Absence of AVP1 transcripts in wild type watermelon scions grafted onto transgenic bottle gourd rootstocks

Byung Oh Kim·Jeung-Sul Han·Kyung Il Park·Su Min Jeon·Chang Kil Kim

B.O. Kim
School of Food science & Biotechnology, Kyungpook National University, Daegu 702-701, Korea

J. S. Han
Department of Ecological Environment, Kyungpook National University, Sangju 742-711, Korea

K. I. Park
Department of Horticulture & Life Science, Yeungnam University, Gyeongsan 712-749, Korea

S. M. Jeon, C. K. Kim*
Department of Horticultural Science, Kyungpook National University, Daegu 702-701, Korea
e-mail: ckkim@knu.ac.kr
Abstract In this study we confirmed the stable integration of *Arabidopsis AVP1* in the genomes of bottle gourd T3 homozygous lines and its transcription, and additionally evaluated possibility of translocation of the *AVP1* mRNA from transgenic bottle gourd rootstocks to wild type watermelon scions. Each *AVP1* gene in two bottle gourd T3 lines is abundantly expressed under a field condition. Given the grafting between wild type watermelon scions and AVP1-expressing bottle gourd rootstocks, no translocation of the *AVP1* mRNA was detected in leaves, both sexual flowers, and fruits of the scions.

Keywords Bottle ground · Gene modified · Graft · RT-PCR · Watermelon

Introduction

Grafting is now a popular technique for the cultivation of the horticultural crops including cucurbitaceae fruit vegetables, which has been developed not only to control growth and development of the scion but also to enhance tolerance against soil-borne diseases and/or abiotic stresses, such as salinity, low temperature and drought (Jang et al. 2012; Kubota et al. 2008; Lee 1994). In some special regions, where land utility is extremely limited, the allied crops are repeatedly cultivated all the year round (Kubota et al. 2008; Lee 1994), which increases specific pathogens and salinity of the rhizosphere. To overcome the disadvantages of the intensive cultivation, improvement of rootstocks by using genetic engineering is being attempted as a solution (Han et al. 2009; Smotka et al. 2010; Wang et al. 2012).

Control of abiotic stresses is an important element to increase total yields in modern agriculture. Plants respond to various abiotic stresses by altering their turgor pressure in vacuoles in order to accomplish selective permeation of solutes through proton pumps (Gaxiola et al. 2001; McNeil 1999). A vacuolar *et al.* H⁺-pyrophosphatase encoded by the *AVP1* gene is one of the proton pumps in *Arabidopsis* (Sarafian, et al. 1992) and generates an H⁺ electrochemical gradient across the tonoplast (Zhen et al. 1997). Several transgenic plants overexpressing AVP1 have been shown to be more tolerant to salt- and drought-stress than their counterparts (Gaxiola et al. 2001; Jeong et al. 2013; Park et al. 2012; Pasapula et al. 2011).
Materials and Methods

Plant materials and transformation

Bottle gourd (Lagenaria siceraria ‘G5’) transformation was performed by means of the Agrobacterium-mediated transformation method using cotyledon explants as described (Han et al. 2004; 2005; 2009). A. tumefaciens strain LBA4404, with the pRG521 plasmid that was generated by replacing the selectable marker cassette of pRG395 plasmid (Park et al. 2005a) with the Nos-pro/Bar/ter of pCB302 plasmid (Xiang et al. 1999), was used for this study. Collectively, the T-DNA region of pRG521 plasmid was consisted of LB/tandem 35S-pro/AVP1/Poly A/Nos-pro/Bar/Nos-tet RB. The T₃ lines, BGA VP05, was developed through phosphinothricin (Duchefa Biochemie, the Netherlands) at 2 mg/L supplementation for selecting T₀ plants in vitro, and herbicide Basta™ (Kyungnoog, Korea) at 0.3%(v/v) treatment and polymerase chain reaction (PCR) analysis for succeeding generations. Final T₃ generation of the BGA VP05 lines and wild type bottle gourd were sown in plastic trays filled with commercial organic soil. After 3 weeks, young plants were transplanted into plastic pots (30×35 cm) and then further grown in a greenhouse at Kyungpook National University located in Daegu, Korea. These plants were then subjected to nucleic acids analyses. Meanwhile, 1 week delayed seedlings of two commercial watermelons (Citrullus vulgaris ‘prince’ and ‘speed’) were grafted onto the two transgenic and wild type bottle gourd lines. After graft unions were stabilized, grafted plants were also transplanted and grown under the same conditions indicated above for non-grafted bottle gourd lines (Fig. 1).

Results and Discussion

Nucleic acids analyses of bottle gourd rootstock lines expressing Arabidopsis AVP1
We obtained T₀ plants, BGA VP05, from different transformation batch according to the same procedure. To confirm copy numbers of AVP₁ in the T₀ plants, DNA gel-blot analysis was conducted. As the recombinant construct for transformation has four multi-cloning sites for total seventeen kinds of restriction enzymes, very limited enzymes were used for the analysis. Among selected four enzymes (Bam HI, Pst I, Spe I, Xba I), the T₀ plants showed distinguishable hybridization patterns with single copy each of AVP₁ in only Xba I and Spe I digestions, respectively: a 3.4 kbp fragment by Xba I in BGA VP18 and a 2.2 kbp fragment by Spe I in BGA VP20 were revealed (data not shown). The independent transgenic plants were self-pollinated to obtain each progeny, after which T₁ and T₂ populations were treated with Basta™ and analyzed by PCR to generate non-segregating homozygous lines (Fig. 2). As rootstocks often help to overcome soil-borne pests and pathogens, many economically important crop species in Solanaceae and Cucurbitaceae are grafted before being transplanted to open fields or greenhouses in order to promote vigorous growth and enhanced yields of scions (Kubota et al. 2008). Genetic improvement of rootstocks is another important issue in modern crop breeding, as useful horticultural traits are being introduced by transgenic approach (Han et al. 2009; Smolka et al. 2010; Wang et al. 2012).

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Fig. 1 Herbicide treatment and polymerase chain reaction (PCR) analysis for bottle gourd T1 plants. (A) Herbicide bioassay for the selectable marker gene Bar. Basta\textsuperscript{TM} (Kyungnog, Korea) was treated at 0.3% (v/v). (B) PCR analysis of the AVP1. PCR products were run on 2% agarose gel and stained with ethidium bromide. WT (G5) and P indicate a non-transgenic wild type plant (negative control) and plasmid possessing full-length AVP1 (positive control), respectively.
Table 1 Effect of salt stress on growth of watermelon scions grafted onto wild-type and transgenic rootstocks.

| Scion cultivar | Rootstock<sup>a</sup> | Scion fresh weight (g/plant) | Scion dry weight (g/plant) | Scion leaf area (cm²/plant) |
|----------------|------------------------|------------------------------|----------------------------|-----------------------------|
| Prince         | Wild type              | 7.3b                         | 0.6b                       | 53.5b                       |
|                | Transgenic             | 11.5a                        | 1.1a                       | 75.7a                       |
| Speed          | Wild type              | 8.1b                         | 0.7b                       | 58.77b                      |
|                | Transgenic             | 13.2a                        | 1.3a                       | 80.3a                       |

Data are means of 15 independent experiments. Means within the same letter are not significantly different by DMRT (P<0.05).

<sup>a</sup>Rootstock means ~

If necessary, you need to give detailed explanations of a, b, c,