Journal of Applied Botany and Food Quality 86, 133 - 137 (2013), DOI:10.5073/JABFQ.2013.086.018

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Effect of sodium chloride, PGDO and Arabic gum in pollen liquid diluent on suspensibility of kiwi pollen

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(Received May 5, 2013)

Summary

This study was conducted to develop the pollen liquid diluent suitable for the artificial pollination of kiwi. The pollen of 'Matua' kiwi was collected at 1 day before flowering. Five concentrations of sodium chloride (0, 50, 150, 250, and 350 mg·L^{-1}), four concentrations of poly (glycolide-co-p-dioxanone) (0, 7, 14, and 21 mg·L^{-1}), and four concentrations of arabic gum (0, 150, 350, and 550 mg·L^{-1}) were tested on an absent condition of each component in the pollen liquid diluent. Twenty mg of pollen was distributed in beakers containing 10 mL of the pollen liquid diluent. Suspensibility of the pollen liquid diluent was measured by the sensory evaluation and particle size analyzer. The addition of sodium chloride in pollen liquid diluent was effectible for the suspensibility improvement and the promotion of pollen growth in kiwi pollen. The kiwi pollen in pollen liquid diluent could be suspended without damage in pollen germination at low concentration of poly (glycolide-co-p-dioxanone) (PGDO), which has been known as a safe surfactant. The addition of Arabic gum would be highly advantageous to the stabilization of the pollen liquid diluent without any contamination for pollen growth. Kiwi fruits were set and grown well by the artificial pollination using the pollen liquid diluent. Therefore, the use of the pollen liquid diluent in the artificial pollination of kiwi fruit should be an effective practice.

Introduction

In Kiwi (*Actinidiu deliciosa*) requiring cross pollination for commercial production, artificial pollination has been developed with equipment for this process (Gonzalez et al., 1998). However, effective pollination periods of kiwi flowers was quite limited with 3~4 days (Sanzol and Herrero, 2001), the artificial pollination, performed as hand pollination, has been formed temporarily as a heavy labor.

As this reason, some growers were spraying pollens from male flowers diluted in a 10 % sucrose solution on female flowers in an attempt to improve fruit set. However, the effectiveness of this practice was incredulous. In grapefruit, pollen germination was poor both for spray and dipping treatments and less than one pollen tube per pistil reached micropyles for any of the pollen concentration used (Kimura et al., 1998).

Pollen liquid diluent maintained pollen in a viable state while in an aqueous liquid, was already developed as named PollenAid® in New Zealand and has been used by kiwi growers. However, since PollenAid® is a commercial product, some information about pollen liquid diluent has never been revealed and reported in the scientific field.

The use of pollen liquid diluent could be an economically viable method for artificial pollination in kiwi production.

Accordingly, this study was conducted to develop the pollen liquid diluent suitable for pollination of kiwi throughout analyzing the effects of sodium chloride as a promoter, poly (glycolide-co-p-dioxanone) (PGDO) as a surfactant (Wang et al., 2012) and arabic gum as a stabilizer (Islam et al., 1997; Zhang and Liu, 2011) in the pollen liquid diluent on pollen growth and pollen suspensibility.

Material and methods

Kiwi pollen

Flowers of 'Matua' kiwi were collected at 1 day before flowering for the pollen collection in the experimental orchard at Chonnam Province Fruit tree Research Institute, which is located in Haenam, Korea. Flowers were brought back to the laboratory, where the anthers were collected from the flower with an anther sifter (MK-600, Mitsuwa, Japan). Roughly selected anthers by the sifter were simply put in the hopper of an anther selecting machine (KBS-6, Mitsuwa, Japan). Finally, the anthers were automatically collected. The collected anthers were scattered on a thick black paper, then maintained for 24 h in an anther opener (M-600D, Mitsuwa, Japan). The anthers, thus dried, were dipped into 99.5 % acetone in a stainless steel bowl, using a sieve (100 meshes). The sieve was removed after a short period of agitation in the bowl to collect pollen grains. The supernatant of the pollen-acetone mixture was then discarded. The pollen was weighed after the volatilization of the remaining acetone, and stored at 4 °C until later use in the germination test.

Basic component of pollen liquid diluent

The component of pollen liquid diluent consisted of basically 2 % of sucrose ($C_{12}H_{22}O_{11}$=342.31), 100 mg·L^{-1} of boric acid (H_3BO_3=61.84), 70 mg·L^{-1} of calcium nitrate ($Ca(NO_3)_2$=164), sodium chloride 250 mg·L^{-1}, arabic gum 350 mg·L^{-1}, PGDO 14 mg·L^{-1} and distilled water; pH adjusted to 7.0. The concentrations of each component was tested already at various concentrations for the determination of the optimal combination and decided through a previous test.

Addition of sodium chloride, PDGO, and arabic gum in pollen liquid diluent

Five concentrations of sodium chloride (0, 50, 150, 250, and 350 mg·L^{-1}), four concentrations of PDGO (0, 7, 14, and 21 mg·L^{-1}), and four concentrations of arabic gum (0, 150, 350, and 550 mg·L^{-1}) were tested on an absent condition of each component based on the basic component of pollen liquid diluent.

Twenty mg of pollen was distributed in beakers containing 10 mL of the pollen liquid diluent.

Pollen germination and pollen tube growth of the pollen liquid diluent

The pollen liquid diluent was cultured for 1,2,3,4, and 5 hours in an incubator maintained at 30 °C. All observations of pollen germination and pollen tube growth were performed at 4 hours after the distribution of the pollen in the pollen liquid diluent. Fifteen µL of pollen liquid diluent was taken and put on the slide glass for the microscopic observation.

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At least 150 pollen grains were counted on 10 random microscopic fields of the optical microscope (X200, Leica, Germany). A pollen grain was judged to germinate when the pollen tube length was greater than the diameter of the pollen grain. Pollen germination and pollen tube growth were measured using an Image Analyzer (IMT, Korea).

The evaluation was carried out with a completely randomized experimental design, with five replicates.

Suspensibility of the pollen liquid diluent

Suspensibility of the pollen liquid diluent was measured by the sensory evaluation and particle size analyzer. For the sensory evaluation, the pollen liquid diluent was stirred during the observation periods and investigated the suspensibility on 5 minute interval for 20 minutes by the sensory evaluation. The appearance score of suspensibility was assessed, using a numerical interval scale from 1 to 5, with 0.1 point intervals. The scale was defined as follows: 5 = very well mixed, 4 = well mixed, 3 = mixed, 2 = minimal mixed, 1 = mass mixed.

The particle size distribution in pollen liquid diluent of the pollens was determined on a particle analyzer (NANOPHOX, Sympatec GmbH, Germany) based on photon cross correlation spectroscopy.

The artificial pollination using the pollen liquid diluent

Artificial pollination was carried out on a netted plastic house for the protection of natural pollination by bees. Four $g \cdot L^{-1}$ pollens of 'Matua' kiwi were placed on the basic component of pollen liquid diluent and suspended by a vortex. The suspended pollen liquid diluent was sprayed at 1, 2, 3, 4, and 5 hours after dipping, respectively. A hand-mister was used to generate the spray. In all treatments, approximately 100 mL of the pollen liquid diluent per tree was used on 5 different 'Hayward' kiwi trees at 2 days after full bloom.

Fruit characteristics by the artificial pollination using the pollen liquid diluent

Fruit set, seed number, and fruit size were investigated from each fruit of 30 flower clusters signed on the pollination time of each tree at 30 days after pollination, when fruit set could be completely confirmed before fruit thinning process.

Results and discussion

Effects of sodium chloride in pollen liquid diluent

The suspensibility of pollens in pollen liquid diluent was improved with an increase of sodium chloride concentration and an increase of dipping time (Tab. 1). These results were similar to the improving effect of the suspensibility in water of dried nanofibrillated cellulose (NFC) by adding sodium chloride at different pH conditions (MISSOUM et al., 2012).

Although the rate of pollen germination was depressed at 350 $mg \cdot L^{-1}$ of sodium chloride, the pollen germination rate by the addition of sodium chloride in pollen diluent solution was increased compared to the control (Fig. 1A).

The pollen tube length was higher at 150 and 250 $mg \cdot L^{-1}$ of sodium chloride than that of the control. However, at 50 and 350 $mg \cdot L^{-1}$ of sodium chloride, pollen tube length demonstrated no difference (Fig. 1B).

The promotion of pollen growth at a low concentration of sodium chloride may be considered as the permeability increase in the exine of pollen grains. However, reducing effects of the pollen growth by the high sodium chloride concentrations was confirmed in rice (ZENG and SHANNON, 2000), tomato (YOKAS et al., 2008), and chickpea (SAMINENI et al., 2011).

Tab. 1: Effect of sodium chloride concentrations in pollen liquid diluent on the suspensibility by sensory evaluation of 'Matua' kiwi pollen.

Sodium chloride ($mg \cdot L^{-1}$)	Pollen suspensibility[z]			
	5 min	10 min	15 min	20 min
0	1.0 b[y]	1.7 c	2.8 c	3.8 c
50	1.0 b	1.8 c	3.0 bc	4.0 bc
150	1.2 b	2.3 b	3.2 b	4.5 b
250	1.8 a	2.8 a	3.8 a	4.7 a
350	2.0 a	3.0 a	4.0 a	4.9 a

[z] The scale was defined as follows: 5 = very well mixed, 4 = well mixed, 3 = mixed, 2 = minimal mixed, 1 = mass mixed. [y]Mean separation within columns by Duncan's multiple range test at 5 % level. Results are from five replicates.

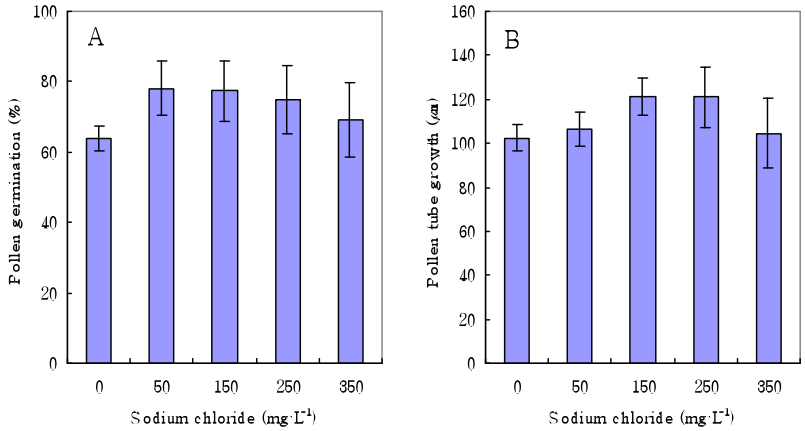

Fig. 1: Effects of sodium chloride concentrations in the pollen liquid diluent on pollen germination rate (A) and pollen tube growth (B) of 'Matua' kiwi in vitro. Vertical bars represent ± SD. Results are from five replicates. Replicates above consist of approximately 50 pollen grains each.

The above mentioned results indicate that the addition of sodium chloride in pollen liquid diluent is effectible for the suspensibility improvement. The optimum dipping condition without a contamination of the pollen germination in pollen liquid diluent should be announced for 20 minutes at 250 mg·L^{-1} of sodium chloride.

Effects of poly (glycolide-co-p-dioxanone) (PGDO) in pollen liquid diluent

The surfactants have been commonly used in a liquid diluent for the suspensibility. We already tested five surfactants which have been known as a possibility for use in pollen liquid diluent and selected PGDO as the adaptable surfactant (data not shown).

The suspensibility of kiwi pollens in pollen liquid diluent was improved with an increase of PGDO concentration. However, the progress of suspensibility in the pollen diluent solution was delayed at an initial time after pollen mixing (Tab. 2).

Tab. 2: Effect of PGDO concentrations in pollen liquid diluent on the suspensibility by sensory evaluation of 'Matua' kiwi pollen.

PGDO (mg·L^{-1})	Pollen suspensibility^{z}			
	5 min	10 min	15 min	20 min
0	1.0 b^{y}	1.5 c	2.5 c	3.5 c
7	1.0 b	1.5 c	3.0 b	4.0 b
14	1.0 b	2.0 b	3.5 ab	4.5 ab
21 2.0 a	3.0 a	4.0 a	5.0 a	

^{z} The scale was defined as follows: 5 = very well mixed, 4 = well mixed, 3 = mixed, 2 = minimal mixed, 1 = mass mixed. ^{y}Mean separation within columns by Duncan's multiple range test at 5% level. Results are from five replicates.

The action of PGDO in pollen liquid diluent was confirmed as the formation of well-defined spherical particles during suspension polymerization in supercritical carbon dioxide (Wang et al., 2012).

The pollen germination rate was depressed significantly by the addition of 21 mg·L^{-1} of PGDO in pollen diluent solution (Fig. 2A). Pollen tube length was also significantly decreased at 21 mg·L^{-1} of PGDO compared to the control (Fig. 2B).

Consequently, the kiwi pollens in pollen liquid diluent could be suspended without damage in pollen germination at low concentration of PGDO, which has been known as a safe surfactant. The optimum condition of PGDO for the improvement of suspensibility as a surfactant in pollen liquid diluent should be announced for 20 minutes at 14 mg·L^{-1}.

Effects of Arabic gum in pollen liquid diluent

The surface dilational rheology of the pollen liquid diluent is importantly considered in the spray process of field work. For the support of suspensibility in pollen liquid diluent, arabic gum was tested at various concentrations.

The suspensibility of pollens in the pollen liquid diluent was observed to gradually increase with dipping time and arabic gum concentration. Pollens in the pollen liquid diluent were quickly suspended in the conditions without arabic gum at the initial time of dipping (Tab. 3).

For adding by arabic gum, pesticide emulsion remained stable for 7 days even at -10 °C. This fact could be explained by a close correlation with arabic gum adsorption at the droplet surface (Zhang and Liu, 2011). Also, the maximum of dilational modulus and dilational elasticity in the trisiloxane surfactant silwet408 was shifted toward higher concentrations when arabic gum of 1 % was added (Cao et al., 2013).

Pollen germination rate and pollen tube growth in the pollen liquid diluent showed no difference with concentrations and dipping times by the addition of arabic gum (Fig. 3). It means that the addition of arabic gum would be highly advantageous to the stabilization of the pollen liquid diluent without any contamination for pollen growth.

Consequently, the addition of arabic gum in the pollen liquid diluent could be considered as an effective method for the advance of surface dilational rheology in the pollen liquid diluent.

Tab. 3: Effect of arabic gum concentrations in pollen liquid diluent on the suspensibility by sensory evaluation of 'Matua' kiwi pollen.

Arabic gum (mg·L^{-1})	Pollen suspensibility^{z}			
	5 min	10 min	15 min	20 min
0	1.7 a^{y}	2.5 b	3.3 b	4.0 b
150	1.7 a	2.5 b	3.5 b	4.5 ab
350	1.7 a	2.7 ab	3.5 ab	4.5 ab
550	1.8 a	3.0 a	4.0 a	4.8 a

^{z} The scale was defined as follows: 5 = very well mixed, 4 = well mixed, 3 = mixed, 2 = minimal mixed, 1 = mass mixed. ^{y}Mean separation within columns by Duncan's multiple range test at 5% level. Results are from five replicates.

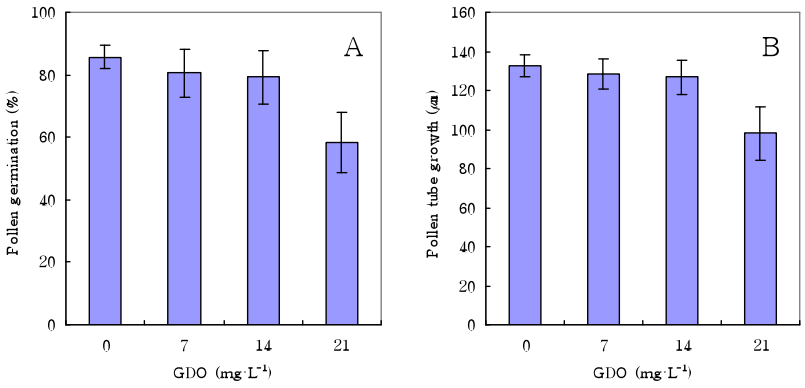

Fig. 2: Effects of PGDO concentrations in the pollen liquid diluent on pollen germination rate (A) and pollen tube growth (B) of 'Matua' kiwi in vitro. Vertical bars represent ± SD. Results are from five replicates. Replicates above consist of approximately 50 pollen grains each.

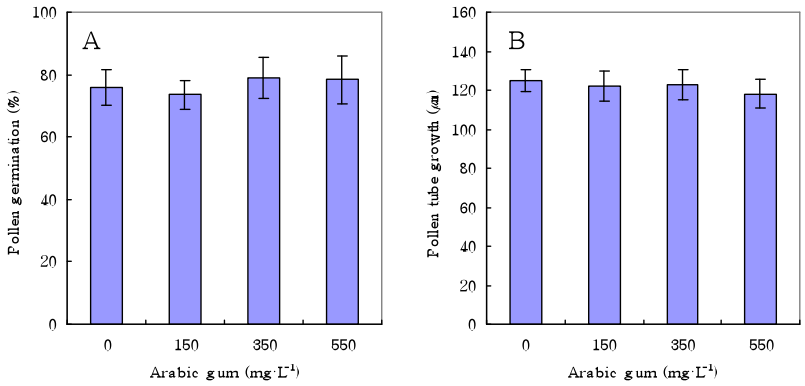

Fig. 3: Effects of arabic gum concentrations in the pollen liquid diluent on pollen germination rate (A) and pollen tube growth (B) of 'Matua' kiwi in vitro. Vertical bars represent ± SD. Results are from five replicates. Replicates above consist of approximately 50 pollen grains each.

Suspensibility analysis of the pollen liquid diluent by the particle analyzer

For suspensibility analysis of the pollen liquid diluent, particle size was observed with or without sodium chloride, PGDO, and arabic gum by the particle analyzer.

The pollen liquid diluent contained sodium chloride, PGDO, and arabic gum showed smaller particle size than pollen liquid diluent which was only manufactured by distilled water. The particle size was the smallest in the pollen liquid diluent without the arabic gum and higher in the pollen liquid diluent without the sodium chloride (Fig. 4).

These results were similar to that of sensory evaluation. Our data showed that the suspensibility of pollen liquid diluent is decreased by the addition of arabic gum and increased by the addition of sodium chloride.

Fruit quality by artificial pollination using the pollen liquid diluent

Fruit set in kiwi trees, pollinated artificially by the pollen liquid diluent was shown to be more than 95 % and demonstrated no difference with dipping times. Although seed number was reduced in

Tab. 4: Effects of the artificial pollination by the pollen diluent solution with dipping time on fruit set, seed number, fruit size, and L/D ratio of 'Hayward' kiwi fruit.

Dipping time (hrs)	Fruit set (%)	Seed number (No/fruit)	Fruit size (mm)		L/D ratio
			Length	Diam	
1	100 a[y]	1,019 a	51.2 a	34.2 a	1.50 a
2	100 a	1,040 a	51.6 a	34.7 a	1.49 a
3	99.0 a	987 a	49.5 a	32.5 a	1.48 a
4	97.7 a	769 b	48.8 ab	33.8 a	1.44 ab
5	96.0 a	636 bc	48.4 ab	33.7 a	1.44 ab

[y]Mean separation within columns by Duncan's multiple range test at 5% level. Results are from five replicates. Replicates above consist of fruits from 30 flower clusters signed on the pollination time of each tree at 30 days after pollination.

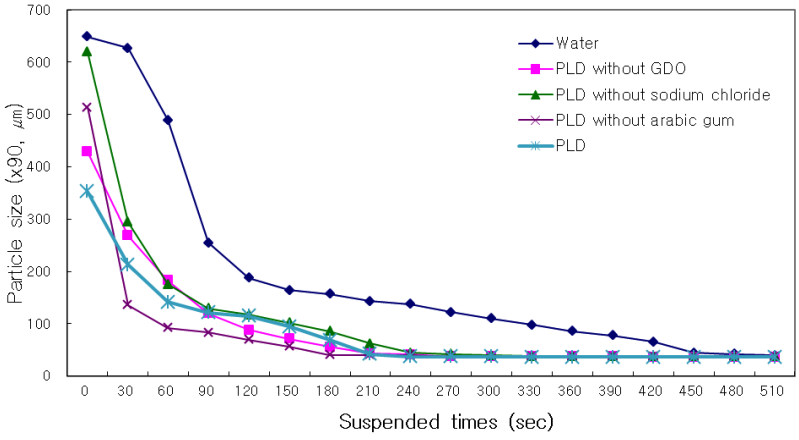

Fig. 4: Changes of particle sizes with compositions of the pollen liquid diluent (PLD) during suspended time.

kiwi trees pollinated at 4 and 5 hours after dipping, fruit size was reduced a little at that time. Consequently, the artificial pollination using the pollen liquid diluent should be a useful method for pollination without a weakness in fruit set and fruit growth of 'Hayward' kiwi.

Conclusion

The addition of sodium chloride in pollen liquid diluent was effectible for the suspensibility improvement. The low concentration of poly (glycolide-co-p-dioxanone) (PGDO) was suitable as surfactant. The addition of Arabic gum related in the dilational elasticity of surfactant should be highly advantageous methods for the stabilization of the pollen liquid diluent without any contamination for pollen growth. Kiwi fruits were set and grown well by the artificial pollination using the pollen liquid diluent.

Therefore, the pollen liquid diluent should be an effective practice in the artificial pollination of kiwi fruit performed temporarily heavy labor.

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