From Herkogamy to Cleistogamy - Development of Cleistogamy in Periwinkle

RAGHAVENDRA N. KULKARNI AND KUPPUSAMY BASKARAN

From the CSIR-Central Institute of Medicinal and Aromatic Plants (Council of Scientific and Industrial Research), Research Centre, Allalasandra, Bangalore 560 065, India.

Address correspondence to Raghavendra N. Kulkarni at the address above, or e-mail: krnpbg@yahoo.com.

Abstract

Periwinkle (Catharanthus roseus [L.] G. Don), an important medicinal plant, is an allogamous species in which the stigma is below the anthers. The receptive portion is at the base of the stigmatic head and thus automatic intra-flower self-pollination is excluded. The structure of the flower is of typical reverse herkogamy and pollination occurs through nectar-seeking insects. A few self-pollinating strains are also reported in which self-pollination is brought about by an increase in length of the style or of the ovary. Self-pollination is governed by allelic duplicate genes recessive to allogamy. An induced monogenic recessive mutant (EMS 17-1) with caducous closed corolla (corolla abscising before anthesis), isolated from variety, Dhawal, was crossed with two self-pollinating strains to study the possibility of obtaining cleistogamous recombinants combining closed corolla and self-pollination traits. Cleistogamous plants were obtained in which development of fruits and seeds occurred without opening of the corolla. Closed corolla and self-pollination were found to be independently inherited. A dominant gene in the parent in which self-pollination occurred due to an increase in length of the ovary, appeared to completely or partially inhibit expression of the gene for closed corolla in homozygous or heterozygous condition, respectively. The genetic basis of development of cleistogamy is described. Cleistogamy in periwinkle would facilitate in ensuring genetic purity, pollen containment, and seed production even in the absence of pollinators. This appears to be the first report on the development of cleistogamous plants in an allogamous species.

Key words: allogamy, genetic purity, inheritance, medicinal plant, pollen containment, self-pollination

Spatial separation of the anthers and stigma within the same flower is known as herkogamy. Two forms of herkogamy are recognized: 1) Approach herkogamy, where the stigma is above the level of the anthers and floral visitors first contact the stigma before removing pollen from the anthers. 2) Reverse herkogamy, where the stigma is below the level of the anthers and floral visitors first contact the anthers before the stigma. It is believed to facilitate greater pollen export than approach herkogamy and is typically associated with lepidopteran (moths and butterflies) pollination.

Cleistogamy refers to the occurrence of self-pollination in unopened flowers. It was first described by Kuhn in 1867 as production of bud-like flowers that do not open but develop into fruit and is known to occur in 693 species, spread across 228 genera and 50 families (Lord 1981; Culley and Klooster 2007). Thus, although cleistogamy has been known for a long time, it has attracted considerable attention in recent years as one of the approaches for gene containment in transgenic crops (Daniell 2002) and being studied in barley (Turuspekova et al. 2004), rice (Maeng et al. 2006; Yoshida et al. 2007), and oil seed rape (Leflon et al. 2010, 2011).

Madagascar periwinkle (Catharanthus roseus [L.] G. Don), an ever-blooming plant belonging to the Apocynacae family, is grown commonly as a garden plant in tropical and subtropical regions. The structure of the periwinkle flower conforms typically to reverse herkogamy, with the stigma being below the anthers. A characteristic feature of the Apocynaceae is the disc-like or otherwise-shaped stigmatic head with a sticky secretion and a brush of hairs, which takes up all pollen as it is shed. The receptive portion is at the base of the stigmatic head and thus automatic intra-flower self-pollination is excluded. Pollination is brought about by nectar-seeking insects, which effect pollination by depositing pollen collected from flowers during previous visits (Knuth 1909; Rendle 1971). However, until recently, periwinkle was considered as an autogamous species (see Kulkarni et al.)
although Darwin and Delpino long ago reported that selfing within individual periwinkle flowers is not automatic and that pollination typically occurs through nectar-seeking insects (Knuth 1909; Rendle 1971). Recent studies have confirmed these observations, further revealing that geitonogamy and phenotypic assortative mating for flower color brought about by pollinating butterflies give a false impression that periwinkle is autogamous when flower color is used as a marker trait to determine the breeding system (Kulkarni 1999; Sreevalli et al. 2000). Although periwinkle is an ever-blooming plant in tropical regions, seed set is dependent on availability of pollinators, whose occurrence may be seasonal. Artificial self-pollination or seed production in isolation is essential for maintenance of genetic purity.

Kulkarni et al. (2001, 2005) reported the existence of a few uncommon autogamous strains in periwinkle and described the mechanism and genetics of their self-pollination. In normal allogamous strains, the stigma remained below the base of the anthers until flower drop, whereas in autogamous strains, self-pollination occurred due to an increase in the length of the gynoecium brought about either by an increase in the length of the style or of the ovary. Self-pollination was found to be under the control of duplicate recessive genes. Seed set in these strains is not dependent on pollinators. Maintenance of their genetic purity, however, is not ensured as self-pollination occurs 1–2 days after anthesis (Kulkarni et al. 2001).

Periwinkle is also an important medicinal plant. Its leaves and roots are, respectively, the source of highly valued anticancer (vincristine and vinblastine) and antihypertension (ajmalicine) alkaloids. Because of the relatively low concentrations of these alkaloids in the plant and their high value, periwinkle has become one of the most intensively investigated medicinal plants after the discovery of these alkaloids in the 1950s, with an average of about 70 publications per year during the period from 1950 to 2001 (van der Heijden et al. 2004). However, most of the work on this plant, particularly after the late 1980s, has been initially on cell and tissue culture aspects (van der Heijden et al. 1989, Moreno et al. 1995). Subsequent work has been on over expression of cloned genes encoding enzymes involved in biosynthesis of periwinkle alkaloids in genetically modified plants. The development of transgenic periwinkle plants for enhancement of production of anticancer alkaloids, vincristine and vinblastine, appears to be a distinct possibility in the near future (Zarate and Verpoorte 2007). However, transgene containment is required when such transgenic periwinkle varieties are developed. Availability of cleistogamous strains in periwinkle would simultaneously facilitate transgene containment, maintenance of genetic purity, and elimination of dependence on pollinators for seed set. However, it is considered to be extremely difficult to create cleistogamous plants in allogamous plant species (Lu 2003). In herkogamous plants, reciprocal anther and stigma positions, generally, tend to be inherited together, as also found earlier in periwinkle (Kulkarni and Baskaran 2008).

During the course of our work on genetic improvement of periwinkle, we isolated a mutant with caducous closed corolla (premature flower bud abscission). The mode of inheritance of this mutant trait and development of cleistogamy using this mutant and earlier reported self-pollinating strains (Kulkarni et al. 2001, 2005) are described here.

**Materials and Methods**

**Inheritance of Caducous Closed Corolla**

The plant materials in this study included strains EMS 17-1, Dhawal, and dw;wy-1.

(i) EMS 17-1: A mutant with caducous closed corolla (premature abscission of corolla) was isolated from M_2 generations raised from ethyl methanesulfonate (EMS)-treated seeds of variety, Dhawal. Seeds of variety, Dhawal, were pre-soaked in water for 19 h before treatment with unbuffered 0.6% aqueous solution of EMS for 4 h. The corolla and the corolla tube of this mutant, EMS 17-1, abscised before anthesis, as against 2–3 days after anthesis in normal periwinkle plants and parental variety, Dhawal. The unopened corolla and the corolla tube were fresh and turgid at the time of abscission and contained style with stigma and undehisced anthers. The corolla appeared to abscise a couple of days before anthesis. The mutant was otherwise phenotypically normal when compared with the parental variety (Figure 1A).

(ii) Variety Dhawal: Developed from the mutant with wavy leaf margin obtained from variety, Nirmal, reported earlier (Kulkarni et al. 1999).

(iii) Strain dw;wy-1: A double mutant recombinant for dwarf and wavy leaf margin mutant traits, developed through several generations of selfing of one of the double mutant recombinant plants obtained from the F2 generation of the cross between dwarf and wavy leaf mutants (Kulkarni et al. 1999; Figure 1B).

CROSSES EMS 17-1 × Dhawal and EMS 17-1 × dw;wy-1 were made to study the mode of inheritance of caducous closed corolla. EMS 17-1 was selfed by pollinating its stigma with its crushed undehisced anthers (as anthers were indehiscent) to study its true breeding behavior. Out of the several pollination attempts, only one fruit with two seeds was obtained from the cross between the mutant, EMS 17-1 × dw;wy-1. Pollinations with parental variety, Dhawal, and self-pollinations were, however, unsuccessful. Out of the two seeds obtained, only one germinated giving one F1 plant. The F1 plant was grown in the glass house, artificially self-pollinated, and F2 seed harvested. Inheritance of caducous closed corolla and its joint segregation with plant height (normal tall vs. dwarf) was studied by raising F2 and F3 generations from seeds produced through artificial self-pollination in the glass house. Plants of F2 generation were raised in the field. Data on corolla type (caducous closed vs. normal) and plant height (normal tall vs. dwarf) were recorded. Plants of F3 generation of a few randomly selected normal F2 plants were raised in polybags in the glass house and scored for mutant traits. Chi-square tests were used to test the goodness-of-fit of observed frequencies of different
Development of Cleistogamy using Genes for Self-pollination Brought about by an Increase in Length of the Style

The plant materials for this study included genotypes \( dw_{1}wycc-1 \) and \( dwarfsp-1 \).

(i) \( dw_{1}wycc-1 \): One of the four recombinant plants with dwarf height, wavy leaf margin, and caducous closed corolla obtained from the above-studied \( F_{2} \) generation of the cross, EMS 17-1 \( \times \) \( dw_{1}wy-1 \) and designated as \( dw_{1}wycc-1 \), was used for development of cleistogamous genotypes (Figure 2A).

(ii) \( dwarfsp-1 \): We had earlier (Kulkarni et al. 2001) isolated self-pollinating dwarf plants from the cross between a dwarf mutant (one of the parental mutants of double mutant recombinant, \( dw_{1}wy-1 \), described above) and a self-pollinating strain OR, in which self-pollination occurred due to an increase in length of the gynoecium brought about by an increase in length of the style (Kulkarni et al. 2005). One of these self-pollinating dwarf plants, designated as \( dwarfsp-1 \), which showed a high percentage of fruit set on natural self-pollination, was advanced to several generations (>\( F_{6} \)) from selfed seed obtained from plants raised in the glass house (Figure 2B).

The recombinant plant \( dw_{1}wycc-1 \) with dwarf height, wavy leaf margin, and caducous closed corolla was crossed with strain \( dwarfsp-1 \) to study the possibility of obtaining cleistogamous plants by combining genes for caducous closed corolla and self-pollination. Again, after several cross-pollinations, only a few seeds and only one \( F_{1} \) plant could be obtained. The \( F_{1} \) plant was raised in the glass house and \( F_{2} \) seeds were produced through manual selfing in the glass house. Plants of the \( F_{2} \) generation were raised in the glass...
house in 9” polybags and scored for plants exhibiting normal open corolla/closed corolla abscising before anthesis, and self-pollinating/nonself-pollinating plants. Although cleistogamy refers to the occurrence of self-pollination before anthesis, in this study only those plants that had closed corolla and showed fruit development were classified as cleistogamous (We did not determine the time of occurrence of self-pollination with reference to anthesis, i.e., before or after anthesis, in plants that had open corolla). Such cleistogamous plants in which fruit development was observed without opening of their corolla were carefully transferred to 12” pots for better growth and seed production for advancement to F3 generation to study their breeding behavior.

Six cleistogamous plants were identified in the F2 generation. These plants showed considerable variation in the percentage of cleistogamous fruit set, which at first few nodes was good but at subsequent nodes later was quite poor or absent. Similar behavior was observed in the F3 generation of these plants. One of these cleistogamous plants designated as dwwycls-1 with highest frequency of fruit set (determined visually) was taken up for further development of cleistogamous genotypes with better cleistogamous fruit set using genes for self-pollination brought about by an increase in length of the ovary.

Development of Cleistogamy using Genes for Self-pollination Brought about by an Increase in Length of the Ovary

The plant materials for this study included genotypes dwwycls-1 and strain MJ.

(i) dwwycls-1: One of the six recombinant plants with dwarf height, wavy leaf margin, and highest cleistogamous fruit set (Figure 3A), isolated from the cross dw1wycc-1 × dwarfsp-1.

(ii) Strain MJ: A self-pollinating strain in which self-pollination is brought about by an increase in length of the ovary. The corolla in this strain was persistent, with corolla tube adhering to the fruit till almost complete development of the fruit (Figure 3B; Kulkarni et al. 2005).

To improve the cleistogamous fruit set and increase the frequency of cleistogamous plants in the F3 generation, one of the cleistogamous recombinant plants, dwwycls-1 derived from the cross dwwycc-1 × dwssp-1, was crossed to the self-pollinating strain MJ. F1 plants were raised in the glass house. F1 plants, as expected, were self-pollinating; however, as the fruit size was small they were manually selfed because the fruit size and the number of seeds per fruit on manual selfing were found to be greater than those obtained on natural self-pollination. Plants of the F2 generation were raised in the glass house in 9” polybags and scored for corolla type (normal open corolla/caducous closed corolla abscising before anthesis), plant height (tall/dwarf), and kind of pollination (self-pollinating/nonself-pollinating) and plants showing cleistogamy, that is, in which fruit development was observed without opening of their corolla. All the dwarf cleistogamous plants were advanced to F3 generation. One tall plant with partially closed corolla was also advanced to F3 generation. Segregation of two of the F3 tall plants with partially closed corolla obtained from the F3 generation of this F2 tall plant with partially closed corolla was studied in F4 generation.

Results

Inheritance of Caducous Closed Corolla

The F1 plant of the cross, EMS 17-1 × dw1wy-1, had normal plant height and open corolla. In the F2 generation, the

Figure 3. Parents used in the development of cleistogamy: (A) dwwycls-1 (arrow indicates cleistogamous fruit set; note closed corolla); (B) MJ (arrows indicate corolla adhering to autogamously developed fruits).
observed ratio of plants with normal open corolla (69) and caducous closed corolla (14) fitted a 3:1 ratio ($\chi^2 = 2.927, P = 0.0871$), suggesting that caducous closed corolla was governed by a single recessive gene. Joint segregation of plant height with corolla type suggested that the genes governing these two traits were independently inherited (56 tall/open corolla:10 tall/closed corolla:13 dwarf/open corolla:4 dwarf/closed corolla; $\chi^2 (9:3:3:1) = 4.539, P = 0.2088$). Only a small F$_1$ population of 48 plants could be raised from 3 normal F$_2$ plants and scored, due to severe damping off of seedlings in many of the progenies. The observed segregation for plant height and corolla type was similar to that observed in the F$_2$ generation ($\chi^2$ open vs. closed corolla $= 0.111, P = 0.739$; $\chi^2$ joint segregation $= 2.815, P = 0.421$).

Development of Cleistogamy using Genes for Self-pollination Brought about by an Increase in Length of the Style

The F$_1$ plant of the cross dw; wycc-1 × dwarfsp-1 had normal open corolla and was nonself-pollinating. A F$_2$ population of 406 plants was raised. The observed ratio of plants with normal open corolla and plants with closed corolla fitted a ratio of 3:1 (Table 1). The observed frequencies of nonself-pollinating and self-pollinating plants fitted an expected ratio of 15:1 (Table 1). Joint segregation for corolla type (open vs. closed) and pollination (self vs. nonself) fitted an expected ratio of 45 (open/nonself):3 (open/self):15 (closed/nonself):1 (closed/self). Six cleistogamous plants were identified in which fruit growth could be observed without opening of their corolla (Table 1).

Development of Cleistogamy using Genes for Self-pollination Brought about by an Increase in Length of the Ovary

The F$_1$ plants of the cross dw; wycc-1 × MJ were self-pollinating. A population of 295 F$_2$ plants was raised in the glasshouse and scored for plant height (tall/dwarf), corolla type (open/closed), and type of pollination (self/nonself). No tall plants with closed corolla were found in the studied population of 295 plants. However, 32 tall plants exhibited partially closed corolla. The observed ratio of tall plants with open corolla to tall plants with partially closed corolla did not fit a ratio of 3:1 (Table 2). In contrast, plants with both open and closed corolla were found among dwarf plants, and the observed ratio of plants with open corolla to tall plants with partially closed corolla were found. The observed ratio of self-pollinating and nonself-pollinating plants fitted a ratio of 10:6 and within both tall and dwarf plants (Table 2).

The observed ratio of tall plants with open corolla and tall plants with partially closed corolla (200:32), however, fitted a ratio of 10:2. Joint segregation for plant height (tall vs. dwarf) with type of corolla (open vs. closed or partially closed) did not fit a 9:3:3:1 ratio but fitted a ratio of 10:2:3:1 (Table 2). Joint segregation of type of corolla (open vs. closed or partially closed) with occurrence or nonoccurrence of self-pollination fitted a ratio of 130:78:20:12:10:6, while joint segregation of plant height (tall vs. dwarf) with type of corolla (open vs. closed or partially closed) and occurrence or nonoccurrence of self-pollination fitted a ratio of 100:60:20:12:30:18:10:6 (Table 2).

<table>
<thead>
<tr>
<th>Generation/Phenotype</th>
<th>Genotype</th>
<th>Expected proportion</th>
<th>Observed frequency</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>dw; wycc-1” Dwarf/closed</td>
<td>dw; wycc-1” Dwarf/closed</td>
<td>dw; wycc-1” Dwarf/closed</td>
</tr>
<tr>
<td>2</td>
<td>Dwarf/open corolla/nonself-</td>
<td>Dwarf/open corolla/nonself-</td>
<td>Dwarf/open corolla/nonself-</td>
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<tr>
<td>3</td>
<td>F$_2$ Dwarf/open corolla/</td>
<td>F$_2$ Dwarf/open corolla/</td>
<td>F$_2$ Dwarf/open corolla/</td>
</tr>
<tr>
<td>4</td>
<td>Dwarf/closed corolla/nonself-</td>
<td>Dwarf/closed corolla/nonself-</td>
<td>Dwarf/closed corolla/nonself-</td>
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<tr>
<td>Grand total</td>
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**Table 1** Phenotypes, genotypes, expected proportions, and observed frequencies in the cross, dw; wycc-1 × dwarf sp-1

<table>
<thead>
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</tr>
<tr>
<td>Grand total</td>
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df, degree of freedom.

*Cleistogamous plants with closed corolla.

(1) $\Sigma_x^2 (3 df) 9$ open corolla:1 closed corolla = 3.156; $P = 0.0756$.

(2) $\Sigma_x^2 (3 df) 5$ non-self-pollinating:1 self-pollinating = 2.286; $P = 0.1305$. 

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Because no tall cleistogamous plants were found, tall F2 plants were not studied further. However, segregation of one of the tall self-pollinating plants with partially closed corolla was studied in F3 generation. This plant segregated into 4 tall plants with open corolla, 10 plants with partially closed corolla, and 4 dwarf plants with closed corolla, which fitted a ratio of 1:2:1 ($\chi^2 = 0.222; P = 0.8949$). Pooled segregation data of two of the F3 tall plants with partially closed corolla, from this single studied F2 tall plant with partially closed corolla, also showed similar segregation in F4 generation (9 tall plants with open corolla:37 tall plants with partially closed corolla:14 dwarf plants with closed corolla; $\chi^2 (1:2:1) = 4.10; P = 0.1287$).

Altogether nine cleistogamous plants were identified among dwarf plants. Because no tall plants were found with closed corolla, there were no tall cleistogamous plants in the studied F2 population of 295 plants. Segregation of six of the nine dwarf cleistogamous plants was studied in F3 generation for cleistogamy. Five of these plants segregated into self-pollinating plants with closed corolla (cleistogamous) and nonself-pollinating plants with closed corolla in the ratio of 3:1 or 10:6. One plant was found to be true breeding for...
cleistogamy (data not shown). Cleistogamous fruit set in two dwarf cleistogamous plants is shown in Figure 4.

Discussion

Inheritance of Closed Corolla

The mutant trait caducous closed corolla of the mutant, EMS 17-1, was found to be inherited as a monogenic recessive trait. Gene symbol cc is proposed for the closed corolla trait. Recently, a monogenic recessive mutant called “bud-flowering” in which flowers do not open was reported in Calluna vulgaris, an important balcony and bedding plant used in Germany (Borchert and Hohe 2009). The mutant lacked stamens but had prolonged flowering period, which is a desirable trait in horticultural plants. In Arabidopsis thaliana, two auxin response factors, ARF6 and ARF8, have been found to regulate both stamen and gynoecium maturation and coordinate the transition from immature to mature fertile flowers. The development of flowers of double mutant arf6 arf8 was arrested as infertile closed buds with immature gynoecia and undehisced anthers that did not release pollen (Nagpal et al. 2005), as found in the periwinkle mutant EMS 17-1 in this study. Defect in anther dehiscence and pollen maturation has also been found to be associated with delayed flower bud opening in Arabidopsis mutant, defective in anther dehiscence, dad1 (Ishiguro et al. 2001). The periwinkle mutant, EMS 17-1, with caducous closed corolla also showed indehiscent anthers with very low pollen germination, which may explain absence of flower bud opening. However, we have reported earlier in periwinkle, a mutant with indehiscent anthers which had normal corolla (Sreevalli et al. 2003). Anther dehiscence in normal periwinkle flowers occurs coupled with anthesis, just before complete anthesis. Therefore, it appears that the mutant either had normal corolla, which abscised much before opening of corolla (i.e., about 2 days before anthesis) because of defective anthers, or it had closed corolla, which abscised without anthesis before another dehiscence. The latter possibility appears to be probable as both self-pollinating and nonself-pollinating plants with closed corolla were obtained in other studied crosses, ruling out the possibility that the corolla in the mutant did not open due to its anthers being defective in dehiscence. In rice, wheat, and barley, mutations affecting morphology of lodicules have been reported to prevent flower opening (Chhabra and Sethi 1991; Honda et al. 2005; Yoshida et al. 2007).

The corolla in the periwinkle mutant, EMS 17-1, abscised prematurely. In the parental variety, Dhawal, pollen germination was not observed in flower buds 2 days before their opening. Thus, it may be inferred that the corolla in the caducous closed corolla mutant, EMS 17-1, abscised at least 2 days before anthesis. Several mutant genes like, dad1 to 3 (delayed in abscission), ctr1 (ethylene response), ein2 (ethylene insensitive), and idle (inflorescence deficient in abscission) involved in floral organ abscission have been identified in Arabidopsis (see Aalen et al. 2006).

The relationship between type of corolla (open/closed) and type of anthers (dehiscent/indehiscent) could not be determined as we did not score for type of anthers in the F2 generation.

Development of Cleistogamy using Genes for Self-pollination Brought about by an Increase in Length of the Style

In the F2 generation consisting of 406 plants, 6 plants were found to be cleistogamous, that is, self-pollinating with closed corolla. The observed ratio of four phenotypic classes viz., open corolla and non-self-pollinating, open corolla and self-pollinating, closed corolla and non-self-pollinating, and closed corolla and self-pollinating, fitted expected ratio of 45:3:15:1, for the two traits, type of corolla, and occurrence or nonoccurrence of self-pollination. This suggested that closed corolla behaved as a single trait in combination occurrence/nonoccurrence of self-pollination, resulting in only...
two phenotypic classes: closed corolla and nonself-pollinating plants, and closed corolla and self-pollinating plants. In other words, the traits closed corolla, it's premature abscission and indehiscent anthers found in the caducous closed corolla parent, EMS 17-1, did not appear to be three separate traits, in which case the observed ratio of four major phenotypic classes in the F2 generation would not have fitted a 45:3:15:1 ratio. Therefore, it appeared that in plants with closed corolla, corolla abscised, if the plants were nonself-pollinating and did not abscise, if the plants were self-pollinating and self-pollination occurred before abscission, resulting in 1) closed corolla and nonself-pollinating plants and 2) cleistogamous closed corolla and self-pollinating plants, respectively. However, as mentioned in Results, the fruit set at first few nodes was good, but at subsequent nodes, it was quite poor or absent. Similar behavior was observed in the F3 generation of these plants. This was probably because in a majority of the flowers of these plants with closed corolla, self-pollination did not occur before abscission of the corolla. The occurrence of cleistogamous plants in the F2 generation indicated that these plants with closed corolla had indehiscent anthers and that closed corolla was not due anthers being defective in anther dehiscence.

Development of Cleistogamy using Genes for Self-pollination Brought about by an Increase in Length of the Ovary

F1 plants were self-pollinating confirming that the cleistogamous recombinant dwwycls-1 carried allelic genes for self-pollination brought about by an increase in length of the style. For sake of simplicity and because the objective of this work was to develop cleistogamous plants, plants of F2 generation of this cross were classified as self-pollinating or nonself-pollinating, and self-pollinating plants were not further distinguished into those in which self-pollination occurred due to an increase in length of the ovary or of the style. Therefore, the expected ratio of self-pollinating: nonself-pollinating plants was 10:6 (instead of 9 [self-pollinating due to increase in length of ovary]:6 [nonself-pollinating]:1[self-pollinating due to increase in length of style]), and a good fit was observed between the observed and expected ratios (Table 2).

No tall plants with closed corolla were found in the studied F2 population of 295 plants. Only 32 plants with partially closed corolla were observed. The absence of tall plants with closed corolla could not be explained due to tight linkage between plant height and type of corolla as in spite of small size of F2 population, the observed frequencies of dwarf plants with open corolla and dwarf plants with closed corolla fitted a 3:1 ratio, which ruled out the possibility of any linkage between plant height and type of corolla. Because no tall plants with closed corolla were found in the studied population, tall plants were not studied further and only the cleistogamous dwarf plants were taken up further to study their breeding behavior. However, the observed segregation of one F2 tall plant with partially closed corolla in F3 generation into tall plants with open corolla, tall plants with partially closed corolla, and dwarf plants with closed corolla in the ratio of 1:2:1 suggested that the F2 plant was heterozygous for plant height. Because no dwarf plants with open corolla were observed, it appeared that the dominant gene for plant height in heterozygous condition partially suppressed the gene for closed corolla resulting in tall plants with partially closed corolla and in homozygous dominant condition probably completely suppressed gene for closed corolla resulting tall plants with open corolla. All the dwarf plants, however, had closed corolla because the recessive allele for plant height did not suppress the gene for closed corolla. To test this hypothesis, progeny of two of the F1 tall plants with partially closed corolla were again studied in F3 generation. Again, although the progeny size was small, the segregation of these plants appeared to support the hypothesis that the dominant gene for plant height in homozygous and heterozygous condition, respectively, completely and partially suppressed the gene for closed corolla, whereas in homozygous recessive condition, it did not suppress the gene for closed corolla resulting in dwarf plants with closed corolla. This hypothesis appeared to explain the observed ratios of all the observed phenotypic classes in the F2 generation. Thus, it appeared that either the dominant allele for plant height in the parent MJ or a dominant gene tightly linked to this allele in parent MJ completely or partially suppressed the gene for closed corolla in homozygous or heterozygous condition, respectively. Because the original mutant EMS 17-1 was of normal plant height (tall) and independent segregation for plant height and type of corolla was observed in the cross EMS 17-1 × dw;wy-1 and no tall plants with partially closed corolla were observed in the F2 generation of this cross, it appears that the allele for normal plant height in the strain MJ may be different from that in the mutant EMS 17-1 with caducous closed corolla obtained from variety, Dhawal or there may be a dominant gene tightly linked to plant height in the strain MJ, which completely or partially suppresses the gene for closed corolla in homozygous and heterozygous condition, respectively.

The expected genotypes of parental strains, F1, and of different phenotypic classes observed in the F2 generation of crosses dw;wycc-1 × dwarfsp-1 and dwwycls-1 × MJ and their expected proportions based on this model are given in Tables 1 and 2.

Cleistogamy in other plants (rice, barley, sorghum, durum wheat, pigeonpea, and soybean) has been found to be governed by one or two recessive or dominant genes and inherited as a single trait (Merwine et al. 1981; Chhabra and Sethi 1991; Saxena et al. 1993; Honda et al. 2005; Maeng et al. 2006; Yoshida et al. 2007). Cleistogamous plants in this study were developed by combining two traits, closed corolla and self-pollination, which were governed by a single recessive gene and duplicate recessive genes, respectively. This appears to be the first report on creation of cleistogamy in an allogamous plant species. Cleistogamy could be combined with other traits of interest in periwinkle for maintenance of genetic purity.

Cleistogamous lines in oil seed rape were found to produce varying proportion (3–33%) of open flowers depending
on genotype, location, and year evaluation (Leflon et al. 2010). In Japan, early maturing land races of soybean have been found to produce open flowers at early flowering stage during years with high temperature. Further, one of the two genes controlling cleistogamy was linked with one of the genes responsible for insensitivity to day length (Takahashi et al. 2001). Some of the cleistogamous genotypes of periwinkle isolated in this study were found to produce a few partially open flowers during some periods. However, self-pollination was found to occur before anthesis in these flowers, as fruit set was observed in flower buds in which stigma and anthers were removed by cutting off the corolla tube (along with stigma and anthers) at the throat region even before separation of corolla lobes had started prior to opening of their corolla. Thus, although cleistogamy of these genotypes may, therefore, be useful for maintenance of genetic purity and seed production independent of prevalence of pollinators, they need to be evaluated for stability of their cleistogamous trait under different environmental conditions to fully understand their utility for pollen containment.

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References


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