

1 [Journal of Plant Biotechnology 영문 투고 규정]

2 **Absence of AVPI transcripts in wild type watermelon scions grafted onto**

3 **transgenic bottle gourd rootstocks**

Title: Times New Roman 14 font size; Bold (Style); Left justified; Capitalize the first letter of the title

4 Line number: Continuously numbering,
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5 **Byung Oh Kim·Jeung-Sul Han·Kyung Il Park·Su Min Jeon·Chang Kil Kim**

Author: Times New Roman 12 font size; Bold (Style); Left justified;
First name-Last name

7 B.O. Kim

8 School of Food science & Biotechnology, Kyungpook National University, Daegu 702-701, Korea

10 J. S. Han

11 Department of

13 K. I. Park

14 Department of

Author and Affiliation: Times New Roman 11 font size; Left justified; 1.15 (Line spacing)

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(e.g) Byung Oh Kim → B.O. Kim

-Affiliation: Department/Institution(University)/City/Postal code/Country

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-Corresponding author must be marked with asterisk, e-mail

16 S. M. Jeon, C. K. Kim*

17 Department of Horticultural Science, Kyungpook National University, Daegu 702-701, Korea

18 e-mail: cckim@knu.ac.kr

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21 **Abstract** In this study we confirmed the stable integration of *Arabidopsis AVPI* in the genomes of bottle

22 Please insert 'Abstract title' in bold size 11 Times New Roman
23 Text: Times New Roman 11 font size); The abstract should not contain more than 1,400
24 to 1,600 letters and contain single paragraph without subheadings. Only SI units are
25 allowed to be used. Please use abbreviation of words (μL) instead of using mark ($\mu\ell$);
26 Abbreviation for Time unit are not allowed [e.g, min. (X) \rightarrow minutes (O)]

25 type watermelon scions and AVPI-expressing bottle gourd rootstocks, no translocation of the *AVPI* mRNA
26 was detected in leaves, both sexual flowers, and fruits of the scions

27 **Keywords:** Times New Roman 11 font size; Bold (Style); Left justified
28 Text: Times New Roman 11 font size; Plain (Style); Provide 5 to 7 keywords;
29 Capitalize the first letter of the word

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

29 Introduction; Times New Roman 14 font size; Bold (Style); Left justified

30 Introduction

31
32 Grafting is now a popular technique for the cultivation of the horticultural crops including cucurbitaceae fruit
33 vegetables, which has been developed not only to control growth and development of the scion but also to

34 Text: Times New Roman 11 font size; Plain (Style); You cannot indent first paragraph, but
35 second paragraph; In text, references should be listed in an alphabetical; Same authors
36 should be listed by year
37 (e.g) In some case ~ (Lee 2014; Kim and Han 2001; Kang *et al.* 2009)
38 (e.g) Kim *et al.* (2010) observed...
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38 a, b, c, etc. should be added after the year
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39 solution (Han *et al.* 2009; Smoika *et al.* 2010; Wang *et al.* 2012).

40 Control of abiotic stresses is an important element to increase total yields in modern agriculture. Plants
41 respond to various abiotic stresses by altering their turgor pressure in vacuoles in order to accomplish
42 selective permeation of solutes through proton pumps (Gaxiola *et al.* 2001; McNeil 1999). A vacuolar *et al.*
43 H^+ -pyrophosphatase encoded by the *AVPI* gene is one of the proton pumps in *Arabidopsis* (Sarafian, *et al.*
44 1992) and generates an H^+ electrochemical gradient across the tonoplast (Zhen *et al.* 1997). Several
45 transgenic plants overexpressing AVPI have been shown to be more tolerant to salt- and drought-stress than
46 their counterparts (Gaxiola *et al.* 2001; Jeong *et al.* 2013; Park *et al.* 2012; Pasapula *et al.* 2011).

47

48 **Materials and Methods**

Materials and Methods: Times New Roman 14 font size; Bold (Style); Left justified

50 **Plant materials and transformation**

Subtitle: Times New Roman 12 font size; Bold (Style); Left location

52 Bottle gourd (*Lagenaria siceraria* ‘G5’) transformation was performed by means of the *Agrobacterium*-
53 mediated transformation using cotyledon explants as described (Han *et al.* 2004; 2005; 2009). A.
54 *ta*... the selectable
55 marker cassette of pRG521 plasmid (Park *et al.* 2005a) with the *nos-pro/Bar/nos-ter* of pCB302 plamid
56 (Xiang *et al.* 1999), was used for this study. Collectively, the T-DNA region of pRG521 plasmid was
57 consisted of *LB/tandem 35S-pro/AVP1/Poly A/Nos-pro/Bar/Nos-ter/RB*. The T₃ lines, BGAVP05, was
58 developed through phosphinothricin (Duchefa Biochemie, the Netherlands) at 2 mg/L supplementation for
59 selecting T₀ plants *in vitro*, and *Arabidopsis*™ (Kvungnoog, Korea) at 0.3%(v/v) treatment and
60 polymerase chain reaction (PCR) analysis. The T₃ lines, BGAVP05
61 lines and wild type bottle gourd were sown in plastic trays filled with commercial organic soil. After 3 weeks,
62 young plants were transplanted into plastic pots (30×35 cm) and then further grown in a greenhouse at
63 Kyungpook National University located in Daegu, Korea. These plants were then subjected to nucleic acids
64 analyses. Meanwhile, 1 week delayed seedlings of two commercial watermelons (*Citrullus vulgaris* ‘prince’
65 and ‘speed’) were grafted onto the two transgenic and wild type bottle gourd lines. After graft unions were
66 stabilized, grafted plants were also transplanted and grown under the same conditions indicated above for
67 non-grafted bottle gourd lines (Fig. 1).

Text: Times New Roman 11 font size; Plain (Style); In addition to SI units, non SI unit such as diagonal line (/) percent (%), degree (°) also can be used. For experimental machine, please mention model, manufacturer, country.

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Results and Discussion: Times New Roman 14 font size; Bold (Style); Left justified

69 **Results and Discussion**

Subtitle: Times New Roman 12 font size; Bold (Style); Left location

71 **Nucleic acids analyses of bottle gourd rootstock lines expressing *Arabidopsis AVP1***

72

73 We obtained T₀ plants, BGAVP05, from different transformation batch according to the same procedure. To
74 confirm the presence of AVP1 in the T₀ plants, DNA gel blot analysis was conducted. As the recombinant
75 constructs were digested with various restriction enzymes, bands of restriction enzymes,
76 *Bam* HI, *Pst* I, *Spe* I, *Xba* I),
77 the T₀ plants showed distinguishable hybridization patterns with single copy each of AVP1 in only *Xba* I and
78 *Spe* I digestions, respectively: a 3.4 kbp fragment by *Xba* I in BGAVP18 and a 2.2 kbp fragment by *Spe* I in
79 BGAVP20 were revealed (data not shown). The independent transgenic plants were self-pollinated to obtain
80 each progeny, after which T₁ and T₂ populations were treated with BastaTM and analyzed by PCR to generate
81 non-segregating homozygous lines (Fig. 2). As rootstocks often help to overcome soil-borne pests and
82 pathogens, many economically important crop species in *Solanaceae* and *Cucurbitaceae* are grafted before
83 being transplanted to open fields or greenhouses in order to promote vigorous growth and enhanced yields of
84 scions (Kubota *et al.* 2008). Genetic improvement of rootstocks is another important issue in modern crop
85 breeding, as useful horticultural traits are being introduced by transgenic approach (Han *et al.* 2009; Smolka
86 *et al.* 2010; Wang *et al.* 2012).

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-In text, table and figures can be mentioned as below;
(ex) Table 3 and Fig. 2 were ...
(ex) Rooting increased under higher light intensities (Fig. 2).

Acknowledgements: Times New Roman 14 font size; Bold (Style); Left justified
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88 **Acknowledgements**

89
90 This study was supported by a grant from the Rural Development Administration in Korea. This research
91 was supported by Kyungpook National University Research Fund, 2012.

92

93 **References**

94 **References: Times New Roman 14 font size); Bold (Style); Left location**

95 Citovsky V, Zambryski P (2000) Systemic transport of RNA in plants. Trends Plant Sci 5:52-54.

96 Edelstein M, Cohen Y, Shriber S (1999) Integrated management of sudden wilt in melons, caused

97 by M **Text: Times New Roman 11 font size; Plain (Style)**

98 1145 **All reference should be listed in an alphabetical order, by author's family name. Same authors should be listed by year. If there are more than one publication in the same year for the same author(s), the letter a, b, c, etc. should be added to the year**

99 Flachow **-Capitalize the first letter of the paper title**

100 medi **-Always use abbreviation of journal in web site ISI Journal Title Abbreviations;**

101 grow **But single title of journal use only full name**

102 Gaxiola RA, Li J, Undurraga S, Dang LM, Allen GJ, Alper SL, Fink GR (2001) Drought- and salt-tolerant
103 plants result from overexpression of the AVP1 H⁺-pump. Proc Natl Acad Sci USA. 98:11444-11449.

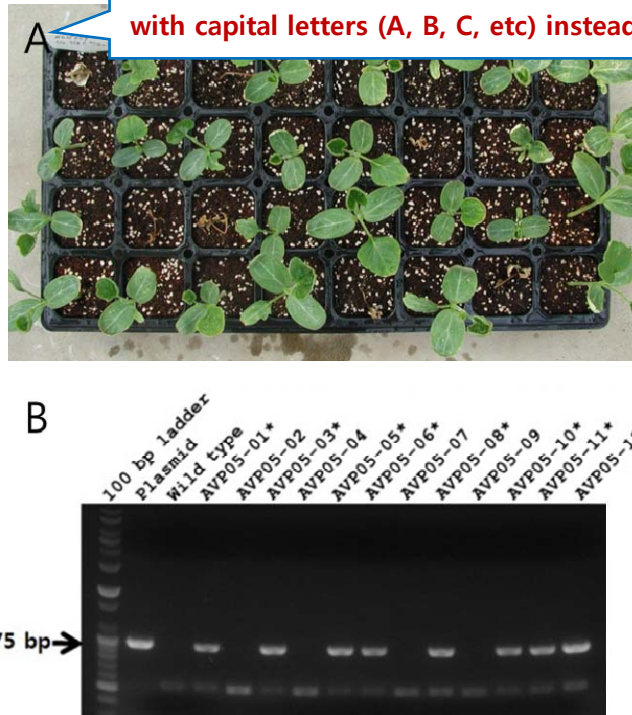
104 Murata K, Nakamura C, Fujiwara M, Mori N, Kaneda C (1997) RFLP mapping of brown planthopper
105 resistance gene in rice. Proc. of the 8th SABRAO Genetal Congress and the Annual Meeting of the Korean
106 **B**reeding **S**ociety. Seoul, Korea. pp.193-194.

107 **Book**

108 **-Capitalize the first letter of all title words of book title, and capitalize only the first letter of the paper's title (or Chapter)**

Tables and Figures:
Each table (figure)
should be placed per
one page

Figure: Should be denoted by upper-left hand corner
with capital letters (A, B, C, etc) instead of a, b, c, etc.



110

111 **Fig. 1** Herbicide treatment and polymerase chain reaction (PCR) analysis for bottle gourd T₁ plants. (A)

112 Herbicide bioassay using selectable marker gene *Bar*. Basta™ (Kyungnong, Korea) was treated at 0.3% (v/v).

113 (B) PCR analysis of the *Bar* gene. Lane 1: 100 bp ladder; Lane 2: plasmid; Lane 3: wild type; Lanes 4-12: AVP05-01* through AVP05-12*.

114 WT (wild type) and AVP05-01* through AVP05-12* (transgenic lines) were used as controls. The expected product length is 975 bp.

115 length AVPI (positive control), respectively.

116

117 **Table 1** Effect of salt stress on growth of watermelon scions grafted onto wild-type and transgenic
 118 rootstocks.

Table: Times New Roman 11 font size; Bold (Style)
Text: Times New Roman 11 font size; Plain (Style)

Scion cultivar	Rootstock ^a	Scion fresh weight (g/plant)	Scion dry weight (g/plant)	Scion leaf area (cm ² /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

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

Data must be placed vertically to compare each result; Capitalize the first letter of the word; Cannot use vertical line; Left justified

121 ^aRootstock means ~

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