Functional analysis of genes in quinoa using a virus vector system

Quinoa (*Chenopodium quinoa* Willd.) is an annual protein-rich pseudocereal native to the Andean region of South America. Quinoa has been recognized as a potentially important crop in terms of global food and nutrition security since it can thrive in harsh environments and has an excellent nutritional profile. JIRCAS and collaborative researchers have been analyzing the complex and heterogeneous allotetraploid genome of quinoa, and have recently overcome the challenges, with the whole genome-sequencing of quinoa and the creation of genotyped inbred lines (Research Highlights 2016, B03: Draft genome sequence of an inbred line of *Chenopodium quinoa*, an allotetraploid pseudocereal crop with high nutritional properties and tolerance to abiotic stresses; Research Highlights 2020, B08: Genetic and phenotypic variation of agronomic traits and salt tolerance among quinoa inbred lines). However, the lack of technology to analyze gene function *in planta* is a major limiting factor in quinoa research.

In this study, we demonstrate that the virus-mediated transient gene expression or repression techniques can be used in quinoa plants (Fig. 1). We show that apple latent spherical virus (ALSV) vector can induce gene silencing of a quinoa carotenoid biosynthesis gene, phytoene desaturase (*CqPDS1*) (Fig. 2). Virus-mediated silencing of *CqPDS1* induces decreased accumulation of carotenoids and causes photobleaching symptoms in quinoa plants (Fig. 3). We also show that ALSV-mediated gene silencing can also be used in a broad range of quinoa inbred lines derived from the northern and southern highland and lowland sub-populations. Our data also indicate that repression of a quinoa 3,4-dihydroxyphenylalanine 4,5-dioxygenase gene (*CqDODA1*) or a cytochrome P450 enzyme gene (*CqCYP76AD1*) reduces accumulation of red-violet betalain pigments in quinoa plants (Fig. 4).

Our data demonstrate that the virus vector system is a useful tool for evaluating gene function in quinoa, where molecular breeding techniques such as genetic transformation have not been developed yet. Functional validation of quinoa genes, utilizing the published genomic information, could provide gene resources for molecular breeding of quinoa.

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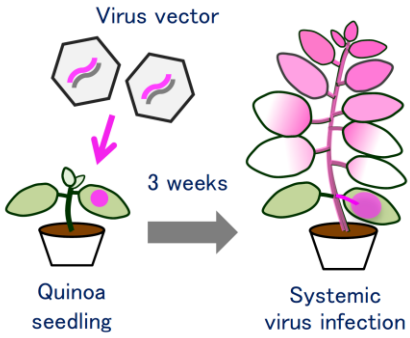

Fig. 1. Schematic of the virus vector system in quinoa plants

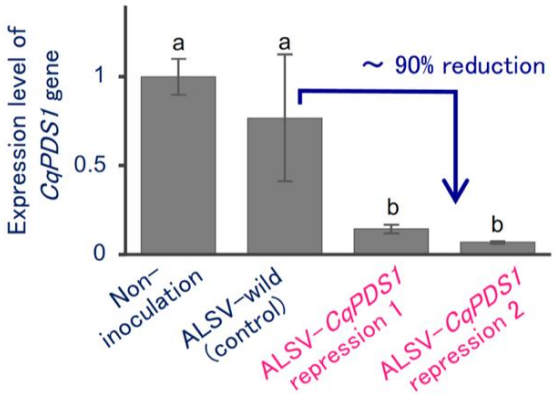

Fig. 2. ALSV induces silencing of carotenoid biosynthesis genes *CqPDS1* in quinoa.
RT-qPCR quantification of *CqPDS1* transcripts in the uninoculated upper leaves of plants inoculated with the indicated inocula. Data are normalized and are shown as means ± SD ($n = 3$). Different letters indicate significant differences ($p < 0.05$).

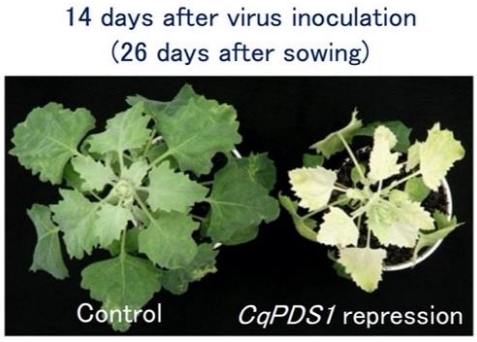

Fig. 3. Silencing of *CqPDS1* induces photobleaching phenotypes in quinoa plants.
A representative image of quinoa plants (inbred Iw line) at 14 days after virus inoculation with ALSV-wild (control) and ALSV-CqPDS1 is shown.

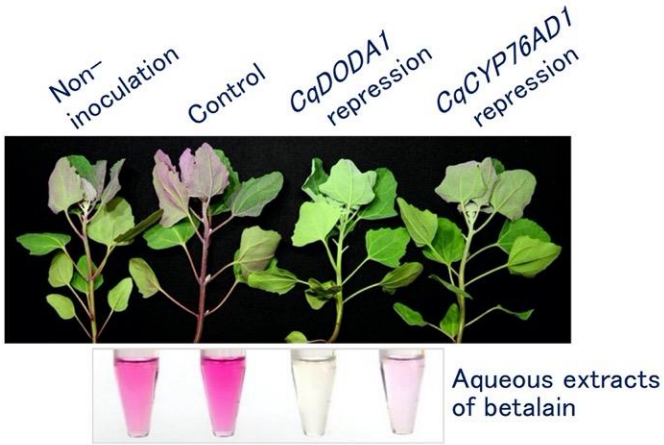

Fig. 4. Functional analysis of genes for betalain pigments biosynthesis in quinoa using the virus vector system
Virus-mediated silencing of *CqDODA1* and *CqCYP76AD1* inhibits betalain production in quinoa. Representative images of quinoa plants (inbred J056 lines) and aqueous extracts from the uninoculated upper leaves are shown. Quinoa plants inoculated with ALSV-wild were used as a control.

Reference: Ogata et al. (2021) *Frontiers in Plant Science* 12: 643499
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