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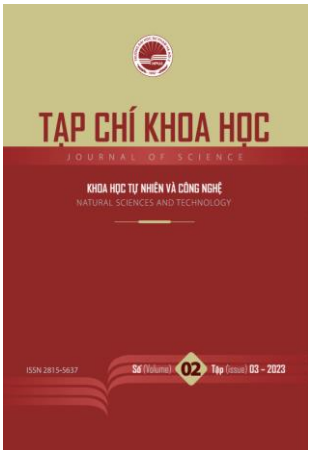

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Identification and characterization of genes encoding phosphoinositide-specific phospholipase C revealed role in drought stress condition in cassava (*Manihot esculenta*)

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Abstract

Phosphoinositide-specific phospholipase C (PI-PLC) has been known as one of the key enzymes that involved in the phospholipid hydrolysis. However, the PI-PLC family in cassava has not been fully recorded. In this study, a comprehensive analysis of the PI-PLC family in cassava assembly has been performed based on various bioinformatics tools. Particularly, a total of seven members of the PI-PLC family has been identified and annotated in the cassava genome. By using the full-length protein sequence of each member of the PI-PLC family in cassava, the properties of these proteins, including the length, size, iso-electric point, instability index, aliphatic index and grand average of hydropathy were analyzed. More interestingly, the expression patterns of genes encoding the PI-PLC family in various major organs/tissues in different conditions were investigated. Taken together, this current study could provide a solid foundation for the PI-PLC family in cassava for further functional characterization towards the improvements of drought stress tolerance in cassava plants.

Keywords: Cassava, identification, gene expression, phosphoinositide-specific phospholipase C, drought stress

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1. Introduction

Cassava (*Manihot esculenta*), a perennial shrub indigenous to the South American region [1, 2], manifests itself as an indispensable alimentary and industrial resource in numerous tropical locales worldwide [1, 3]. Serving as a cardinal source of carbohydrates, it underpins the nutritional framework of over half a billion individuals, particularly in Africa and Asian territories, thus offering a bulwark against pervasive food insecurities [4, 5]. Beyond its nutritional valence, cassava is esteemed for its versatile starch, which finds multifaceted applications across varied sectors, including, but not limited to, the food, textile, and adhesive industries [5-7]. This tuberous plant, through its historical and contemporary significance, perpetuates its role as a linchpin, anchoring both dietary and industrial applications in numerous global contexts. However, the effects of water limitation on the growth and development of cassava can be both multifaceted and profound, given the plant's inherent susceptibility and responsiveness to variations in its growth environment [8]. While cassava is often lauded for its drought-tolerance relative to other staple crops, protracted periods of insufficient rainfall or moisture can deleteriously impact its physiological processes, morphological characteristics, and overall productivity. In Vietnam, cassava plays an important socio-economic role as a secondary crop. In the North, the crop is an important source of food and feed at the household level; in the south mainly as a source of cash income. Cassava and cassava products are one of 13 key agricultural products for export of Vietnam with an export turnover of 1.35 billion USD/year, ranking 2nd in the world, only after Thailand. Thus, it would be significant to understand the molecular mechanism of cassava plant growth, development and regulation of responses to adverse environmental conditions.

In plants, phospholipids have been demonstrated to be an important component of cytoplasmic membranes [9], and they play a key role in various biological processes [10, 11]. Particularly, phospholipases, including phospholipase A1, phospholipase A2, phospholipase C (PLC) and phospholipase D, are responsible for phospholipid hydrolysis [11]. Among them, PLCs are classified into two sub-families based on their substrates, namely phosphatidylinositol specific PLC (PI-PLC) and non-specific PLC [12, 13]. Phosphatidylinositol 4,5-bisphosphate can be hydrolyzed by PI-PLC to produce inositol triphosphate and diacylglycerol, which then release cellular Ca^{2+} and activate protein kinase C [13]. Great efforts have been made to report the functions of PI-PLC in plant growth and development and members of the PI-PLC families were identified and characterized in various higher plant species, like *Arabidopsis thaliana* [14], rice (*Oryza sativa*) [15, 16], soybean (*Glycine max*) [17], maize (*Zea mays*) [18], cotton (*Gossypium* spp.) [19] and wheat (*Triticum aestivum*) [20]. However, the PI-PLC proteins in cassava has not been reported.

The aim of this recent study was to provide a comprehensive analysis of the PI-PLC proteins in cassava under developmental and drought stress conditions. Particularly, all putative PI-PLC proteins were identified in the cassava assembly based on the bioinformatics tool. The protein features of each member of the PI-PLC family in cassava were then analyzed. The expression patterns of genes encoding the PI-PLC family in various organs were explored based on the previous transcriptome atlas.

2. Materials and Methods

2.1. Materials

Well-characterized PI-PLC proteins in *Arabidopsis* available in the previous report [14] were downloaded for further screening and validation of the PI-PLC proteins in cassava.

Recent cassava assembly (NCBI RefSeq assembly: GCF_001659605.2), including genome and proteome [21] in the Phytozome [22] and NCBI databases was used for all *in silico* analyses in this study.

Available transcriptome databases of cassava plants, including GSE82279 [23] and GSE98537 [33] available in the GEO NCBI [24] portal were explored to analyze the expression profiles of genes encoding the PI-PLC family in cassava.

2.2. Screening and validation of the PI-PLC proteins in cassava

The well-characterized PI-PLC proteins in *Arabidopsis* were used to perform a Blast search against the current cassava assembly [21] in the Phytozome [22]. All obtained proteins were then validated by the Pfam tool [25]. Annotation of each member of the PI-PLC family in cassava, including gene identifier (geneID), protein identifier (proteinID), transcript identifier (transcriptID) and locus identifier (locusID) were obtained by exploring in the NCBI database. Finally, the full-length protein sequence, genomic DNA sequence and coding DNA sequence of each member of the PI-PLC family were collected for further characterization.

2.3. Analysis of the general properties of the PI-PLC proteins in cassava

Full-length protein sequence of each member of the PI-PLC family in cassava was used to analyze the physico-chemical features as previously reported [26, 27]. Particularly, the Expasy Protparam tool [28, 29] was used to analyze several general characteristics of each protein sequence, including size (amino acid residues), mass (kDa), theoretical iso-electric point (acidic, neutral and basic), instablity index (instability and stability), aliphatic index and grand average of hydropathy (hydrophilic and hydrophobic).

2.4. Analysis of expresison profiles of genes encoding the PI-PLC proteins in cassava

To analyze the expression levels of genes encoding the PI-PLC family in cassava, the previous RNA-Seq datasets were explored in the NCBI GEO [30] as previously described [31, 32]. Particularly, the GSE82279 dataset reported in the previous study [23] was downloaded to re-analyze the expression patterns of genes encoding the PI-PLC family during the growth and development processes. Gene identifier of each member of the PI-PLC family in cassava was used to search its corresponding expression levels in major organs/tissues, including leaf blade, leaf mid-vein, petiole, stem, lateral bud, storage root, fibrous root [23]. Additionally, the GSE98537 dataset related to drought stress condition as previously described [33] was also analyzed. More specifically, leaf samples were collected for drought treatment [33]. The fold-change of each gene was estimated by comparing between drought treatment and control [33]. A heat map of all expression levels in these datasets were illustrated by using the R script.

3. Results and Discussion

3.1. Survey of the PI-PLC family in cassava

In order to identify the PI-PLC proteins in cassava, well-characterized PI-PLC members in *Arabidopsis* were used search against the current assemblies [21] in the Phytozome [22] and NCBI databases. As the results, a total of seven members of the PI-PLC family has been found in the cassava assembly (Table 1). All annotations of each member of the PI-PLC family, including geneID, transcriptID, proteinID and locusID were then fully provided in Table 1.

Table 1. Annotation of the PI-PLC family in cassava

#	GeneID	TranscriptID	ProteinID	LocusID
1	Manes.01G161000	XM_021764955	XP_021620647	LOC110620987
2	Manes.02G118900	XM_021747892	XP_021603584	LOC110608625
3	Manes.02G119000	XM_021747838	XP_021603530	LOC110608574
4	Manes.02G119100	XM_021750019	XP_021605711	LOC110610146
5	Manes.04G025600	XM_021755289	XP_021610981	LOC110613893
6	Manes.10G012700	XM_021770314	XP_021626006	LOC110624891
7	Manes.10G012800	XM_021769869	XP_021625561	LOC110624649

Previously, a number of the PI-PLC families has been identified in higher plant spcies. For example, at least six members of the PI-PLC family, namely from AtPI-PLC01 to AtPI-PLC07 have been found in *Arabidopsis* [14]. A total of four PI-PLC proteins was identified in rice [15, 16], while the PI-PLC family in maize contained five members [18]. Recently, 12 members of the PI-PLC family have been reported in cotton [19], while 11 members of the PI-PLC family in wheat have been well-characterized [20]. In constrast, very few members have been reported in chlorophyta [19]. Specifically, only one PI-PLC protein were found to exist in *Ostreococcus lucimarinus*, *Chlamydomonas reinhardtii* and *Volvox carteri* [19]. In moss species, a total of seven members of the PI-PLC family has been found in *Physcomitrella patens* [19]. In this study, according to the recent assembly of cassava, seven members of the PI-PLC family were found (Table 1). Taken together, this analysis revealed that the PI-PLC proteins in higher plant species is multi-gene family.

3.2. Properties of the PI-PLC family in cassava

In order to investigate the features of the PI-PLC family in cassava, the full-length protein sequence of each member was analyzed by using the Expasy Protparam tool [28, 29] as previously reported [26, 27]. As the results, six common properties of proteins, including protein size, protein mass, theoretical iso-electric point, instablity index, aliphatic index and grand average of hydropathy were explored. Table 2 provided a detailed information of the characteristics of the PI-PLC family in cassava.

Table 2. Characteristics of the PI-PLC family in cassava

#	PI-PLC family	Size	Mass	pI	II	AI	GRAVY
1	Manes.01G161000	584	66.76	7.14	39.61	72.07	-0.54
2	Manes.02G118900	568	65.42	6.75	49.32	78.93	-0.51
3	Manes.02G119000	594	67.25	7.91	40.68	75.32	-0.46
4	Manes.02G119100	615	70.19	6.81	39.53	76.08	-0.57
5	Manes.04G025600	539	61.26	5.12	42.16	81.74	-0.42
6	Manes.10G012700	592	67.70	6.22	50.60	82.33	-0.46
7	Manes.10G012800	600	68.78	8.23	48.59	76.53	-0.51

Note: pI - Theoretical iso-electric point, II - Instability index, AI - Aliphatic index, GRAVY - Grand average of hydropathy.

According to Table 2, the protein size of the PI-PLC family in cassava ranged from 539 (Manes.04G025600) to 615 (Manes.02G119100) amino acid residues. The PI-PLC family in cassava were varied from 61.26 (Manes.04G025600) to 70.19 (Manes.02G119100) kDa in masses (Table 2).

Next, four (out of seven) members of the PI-PLC family in cassava, including Manes.02G118900, Manes.02G119100, Manes.04G025600 and Manes.10G012700 exhibited scores of iso-electric point less than 7.0 (acidic), whereas three remaining PI-PLC proteins, including Manes.01G161000, Manes.02G119000 and Manes.10G012800 were basic (iso-electric point more than 7.0) (Table 2). Based on the scores of instablity index, it has been found that only two members of the PI-PLC family in cassava, including Manes.01G161000 and Manes.02G119100 were stable (instablity index less than 40.0), whereas five (out of seven) members of the PI-PLC family in cassava, including Manes.02G118900, Manes.02G119000, Manes.04G025600, Manes.10G012700 and Manes.10G012800 were unstable in the test tube (instablity index more than 40.0) (Table 2). Additionally, the scores of aliphatic index of all members of the PI-PLC family in cassava were varied from 72.07 (Manes.01G161000) to 82.33 (Manes.10G012700) (Table 2). Finally, the grand average of hydropathicity values of all proteins were less than 0 indicated that all members of the PI-PLC family in cassava are hydrophilic (Table 2).

Previously, characteristics of the PI-PLC families in higher plant species have been comprehensively reported. For example, the PI-PLC proteins in wheat ranged from 585 - 733 amino acid residues, with masses of 65.7 to 71.1 kDa [20]. The iso-electric points of the PI-PLC proteins in wheat were less than 7.0, suggested that these proteins were slightly acidic [20]. The grand average of hydropathy of the PI-PLC proteins were also less than 0, revealing that members of the PI-PLC family in wheat were hydrophilic [20]. Similarly, the protein sizes of the PI-PLC proteins in rice were reported to be comparable, ranging from 591 - 599 amino acid residues, except for OsPLC2 (491 amino acid residues) [15]. In cotton, the PI-PLS proteins were varied from 541 to 1076 amino acid residues in lengths and from 61.44 to 122.50 kDa in weights [19]. Only five (out of 12) PI-PLC proteins in cotton were basic (iso-electric points more than 7.0), whereas seven (out of 12) PI-PLC proteins were acidic (iso-electric points less than 7.0) [19]. Taken together, our comparisons suggested that the PI-PLC families in higher plant species exhibited a slightly variable characteristics.

3.3. Expression patterns of genes encoding the PI-PLC family in cassava during the developmental process

In order to get insight into the function of genes encoding the PI-PLC family in cassava, their expression levels in various organs/tissues during the growth and development of cassava plants were re-analyzed based on the available RNA-Seq dataset [23]. As provided in Figure 1, all genes encoding the PI-PLC family in cassava exhibited diferential expression levels in various major organs/tissues. Particularly, the expression patterns of three genes, including *Manes.01G161000*, *Manes.02G118900* and *Manes.02G119000* in all tested organs were not expressed or below the limits of detectable expression (Figure 1). It found that *Manes.02G119100* was tend to express in lateral bud, while *Manes.04G025600* was noted to be mainly expressed in leaf blade and leaf mid-vein samples (Figure 1). Interestingly, two remaining genes, *Manes.10G012700* and *Manes.10G012800* were specifically expressed in lateral bud and storage root (Figure 1).

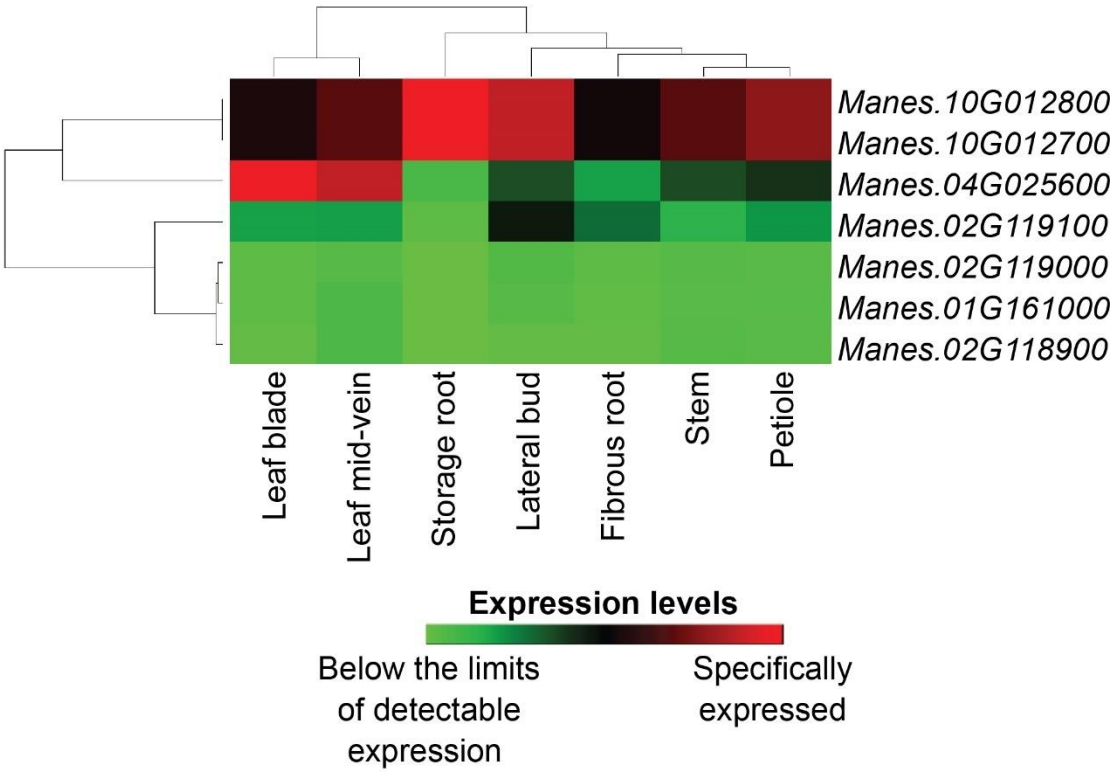

Figure 1. Expression profiles of genes encoding the PI-PLC family in various organs of cassava plants

Previously, the expression patterns of genes encoding the PI-PLC families in different tissues and organs have been performed in the previous studies. For example, four genes encoding the PI-PLC family in wheat, namely *TaPI-PLC1-2A*, *1-2B*, *1-2D* and *2-1D* were specifically expressed in root and stem tissues, whereas three genes, including *TaPI-PLC4-5A*, *4-5B* and *4-5D* were noted to be mostly expressed in root, stem and grain samples [20]. Additionally, genes encoding the PI-PLC family in cotton had different expression patterns in main organs [19]. Particularly, two genes, like *GhPIPLC5* and *11* were mostly expressed in all tested tissues. It has been realized that four genes encoding the PI-PLC family in cotton, including *GhPIPLC1*, *2*, *6*, *7* were weakly expressed in the petal, stamen and 20 days post anthesis fiber, whereas *GhPIPLC4* gene was preferentially expressed in the 5 days post anthesis fiber and stem [19]. Our re-analysis suggested that genes encoding the PI-PLC family might play important roles in the organ development of cassava plants.

3.4. Expression patterns of genes encoding the PI-PLC family in cassava in response to drought stress

It has been believed that the PI-PLC proteisn may play a key role in various biological processes, particularly in regulation of stress responses [14-20]. Thus, it would be very interesting to investigate the potential function of genes encoding the PI-PLC family in cassava related to stress conditions. Here, the GSE98537 dataset related to drought stress condition was re-analyzed as previously described [33]. All expression patterns of genes encoding the PI-PLC family in drought-treated leaf samples were then provided in Figure 2.

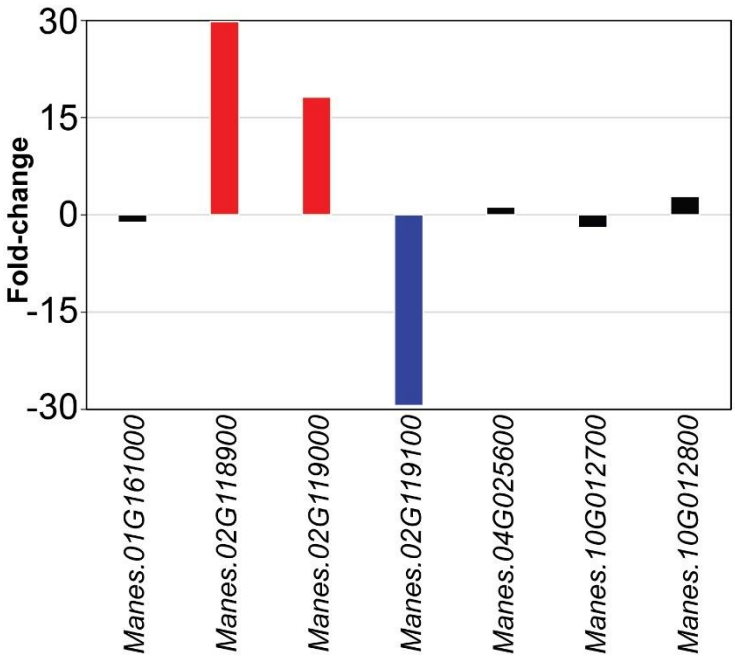

Figure 2. Expression profiles of genes encoding the PI-PLC family in leaves under the drought stress condition

Paricularly, it has been revealed that two genes encoding the PI-PLC family in cassava, including *Manes.02G118900* and *Manes.02G119000* were up-regulated in leaves under drought stress condition (Figure 2). Only one gene, namely *Manes.02G119100* was noted to reduce in drought-treated leaf samples (Figure 2). Meanwhile, four remaining genes encoding the PI-PLC family in cassava, including *Manes.01G161000*, *Manes.04G025600*, *Manes.10G012700* and *Manes.10G012800* were not differentially expressed in leaf samples under the drought stress condition (Figure 2). To sum up, this current study proposed three drought-responsive genes, including *Manes.02G118900*, *Manes.02G119000* and *Manes.02G119100* for further functional characterizations.

Previously, two genes encoding the PI-PLC family in cotton, like *GhPIPLC5* and *11* exhibited no significant changes under stress conditions [19]. Two other genes, like *GhPIPLC1* and *6* were weakly expressed under cold stress, whereas *GhPIPLC2* and *7* were up-regulated under heat stress [19]. Next, *GhPIPLC8* was preferentially expressed under salt and drought stress conditions [19]. In *Arabidopsis*, two genes, including *AtPIPLC3* and *9* have been demonstrated to play roles in heat tolerance [14]. In the case of wheat plants, the expressions of *TaPI-PLC1-2B*, *2-1D* and *3-4A* were induced in leaf samples under drought stress condition [20]. Taken together, the different levels of expression under various stress conditions suggested that genes encoding the PI-PLC families differ in their responses and regulatory mechanisms when exposed to conditions of adverse environmental conditions.

4. Conclusions

In this study, a total of seven members of the PI-PLC family has been identified and well-characterized in cassava based on various bioinformatics tools. Based on the full-length protein sequences, this study demonstrated that the PI-PLC members in cassava were varied from 539 to 615 amino acid residues in lengths and 61.26 to 70.19 kDa in weights. The scores of iso-electric point, instability index and aliphatic index of the members of the PI-PLC family in cassava were variable. All PI-PLC proteins in cassava were hydrophilic. Interestingly, the expression profiles of genes encoding the PI-PLC family were greatly variable in various organs/tissues in cassava plants. Among

them, *Manes.02G118900* and *Manes.02G119000* were induced in drought-treated leaf samples, whereas *Manes.02G119100* was reduced in drought-treated leaf samples.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

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