Isolation and characterization of ethylene receptor genes in *Dendrobium Pompadour*

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Abstract. The generally long life span, unique appearance and attractive colours have transformed orchids from being an exquisite plant enjoyed only by collectors to a plant found in almost every household, airport, hotel and restaurant. However, wounding, pollination and exposure to exogenous ethylene or cigarette smoke may reduce the shelf-life of some orchid species rendering them unmarketable. Therefore, there is a crucial need to circumvent this problem to ensure better quality and commercial value for the sustainability of the orchid industry and competition in the world market. The advent of biotechnology has allowed for genetic engineering of senescence controlling genes in flowers like carnations, resulting in extended shelf-life. The success of this genetic approach is a stepping-stone in the development of transgenic orchids and needs to be buttressed by a strong understanding of the ethylene-related genes involved in senescence. In this paper, we report the cloning and characterization of four known ethylene receptors, ETR1, ETR2, ERS1 and ERS2 from the popular tropical orchid genus *Dendrobium*, as part of initial steps towards using these genes (sequences) to regulate ethylene sensitivity for greater longevity of pollinated orchid flowers. Physiological studies have shown that within 24 hours, pollinated *Dendrobium* flowers display an increase in ethylene production, alongside distinct visual changes. The presence of these genes in pollinated and unpollinated flowers was detected using RT-PCR. Detailed analysis, with Scan Prosite, showed that the RT-PCR product of ETR1 shares more than 60% nucleotide sequence homology with delphinium, as well as tobacco, rice, petunia and carnation ERS 1 primers produced a product with more than 80% homology with delphinium, as well as a petunia hybrid and *Oncidium* Gower Ramsey. Sequence results for ETR2 and ERS2 were compared and characterized using bioinformatics tools will also be presented.

Keywords: *Dendrobium* Pompadour; Ethylene receptors; Orchid; Senescence.

**INTRODUCTION**

The popularity of orchids has transformed these plants from once being an exotic plant found only in the wild into one of the most demanded potted plants or cut flowers on an international scale making it a world’s US$2 billion industry. For the cut flower industry, the major chemical pollutant affecting shelf life is ethylene ( Haley and Mayak, 1979). Pollination is the most striking ethylene producing phenomena, thus shortening their life span and decreasing their value to the cut flower industry. Petal senescence is a process regulated by a complex of ethylene biosynthesis and perception. The proteins responsible for the response to ethylene are the ethylene receptors, proteins which bind to ethylene. To date, five members of the ethylene receptor family have been identified in Arabidopsis thaliana; ETR1, ERS1, ETR2, ERS2 and EIN4. The first and most widely studied ethylene receptor is the Ethylene Receptor 1, (ETR1), reported in Arabidopsis (Bleecker et al., 1988 and Chang et al., 1993), tobacco (Knoester et al., 1997), rice (Yau and Yip, 1997) and Phalaenopsis (Do et al., 1999). Subsequently, ETR2, ERS1, ERS2 and EIN4 were also identified as the ethylene receptors involved in ethylene perception (Hua et al., 1995, 1998; Sakai et al., 1998). Ethylene receptor genes with homology to receptors found in Arabidopsis also have been identified in flowers such as carnation, rose, delphinium and geranium. These receptors function as negative regulators as the binding of ethylene results in the inactivation of receptor function. An advocated strategy is therefore to inhibit ethylene receptor genes (Bouzayen and Pech, 1997). When a mutant of ETR, viz etr1-1 gene was inserted into petunia, this genetic manipulation rendered the transgenic flowers ethylene-insensitive thus delaying senescence, and extending shelf life of these ephemeral flowers by more than a week (Wilkinsen et al., 1997). In this paper, we report the isolation and characterization of five ethylene receptors, ETR1, ERS1, ETR2 and ERS2 from a popular tropical orchid genus, *Dendrobium*, as a foundation for future gene manipulation for greater longevity of flowers.

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MATERIALS AND METHODS

**RNA Extraction and RT-PCR Analysis.** Total RNA from *Dendrobium* flowers (pollinated/unpollinated) was extracted at specific time intervals, using an RNA extraction procedure, modified from Liu et al., 1998. The quality of extracted RNA samples was checked by visualizing fractionated and ethidium bromide-stained samples on agarose gels, prior to RT-PCR procedures. For RT-PCR, the AccessQuickTM RT-PCR System (Promega) was used to detect the genes. Appropriate primers were designed for ETR1 gene using the sequences from *Arabidopsis* (Chang et al., 1993) sequences from delphinium (Tanase and Ichimura, 2005) for the ERS1 gene. The primers for ETR2 and ERS2 were designed via multiple alignments.

**Sequencing.** The RT-PCR product was sent for sequencing by First Base Laboratories Sdn Bhd, BST Techlab, Kuala Lumpur. Sequence analysis and multiple sequence alignment were carried out using the CLUSTAL W (1.82) program. A detailed analysis of protein structure was also conducted in order to identify the specific regions characteristic to, ETR1, ERS1, ETR2 and ERS1 proteins.

RESULTS AND DISCUSSION

The ETR1 gene isolated and sequenced was 744 bp in length with more than 65% homology with ETR1 sequences of tobacco (Knoester et al., 1997), rice (Yau and Yip, 1997), petunia (Lai and Shaw, 2001), *Phalaenopsis* (Do et al., 1999) and carnation (Shibuya et al., 1998). The RT-PCR product predicted a sequence of about 248 amino acids. The Scan Prosite results identified a domain characteristic to the ETR1 protein family, viz. one histidine kinase domain. Other sites that were commonly found in ETR1 receptors were also present i.e. one N-glycosylation site, three protein kinase C phosphorylation sites, three Caspase III phosphorylation sites, and two N-myristoylation sites. ERS 1 primers produced a bigger product of 1.2kb, compared to that of ETR1 with more than 60% homology with sequences of petunia (Wang and Kumar, 2005), cucumber (Yamasaki et al., 2000), apple (Wiersma et al., 2007), rice (Yau et al.) and Arabidopsis (Sakai et al., 1998). Scan Prosite analysis showed that the presence of a histidine kinase domain. Other sites present included two N-myristoylation sites, two N-glycosylation sites, three Protein kinase C phosphorylation site and seven Casein kinase II phosphorylation sites.

In conclusion, this study has resulted in the isolation of good size genes which are comparable to genes cloned in the databank. The isolation method proved adequate to fish out genes to prepare plasmid cassettes for the transformation of *Dendrobium* Pompadour in the quest for extending flower longevity.

REFERENCES


