Hermaphrodite Flower Induction by Silver Nitrate (AgNO₃) Application and Possibility of Crossbreeding in Pointed Gourd (Trichosanthes dioica Roxb.)

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This study aimed to reveal the effect of silver nitrate on sex modification in dioecious pointed gourd (Trichosanthes dioica Roxb.) originated in South Asia, and to explore the possibility of crossing among female plants. Three concentrations (50, 100, and 200 ppm) of silver nitrate were applied as foliar spray on female vines of three pointed gourd accessions. Application of 50 ppm silver nitrate found as effect to induce maximum proportion of hermaphrodite flowers with expanded duration irrespective of genotypes. The percentage of hermaphrodite flower induction was decreased with the increase of silver nitrate concentration. Viability of pollen grain (92.6–94.0%) in 100 ppm silver nitrate induced hermaphrodite flowers were as similar as that of normal male flowers (95.3%). Crossing among different female genotypes with pollen taken from the induced hermaphrodite flowers, produced same size of fruits of normal crossing. Seeds obtained from such crossing germinated successfully and seedlings grew vigorously as that of normal seeds. This technique seems to be useful for crossings among female plants that have suitable characters and facilitate a breeding of new variety in pointed gourd.

Key Words: dioecious plant, hermaphrodite flower, sex modification, silver nitrate, Trichosanthes dioica.

Introduction

Recently, the annual mean temperature in Japan fluctuates on different time scales. The air temperature is getting high and the daily maximum temperature is 25°C or higher in summer season (Fujime, 2012). This temperature variations bring the serious problems in the vegetable production. For example, poor fruit bearing, cracking, poor coloring of vegetables is commonly observed due to high temperature in the summer season. In order to solve these problems, it seems that the introduction of the vegetables that originated in tropical and subtropical regions should be considered.

Pointed gourd (Trichosanthes dioica Roxb.) which is originated in Indian subcontinent is one of the most important summer vegetables in India and Bangladesh. This species belong to Cucurbitaceae and is locally known as “Potol” or “Parwal” (Kumar and Singh, 2012). Young fruit which is 10–12 cm length (30–50 g) at green stage is the main edible size and cooked as curry, fried, and pickles (Fig. 1). This species is a dioecious and climbing type plant, propagated using its vine cuttings. Among the cucurbitaceous vegetables, probably, pointed gourd has not been received much more attention for its breeding and subsequent improvement.

Since pointed gourd is usually grown in open field under high temperature condition of its origin countries, this vegetable seems to have a potential to become a new summer vegetable in Japan. However, this species is a dioecious plant, therefore it is impossible to crossbreeding among female plants. Dioecious characteristics bring the difficulties of the advancement of generations. Thus, this obstacle can be overcome by the sex modification to induce bisexual flowers in female plants.

Sex modification in plants can often be achieved by application of plant growth regulators (Das and Mukherjee, 1986; Marchetti et al., 1992). Ethylene is the principal hormone regulating sex expression in the Cucurbitaceae family. Thus, treatments with inhibitors of ethylene biosynthesis and controlling ethylene action, such as aminoethoxyvinylglycine or silver nitrate or silver thiosulphate have been reported as to increase the number of male flowers per plant and converted sex expression from female flowers into hermaphrodites.
Ali et al. (1991) and Sanwal et al. (2011) worked on *Momordica dioica* and *Momordica cochinchinensis* as dioecious species and reported that crossing between two female genotypes is possible through the induction of hermaphrodite flowers in one or both of the female plants by application of silver nitrate. Hoque et al. (2002) first reported that the success of the conversion of female flowers to hermaphrodite flower by application of 800 or 1000 mg·L\(^{-1}\) AgNO\(_3\) treatment in pointed gourd. However, the crossings among female plants were not able to be carried out because the hermaphrodite flowers didn’t produce any pollen grains. Thus, further studies should be done not only considering AgNO\(_3\) concentration but also suitable plant growth stage and frequency of application.

We collected and investigated 33 (24 female, 9 male) accessions of pointed gourd, distributed in different locations of Bangladesh. Among these, some accessions showed early flowering, large fruit, high yield and high fruit set rate (unpublished). Thus, the silver nitrate application might be expected to induce hermaphrodite flower with viable pollen in pointed gourd for the recombination of desirable characters.

In this study, the effect of the application of silver nitrate for hermaphrodite flower induction and the possibility of crossbreeding among female plants in pointed gourd were investigated.

**Materials and Methods**

*Plant materials*

Mature vines of one male (PGM03) and three female accessions (PGF01, PGF02, and PGF03) of pointed gourd were collected from North-Eastern part of Bangladesh on November 2016 and used for this study. The vines were planted on the soil in a plastic tray and kept on the electric-heated hotbed in the glasshouse in Hakozaki campus (lat. 33°37’ N; long. 130°25’ E), Kyushu University, Japan. The vines began to sprout at the end of March 2017. The sprouted vines of each accession were transplanted to the individual plastic pot on first week of May 2017 for further growth.

*Silver nitrate application*

In the previous report in pointed gourd, silver nitrate (50–2000 ppm) were applied on the female plants with 12–22 leaves (from the tip) at the time of flowering (Hoque et al., 2002). Consequently, very few hermaphrodite flowers (2–6) were produced without viable pollen at 800 and 1000 ppm. Therefore, a modification was done in this study for application frequency and plant growth stage of application with three silver nitrate concentrations.

The female vines were treated two times with three concentrations of silver nitrate (50, 100, and 200 ppm) and added a few drops of Tween20. First spray was done as foliar spray at 4–5 leaf of fully expanded stage when the 4th leaf size was about 3 cm in diameter. The second spray was done 7–10 days after first spray. It was done manually with a plastic hand sprayer aimed to ensure the solution ran off the shoots properly. Silver nitrate solutions were prepared with deionized distilled water.

The total number of female and hermaphrodite flowers appearing on each node were recorded daily until the end of the trial. Period from the first spraying date to the initial and final date of hermaphrodite flower induction per accession was also recorded.

*Pollen grain viability and crossing among different sex types for hybridization*

Fresh pollen grains were collected from the induced hermaphrodite and normal male flowers and stained with 1% acetocarmine for 10–15 minutes on a glass slides. The slides were observed under an optical microscope (Leica DM2500; Leica Microsystems GmbH, Wetzlar, Germany). The percentage of viable pollen was determined from three (3) randomly focused fields and 100 pollen grains were counted in each microscopic field. A total of 300 pollen grains were evaluated for their viability assessment.

Homo and heterosexual crossing among flowers of different sex forms (male, female, and induced hermaphrodite) was conducted as following hybridization technique (Hussain and Rashid, 1974). Normal crossing was done between the female flowers of female plant and male flowers of male plant (Fig. 2). Selfing within and between the flowers of the same clone was done using female and hermaphrodite flowers (Fig. 2). Homosexual crossing was done between the female flowers and the pollen from different hermaphrodite flowers induced in other female accessions (Fig. 2). Heterosexual crossing was done between the emascu-

![Fig. 1. Pointed gourd fruits at edible stage (Bar = 1 cm). Harvested at 18 days after crossings.](image-url)
lated hermaphrodite flower and the pollen of normal male flower (Fig. 2). Ten (10) female flowers were used for each crossing and repeated for three times. The edible stage fruits were harvested at 15–18 days after crossing and several fruit characteristics (fruit length, fruit diameter, and fruit weight) were measured.

Seed germination test

The germination test of the seeds obtained from self, homosexual, heterosexual and normal crossing was conducted in the incubator at 25°C under 24 h dark condition. Fruits were harvested at fully ripening stage and seeds were taken out manually, washed and dried at room temperature before storage. Thirty seeds of each crossing were used for germination test. Seeds were soaked overnight (about 12 h) in tap water. Afterwards, seeds were air dried and placed in the petri dishes with two layers of whatman filter paper (No. 2, 90 mm diameter) moistened with benlate solution (3–5 mL) to avoid fungal contamination. Number of germinated seeds were counted at daily and continued up to 30 days after sowing.

Results

Silver nitrate application

Though the normal female and male flower has only stigma and ovary and anther, respectively (Fig. 3A, B), the application of silver nitrate successfully induced male organ in the female flowers regardless of the concentration in all the treated vines (Fig. 3C). The onset of hermaphrodite flowers started 13–20 days after silver nitrate spray and continued up to 5–36 days, depending on the silver nitrate concentrations and accessions variations (Table 1). Hermaphrodite flowering continued at the best for 36, 22, and 19 days in the PGF03, PGF01, and PGF02 accessions treated with 50 ppm silver nitrate and sharply declined that duration with the increase of silver nitrate concentration in case of all accessions (Table 1). Furthermore, the node number of the first hermaphrodite flower and the last hermaphrodite flower was ranged from 14–21 and 27–54, respectively (Table 1). Application of 50 ppm silver nitrate showed significantly superior to provide hermaphrodite flowers at higher position of nodes at 54, 46, and 42 and the range of nodes of hermaphrodite flower induced was 38, 26, 21 in PGF03, PGF01, and PGF02, respectively (Table 1).

The number of induced hermaphrodite flowers in PGF03 was the highest (23.7) when treated with 50 ppm silver nitrate followed by PGF01 (22.3) and PGF02 (19.0) (Table 2). The consistent of this result was also reflected in hermaphrodite induction (%) ability where 31.5% flowers were altered to hermaphrodite in PGF03 with 50 ppm silver nitrate whereas only 11.3% altered flowers were observed in PGF01 with 200 ppm silver nitrate (Table 2).

From these findings, it has been realized that with an increase in concentration from 50 to 200 ppm of silver nitrate, there was a dramatic reduction observed in the number of induced hermaphrodite flowers, hermaphrodite induction ability (%) and total number of female flower production in all the accessions with the exception of female flower production in PGF03.

Pollen grain viability and crossing among different sex types for hybridization

Pollen grain viability of 100 ppm silver nitrate induced hermaphrodite flowers in PGF01 (92.6%),
PGF02 (94.0%), and PGF03 (93.0%) were statistically identical with that of normal male (95.3%) (Fig. 4A). The pollen size of hermaphrodite flowers (60–63 μm) induced by 50 and 100 ppm silver nitrate was also very close to normal male flower pollen (65.4 μm) while remarkably smaller size pollen (53–57 μm) was observed in 200 ppm silver nitrate application (Fig. 4B).

Among the 15 cross combinations, 9 crosses were made by pollen from hermaphrodite flowers (as selfing and homosexual cross) and 6 were made by pollen from the normal male flower (as heterosexual cross). The highest fruit setting rate (96.6%) was observed when PGF03 was crossed with PGM03 normal male pollen and it was statistically identical with PGF02 × PGM03 (90.0%) followed by PGF01 × PGM03 (86.6%) (Table 3). The similar pattern of fruit setting rates were also found in the hermaphrodite flowers produced female parents PGF01, PGF02, PGF03 crossed with normal male pollen PGM03 (Table 3). Moreover, selfing and homosexual crossing among the combinations of hermaphrodite flower producing female genotypes had the lower fruit setting rates ranging from 20.0–23.3% and

| Accessions | Silver nitrate concentration (ppm) | Days to Induction | No. of hermaphrodite flower/plant | No. of female flower/plant | Hermaphrodite flower induction rate (%) |
|------------|---------------------------------|-------------------|---------------------------------|---------------------------|----------------------------------|
| PGF01      | 0                               | —                 | —                               | —                         | —                                |
|            | 50                              | 19.6±0.5 a        | 41.3±1.5 b                      | 21.7±1.5 b                | 20.3±1.5 NS                      |
|            | 100                             | 18.0±4.0 ab       | 30.0±2.6 cd                     | 12.0±2.6 bc               | 17.0±1.0                        |
|            | 200                             | 18.7±1.1 ab       | 23.3±2.5 d                      | 4.6±1.5 e                 | 14.3±2.0                        |
| PGF02      | 0                               | —                 | —                               | —                         | —                                |
|            | 50                              | 17.6±2.0 ab       | 36.7±3.0 bc                     | 19.1±1.0 b                | 21.0±1.7 NS                      |
|            | 100                             | 13.3±0.5 b        | 31.0±4.5 bcd                    | 17.7±5.0 b               | 16.0±3.6                        |
|            | 200                             | 13.3±1.1 b        | 28.3±3.7 cd                     | 15.0±4.3 b               | 14.3±2.5                        |
| PGF03      | 0                               | —                 | —                               | —                         | —                                |
|            | 50                              | 16.6±1.5 ab       | 53.0±3.6 a                      | 36.4±4.6 a                | 16.3±1.5 NS                      |
|            | 100                             | 15.6±2.5 ab       | 36.7±5.0 b                      | 21.1±2.6 b               | 17.6±1.5                        |
|            | 200                             | 13.6±2.0 b        | 27.3±3.5 cd                     | 13.7±3.7 bc              | 14.6±3.0                        |

Mean±Standard Error, NS=Not significant.

Different letters within a column are significantly different at P<0.05, according to HSD Tukey’s test.
50.0–73.3%, respectively (Table 3). Homosexual crossings produced maximum fruit length and fruit diameter compared to that of normal crossing (Table 3). Fruit weight was also recorded higher in homosexual crossings than normal crossing. Meanwhile, comparatively minimum fruit length, fruit diameter and lower fruit weight was observed in selfing (Table 3).

**Seed germination test**

Number of seeds per fruit were almost same between normal crossing and homosexual crossing, whereas few seeds were obtained by selfing (Table 4). The germination of the seeds obtained from normal crossing was 72.2 to 80.0% (Table 4). Seeds of heterosexual crossing showed 63.3 to 68.8% of the germination rate and it was similar with those of self pollinated seeds (55.5 to 68.8%), whereas homosexual crossing had the lowest germination ranging from 33.3 to 50.0% (Table 4).

**Discussion**

Beyer (1976) postulated that silver nitrate induced staminate flower production by blocking ethylene action. Ethylene has been described as the principal regulator of sexual expression in different cultivated species of the Cucurbitaceae family, including cucumber, melon, watermelon and zucchini (Rudich, 1990; Tongjia and Quinn, 1995; Yamasaki et al., 2005). This hormone is able to control the duration of the sexual phases of development in the species, as well as the proportion of male and female flowers per plant (Manzano et al., 2011). Our findings indicate that all female accessions were sensitive to silver nitrate and successfully altered female flowers to hermaphrodite flower. This fact is that silver ion (Ag⁺) interferes the

![Fig. 4. Pollen grain viability (A) and pollen size (B) of normal male (PGM03) and hermaphrodite flowers (PGF01, PGF02, PGF03) induced by different concentration of silver nitrate (50, 100, 200 ppm). Straight line on each bar is ± Standard Error of Mean.](image-url)

**Table 3.** Fruit characteristics of normal, self, homo and heterosexual crossings among different sex types of pointed gourd.

| Type of crossing  | Pistillate parent⁶ | Pollen parent⁷ | % of fruits obtained | Fruit length ⁸ (cm) | Fruit diameter ⁸ (cm) | Fruit weight ⁸ (g) |
|-------------------|--------------------|----------------|---------------------|----------------------|----------------------|------------------|
| Normal crossing   | PGF01              | PGM03          | 86.6 (8.6 ± 0.5)    | 10.2 ± 0.1 ef₉       | 4.1 ± 0.2 ab        | 44.6 ± 1.2 bc     |
|                   | PGF02              | PGM03          | 90.0 (9.0 ± 1.0)    | 10.7 ± 0.5 def       | 3.2 ± 0.2 def       | 33.3 ± 1.3 ef     |
|                   | PGF03              | PGM03          | 96.6 (9.6 ± 0.5)    | 9.5 ± 0.5 fg         | 2.9 ± 0.2 f         | 32.6 ± 2.0 ef     |
| Selfing           | PGF01-H            | PGF01-H        | 20.0 (2.0 ± 1.0)    | 9.7 ± 0.3 fg         | 3.4 ± 0.2 cdef      | 27.5 ± 1.3 fg     |
|                   | PGF02-H            | PGF02-H        | 33.3 (3.3 ± 1.1)    | 7.5 ± 0.3 h          | 3.0 ± 0.1 ef        | 21.6 ± 1.6 g      |
|                   | PGF03-H            | PGF03-H        | 23.3 (2.3 ± 0.5)    | 9.1 ± 0.1 g          | 3.2 ± 0.1 def       | 24.6 ± 1.3 g      |
| Homosexual crossing | PGF01-H           | PGF02-H        | 50.0 (5.0 ± 1.0)    | 13.2 ± 0.2 a         | 4.3 ± 0.1 a         | 59.3 ± 1.4 a      |
|                   | PGF03-H            | PGF03-H        | 60.0 (6.0 ± 1.0)    | 12.8 ± 0.2 ab        | 3.9 ± 0.2 abc       | 58.4 ± 1.7 a      |
| Heterosexual crossing | PGF01-H          | PGF01-H        | 63.3 (6.3 ± 0.5)    | 12.5 ± 0.1 abc       | 4.2 ± 0.1 a         | 48.9 ± 1.3 b      |
|                   | PGF03              | PGF01-H        | 73.3 (7.3 ± 0.5)    | 12.3 ± 0.1 abc       | 3.7 ± 0.1 abcd      | 44.9 ± 2.3 bc     |
|                   | PGF02              | PGF01-H        | 73.3 (7.3 ± 0.5)    | 12.3 ± 0.1 abc       | 3.7 ± 0.1 abcd      | 44.9 ± 2.3 bc     |
|                   | PGF03              | PGF01-H        | 87.7 (8.7 ± 0.5)    | 13.4 ± 1.2 a         | 3.6 ± 0.1 bcd       | 46.5 ± 2.2 b      |
|                   | PGF02              | PGF01-H        | 90.0 (9.0 ± 1.0)    | 11.4 ± 0.5 cde       | 3.4 ± 0.3 cde       | 31.0 ± 3.3 f      |
|                   | PGF03              | PGF01-H        | 93.3 (9.3 ± 1.1)    | 10.0 ± 0.1 fg        | 3.4 ± 0.2 cdef      | 30.8 ± 1.3 f      |

⁶ PGF: Normal female flower, PGF-H: Hermaphrodite flowers which removed anthers.
⁷ PGM: Normal male flower, PGF-H: Pollen from hermaphrodite flowers.
⁸ Ten (10) flowers were used for each cross and repeated for three times.
⁹ Different letters within a column are significantly different at P<0.05, according to HSD Tukey’s test.
cyclic event of ethylene biosynthesis in plant body resulting in a decrease of ethylene concentration and promotes to produce male sex organ in genetically female plant as hermaphrodite flower (Hossain et al., 1996; Tolla and Peterson, 1979). This action might be varied depending on the strength of silver nitrate. As we observed, the proportion of induced hermaphrodite flowers, hermaphrodite induction rate (%) and duration of induction were found as the highest in all accessions depending on the strength of silver nitrate. As we observed, the proportion of induced hermaphrodite flowers, hermaphrodite induction rate (%) and duration of induction were found as the highest in all accessions when treated with 50 ppm silver nitrate. On the other hand, there was a sharp decline observed in the foresaid traits with an increase in concentration and the lowest result was found at 200 ppm silver nitrate. The present findings contradict Hoque et al. (2002), who observed a shift of female flowers to hermaphrodite in pointed gourd treated with 800 and 1000 ppm silver nitrate at 10–14 days of application. But, Hoque et al. (2002) proposed results. However, induced hermaphrodite flowers produced good size and viable pollen comparable with normal male in the present study, whereas it is not occurred in former study (Hoque et al., 2002). These pollen grains were subsequently applied in conducting hybridization among the different accessions. In that case, crossing among female genotypes (homosexual crossing) produced better fruit size and weight than selfing and heterosexual crosses. A similar observation has also been made for sweet gourd (Sanwal et al., 2011) while disputes for kakrol (Hossain et al., 1996).

In conclusion, silver nitrate at 50 ppm appears to be the best for induction of hermaphrodite flowers with maximum viable pollen at 100 ppm in pointed gourd. Therefore, the crossbreeding of the varieties having different traits with an increase in concentration and the lowest result was found at 200 ppm silver nitrate. The present findings contradict Hoque et al. (2002), who observed a shift of female flowers to hermaphrodite in pointed gourd treated with 800 and 1000 ppm silver nitrate at 10–14 days of application. But, Hoque et al. (2002) proposed results. However, induced hermaphrodite flowers produced good size and viable pollen comparable with normal male in the present study, whereas it is not occurred in former study (Hoque et al., 2002). These pollen grains were subsequently applied in conducting hybridization among the different accessions. In that case, crossing among female genotypes (homosexual crossing) produced better fruit size and weight than selfing and heterosexual crosses. A similar observation has also been made for sweet gourd (Sanwal et al., 2011) while disputes for kakrol (Hossain et al., 1996).

In addition, seeds obtained from the all crossings among female genotypes in pointed gourd were successfully germinated and the seedlings were grown vigorously (data not shown). According to our study, application of silver nitrate at 200 ppm showed stunted growth or sunburn in the treated leaves or vines as toxic effect. However, these symptoms were resumed after 10–14 days of application. But, Hoque et al. (2002) noticed the toxicity where the treated vines were died at 1200–2000 ppm silver nitrate and it was quite different compared to us.

In conclusion, silver nitrate at 50 ppm appears to be the best for induction of hermaphrodite flowers with maximum viable pollen at 100 ppm in pointed gourd.

### Table 4. No. of seeds and seed germination of normal, self, homo and heterosexual crossings among different sex types of pointed gourd.

| Type of crossing     | Pistillate parent<sup>a</sup> | Pollen parent<sup>a</sup> | No. of seeds/fruit | % of seeds germinated |
|----------------------|-------------------------------|--------------------------|--------------------|----------------------|
| Normal crossing      | PGF01                         | PGM03                    | 23.0 ± 1.0 bcd     | 74.4 (22.3 ± 6.9)<sup>y</sup> |
|                      | PGF02                         | PGM03                    | 30.3 ± 1.5 a       | 80.0 (20.4 ± 5.7)    |
|                      | PGF03                         | PGM03                    | 24.6 ± 2.5 abc     | 72.2 (21.7 ± 5.0)    |
| Selfing              | PGF01-H                       | PGF01-H                  | 13.0 ± 1.0 f       | 65.5 (19.7 ± 6.9)    |
|                      | PGF02-H                       | PGF02-H                  | 16.3 ± 1.5 ef      | 68.8 (20.7 ± 8.3)    |
|                      | PGF03-H                       | PGF03-H                  | 13.3 ± 3.0 f       | 55.5 (16.7 ± 3.8)    |
| Homosexual crossing  | PGF01                         | PGF01-H                  | 25.6 ± 1.5 ab      | 47.7 (14.3 ± 1.9)    |
|                      |                                | PGF03-H                  | 26.6 ± 2.0 ab      | 35.5 (10.7 ± 5.1)    |
|                      | PGF02                         | PGF01-H                  | 24.6 ± 3.5 abc     | 50.0 (15.0 ± 10.0)   |
|                      |                                | PGF03-H                  | 21.6 ± 3.0 bcde    | 33.3 (10.0 ± 6.6)    |
|                      | PGF03                         | PGF01-H                  | 24.6 ± 2.5 abc     | 44.4 (13.3 ± 10.1)   |
|                      |                                | PGF02-H                  | 18.0 ± 2.0 def     | 48.8 (14.7 ± 13.8)   |
| Heterosexual crossing| PGF01-H                       | PGM03                    | 22.3 ± 1.1 bcede   | 63.3 (19.0 ± 5.7)    |
|                      | PGF02-H                       | PGM03                    | 21.0 ± 1.0 bcde    | 66.6 (20.0 ± 3.3)    |
|                      | PGF03-H                       | PGM03                    | 18.3 ± 1.5 cdef    | 68.8 (20.7 ± 12.6)   |

<sup>a</sup> PGF: Normal female flower, PGF-H: Hermaphrodite flowers which removed anthers.
<sup>y</sup> PGM: Normal male flower, PGF-H: Pollen from hermaphrodite flowers.
<sup>x</sup> Thirty (30) seeds were sown for germination of each cross and repeated for three times.
<sup>w</sup> Different letters within a column are significantly different at <i>P</i>&lt;0.05, according to HSD Tukey’s test.
suitable characteristics and heat tolerance seems to be possible through the induction of hermaphrodite flower in pointed gourd.

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