Isolation and characterization of an ethylene biosynthesis gene in response to waterlogging in chrysanthemum

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Abstract Waterlogging has major effects on natural vegetation and agricultural crops, limiting the growth of many plants mainly in humid regions. The phytohormone ethylene is known to mediate a diverse array of signaling processes in response to various abiotic stresses. To better understand the roles of ethylene in response to waterlogging, a full-length *CgACO* cDNA (GenBank accession KT438660) was isolated from chrysanthemum and its expression patterns were examined in two *Chrysanthemum* species (*Chrysanthemum zawadskii* and *C. nankingense*) with different tolerance to waterlogging. In the sensitive one (*C. nankingense)*, the transcriptional levels of the *CgACO* were increased rapidly but only transiently (12hrs after treatment) in the roots, while in tolerant one (*C. zawadskii*), the expression of the *CgACO* increased rapidly and maintained over a longer time frame (up to 48hrs after treatment). *CgACO* expression was not induced by exogenous ethylene application in both species. The ethylene inhibitor, 1-methylcyclopropene (1-MCP) enhanced *CgACO* expression after 12h of waterlogging in tolerant one but much less in the sensitive species. Higher expression of the *CgACO* gene correlated with higher accumulation of ethylene in the tolerant *C. zawadskii*. This supports the previously observed phenomenon that *C. zawadskii* exhibits well-developed aerenchyma adaptations to cope with waterlogged conditions.

Introduction

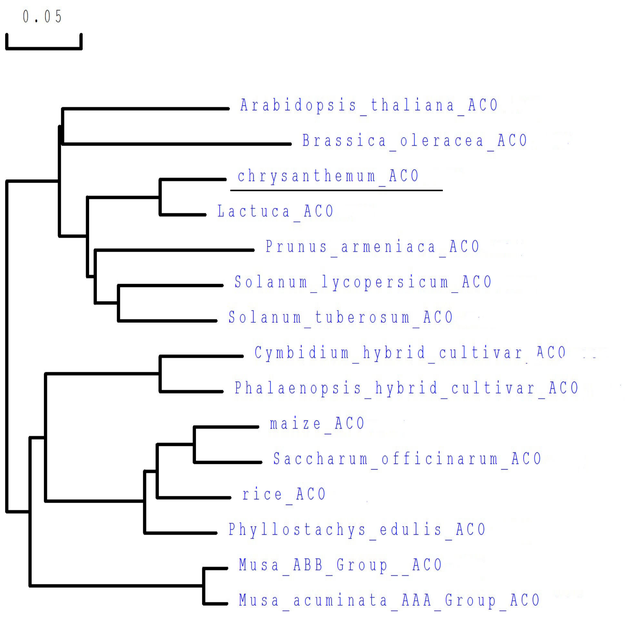
Ethylene, a gaseous plant hormone, plays an important role in plant growth, differentiation, and development in the tolerance and acclimation of plants to various environmental stresses. Of particular importance is the role ethylene plays in the acclimating responses to waterlogging. ACC oxidase (ACO) is involved in the final step of ethylene production in plant tissues. There is a strong evidence that ACO gene expression is positively correlated to the ethylene production rates under developmental and environmental control. Thus, the regulation of ACO may play a critical role in controlling of ethylene biosynthesis in higher plants. Chrysanthemum (*Chrysanthemum grandiflorum*) is an important cut flower and pot plant. A likely scenario arising from climate change includes a higher incidence of extreme rainfall events, increasing the need to improve the tolerance of plants to waterlogging. Here we report the isolation of a *CgACO* gene from chrysanthemum and its expression patterns in two *Chrysanthemum* species with different tolerance.

Materials and Methods

C. nankingense and *C. zawadskii* plants were obtained from the Chrysanthemum Germplasm Resource Preserving Centre (Nanjing Agricultural University, China). Plants at the eight node stage were flooded by placing the pots in a container filled with tap water to a level of 2.5cm above the soil surface. For the ethylene or 1-MCP treatments, the pots were sealed in an 8.5L chamber held at 25°C into which either 340μL ethylene or 34μL 1-MCP was then introduced. A 1M solution of NaOH was placed on the floor of the chamber to prevent the accumulation of CO2. As a control, both waterlogged and well-watered (60% soil moisture) plants were sealed in an identical chamber without the addition of either ethylene or 1-MCP. Fresh root sections (3.0–5.0 cm long, weight ~0.5 g) were harvested at 0, 6, 12,24, 48 and 72 h after treatments, immediately frozen in liquid nitrogen and then stored at −80ºC until use. Total RNA was extracted from tissues and qRT-PCR was used to compare the levels of *ACO* transcripts.

Results

Fig.1. Phylogenetic analysis of ACO gene family from different plant species. The chrysanthemum *CgACO* gene putatively encodes a 227 residue polypeptide, which shares 92% homology with lettuce (*Lactuca sativa*) ACC oxidase 2 (BAH15312.1).



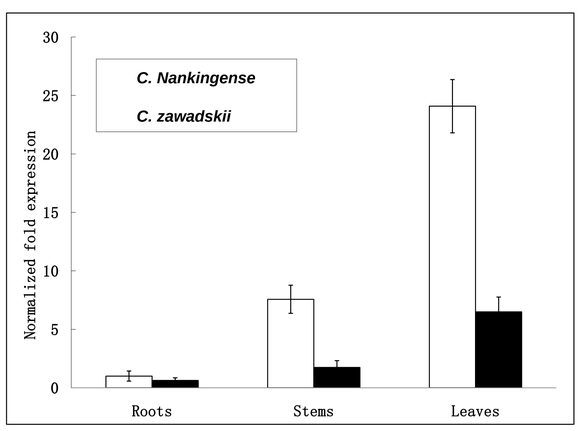


Fig.2 *CgACO* expression in different tissues as quantified by qRT-PCR. The vertical scale shows the relative amounts of transcript, as calibrated against the expression of the constitutively expressed *actin* gene.

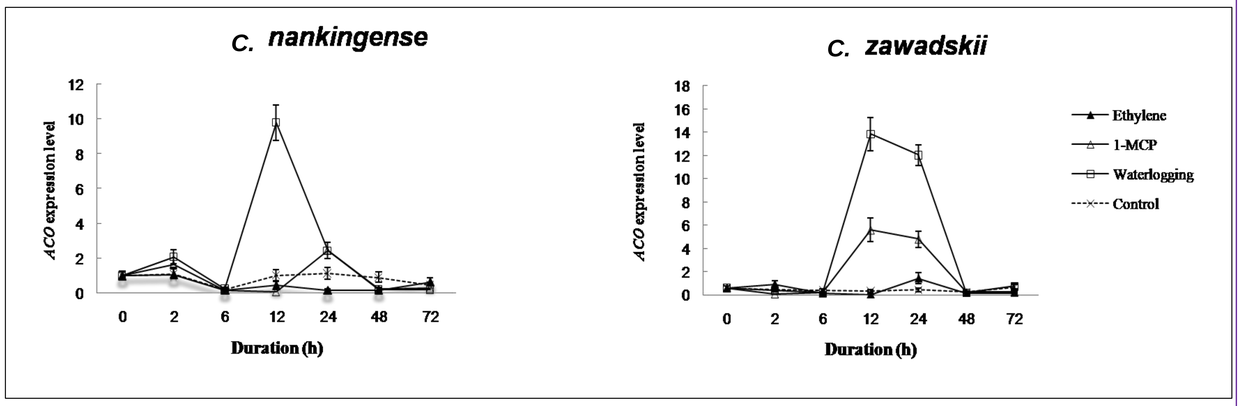


Fig.3 *CgACO* expression in response to waterlogging in two *Chrysanthemum* species (*Chrysanthemum zawadskii* and *C. nankingense*) with different tolerance to waterlogging. Four treatments were applied to plants at the eight-node stage. Control: well-watered plants; waterlogging: soil level covered by 2.5 cm of water; Ethylene : waterlogged plants exposed to ethylene; 1-MCP: waterlogged plants exposed to 1-MCP. Data represent the mean ± standard error from three independent replicates.

Fig 4. In the tolerant *Chrysanthemum* species, ethylene appeared to signal an acceleration of both waterlogging-induced programmed cell death and aerenchyma formation and to alleviate ethanolic fermentation, whereas in the sensitive species ethylene activated fermentation and increased the release of ethanol and acetaldehyde, which are by-products probably responsible for the collapse of the waterlogging-damaged root.

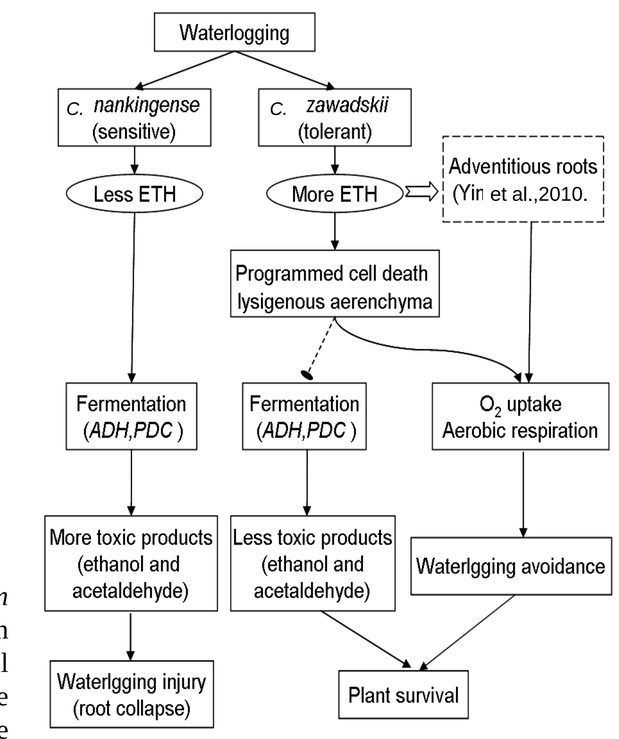


Fig.5. Proposed model for ethylene-mediated adaptative responses of chrysanthemum to waterlogging.

Conclusions

This study confirmed the sensitivity of *CgACO* to external factors and its response to waterlogging. We conclude that higher expression of the *CgACO* accounts at least in part for the relatively better tolerance of *C. zawadskii* to waterlogged conditions. Ethylene regulates plant growth in response to many adverse environmental conditions, including the induction of aerenchyma, i.e. the formation of air spaces, in flooded roots in an effort to maintain oxygen levels and thus alleviates hypoxia. In this way, the stress is avoided, and plant survival is promoted. Determination of the effect of ethylene synthase genes (ACC synthase) on waterlogging and even the enzymatic activities of the ACO and ACS should be a promising task in the near future.

Acknowledgments

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