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A novel ethylene receptor homolog gene isolated from ethylene-insensitive flowers of gladiolus (*Gladiolus grandiflora* hort.)

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Abstract

Gladiolus is an ethylene insensitive flower whose exogenous ethylene and ethylene inhibitors have no effect on the petal senescence process. To study which processes in gladiolus are associated with changes in ethylene perception, two types of gladiolus genes, named *GgERS1a* and *GgERS1b*, respectively, homologous to the Arabidopsis ethylene receptor gene *ERS1* were isolated. *GgERS1a* is conserved in terms of exon numbers and intron positions, whereas *GgERS1b* is almost same with *GgERS1a* except lacking 636 nucleotide encoding first and second histidine kinase (HisKA) motifs. The sequence data on full length genomic DNA indicated that both *GgERS1a* and *b* were spliced from different genomic DNA. As the result of mRNA expression study, in spite of lacking the two significant motifs, the expression of *GgERS1b* dramatically changed with advance in petal senescence, whereas the level of *GgERS1a* expressed highly and constitutively. The result suggests that both the genes possess a significant role for the subfunctionalization process to provide ethylene insensitivity in gladiolus flowers.

Keywords: Ethylene insensitive; ERS1; Gladiolus; Ethylene receptor; Senescence

The ethylene plays a central role in physiological and developmental processes of plant, such as germination, growth, flower initiation, senescence of leaves and flowers, organ abscission, and fruit ripening [1,2]. Ethylene perception in plant tissue requires specific receptors and a signal transduction pathway to coordinate downstream responses. Hua and Meyerowitz [3] showed that the ethylene receptors positively regulate CTR1, a RAF kinase-like protein that acts as a negative regulator of the ethylene transduction pathway [4], in the absence of ethylene and that ethylene binding inhibits this interaction. Ethylene receptors have a central role on the ethylene signal transduction pathway.

Since the initial discovery of ethylene receptor gene ETR1 in Arabidopsis, many homologues have been isolated from various plants, but all these reports belong to ethylene-sensitive or climacteric plants. Most information on the biology of flower senescence was obtained from the study on flowers where the plant hormone ethylene coordinates the entire perception process. However, there is no report about ethylene perception mechanism from ethylene insensitive flowers. This is the first study to isolate and characterize the ethylene receptors from ethylene insensitive flower. Gladiolus is a cut flower whose vase life is approximately 5–6 days. A response to ethylene in ethylene sensitive flower is regulated by the production of ethylene receptors whose expression may be regulated by the ethylene production [10]. However, exogenous ethylene and ethylene inhibitors have no effect on petal senescence of gladiolus [11,12]. The results indicated that the fundamental factor for ethylene insensitiveness might not play a role in the ethylene biosynthesis process, but in the ethylene perception itself or the downstream response. In this study,

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to identify what processes in gladiolus were associated with changes in ethylene insensitiveness, we isolated two gladiolus full-length homologues of the Arabidopsis ethylene receptor genes, designated as *GgERS1a* and *GgERS1b*, that seemed to be generated by gene duplication and splicing. The expression studies showed that both the genes were expressing well, which indicated that both the genes include some significant role for the subfunctionalization process to provide ethylene insensitivity in gladiolus flowers.

Materials and methods

Plant materials and genomic DNA extraction. Gladiolus grandiflora hort cv. Traveler. ($4x = 2n = 60$) were grown in compost in a greenhouse condition. For expression analysis, flowers, at five distinct developmental stages from buds open to senescence, were collected and the different tissues were frozen in liquid nitrogen and stored at −80 °C until each analysis. Extraction of genomic DNA from young leaves of gladiolus was performed by DNeasy Plant Maxi Kit (Qiagen Co., Hilden, Germany) according to the manufacturer's instruction.

Isolation of ethylene receptor and actin gene fragment by RT-PCR. Total RNA was isolated using RNeasy Mini Kit (Qiagen, Hilden), and reverse transcription polymerase chain reaction (RT-PCR) was performed with 1 μl total RNA by SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, CA). These samples were fractionated on agarose gels, stained with EtBr, and quantitated on the Image Analyzer to determine optimum cycle number. It was determined 28 cycles of PCR were in the linear response range.

The primers used for the isolation of gladiolus ethylene receptors and actin gene fragment were as follows: Degenerate Actin primers F-5′-GTATTGTBHTNGAYTCHGGDGATGGTGT-3′ and R-5′-GADGCHARRATDGANCCWCCRATCCAVAC-3′ were amplified at 52 °C annealing temperature with 540 bp PCR product and the gene specific primers *GgActin* F-5′-GGGTGACTCATACTGTTCCAATTTACGAA-3′ and *GgActin* R-5′-GCGACCCCCTTAATTTTCATGCTGCTG-3′ at 55 °C annealing temperature with 28 PCR cycles and the size of PCR product was 520 bp. The Degenerate Ethylene receptor primer sequences F-5′-AGRATGYTNACNCATGARAT-3′ and R-5′-TTTGWRAAYTTHACAGCATT-3′ were amplified at 40 °C annealing temperature with 1013 bp was the size of the PCR product and the gene specific sequences *GgERS1* F-5′-ATGGAGGGATGTGATTGCAT-3′ and GgERS1 R-5′-TTATACACTGAGGTATCGGA-3′ were amplified at 55 °C annealing temperature with 35 PCR cycles and the size of PCR product was 975 bp.

In brief, poly $(A)^+$ RNA was isolated from petal of flower at half opening stage and the corresponding cDNA was synthesized. The 5′ and 3′ ends of fragments of cDNA for the ethylene receptor, which remained to be characterized after RT-PCR, were isolated by RACE-PCR. Gene-specific primers were designed to identify 5′ and 3′ rapid amplification of cDNA end (RACE) products. cDNA products were generated from wild-type gladiolus inflorescence and young seedling double-stranded cDNAs. Plant sequences flanking the T-DNA generated the 3′cDNA (3′RACE) primer, 5′-TCTGTTTTCTTCTTACAAGATGGGAAGATTCG-3′. Plant sequences in DELAYED DEHISCENCE1 exon 4 generated the 5′cDNA (5′RACE) primer, 5′-GCAATAATCCTCCACCACCCGAGGTATC-3′. The RACE primers were designed to match the Marathon (Clontech Laboratories Inc., Palo Alto, CA, USA) cDNA amplification protocol specifications. The 3′cDNAs were reamplified, gel-purified (GeneClean; Bio101, Vista, CA, USA), and cloned into the plasmid vectors (Topo 2.1; Invitrogen, Carlsbad, CA, USA).

Genomic Southern analysis. Ten micrograms of genomic DNA was digested with the restriction enzymes, *Eco*RV or *Bam*HI, respectively, and resolved on 0.8% (w/v) agarose gels. The genomic DNA was transferred to

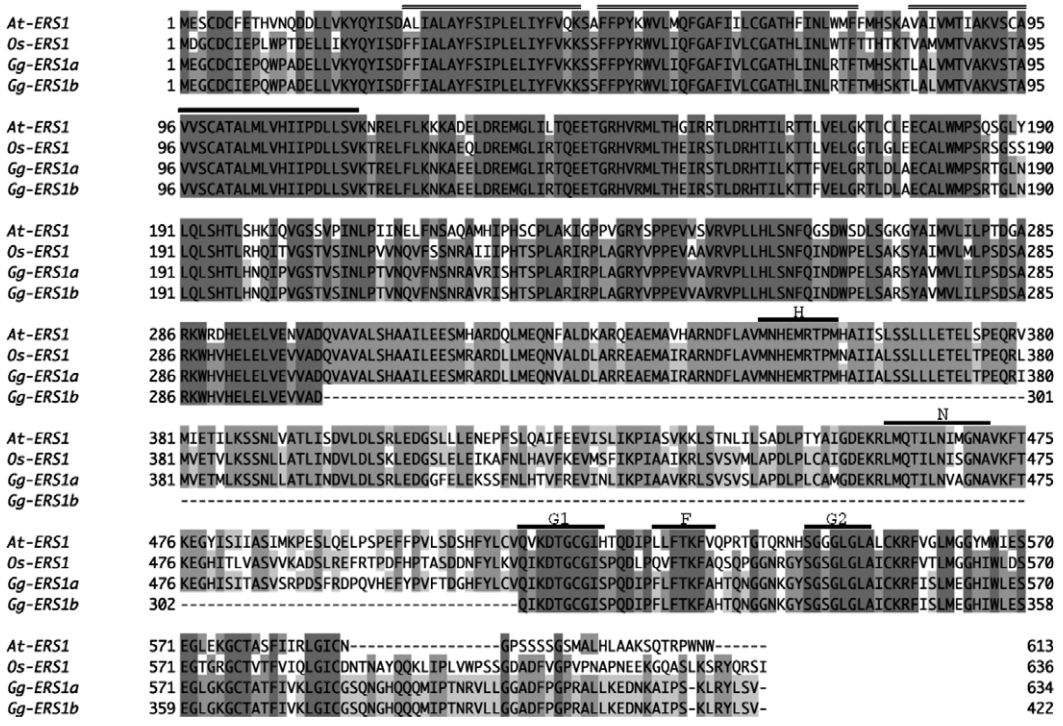

Fig. 1. Amino acid sequence alignment of ethylene receptor gene, *ERS1* in *Arabidopsis thaliana*, *Oryza sativa*, and *Gradiolus grandiflora*. *GgERS1b* lacks 636 nucleotide (907–1542) of *GgERS1a*. Sequences were aligned by the ClustalX. Conserved amino acids in all sequences are colored dark gray. Middle gray shading indicates similar residues in three out of four of the sequences and clear gray shading indicates similar residues in two out of four of the sequences. Conserved motifs are indicated above the alignment. Double vertical bars indicate putative regions of transmembrane domain. Single vertical bars indicate HisKA motifs (H, N, G1, F, and G2).