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In silico characterization and transcriptional modulation of phenylalanine ammonia lyase (PAL) by abiotic stresses in the medicinal orchid *Vanda coerulea* Griff. ex Lindl.

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ABSTRACT

Phenylalanine ammonia lyase (PAL) is the first enzyme of phenylpropanoid pathway. In the present study, a full-length *PAL* transcript from *Vanda coerulea* Griff. ex Lindl. (Family: Orchidaceae) was isolated and characterized. It was found that complete *PAL* transcript of *V. coerulea* (*VcPAL*; Gene Bank no. MG745168) contained 2175 bp with the open reading frame (ORF) of 2112 bp, encoding 703 amino acid residues. The multiple sequence alignment showed that VcPAL protein had 81% identity with that of the orchid, *Bromheadia finlaysoniana*. Phylogenetic analysis also disclosed that VcPAL shared the same evolutionary relationship with PAL proteins of other orchid species and to be closely related to that of other angiosperm species as well. The three-dimensional structure of VcPAL was found to be homo-tetrameric in nature consisting of four identical subunits with a molecular mass of 75 kDa per subunit. *In silico* characterization revealed the deduced protein to be a stable protein, comprising three major functional domains as reported in PAL proteins of other species. The transcription profiling of *VcPAL* exhibited the highest expression level to be present in the *in vitro* - raised leaf and root samples as compared to that of the *ex vitro* plant. The differential expression of *VcPAL* transcript was observed to be up-regulated by different types of abiotic stresses like wounding, cold, UV-B, salinity, and down-regulated by dark treatment. The study also exhibited that the VcPAL enzyme activity was directly proportional to the gene expression after the tissues were subjected to salinity and wounding stresses wherein a 1.7- fold increase in the enzyme activity was recorded in the leaf tissues exposed to salinity stress. A positive correlation could be found between the enzyme activity and the accumulation of phenylpropanoids such as total phenolic and flavonoid contents with $R^2 = 0.85$ and 0.842 respectively.

1. Introduction

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), the key enzyme of phenylpropanoid pathway catalyzes the deamination of phenylalanine to *trans*-cinnamic acid and converts primary metabolites into specialized metabolites (Dixon et al., 2002). Phenylpropanoid pathway leads to synthesis of various biologically important metabolites such as lignin, anthocyanins, alkaloids, flavonoids, isoflavonoids, ultraviolet protectants, antimicrobial, phytoalexins, coumarins, caffeic acid, chlorogenic acid, stilbenoids, wound phenolic esters and so on (Hahlbrock and Scheel, 1989; Dixon and Paiva, 1995). Besides their roles in plants' development and defense mechanism, many phenylpropanoid compounds are major active molecules having different medicinal properties (Bourgaud et al., 2001; Ma et al., 2013). Generally, PAL is a homo - tetrameric protein with a molecular mass ranging from 270 to 330 kDa (Kong, 2015). The enzyme is widely present in almost all higher plants, some fungi, and yeasts but absent in true bacteria and animals (MacDonald and D'Cunha, 2007). Generally, PAL proteins are hydrophobic in nature and the subcellular location of PAL is mainly considered to be cytoplasmic (Dubery and Schabort, 1986). However, biochemical fractionation studies have suggested an association of PAL protein with endoplasmic reticulum membranes (Achnine et al., 2004). The subcellular fractionation and protein gel blot analysis in case of transgenic tobacco (*Nicotiana tabacum*) revealed that PAL1 isoform was located in both microsomal and cytosolic regions where as PAL2 isoform was confined to only cytosol (Rasmussen and Dixon, 1999; Achnine et al., 2004). Due to its immense role in synthesis of various specialized metabolites, *PAL* gene and its expression has been studied in various medicinal plants *viz.*, *Ephedra sinica* (Okada et al., 2008), *Gingo biloba* (Xu et al., 2008), *Salvia miltiorrhiza* (Song and

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Wang, 2009), *Scutellaria baicalensis* (Xu et al., 2010), *Lycoris radiate* (Jiang et al., 2011), *Rhus chinensis* (Ma et al., 2013), edible plants like, *Oryza sativa* (Minami et al., 1989), *Petroselinum crispum* (Lois et al., 1989), *Lactuca sativa* (Campos et al., 2004), *Juglans regia* (Xu et al., 2012), and various orchid species such as *Bromheadia finlaysoniana* (Liew et al., 1996), *Phalaenopsis* x *Doritaenopsis* hybrid (Su and Hsu, 2003) and *Dendrobium candidum* (Jin et al., 2013). The expression of *PAL* gene can be induced by various biotic as well as abiotic stresses such as tissue wounding, UV irradiation, low temperature, salinity, fungal infection, plant growth regulators, jasmonic acid, salicylic acid and chitosan (Kong, 2015).

Vanda coerulea Griff. ex Lindl. (Family: Orchidaceae), commonly known as 'blue *Vanda*' due to its blue colored flowers, is one of the most expensive, rare and medicinally important orchid. Different parts of this orchid have been reported to be used in the traditional medical system to cure diseases like diarrhea, dysentery, dermal disorders, glaucoma, cataract and blindness (Medhi and Chakrabarti, 2009; Priya et al., 2011). The medicinal values may be attributed to the presence of specialized metabolites such as alkaloids, flavonoids, stilbenoids, gigantol, flavidin, methoxycoelonin, coelonin which are synthesized via phenylpropanoid pathway. The compounds stilbenoids and flavonoids have been reported to be important for their antioxidant, anti-inflammatory and anti-cancerous activities (Simmler et al., 2010). On the other hand, coelonin, a 9, 10 - dihydrophenanthrene is found to act as phytoalexin and can be used as antimicrobial and antifungal agent (Majumder et al., 1982). But so far, *PAL* transcript of *V. coerulea* has not been explored. The present study deals with the isolation of *VcPAL* transcript from leaf sample of *V. coerulea* and *in silico* characterization of the deduced protein for structural and physicochemical properties. Differential expression of *VcPAL* transcript under various abiotic stresses has been studied in detail. And, to acquire more information on enhancement of *PAL* transcript expression by abiotic stresses, a correlation between the accumulation of phenylpropanoids with PAL enzyme activity has been found out.

2. Results and discussion

2.1. Isolation and characterization of VcPAL gene

The sequence of full-length of cDNA from *V. coerulea* was designated as *VcPAL* and was submitted to NCBI databank (GeneBank accession no. MG745168). From overlapping sequences of three fragments, a putative sequence of 2175 bp along with poly-A tail was constructed and identified. Sequence analysis revealed the presence of open reading frame (ORF) comprising 2112 bp (encoding 703 amino acid residues) which was flanked by 14 bp of 5′ untranslated region (UTR) from the start codon and 49 bp of 3′ UTR from the stop codon (Fig. 2). Studies have shown that the 5′ UTR of *PAL* transcript in case of orchids, *Bromheadia finlaysoniana* and *Phalaenopsis* species is relatively smaller in size (77 bp) as compared to that of other plant species (Liew et al., 1996; Su and Hsu, 2003). In the present study, the obtained flanking 14 bp 5′ UTR in putative transcript of *VcPAL* is an important part of start codon initiation in its complete ORF. However, it may be essential to explore a complete 5′ UTR before cloning this *VcPAL* transcript for expression studies. Protein sequence alignment analysis (BLASTp) indicated that the deduced VcPAL protein shared maximum identities (75–81%) with PALs of other orchids *viz., Bromheadia finlaysoniana*, *Phalaenopsis* X *Doritaenopsis* hybrid cultivar, *Phalaenopsis* equestris, *Dendrobium catinatum, D. candidum* and *Apostasia shenzhenica*. Apart from orchids, VcPAL showed 72% identity with *Musa acuminate,* 71% with *Lycoris radiata*, 70% identity with both *Arabidopsis thaliana* and *Capsicum chinensis*. Multiple sequence alignment revealed that the putative full coding region of VcPAL was highly similar to the reported PAL proteins of other plant species (Fig. 3). Further, the conserved motif Ala- Ser- Gly (ASG) triad was also found to be present in VcPAL between 185 and 192 amino acid residues (GTIT**ASG**D). BLASTp sequence alignment also showed that VcPAL contained conserved catalytic active sites in the regions *viz.,* 243 to 249, GLALVNG; 370–372 NDN; 474–477 HNQD (Fig. 3). The presence of PAL protein finger motif tags (ASG) and conserved catalytic regions suggests that VcPAL may have similar function as that reported for other PAL proteins (Jin et al., 2013; Xu et al., 2012).

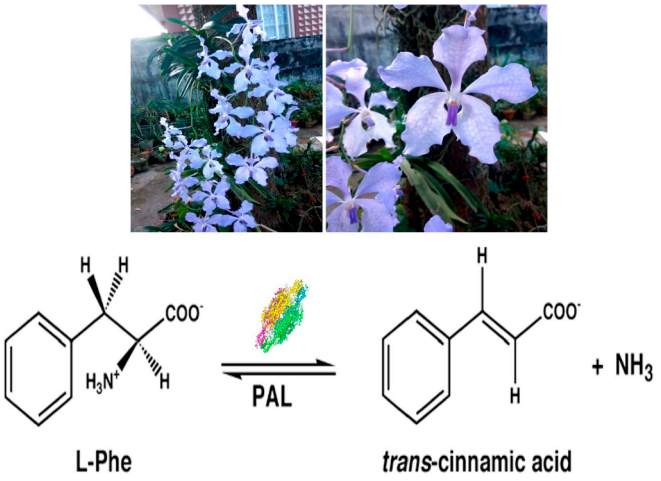

Fig. 1. *Vanda coerulea* plant with flowers; schematic representation of phenylalanine ammonia lyase enzyme in conversion of L-phenylalanine to trans-cinnamic acid production, the main source of specialized metabolite synthesis.

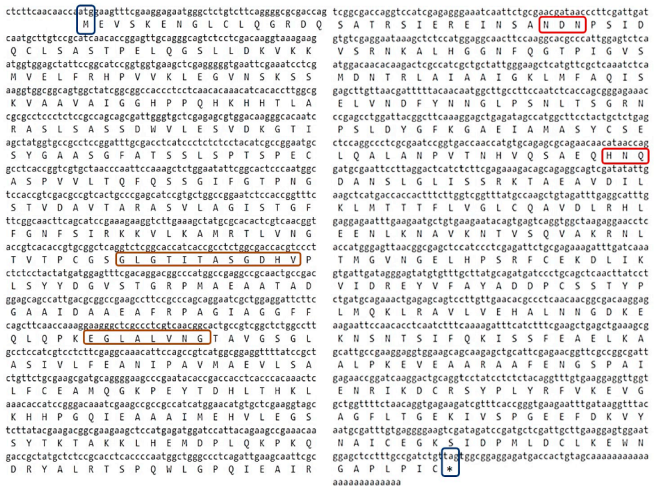

Fig. 2. Complete nucleotide sequence along with open reading frame (ORF) of VcPAL protein. Boxes showing the active residues present in complete ORF. Start codon M (ATG) and stop codon * (TAG) showing in blue colored boxes.

2.2. Three dimensional deduced VcPAL protein analyses

To design three-dimensional structure of VcPAL protein, 3D structure of *Petroselinum crispum* PAL protein (PDB No. 1w27A) was used as template and homology modeling was performed using SWISS- MODEL (http://www.swissmodel.expasy.org) (Fig. 4). Using the online tool PDBsum, it was found that the deduced VcPAL protein consisted of α-helices (51.9%), 3–10 helices (3.7%), β, γ-turns and random coils (41.2%), and extended strands (3.2%). Based on the earlier report on PAL protein of *Petroselinum crispum*, the structures of PALs were assumed to be 'sea horse'- shaped and comprised precursor binding domain (4-methylidene-imidazole-5 one; MIO), core domain and inserted shielding domain (Ritter and Schulz, 2004). VcPAL also showed a similar structure containing MIO domain a highly conserved domain in all plant PALs enzyme (Fig. 4). The docking results revealed amino acid residues present in the active sites of VcPAL enzyme to be Asn-479, Gln-476, Tyr- 339, Ala-190, Ser-191, Gly-192, Gln-389 and Phe-388. The