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SHORT COMMUNICATION

Detection of Gene Flow from GM to non-GM Watermelon in a Field Trial

Chang-Gi Kim[1], Bumkyu Lee[1], Dae In Kim[1], Ji Eun Park[1], Hyo-Jeong Kim[1], Kee Woong Park[1], Hoonbok Yi[1], Soon-Chun Jeong[1], Won Kee Yoon[1], Chee Hark Harn[2] and Hwan Mook Kim[1]*

[1]Bio-Evaluation Center, KRIBB, Cheongwon 363-883, Korea
[2]Biotechnology Institute, Nongwoo Bio Co., Ltd., Yeoju 469-885 Korea

Gene flow from genetically modified (GM) crops to conventional non-GM crops is a serious concern for protection of conventional and organic farming. Gene flow from GM watermelon developed for rootstock use, containing cucumber green mottle mosaic virus (CGMMV)-coat protein (CP) gene, to a non-GM isogenic control variety 'Chalteok' and grafted watermelon 'Keumcheon' was investigated in a small scale field trial as a pilot study. Hybrids between GM and non-GM watermelons were screened from 1304 'Chalteok' seeds and 856 'Keumcheon' seeds using the duplex PCR method targeting the *CGMMV-CP* gene as a marker. Hybrids were found in all pollen recipient plots. The gene flow frequencies were greater for 'Chalteok' than for 'Keumcheon'; with 75% outcrossing in the 'Chalteok' plot at the closest distance (0.8 m) to the GM plot. A much larger scale field trial is necessary to identify the isolation distance between GM and non-GM watermelon, as the behaviors of insect pollinators needs to be clarified in Korea.

Keywords: Citrullus lanatus; gene flow; genetically modified crop; watermelon

Gene flow from genetically modified (GM) crops to wild and weedy relatives via pollen and seed dispersal is a major concern regarding environmental release of GM plants (Chapman and Burke, 2006). Although the ecological consequences of crop-to-crop gene flow are beginning to be investigated (Snow et al., 2005), it is a concern for the protection of conventional and organic farming (Messeguer, 2003). Pollen flow from GM crops to neighboring non-GM crops might even cause a legal problem because the spread of transgenes can be considered to damage the property of farmers cultivating non-GM crops (Kershen, 2004).

Most watermelons (*Citrullus lanatus*) are usually cultivated using grafted seedlings in Korea (Lee, 1994). Recently, a GM watermelon for rootstock resistant to cucumber green mottle mosaic virus (CGMMV), which has caused considerable reduction in yields of Cucurbitaceae crops, has been developed (Park et al., 2005) and field tests reported (Park et al., 2007). This GM watermelon was used in the present study.

Southern Africa is considered the center of origin and diversity for watermelon (Wehner et al., 2001). Watermelon will cross with five other *Citrullus* spp. worldwide (Fehér, 1992). It will not cross with other cucurbits (Wehner et al., 2001), such as cucumber (*Cucumis sativus*), melon (*Cucumis melo*), pumpkin (*Cucurbita pepo*) and gourd (*Lagenaria siceraria*). *Citrullus lanatus* is the only member of the genus grown for agricultural purposes in Korea and wild watermelons are absent in the natural environment.

The objective of the present study was to investigate gene flow from GM watermelon to the non-GM isogenic variety 'Chalteok' and grafted watermelon 'Keumcheon' using a transgene as a tracer marker in a small-scale field trial as a pilot study. We did not suggest an isolation distance between GM and non-GM watermelon, which should be investigated in a much larger scale field study. Crop-to-crop gene flow has been reported for GM cotton (Umbeck et al., 1991), potato (McPartlan and Dale, 1994) and rice (Messeguer et al., 2001), however, we are not aware of any gene flow studies of GM watermelon.

*Corresponding author; fax +82-43-240-6519
e-mail Hwanmook@kribb.re.kr

MATERIALS AND METHODS

Plant materials

A GM watermelon line, derived from the isogenic variety 'Chalteok', was developed using *Agrobacterium tumefaciens*-mediated transformation (Park et al., 2005). The plant contained the *CGMMV* coat protein (*CGMMV-CP*) gene under the control of the cauliflower mosaic virus (CaMV) 35S promoter, *nos* terminator and hygromycin phosphotransferase (*hpt*) gene for hygromycin selection. The T_5 generation of the GM watermelon, non-GM watermelon 'Chalteok' and grafted watermelon 'Keumcheon' (the scion 'Keumcheon' grafted onto the rootstock 'Chalteok') were used for a field trial.

Field trial

The field trial was conducted in a confined field located at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cheongwon-gun, Chungcheongbuk-do, Korea (36°43'04"N, 127°26'07"E, elevation 37 m). An 11 m × 30 m plot was established with north to south orientation (Fig. 1). The plot was divided into 6 plots (5 m × 10 m) with a 1-m alley left unplanted between the plots. GM and non-GM watermelons were transplanted into the plastic-mulched plot with 60 cm spacing between plants. Thirty GM watermelons were planted in the two central 5 m × 10 m plots. Both non-GM 'Chalteok' and 'Keumcheon' were planted in the north and south plots (Fig. 1), with 15 seedlings per plot. In the north and south plots, the closest plants to the

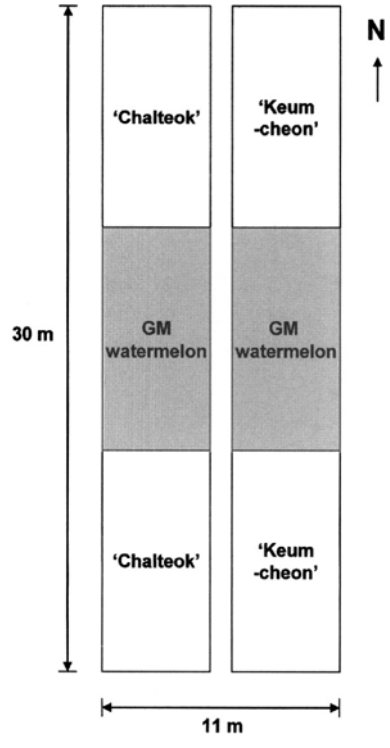

Figure 1. An experimental design of the field trial. GM watermelon was planted in gray rectangles. Both non-GM isogenic variety 'Chalteok' and non-GM grafted watermelon 'Keumcheon' were planted in the north and south plots.

central GM watermelon plots were at a distance of 0.8 m and the farthest 9.2 m. Watermelon was cultivated according to the conventional practice for 17 weeks. During the cultivation period, the mean air temperature was 23.6°C and total rainfall was 694.4 mm. All fruit on the vines was retained until harvest.

Seed sampling

GM and non-GM watermelons flowered from June until September. The ripened fruit of 'Chalteok' and 'Keumcheon' were harvested twice, on 11 August and 26 September. The collected fruits were matured in a contained greenhouse and the seeds were separated. All seeds from each fruit were collected, counted and stored at 4°C until analyzed.

Screening for hybrids

The fruits with the largest seed numbers from each pollen recipient plants were used for the hybrid screening. Hygromycin resistance at a concentration of 30 mg/L was tested to screen seedlings. However, hygromycin-resistant and hygromycin-sensitive seedlings could not be separated, as the growth reduction in hygromycin-sensitive seedlings was not conspicuous. Therefore, PCR targeting the *CGMMV-CP* gene was used to test for the presence of transgenes in the hybrids. PCR has been widely used to detect the presence of transgenes in the GM soybean and maize (Querci et al., 2004; Kim et al., 2006).

Fifty seeds collected from each plant were sown on 20 July 2007 in a 50-hole plastic tray (5 rows × 10 holes) filled with soil and grown in a contained greenhouse. Three weeks after sowing, newly grown petioles were collected from the seedlings. Ten petioles collected from a row of a tray were crushed on a PlantSaver™ FTA card (Whatman, USA) to make a bulk sample. The FTA cards containing DNA samples were stored at room temperature until PCR was completed in October 2007.

The duplex PCR method was used to detect hybrids. A forward primer CGMMV-37F (CTT ACA ATC CGA TCA CAC CT) and a reverse primer CGMMV-212R (CTA CGA CAG ACG AGG GTA AC) were designed to detect the *CGMMV-CP* gene (size: 176 bp). Actin-F (TGG ACT CTG GTG ATG GTG TC) and Actin-R (CCT CCA ATC CAA ACA CTG TA) were designed to detect the *actin* gene (size: 560 bp) as a PCR positive control. The primers were synthesized by Bioneer Co. (Daejeon, Korea). Two of 2-mm discs of a FTA card containing plant tissue were removed and transferred into a 1.5-mL tube. A volume of 400 µL FTA purification buffer (Whatman, USA) was added to each tube and incubated for 5 min at room temperature. The buffer was removed by micro pipette and discarded, the purification was repeated and the buffer removed again. Washing was then performed using 400 µL TE buffer (10 mM Tris-HCl; pH 7.5, 0.1 mM EDTA; pH 8.0) for 5 min, and the FTA card disks were dried at room temperature for 1 h before PCR was performed. The duplex PCR was performed with a final volume of 25 µL containing two disks of the FTA card, 0.5 µL of a 10 mM dNTP mixture, 0.3 µL of Taq DNA polymerase, 2.5 µL of 10X Taq buffer, 2 µL of 10 pmole of each primer. The PCR conditions for the amplifications were as follows: initial denaturation at 94°C for 3 min, 37 cycles of denaturation at 94°C for 1 min, annealing at 55°C.

If PCR-positive bulk samples were found, each individual plant in the bulk sample was separately sampled on a FTA card and PCR was conducted by the method described above. The PCR results targeting *CGMMV-CP* gene were confirmed by the PCR targeting *hpt* gene (size: 544 bp) using the forward primer Hygro-546F (GTG TCG TCC ATC ACA GTT T) and the reverse primer Hygro-3R (GAA AAA GCC TGA ACT CAC C) (data not shown). The gene flow frequencies were calculated as the percentages of the number of transgene-detected plants per the number of germinated seedlings.

RESULTS AND DISCUSSION

Flowering of GM and non-GM watermelons was synchronous from June to September. Mean number of fruits per plant was greater in 'Chalteok' (2.4 ± 1.5, mean ± SD) than 'Keumcheon' (1.6 ± 1.7). The mean number of seeds per fruit was also greater in 'Chalteok' (601.9 ± 216.0) than 'Keumcheon' (239.6 ± 99.8). Although 30 pollen recipient plants were planted in the 'Chalteok' and 'Keumcheon' plots, respectively, viable seeds were obtained from only 27 'Chalteok' and 20 'Keumcheon' plants, because the other plants were either dead or did not maintain fruits until harvest.

Of 1304 seedlings tested, there were 42 hybrids between

Table 1. Frequencies of gene flow from GM watermelon to 'Chalteok' and 'Keumcheon' at increasing distances from the GM plots. Values shown are the number of PCR-positive seedlings/ number of germinated seedlings and values in parentheses are the gene flow frequencies.

Distance from GM plot (m)	'Chalteok'		'Keumchon'	
	North plot	South plot	North plot	South plot
0.8	3/50 (6.00%)	37/49 (75.51%)	0/44	0/31
1.4	0/50	0/49	0/41	2/43 (4.65%)
2.0	0/44	0/50	0/37	ND
2.6	0/50	0/47	0/44	0/47
3.2	0/50	0/33	0/49	0/43
3.8	2/49 (4.08%)	ND	0/45	ND
4.4	0/46	0/50	0/49	ND
5.0	0/49	0/50	ND	ND
5.6	0/49	0/50	ND	ND
6.2	0/47	0/50	0/48	0/46
6.8	0/50	0/50	ND	0/50
7.4	0/45	0/50	ND	0/43
8.0	ND	0/49	ND	0/41
8.6	ND	0/48	0/49	0/15
9.2	0/50	0/50	0/43	0/48
Total	42/1304 (3.22%)		2/856 (0.23%)	

ND: no data

GM watermelon and 'Chalteok' (Table 1); these hybrids were at the closest distance in both north and south plots and were also up to 3.8 m from the GM plots. In the south plot, at 0.8 m the outcrossing rate was as high as 76%. PCR analysis showed the presence of *CGMMV-CP* fragment in the bulk (Fig. 2A, lane 5) and individual samples (Fig. 2B, lanes 4 and 7).

There were only two hybrids between GM watermelon and non-GM watermelon 'Keumcheon' out of 856 seedlings (Table 1) and the presence of *CGMMV-CP* fragment was confirmed by PCR (Fig. 3, lanes 1 and 2). The hybrids between GM watermelon and 'Keumcheon' were only found at 1.4 m distance in the south plot. Although the outcrossing rate was very low in the present study, the commercial watermelon variety did cross with GM watermelon derived from 'Chalteok'. This was contrary to the claims of Park et al. (2005) that the watermelon for rootstock, 'Chalteok', would not cross with most watermelon lines.

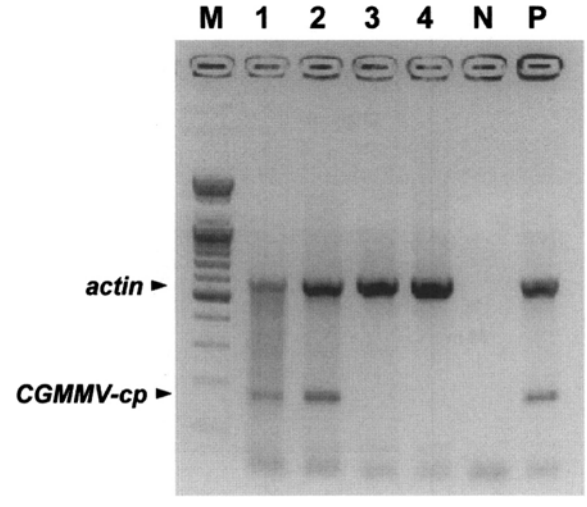

Figure 3. Agarose gel electrophoresis patterns of PCR products amplified from *actin* and *CGMMV-CP* genes of 'Keumcheon' samples obtained from the south plot at 1.4 m distance. M: 100-bp DNA ladder; Lanes 1-4: individual samples; N: negative control; P: positive control.

Maize and sugar beet are almost entirely pollinated by the wind (Hoyle and Cresswell, 2007), however, insects are the main vector for watermelon pollination. Insect visitation on the flowers is essential for fruit formation of watermelon. Among pollinators of watermelon, honey bees and bumble bees are considered the most effective (McGregor, 1976; Delaplane and Mayer, 2000). Therefore, these bees have frequently been applied to pollinate watermelon in both open conditions and protected cultivation.

To restrict gene flow from GM to non-GM plants, the isolation of the GM plants by distance and border rows, genetic isolation and temporal separation have frequently been used (Hokanson et al., 1997; Damgaard and Kjellsson,

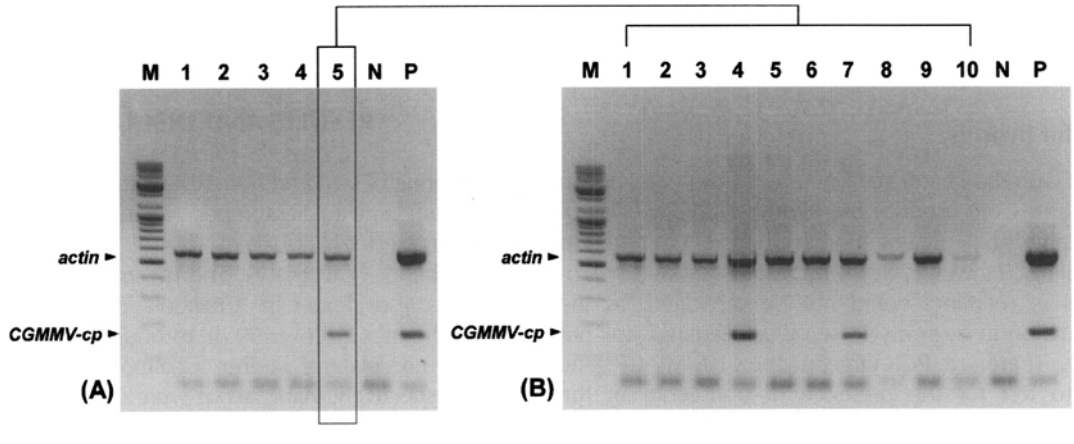

Figure 2. Agarose gel electrophoresis patterns of PCR products amplified from *actin* and *CGMMV-CP* genes of 'Chalteok' samples. (A) PCR of bulk samples formed by ten recipient plants obtained from the north plot at 0.8 m distance. M: 100-bp DNA ladder; Lanes 1-5: bulk samples; N: negative control; P: positive control (a mixture of samples from one GM plant and nine non-GM plants). (B) PCR of individual samples for a positive bulk sample (Lane 5 of Fig.2(A)). Lanes 1-10: individual samples.

2005; Halsey et al., 2005). The minimum isolation distance for foundation watermelon seed production is 800–1000 m (Kim et al., 1979; Traynor et al., 2001) due to the behavior of insect pollinators. Because seeds of watermelon rootstock are mostly produced by open pollination, gene flow from GM to non-GM watermelon rootstock should be assessed up to 1000 m, depending on the size of GM pollen donor plots. Gene flow of insect pollinated crops has been assessed up to 1600 m for GM cotton in the USA (Van Deynze et al , 2005) and 500–800 m for GM canola in Canada (Beckie et al., 2003; Morandin and Winston, 2005).

Lee et al. (2006) reported the roles of *Apis mellifera* on the pollination of watermelons under protected cultivation in Korea. However, there is little information on open pollination. Therefore, further studies on major insect pollinators' behavior in open fields of watermelon and distances of their pollen dispersal are required.

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