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Preliminary *In Silico* Analysis of *CHS1* Gene in Commelinids Clade: Family Zingiberaceae, Costaceae, and Poaceae

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ABSTRACT

The chalcone synthase (*CHS*) gene families are known to be conserved in plants and have been well-studied in many plants, and they have an important role in the physiological and biological processes of plants. One of the studied *CHS* gene families is the *CHS1* gene. *CHS1* gene is known for its function in the flavonoid biosynthetic pathway. However, not many studies have been reported on the *CHS1* gene in the Commelinids clade, especially the evolution of this gene within three families: Zingiberaceae, Costaceae, and Poaceae. Thus, this study aimed to perform a preliminary *in silico* comparative analysis of the *CHS1* gene across these three families. Through this *in silico* comparative analysis, 20 partial sequences of the *CHS1* gene, which are restricted to 565 bp regions, were analysed. The partial sequences were extracted from the National Center for Biotechnology Information database comprised of 16 Zingiberaceae species, three Costaceae species, and one Poaceae species. From the analysis, these targeted regions showed a low polymorphic site (18.23%) with 103 positions of single nucleotide polymorphisms and three mutations (substitution, insertion, and deletion). Meanwhile, phylogenetic analysis showed no clear evolutionary pattern within the three studied families. In conclusion, the studied partial sequences of the *CHS1* gene in Zingiberaceae, Costaceae, and Poaceae showed that the gene is conserved within the Commelinids clade. Further studies to understand the consequences of low polymorphism and mutations as well as adaptive evolution in the *CHS1* gene,

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accompanied by biochemistry and gene expression studies, should be done in these 20 species of Zingiberaceae, Costaceae, and Poaceae.

Keywords: *CHS1* gene, commelinids, Costaceae, evolution, Poaceae, Zingiberaceae

INTRODUCTION

A group of genes can be grouped into a family according to their high sequence similarity, but adaptation or speciation can contribute to gene diversification (gene polymorphism) and evolution (Lynch & Conery, 2000; Nei & Rooney, 2005). The mutation is a factor that induces gene diversification and evolution. Gene diversification has largely arisen from duplication followed by functional divergence (Reams & Roth, 2015). Many duplicates are immediately lost during a gene duplication event because mutations accumulate in duplicated genes with redundant functions (Innan & Kondrashov, 2010). However, some mutations can lead to a higher degree of functional divergence of duplicates, which can be advantageous for adaptive evolution (Ezoe et al., 2021), consistent with the neutral theory of molecular evolution (Kimura, 1983). Gene divergence could be measured in multigene families through unequal crossing-over rate, mutation rate, gene conversion rate, and selection coefficient of the locus (Matsuo & Yamazaki, 1989).

The chalcone synthase (*CHS*) gene families are among the important genes continuously studied in many plants to understand their evolutionary patterns (Durbin et al., 2000; Glagoleva et al., 2019). The *CHS* gene families express enzymes that belong to type III polyketide synthases and are involved in flavonoid biosynthesis (Roslan, Huy, Kee et al., 2020; Roslan, Huy, Ming et al., 2020; Yuan et al., 2021). The *CHS* genes have been studied in many plants and showed up to 60% homologues sequences (Jiang & Cao, 2008). The *CHS* genes have been studied in *Arabidopsis thaliana* (Dao et al., 2011), *Juglans regia* (Cheniany et al., 2012), *Oncidium* Gower Ramsay (Liu et al., 2012), *Malus domestica* (Dare et al., 2013), *Garbera hybrida* (Deng et al., 2014), *Triticum aestivum* (Trojan et al., 2014), and some species of Zingiberaceae such as *Curcuma longa* (Ayer et al., 20108; Deepa et al., 2017; Resmi & Soniya, 2012), *Boesenbergia rotunda* (Chia et al., 2020; Roslan, Huy, Kee, et al., 2020; Roslan, Huy, Ming, et al., 2020), and *Alpinia oxyphylla* (Yuan et al., 2021). Comparative analysis of the *CHS* gene between and among families of plants to understand the evolutionary pattern of the *CHS* gene is still scarce, and no study has been done on the *CHS1* gene.

The *CHS1* gene involves in the flavonoid biosynthetic pathway. Flavonoid biosynthetic pathway genes (structural and regulatory genes) presented a model system to understand the variety of evolutionary processes, such as causes of evolutionary variation rate among genes, duplicated genes that presented an evolution of novel characters, and the relative importance of structural and regulatory genes that involved in important ecological characters (Rausher, 2006). Each of these processes

is important for plant adaptation, and it has been presumed that due to this reason, selection played a determining role in the evolution of the genes (Yang et al., 2004). However, *CHS* genes varied in plants across taxa leading to the evolution of those genes involved in the flavonoid pathway. Therefore, *in silico* comparative analysis of the *CHS1* gene between the Zingiberaceae, Costaceae, and Poaceae was done as a preliminary study to provide a fundamental idea of the evolutionary pattern in the *CHS1* gene within the Commelinids clade.

MATERIALS AND METHODS

A total of 22 *CHS1* sequences were downloaded from the National Center for Biotechnology Information (NCBI) database (Table 1; https://www.ncbi.nlm.nih.gov/genbank/). Those *CHS1* sequences belong to 16 species of Zingiberaceae (*Curcuma longa*, *Alpinia galanga*, *Alpinia luteocarpa*, *Alpinia vittata*, *Alpinia zerumbet*, *Curcuma amada*, *Curcuma aromatica*, *Curcuma caesia*, *Elettaria cardamomum*, *Globba marantina*, *Hedychium coronorium*, *Kaempferia elegans*, *Kaempferia galanga*, *Kaempferia rotunda*, *Etlingera elatior*, and *Zingiber officinale*), five species of Costaceae and only one Poaceae species. Of those *CHS1* sequences, two were complete *CHS1* sequences, and 20 were partial *CHS1* sequences, as shown in Table 1. All *CHS1* sequences belonging to Costaceae and Poaceae were subjected to dataset selection. The sequences were checked for their percentage of identity, query cover, and E-value with *CHS1* sequences of Zingiberaceae taxid using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Two *CHS1* sequences belonging to Costaceae (HM161806.1 and HM161808.1) were excluded from the final dataset because they showed no similarity with *CHS1* sequences belonging to the Zingiberaceae taxid (Table 2).

Thus, the final dataset consisted of 16 *CHS1* sequences belonging to Zingiberaceae, three *CHS1* sequences belonging to Costaceae, and one *CHS1* sequence belonging to Poaceae (Table 3). Then, multiple sequence alignment was performed using BioEdit 7.2 software (Hall, 1999), and all sequences were adjusted to well match each other by restricting the sequence size to 565 bp (Figure 2). Overall nucleotide dissimilarities among 20 *CHS1* sequences were manually determined to look for mutation and were recorded.

As shown in Figure 1, a similarity matrix was computed using MEGA X software (Kumar et al., 2018). After that, phylogenetic trees were constructed using unrooted neighbour-joining (NJ) trees with two iterations (100 and 1,000). Both iterations constructed similar trees. Hence, the phylogenetic tree constructed with 1,000 iterations was selected to show the *CHS1* evolutionary pattern within the Commelinids clade: Zingiberaceae, Costaceae, and Poaceae.

RESULTS

Twenty *CHS1* sequences belonging to Zingiberaceae, Costaceae, and Poaceae were aligned, and nucleotide dissimilarities

Table 1
List of CHS1 *gene sequences of Zingiberaceae, Costaceae, and Poaceae from the NCBI database*

Family	Species	Accession number	Sequence length (bp)	Sequence status
Zingiberaceae	*Curcuma longa*	AB573020.1	2,107	Complete
	Alpinia galanga	HM161832.1	568	Partial
	Alpinia luteocarpa	HM161830.1	568	
	Alpinia vittata	HM161834.1	568	
	Alpinia zerumbet	HM161836.1	568	
	Curcuma amada	HM161809.1	568	
	Curcuma aromatica	HM161810.1	568	
	Curcuma caesia	HM161811.1	568	
	Elettaria cardamomum	HM161813.1	568	
	Globba marantina	HM161814.1	568	
	Hedychium coronarium	HM161815.1	568	
	Kaempferia elegans	HM161819.1	565	
	Kaempferia galanga	HM161816.1	568	
	Kaempferia rotunda	HM161820.1	568	
	Etlingera elatior	HM161821.1	571	
	Zingiber officinale	DQ089697.2	578	
Costaceae	*Costus erythrophyllus*	HM161829.1	568	
	Costus malortieanus	HM161838.1	568	
	Costus pulverulentus	HM161806.1	568	
	Costus pictus	HM161826.1	568	
	Cheilocostus speciosus	HM161808.1	568	
Poaceae	*Sorghum bicolor*	AF152548.1	2,078	Complete

Table 2
The similarity index of CHS1 *sequences of Costaceae and Poaceae with Zingiberaceae taxid using BLASTn*

Query sequence	Match sequence	Query cover	E-value	Percentage of identity
HM161829.1	HM161832.1 (*Alpinia galanga* chalcone synthase (*CHS1*) gene, partial cds)	100%	6e-159	84.51%
HM161838.1	HM161830.1 (*Alpinia luteocarpa* chalcone synthase (*CHS1*) gene, partial cds)	97%	6e-134	82.29%
HM161806.1	-	-	-	-
HM161826.1	HM161819.1 (*Kaempferia elegans* chalcone synthase (*CHS1*) gene, partial cds)	100%	9e-123	80.84%
HM161808.1	-	-	-	-
AF152548.1	MT811929.1 (*Curcuma alismatifolia CHS1* mRNA, complete cds)	47%	0.0	79.90%

Note. Cds = Coding sequences

Table 3
The final dataset of CHS1 *gene sequences for in silico comparative* CHS1 *gene study*

Family	Species	Accession number
Zingiberaceae	*Curcuma longa*	AB573020.1
	Alpinia galanga	HM161832.1
	Alpinia luteocarpa	HM161830.1
	Alpinia vittata	HM161834.1
	Alpinia zerumbet	HM161836.1
	Curcuma amada	HM161809.1
	Curcuma aromatica	HM161810.1
	Curcuma caesia	HM161811.1
	Elettaria cardamomum	HM161813.1
	Globba marantina	HM161814.1
	Hedychium coronarium	HM161815.1
	Kaempferia elegans	HM161819.1
	Kaempferia galanga	HM161816.1
	Kaempferia rotunda	HM161820.1
	Etlingera elatior	HM161821.1
	Zingiber officinale	DQ089697.2
Costaceae	*Costus erythrophyllus*	HM161829.1
	Costus malortieanus	HM161838.1
	Costus pictus	HM161826.1
Poaceae	*Sorghum bicolor*	AF152548.1

	[Cl]	[Ag]	[Al]	[Av]	[Az]	[Ca]	[Cr]	[Cc]	[Ec]	[Gm]	[Hc]	[Ke]	[Kr]	[Ne]	[Zo]	[Ce]	[Cm]	[Cp]	[Sb]	[Kg]
[Cl]																				
[Ag]	0.4705																			
[Al]	0.5138	0.4525																		
[Av]	0.5155	0.4443	0.3651																	
[Az]	0.4913	0.0348	0.4865	0.4605																
[Ca]	0.4796	0.4412	0.3245	0.0519	0.4676															
[Cr]	0.4934	0.4444	0.3188	0.0462	0.4676	0.0145														
[Cc]	0.4861	0.4612	0.3216	0.0519	0.4849	0.0218	0.0181													
[Ec]	0.4775	0.0181	0.4593	0.4540	0.0348	0.4509	0.4509	0.4712												
[Gm]	0.4759	0.4580	0.3358	0.0813	0.4853	0.0558	0.0558	0.0675	0.4647											
[Hc]	0.4899	0.4476	0.3245	0.0635	0.4675	0.0386	0.0367	0.0442	0.4542	0.0558										
[Ke]	0.5031	0.4519	0.4855	0.5080	0.4720	0.4729	0.4794	0.4795	0.4584	0.4662	0.4691									
[Kr]	0.4791	0.4477	0.3358	0.0713	0.4710	0.0480	0.0461	0.0576	0.4543	0.0615	0.0385	0.4660								
[Ne]	0.5214	0.4355	0.4859	0.4624	0.4585	0.4427	0.4297	0.4394	0.4325	0.4493	0.4489	0.2356	0.4395							
[Zo]	0.5507	0.5137	0.5321	0.5481	0.5281	0.5110	0.5107	0.5145	0.5280	0.5187	0.5108	0.0836	0.5182	0.2737						
[Ce]	0.4841	0.1756	0.4696	0.4303	0.1848	0.4274	0.4240	0.4339	0.1779	0.4276	0.4208	0.4654	0.4115	0.4196	0.4852					
[Cm]	0.5178	0.4367	0.2171	0.3303	0.4629	0.3107	0.3107	0.3190	0.4399	0.3332	0.3219	0.4316	0.3218	0.4447	0.4780	0.4458				
[Cp]	0.4905	0.4149	0.4555	0.4509	0.4276	0.4310	0.4210	0.4307	0.4308	0.4212	0.4242	0.2239	0.4182	0.2075	0.2511	0.3837	0.4361			
[Sb]	0.5156	0.2969	0.4865	0.5023	0.2996	0.5207	0.5245	0.5356	0.3051	0.5096	0.5244	0.4974	0.5132	0.4489	0.5113	0.2600	0.4694	0.4436		
[Kg]	0.4830	0.4475	0.3301	0.0773	0.4710	0.0538	0.0519	0.0635	0.4542	0.0714	0.0442	0.4902	0.0386	0.4489	0.5405	0.4113	0.3274	0.4273	0.5131	

Figure 1. Similarity matrices of 20 *CHS1* sequences belong to 16 Zingiberaceae species, three Costaceae species, and one Poaceae species
Note. [Cl] *Curcuma longa*; [Ag] *Alpinia galanga*; [Al] *Alpinia luteocarpa*; [Av] *Alpinia vittate*; [Az] *Alpinia zerumbet*; [Ca] *Curcuma amada*; [Cr] *Curcuma aromatica*; [Cc] *Curcuma caesia*; [Ec] *Elettaria cardamomum*; [Gm] *Globba marantina*; [Hc] *Hedychium coronarium*; [Ke] *Kaempferia elegans*; [Kr] *Kaempferia rotunda*; [Ne] *Etlingera elatior*; [Zo] *Zingiber officinale*; [Ce] *Costus erythrophyllus*; [Cm] *Costus malortieanus*; [Cp] *Costus pictus*; [Sb] *Sorghum bicolor*; [Kg] *Kaempferia galanga*

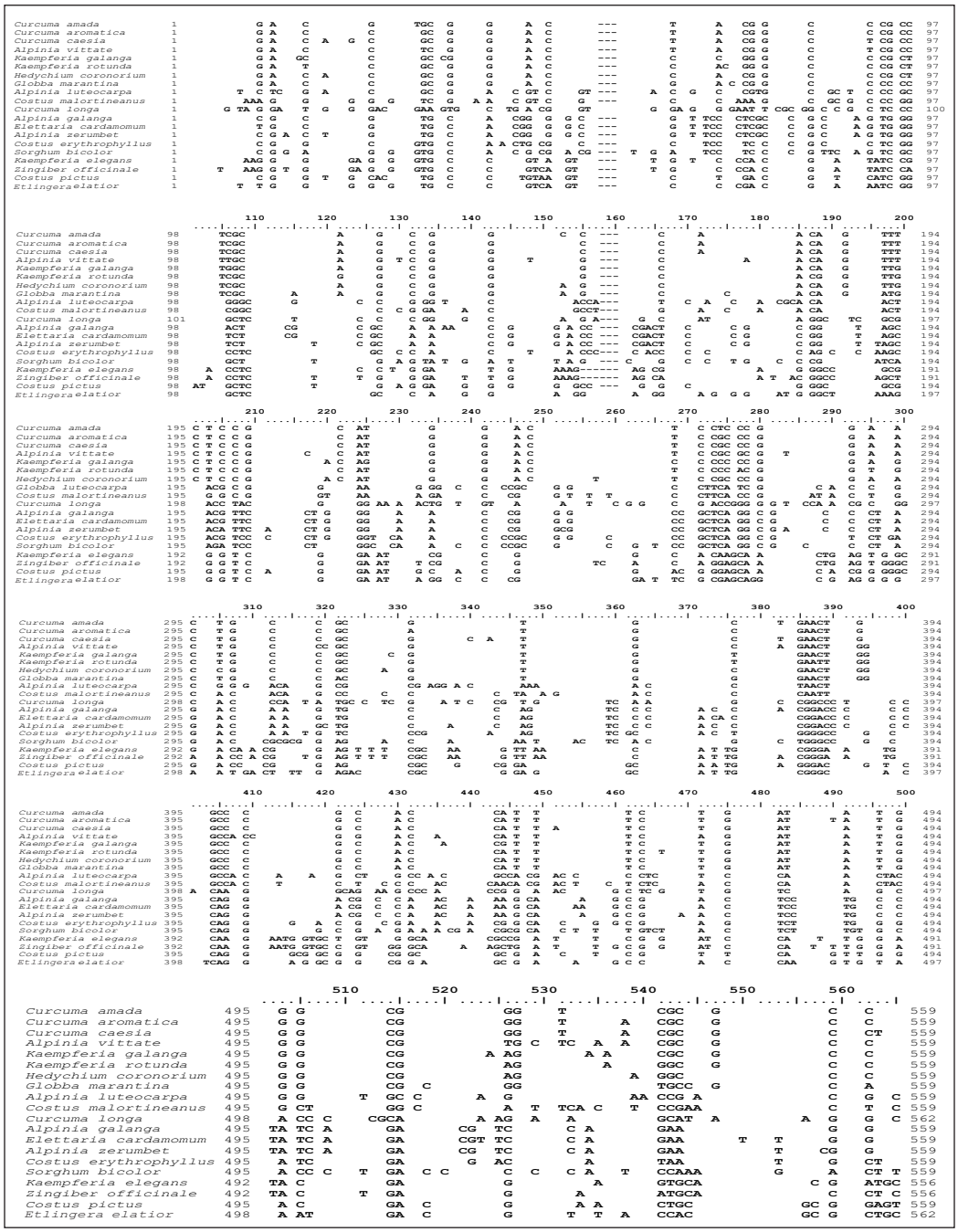

Figure 2. The 20 *CHS1* sequences aligned using BioEdit 7.0 software. The 16 Zingiberaceae species: *Curcuma longa*, *Alpinia galanga*, *Alpinia luteocarpa*, *Alpinia vittata*, *Alpinia zerumbet*, *Curcuma amada*, *Curcuma aromatica*, *Curcuma caesia*, *Elettaria cardamomum*, *Globba marantina*, *Hedychium coronorium*, *Kaempferia elegans*, *Kaempferia galanga*, *Kaempferia rotunda*, *Etlingera elatior*, and *Zingiber officinale*, three Costaceae species: *Costus erythrophyllus*, *Costus malortineanus*, and *Costus pictus*, and one Poaceae species: *Sorghum bicolor*

were observed among them (Figure 2). The observed nucleotide dissimilarities proposed three mutations, which were deletion, insertion, and substitution. Two minor deletions were observed at 155 to 157 in two species (*Kaempferia elegans* and *Zingiber officinale*). Insertion mutations of three nucleotides were observed in two sequences at two different positions in two species: (i) CTT were inserted at positions 57 to 59 in *Curcuma longa*, while (ii) GTC were inserted at positions 158 to 160 in *Etlingera elatior*. Substitution mutations were observed at 285 positions, equivalent to 50.44% of sequence variation. Moreover, 103 positions showed single nucleotide polymorphisms (SNPs). Hence, the results propose the studied 565 bp *CHS1* gene regions within the Commelinids clade; the Zingiberaceae, Costaceae, and Poaceae are conserved *CHS1* gene regions with low polymorphism (only 18.23% of SNPs).

Moreover, the evolutionary pattern of the *CHS1* gene within the Commelinids clade showed no clear evolutionary pattern (Figure 3), and this supports the previous results, which found *CHS1* gene is conserved within the Commelinids clade. The constructed phylogenetic tree showed two clades (Clade i and Clade ii). Clade i comprised 11 species, and Clade ii comprised two subclusters with nine species. In Clade i, 10 species belong to Zingiberaceae, while one belongs to Costaceae. In Clade ii, subcluster

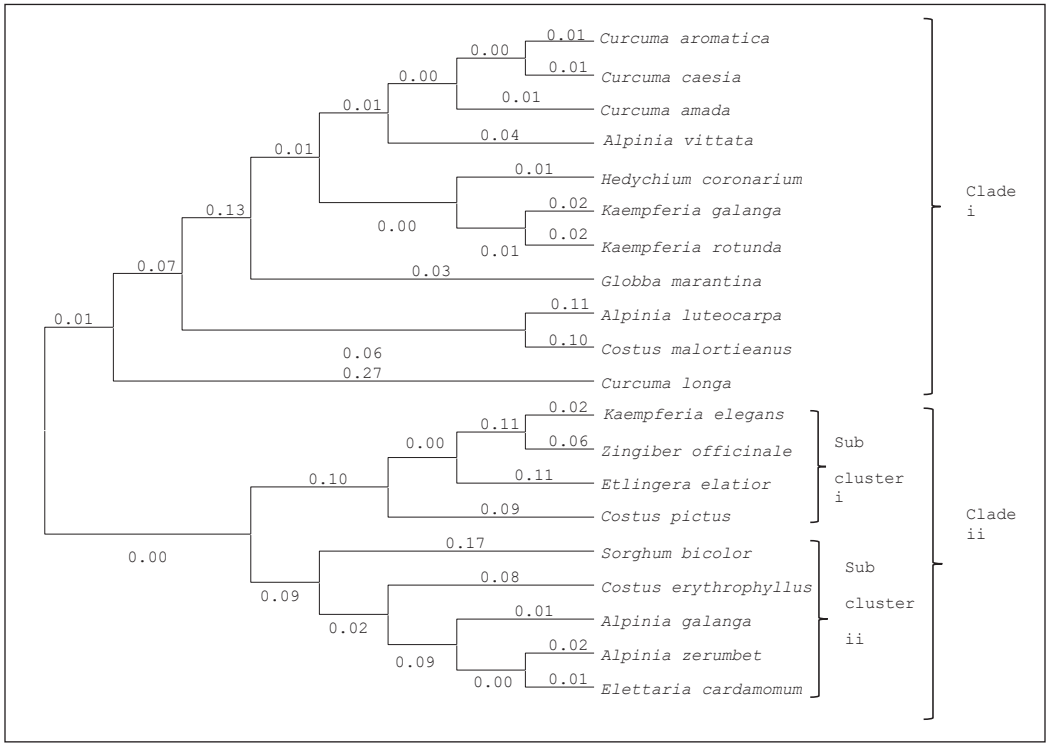

Figure 3. An unrooted neighbour joining (NJ) tree with 1,000 iterations showed an unclear evolutionary pattern of the *CHS1* gene within the 20 studied sequences of the Commelinids clade

i comprises three species that belong to Zingiberaceae, while only one species belongs to Costaceae. Whereas subcluster ii comprised three species belonging to Zingiberaceae, one species belonging to Costaceae, and one to Poaceae.

DISCUSSION

The present study investigated the evolutionary pattern in the *CHS1* gene of the Commelinids clade using 20 species belonging to Zingiberaceae, Poaceae, and Costaceae at a preliminary stage. Nucleotide dissimilarities were observed due to three mutations: substitution, insertion, and deletion. These mutations contribute to low polymorphism in the *CHS1* gene. In addition, the unrooted NJ tree showed no clear evolutionary pattern of the *CHS1* gene and supported the *CHS1* gene as a conserved gene with low polymorphism due to several mutations.

Two of the three mutations (i.e., substitution and deletion) observed in this study have been reported in previous *CHS* gene studies of different plant families. Deletion in the *CHS* gene has been reported at the promotor region of the *CHS4* gene in *Glycine max* (Tuteja et al., 2004). Short frameshift deletions in protein-coding regions of *CHS* genes (*Chs-A4T*, *Chs-A3*, and *Chs-A4T*) have been found in *Triticum aestivum* (Glagoleva et al., 2019). Truncated *CHS3-ICHS1* is presented in mutant soybean (*Glycine max*) due to deletion at 5' flanking or coding region of *ICHS1* in Ms-m mutant (Senda et al., 2002). Meanwhile, Jiang and Cao (2008) have observed nucleotide substitutions in *BcCHS-wf* at two positions (i.e., A to G at 37 and 970 bp, respectively) in both wild and mutant types of Chinese cabbage-Pak choi (*Brassica campestris* spp. *chinensis*). Another mutation reported in the *CHS* gene is duplication (Clegg et al., 1997; Lynch & Conery, 2000; Vision et al., 2000). It has been found in *Arabidopsis thaliana* (Lynch & Conery, 2000; Vision et al., 2000). However, duplication was not found in the present study. Duplication in a gene indicates polyploidisation, which was known to occur 100 million years ago (Durbin et al., 2000). Mutations at nucleotide sequences or amino acids are a primary step in gene evolution (Durbin et al., 2000; Vision et al., 2000). Mutations can lead to the divergence of gene families due to evolutionary forces, such as demographic history, mating system, and natural selection (Chiang et al., 2003; Chiang et al., 2004; Huang et al., 2004).

The *CHS* gene families are functional genes that control flavonoid production and are conserved genes that portray an adaptive evolution (De Meaux et al., 2006; Johnson & Dowd, 2004). For example, the *CHS* genes are structurally and functionally conserved in flowering plants, such as *Antirrhinum majus* (Sommer & Saedler, 1986). In functional and conserved genes, mutations usually occur in intergenic regions, including insertion, deletion, and a large amount of substitution (Mitchell-Olds, 2001). Previous studies also showed the *CHS* genes are conserved in many plant genera and families (Austin & Neol, 2003; Clegg et al., 1997; Durbin et al., 2000;

Huang et al., 2004; Koch et al., 2000; Koes et al., 1989; Yang et al., 2002) as a single gene (Feinbaum & Ausubel, 1988; Kreuzaler, 1983) or as multigene families (Anguraj et al., 2018; Christensen et al., 1998; Deng et al., 2014; Durbin et al., 2000; Glagoleva et al., 2019; Han et al., 2016, 2017; Koes et al., 1987, 1989; Ito et al., 1997; Radhakrishnan & Soniya, 2009; Schroder et al., 1998; Tuteja et al., 2004). For example, barley has seven copies of *CHS* genes (Christensen et al., 1998), *Pinus* has two copies of *CHS* genes (Schroder et al., 1998), and *Petunia hybrida* has eight complete and four partial *CHS* genes that are expressed in floral tissues and seedlings but not present in leaf, root, and stem (Koes et al., 1987).

CONCLUSION

The *in silico* comparative *CHS1* gene study within the Commelinids clade using 20 partial sequences (565 bp) belonging to 16 Zingiberaceae species, three Costaceae species, and one Poaceae species showed the *CHS1* gene is conserved with no clear evolutionary pattern. However, low polymorphism (18.23% of SNPs) and a few mutations, substitution, insertion, and deletion were observed. Hence, further studies are needed to explain the possible consequences of low polymorphism and mutations in the *CHS1* gene within the Commelinids clade. Understanding this might elucidate adaptive evolution in the *CHS1* gene. In addition, more species under the Commelinids clade should also be studied for their *CHS1* gene evolution.

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