Ⅲ-2

Development of a transgenic potato accumulating high content of vitamin C through the introduction of Dehydroascorbate Reductase cDNA derived from hairy root culture of Sesame

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Objectives

Increasing nutritional value and resistance to various environmental stresses of potato crops accumulating higher content of vitamin C through the enhanced recycling activity of dehydroascorbate reductase.

Materials and Methods

○ Materials : *Solanum tuberosum* cv. Jowon, constitutive expression promoter(CaMV35S) and tuber-specific (patatin) promoter

○ Methods : *Agrobacterium*-mediated transformation, southern and northern blot hybridization and western blot hybridization were conducted. DHAR enzyme activity was determined as described by Hossain and Asda(1984) and Ascorbic acid was determined by HPLC.

Results

DHAR cDNA, isolated from sesame hairy roots, was inserted into two plant expression vector systems with the CaMV35S promoter (CaMV35S::DHAR) and a potato tuber-specific promoter, Patatin (Patatin::DHAR). Southern and northern blot hybridization analyses indicated that DHAR cDNA was successfully integrated into the potato genome and actively transcribed. High levels of sesame DHAR transcript and DHAR enzyme activity were determined, by the Patatin promoter, in regenerated potato tubers, but their levels in leaves were very low. In contrast, much higher amounts of transcript were accumulated in the leaves of CaMV35S::DHAR regenerants than in the tubers while the activity of DHAR enzyme was higher in the latter. AsA content in the tubers of Patatin::DHAR transgenic lines was also increased (1.1- to 1.3-fold) compared with that of non-transgenic plants. However, this was not true for the transgenic leaves. In contrast, the CaMV35S promoter was associated with AsA accumulations in both the tubers (up to 1.6-fold) and the leaves (up to 1.5-fold). However, more detailed analyses indicated that this increased enzyme activity was not always accompanied by an elevation in AsA content from transgenic plants. We now begin to analyze their behaviour to various abiotic environmental stresses.

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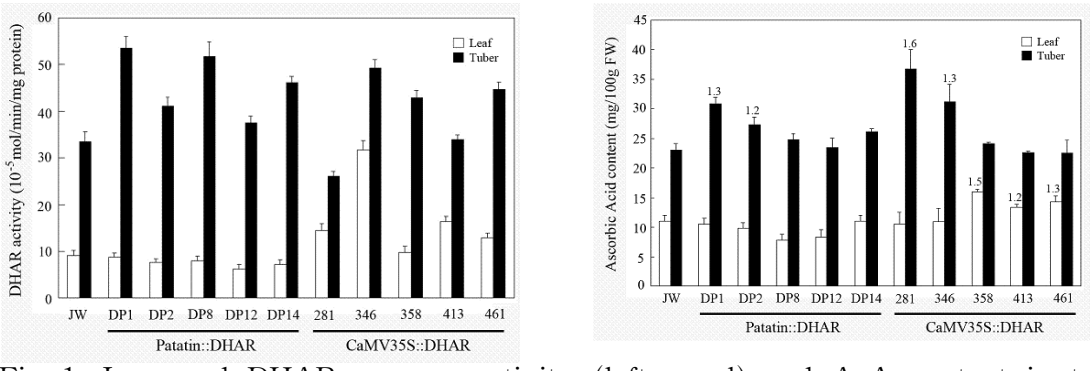

Fig 1. Increased DHAR enzyme activity (left panel) and AsA content in transgenic potato leaves (white histograms) and tubers (black histograms). JW indicates non-transgenic plant (S. tuberosum cv. Jowon). DP1, DP2, DP8,DP12, and DP14 are transgenic lines with Patatin::DHAR; Lines 281, 346, 358, 413, and 461 contain CaMV35S::DHAR construct. DHAR enzyme activity was defined as increase in absorbance at 290 nmupon converting DHA to AsA by DHAR enzyme, with GSH as an electron donor. AsA contents were determined by HPLC. Fold increases are stated relative to control, non-transgenic plants, Means and SDs are reported for 3 independent measurements.

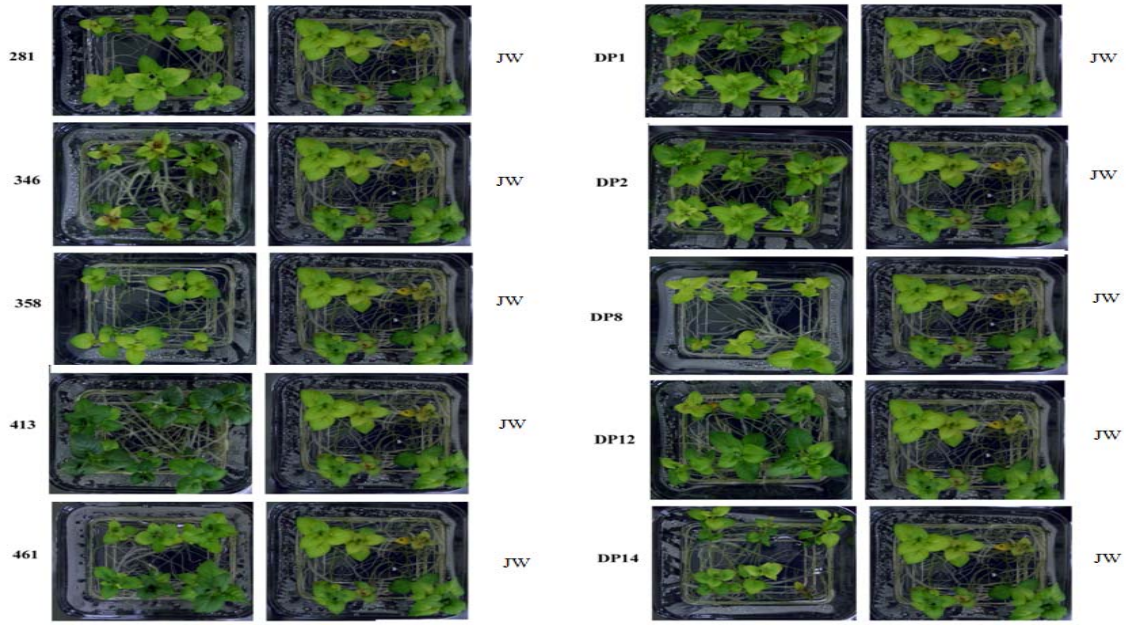

Fig 2. Increased resistnace to cadmium ion. Plants were treated in 75 uM for one month.