials and Methods

2.1 Plant materials and fungal materials

Jao Hom Nin (JHN), a non-glutinous Thai rice variety, was used in this experiment. Khao Dawk Mali 105 (KDML105), a famous Thai aromatic rice variety, was used as the susceptible variety or control. The rice seeds were soaked in room temperature for 7 days, then germinated and grown in soil. Three-week-old rice seedlings were used for rice inoculation.

Two rice blast fungal isolates namely: CCO56001 and TH196031, were used for the rice inoculation experiment. CCO56001 is collected from Chachoengsao of Thailand. It can cause blast disease on KDML105 but cannot cause blast disease on JHN. On the other hand, TH196031 is collected from Ubon Ratchathani of Thailand, can cause blast disease on both KDML105 and JHN.

2.2 Fungal culture and rice inoculation

Each of the fungal isolates was cultured on rice flour agar (RFA; Rice 2.0 %, agar 2.0 % and yeast extract 0.4 %) in the 9-cm petri dish and incubated the fungus in the incubator at 28 ˚C for 1 week. The fungus was sub-cultured after 1 week, scrapped the surface of fungus mycelium using spreader glass, and transferred to an ultraviolet-light chamber for 2 days to enhance sporulation. Then, the fungal conidia were scraped off the fungal surface using spreader glass. The fungal spore solution was mixed with 7.5 mL of 2.5 % (v/w) gelatin solution and 5 mL of distilled water. Spores were counted under the light microscope using hemocytometer and adjusted the concentration to 5×10^4 conidia/mL. The 20 mL of spore solution was used to spray a rice seedling. The inoculated rice was kept in the dark at 80-100 % humidity 25 ˚C for 24 hours and moved to the greenhouse. The leaf samples were collected at 0, 6, 12, 24 and 36 hours after inoculation by cutting rice leaves and immediately immerse in the liquid nitrogen. The samples were stored at -80˚C until RNA extraction step.

2.3 RNA extraction and cDNA synthesis

Total RNA was extracted from the leaf samples using RNA Extraction Kit (Vivantis, USA). Extraction was performed as described in the manufacturer's instructions. Each RNA samples was quantified using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Two µg of total RNA were converted to cDNA using the MMuLV Reverse Transcriptase (Biotechrabbit, Germany) according to the manufacturer’s instructions. cDNA solution was diluted to 1 in 20 for the gene expression analysis.

2.4 Gene expression analysis by semi-quantitative RT-PCR

cDNAs were used as a template for RT-PCR amplification with *OsVTC1-1*_F (5′-GTC ATGTGAACTAACCCTCC-3′) and *OsVTC1-1*_R (5′-GAGTTTCTTCTGGTCCTCTTG-3′) primers (Qin *et al*., 2016). Total volume of reaction was 10 µL. PCR reaction includes 0.5 µL of cDNA,

10 mM dNTP mix, 10x Buffer A, 50 mM $MgCl_2$, 0.2 U *Taq* DNA polymerase enzyme (Vivantis, USA) and dH_2O. PCR Reactions were performed using following condition by initial denaturation at 95 ˚C for 5 min, followed by 30 cycles of denaturation at 95 ˚C for 15 seconds, annealing at 58 ˚C for 15 seconds off, extension at 72 ˚C for 15 seconds and a final extension at 72 ˚C for 7 minutes. The PCR products were observed using 1 % agarose gel electrophoresis at 100 Volts for 35 minutes. The agarose gels were photographed under ultraviolet light. *OsActin* gene (XM_015774830.1) was used as an internal control to normalize gene expression. *OsActin*_F (5′-TCCATCTTGGCATCTCTCA-3′) and *OsActin*_R (5′-GTACCCTCATCAGGCAT CTG-3′) primers were used for amplification *OsActin* gene. PCR reaction was performed 28 cycles. Each experiment was performed at least twice with two different biological samples.

2.5 *OsVTC1-1* gene cloning

From the gene analysis results, the highest expression of *OsVTC1-1* gene was at 12 hours after inoculation therefore, we used cDNA of this time for gene cloning. PCR reaction was performed same as previous experiment with a total volume of 50 µL reaction. *OsVTC1-1*_F (5′-ATGAAGGCGCTCATTCTTGTTG-3′) and *OsV TC1-1*_R (5′-TCACATGACAATCTCAGGCT-3′) primers were used for amplification coding sequences of *OsVTC1-1* gene. The PCR products were observed using 1 % agarose gel electrophoresis at 100 Volts for 35 minutes. Then, the specific PCR band was cut and purified using GF-1 AmbiClean Kit (Gel & PCR) (Vivantis, USA). *OsVTC1-1* gene was cloned into pGEM®-T Easy vector (Promega, Wisconsin, USA) and transformed to *E. coli* DH5α by heat shock. The recombinant clones were selected using ampicillin 100 mg/mL, 0.1 M IPTG and 20 mg/mL X-Gal. The correct clones were checked using PCR with T7 (5′-AATACGACTCACTAT AG-3′) and SP6 primer (5′-ATTTAGGTGACAC TATAG-3′). PCR products were submitted for sequencing by Macrogen, Inc. (Korea). After that, the nucleotide sequences were aligned with NCBI database for construct gene structure.

2.6 Phylogenetic analysis

Amino acid of *OsVTC1-1* gene of rice and other plant species were downloaded from NCBI database (Table 1). Multiple sequence alignment and sequence analysis were conducted by MAFFT software program (http:// mafft.cbrc.jp/alignment/server). The phylogenetic analysis was analyzed using Maximum likelihood methods. The robustness of the nodes was assessed by bootstrap proportion (BP) analysis computed from 1,000 replicates. The phylogenetic tree was built using the MEGA version 7.0 software program (http://www.mega software.net/) (Tamura *et al*., 2013).

3. Results

3.1 Gene expression analysis

OsVTC1 is a key gene in ascorbic biosynthesis pathway in plants. To examined the expression of *OsVTC1-1 gene* in rice. We collected leaf samples at 6, 12, 24 and 36 hours after inoculation with blast fungus. Then, the expression level was determined using semi-

quantitative RT-PCR. The results showed that the highest expression level of *OsVTC1-1* was at 12 hours after inoculation and then decreased to normal levels after 12 hours. The pattern of gene expression was similar in both resistance and susceptible reactions (Figure 1). These results suggested that *OsVTC1-1* involved in rice blast responsive systems.

Table 1 Data of amino acid sequences used in genetic relationship analysis of *OsVTC1-1* gene from 11 plant species.

No.	Common name	Scientific name	Accession No.
1	kiwi	*Actinidia latifolia*	ACN38266.1
2	Arabidopsis	*Arabidopsis thaliana*	NP_181507.1
3	peanut	*Arachis duranensis*	XP_015931420.1
4	pigeonpea	*Cajanus cajan*	XP_020208248.1
5	tea	*Camellia sinensis*	AGI78460.1
6	soybean	*Glycine max*	ACW84415.1
7	rice	*Oryza sativa*	VTC1-1; XP_015622139
			VTC1-3; XP_015632712
			VTC1-8; Q6Z9A3
8	tomato	*Solanum lycopersicum*	NP_001234025.1
9	sorghum	*Sorghum bicolor*	VTC1-1; XP_021306382
			VTC1-3; XP_002456631
10	corn	*Zea mays*	VTC1-1; NP_001142215.1
			VTC1-3; NP_001306688
			VTC1-8; NP_001142302
11	jujube	*Ziziphus jujuba*	NP_001310790.1

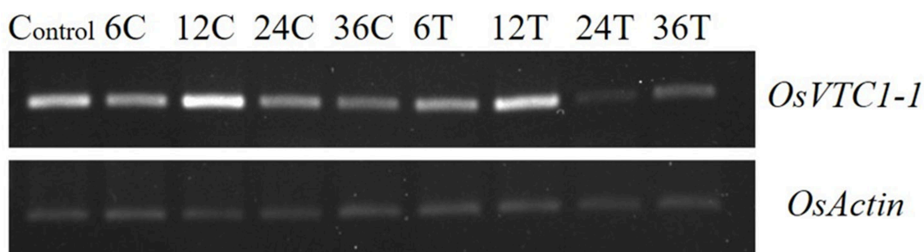

Figure 1 1 % agarose gel showing transcript levels of *OsVTC1* genes in different times including; 6, 12, 24 and 36 h after inoculation with two rice blast fungus isolates (C; CCO56001 and T; TH196031). *OsActin* gene was used as internal control.

3.2 Gene structure of *OSVTC1-1* gene

OsVTC1-1 gene showed the highest transcript level at 12 hours after inoculation with blast fungus. We used cDNA of this time point for gene cloning and submitted for the sequencing. The nucleotide sequence was aligned with rice genome database for construct gene structure. The result showed that *OsVTC1-1* gene located on chromosome 1 of rice. The nucleotide length of this gene is 1,086 bp, consists of 4 exons and 3 introns and encodes for a 362 amino acid polypeptide chain (Figure 2).

```
CDS atgaaggcgctcattcttgttggaggcttcggcactcgccttcggcctttgacgctcagt 60
aa   M  K  A  L  I  L  V  G  G  F  G  T  R  L  R  P  L  T  L  S  20
CDS ttcccaaagcctcttgttgatttcgcgaacaagcccatgattctgcatcagattgaggcc 120
aa   F  P  K  P  L  V  D  F  A  N  K  P  M  I  L  H  Q  I  E  A  40
CDS ttgaaagaagttggagtaacagaagttgttttagccatcaactaccgaccggaggtaatg 180
aa   L  K  E  V  G  V  T  E  V  V  L  A  I  N  Y  R  P  E  V  M  60
CDS ctcaatttcctgaaggactttgaggataagcttggcatcacaatcacgtgttcccaagag 240
aa   L  N  F  L  K  D  F  E  D  K  L  G  I  T  I  T  C  S  Q  E  80
CDS actgagcccttgggaactgctggccctcttgctctagcaagggacaagcttgtggatgga 300
aa   T  E  P  L  G  T  A  G  P  L  A  L  A  R  D  K  L  V  D  G  100
CDS tctggtgagccattctttgtcctcaacagtgacgtcataagtgaatacccttttgctgag 360
aa   S  G  E  P  F  F  V  L  N  S  D  V  I  S  E  Y  P  F  A  E  120
CDS ctcataaaatttcacaagagccatggtggtgaggcaacgattatggtcaccaaggtggac 420
aa   L  I  K  F  H  K  S  H  G  G  E  A  T  I  M  V  T  K  V  D  140
CDS gaaccatcaaaatatggtgttgtggttatggaggaggtcactggaatggtggaaaaattt 480
aa   E  P  S  K  Y  G  V  V  V  M  E  E  V  T  G  M  V  E  K  F  160
CDS gttgagaaaccaaaaatatttgtaggcaacaagatcaatgcgggaatttacttgttgaat 540
aa   V  E  K  P  K  I  F  V  G  N  K  I  N  A  G  I  Y  L  L  N  180
CDS ccatctgtcctggaccgcatcgagctgaagccaacttcaattgagaaagaggtctttcct 600
aa   P  S  V  L  D  R  I  E  L  K  P  T  S  I  E  K  E  V  F  P  200
CDS cgaattgcatctgatgcaaagctctttgctctggtccttccaggtttttggatggatgtt 660
aa   R  I  A  S  D  A  K  L  F  A  L  V  L  P  G  F  W  M  D  V  220
CDS ggccaaccaagggattacattacaggcttgcgcctttatctggattcacttaggaagaga 720
aa   G  Q  P  R  D  Y  I  T  G  L  R  L  Y  L  D  S  L  R  K  R  240
CDS tcaaccaacaggttagccactggagcacacattgttgggaatgtgctagttcacgagagt 780
aa   S  T  N  R  L  A  T  G  A  H  I  V  G  N  V  L  V  H  E  S  260
CDS gccaagattggcgaaggctgtctgattggtcctgatgttgctatcggtcctggatgcgtc 840
aa   A  K  I  G  E  G  C  L  I  G  P  D  V  A  I  G  P  G  C  V  280
CDS gtggaggatggtgtgaggctttcccgttgcacggtgatgcgtggcgtgcacattaagaag 900
aa   V  E  D  G  V  R  L  S  R  C  T  V  M  R  G  V  H  I  K  K  300
CDS catgcttgcatatcaaacagcattattggatggcactcaactgttggacaatgggcacgg 960
aa   H  A  C  I  S  N  S  I  I  G  W  H  S  T  V  G  Q  W  A  R  320
CDS atagaaaatatgactatcctgggagaggacgtacatgtaggtgatgaggtctataccaac 1020
aa   I  E  N  M  T  I  L  G  E  D  V  H  V  G  D  E  V  Y  T  N  340
CDS ggcggtgttgttctcccgcacaaagagatcaagtcaagcatcctgaagcctgagattgtc 1080
aa   G  G  V  V  L  P  H  K  E  I  K  S  S  I  L  K  P  E  I  V  360
CDS atgtga 1086
aa   M  -  362
```

Figure 2 Gene structure of *OsVTC1-1* gene. (A) *OsVTC1-1* gene consists of 1,086 bp including 4 exons and 3 introns and (B) Coding sequence and amino acid sequence of *OsVTC1-1* gene. (-) refers to stop codon.

3.3 Phylogenetic analysis of *OsVTC1-1* gene

To investigate the genetic relationship of *OsVTC1-1* gene among 11 plant species, amino acid sequences of *VTC1* genes were used. A phylogenetic tree was constructed using Maximum likelihood methods of MEGA version 7.0 software program. The result revealed that phylogenetic tree based on *VTC1* sequences of 11 plant species were clustered into two groups, for instance, Group A and Group B. Group A comprised all dicotyledon species, such as kiwi (*Actinidia latifolia*), Arabidopsis (*Arabidopsis thaliana*), peanut (*Arachis duranensis*), pigeonpea (*Cajanus cajan*), tea (*Camellia sinensis*), soybean (*Glycine max*), tomato (*Solanum lycopersicum*) and jujube (*Ziziphus jujuba*). Group B composed of monocotyledon species, such as rice (*Oryza sativa*), sorghum (*Sorghum bicolor*) and corn (*Zea mays*). The genome of dicotyledon species contains only one copy of *VTC1* gene, whereas the genome of monocotyledon species consists of two to three copies of *VTC1* gene.

Rice (*Oryza sativa*) was grouped together with corn (*Zea mays*) and sorghum (*Sorghum bicolor*). Moreover, the phylogenetic tree showed that corn (*Zea mays*) and rice (*Oryza sativa*) encoded three homolog genes including *VTC1-1*, *VTC1-3* and *VTC1-8*. In contrast, sorghum (*Sorghum bicolor*) contains two homolog genes: *VTC1-1* and *VTC1-3.* From the three copies of *VTC1* in rice, *OsVTC1-1* and *OsVTC1-8* were close relationship to each other more than *OsVTC1-3* (Figure 3).

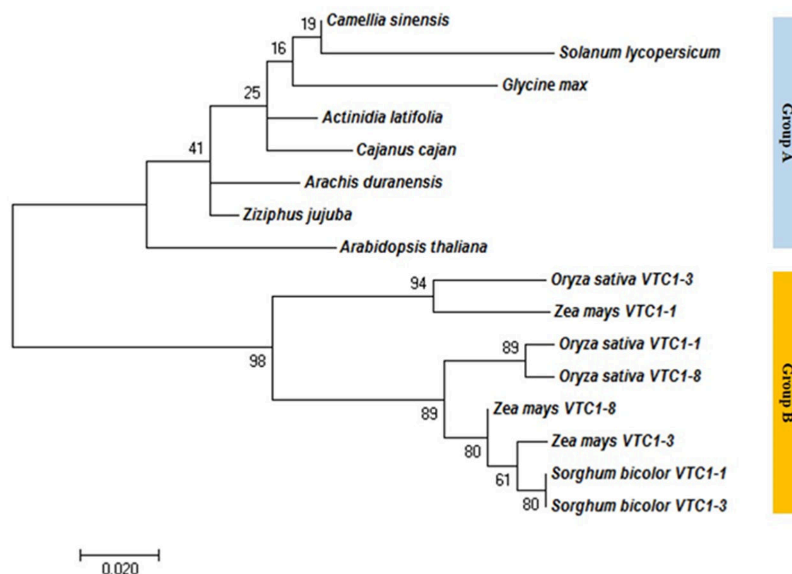

Figure 3 The phylogenetic tree of *VTC1* gene among 11 plant species using maximum likelihood methods of MEGA version 7.0 software program based on amino acids sequence. The common name and accession numbers are showed in follows (Table 1).

4. Discussion

One of the devastating diseases in rice production worldwide is blast disease. This disease caused by the ascomycete fungus *Magnaporthe oryzae*. It caused low production in rice. However, plants have many strategies to detect an attacker and stop growth before it damages to plants. Many plant species have modified cells to thorns, spines, trichomes, and prickles (Simpson, 2010). These structures can kill or inhibit the development of herbivores. The cell wall is also one of the barriers that pathogen need to penetrate to colonize in plant cells (Miedes *et al.*, 2014). Nonetheless, hypersensitive response (HR) is the most efficient defense response in plants. It regulates oxidative stress or accumulation of reactive oxygen species (ROS), which can inhibit the spread of pathogen (Nanda *et al.*, 2010).

In our study, we examined the expression of *OsVTC1* gene during rice blast fungus infection. This gene catalyzes the conversion D-mannose-1-phosphate to GDP-D-mannose, which is a precursor for ascorbic synthesis. The result revealed that *OsVTC1* had the highest transcript level at 12 hours post-inoculation. The time of the highest expression was correlated with the time that rice blast spores produce appressoria (fungal specialized structure use for the penetration of plant cell) and start penetrating the rice leave surface at 12 hours post inoculation. Our data was supported by the report of Rajput *et al*. (2017), which reported that spores of *M. oryzae* were developed to 6 hours and leads to produce appressoria, then germ tube were grown rapidly at 12 hours. The stress leads to the production of ROS, which are very toxic to plant cells. Similar to the study by Jo *et al*. (2006) that peroxidase which catalyzes the reaction of H_2O_2 increased at 12 hours after inoculation. Similarly, the expression of β-1,3-glucanase which can kill pathogens were also induced at 12 hours after inoculation. Thus, some defense mechanism may start action at this time point. Ascorbic acid is a strong antioxidant, may play an important role at this stage to protect cell from damage and help to defense against the oxidative stress. Moreover, previous studies showed that ascorbic acid is an important component in plant defense mechanism. The mutation of *vtc1* gene in *Arabidopsis* activated the expression of pathogenesis-related genes (PR) (Kiddle *et al*., 2003). Similarly, the *Arabidopsis* mutants, *vtc1* and *vtc2* showed resistance against pathogen after infection by *Pseudomonas syringae*. The mutants appeared microlesions and induced the expression of PR genes (Pavet *et al*., 2005). This suggested that ascorbic acid may involve in plant defense response.

From the gene structure of *OsVTC1* gene, it contains 1,086 bp in length and encodes for 362 amino acids similar to the other plant species. For phylogenetic tree analysis, rice *OsVTC1* was closely related to other monocot species. In monocot, *VTC1* contains of two to three homolog genes. Previous report by Qin *et al*. (2017) showed that different *VTC1* genes in rice expressed in different organs. They found that the expressions of *OsVTC1-1* and *OsVTC1-*

3 were highest in leaves, while the expression of *OsVTC1-8* was highest in roots. However, we found *OsVTC1-1* has the close relationship with *OsVTC1-8* but not close with *OsVTC1-3*. This founding might indicated that *OsVTC1-3* may play a different function from the *OsVTC1-1* and *OsVTC1-8* genes.

Our result revealed that *OsVTC1* gene had the highest expression at 12 hours post-inoculation. Therefore, this gene may involve in plant defense mechanism, which can lead to study of the function of the ascorbic acid synthesis genes during rice blast interaction. The results from this study may be useful for assisting the knowledge of crops breeding and improving the rice crops in the future.

5. Acknowledgements

This research was supported from graduate scholarship provided by the National Research Council of Thailand (NRCT) as of fiscal year 2018 and Development and Promotion of Science and Technology Talents Project (DPST).

6. References

Dutta, A., Chan, S.H.P., Pauli, N.T. and Raina, R., 2015, Hypersensitive response-like lesions 1 codes for *AtPPT1* and regulates accumulation of ROS and defense against bacterial pathogen *Pseudomonas syringae* in *Arabidopsis thaliana*, Antioxid. Redox Signal. 22: 785-796.

Herdt, R.W., 1991, Research priorities for rice biotechnology, In Khush, G.S., Toenniessen, G.H. (Eds.). Rice Biotechnology. Alden Press, Ltd., London, 35-37.

Imai, T., Ban, Y., Yamamoto, T. and Moriguchi, T., 2012, Ectopic overexpression of peach GDP-D-mannose pyrophosphorylase and GDP-D-mannose-3, 5′ epimerase in transgenic tobacco, Plant Cell Tiss Organ Cult. 111: 1-13.

Jo, Y.K., Wang, G.L. and Boehm, M.J., 2007, Expression analysis of rice defense-related genes in turfgrass in response to *Magnaporthe grisea*, Phytopathology 97: 170-178.

Kiddle, G., Pastori, G.M., Bernard, S., Pignocchi, C., Antoniw, J., Verrier, P.J. and Foyer, C.H., 2003, Effects of leaf ascorbate content on defense and photosynthesis gene expression in *Arabidopsis thaliana*, Antioxid. Redox Signal. 5: 23-32.

Miedes, E., Vanholme, R., Boerjan, W. and Molina, A., 2014, The role of the secondary cell wall in plant resistance to pathogens, Front. Plant Sci. 5: 1-13.

Nanda, A.K., Andrio, E., Marino, D., Pauly, N. and Dunand, C., 2010, Reactive oxygen species during plant-microorganism early interactions, J. Integr. Plant Biol. 52: 195-204.

Ohyanagi, H., Ebata, T., Huang, X., Gong, H., Fujita, M., Mochizuki, T., Toyoda, A., Fujiyama, A., Kaminuma, E., Nakamura, Y., Feng, Q., Wang, Z.X., Han, B. and Kurata, N., 2016, Oryzagenome: genome diversity database of wild *Oryza* species, Plant Cell Physiol. 57: 1-7.

Pavet, V., Olmos, E., Kiddle, G., Mowla, S., Kumar, S., Antoniw, J., Alvarez, M.E. and Foyer, C.H., 2005, Ascorbic acid deficiency activates cell death and disease resistance responses in *Arabidopsis*, Plant Physiol. 139: 1291-1303.

Prasertsri, P., 2017, Grain and Feed Annual, In USDA gain Report.

Qin, H., Deng, Z., Zhang, C., Wang, Y., Wang, J., Liu, H., Zhang, Z., Huang, R. and Zhang, Z., 2016, Rice GDP-mannose pyrophosphorylase *OsVTC1-1* and *OsVTC1-3* play different roles in ascorbic acid synthesis, Plant Mol. Biol. 90: 317-327.

Rajput, L.S., Sharma, T., Madhusudhan, P. and Sinha, P., 2017, Effect of temperature on rice blast infection process with emphasis on appressoria formation by *Magnaporthe oryzae*, Int. J. Curr. Microbiol. App. Sci. 6: 1931-1939.

Simpson, M.G., 2010, Plant Systematics. 2nd Ed., Elsevier Academic Press, China.

Sreewongchai, T., Sriprakhon, S., Wongsaprom, C., Vanavichit, A., Toojinda, T., Tharreau, D. and Sirithunya, P., 2009, Genetic mapping of *Magnaporthe grisea* avirulence gene corresponding to leaf and panicle blast resistant QTLs in Jao Hom Nin rice cultivar, Phytopathology 157: 338-343.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013, Mega6: Molecular Evolutionary Genetics Analysis Version 6.0, Mol. Biol. Evol. 30: 2725-2729.

Titapiwatanakun, B., 2012, The Rice Situation in Thailand, In ADB Technical Assistance Consultant’s Report, 1: 1-29.

Zhang, Y., 2013, Ascorbic acid in plants, Springer-Verlag, New York.