

## Adopting slight changes in agronomic practices to obtain bigger impacts on yield and yield quality: hybrid seed production of *Platycodon Grandiflorus* under tropical greenhouse conditions

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**Abstract:** Recommendations given based on the performance of exotic crop species in the country of their origin has not given expected results under Sri Lankan conditions. Therefore, efforts have been taken to address major issues in export-oriented floricultural enterprises in Sri Lanka by adopting slight changes in agronomic practices targeting larger impacts on yield and yield quality leading to higher profits; more buyers and more exports. The present study is one such attempt of a series of studies conducted to find customized solutions for real problems specified by the crop species, management practices and environmental conditions. *Platycodon grandiflorus* is one of the major flower plant species cultivated in Sri Lanka for the exportation of F<sub>1</sub> hybrid seeds. Comprehensive knowledge on floral biology (i.e. flowering behavior, time of anthesis, pollen viability, receptive stage and receptive time of the stigma) of parent lines is very important to produce F<sub>1</sub> hybrid seeds. However, unavailability of information regarding best receptive stage and receptive time of stigma is a limitation and it has led to reduced seed yield. The present experiment was conducted to study the effective receptive stage and receptive time of the day of *Platycodon grandiflorus* under tropical greenhouse conditions. Average yield obtained under the current pollination practice at the existing growth stage is 42 seeds/ pod. A combination of parent lines 2012/99 (Female) and 2026/99 (Male) was used to produce F<sub>1</sub> hybrid seeds of SPD 2008 variety. All female parent flowers were emasculated three days before pollination. Pollination treatment was started three days after emasculation and continued up to the seventh day. Artificial pollination was done either in the morning or in the afternoon on each day. The pollination days after emasculation in combination with the pollination time of the day were used as treatments. The ten treatments were replicated 15 times. Self-pollinating flowers were used as the control treatment. The research was conducted in Complete Randomized Design (CRD) and data were analyzed using Excel and Statistical Analytical Software (SAS) packages. The maximum seed yield of 81 per pod was recorded in the treatment of artificial pollination after five days of emasculation. Pollination between 5-7 days of emasculation had no significant difference in seed yield. The artificial pollination in the afternoon between 3-4 days of emasculation is significantly better than artificial pollination in the morning in terms of yield. There was an inverse relationship between seed weight and number of quality seeds per pod. Pollinating *Platycodon grandiflorus* flowers either in the morning or in the afternoon, 5-7 days after emasculation gives nearly a two-times higher yield of quality seeds than that of contemporary pollination practices under tropical greenhouse conditions.

**Keywords:** hybrid seed production, *Platycodon grandiflorus*, receptive time and receptive stage of stigma, seed yield and quality.

## Introduction

Recommendations given based on the performance of exotic crop species in the country of their origin has not given expected results under Sri Lankan conditions. Therefore, efforts have been taken to address major issues in export-oriented floricultural enterprises in Sri Lanka by adopting slight changes in agronomic practices targeting larger impacts on yield and yield quality leading to higher profits; more buyers and more exports. The present study is one such attempt of a series of studies conducted to find customized solutions for real problems specified by the crop species, management practices and environmental conditions.

Hybrid flower seed production is a lucrative business with a high potential return in the world. Now, F<sub>1</sub>-hybrids are available in many flowers from A (*Antirrhinum*) to Z (*zinnia*) [1]. The one and only company which has over three decades of experience in F<sub>1</sub> hybrid flower seed production in Sri Lanka deals with top seed breeding companies around the world. Therefore, it is of great importance to produce high quality, standard hybrid seeds for their customers. *Platycodon grandiflorus* has become one of the major export earning crops of the company. *Platycodon* plant which is commonly known as “Balloon Flower” belongs to the family Campanulaceae. It is a monotypic species of the bellflower family, which is native to Asia. *Platycodons* are commonly used as versatile, attractive and ornamental perennials in gardens. They are utilized by gardeners and landscapers as accent plants, border plants, potted plants, container plantings and in rock gardens. And also the root of balloon flower is also used as a vegetable. It is widely used as a traditional oriental medicine to treat coughs, colds, upper respiratory tract infections, sore throats, tonsillitis, and chest congestion. It has a traditional historical importance in China for over 2,000 years. Recently, the historical importance was proven in modern medicine with the scientific evidence [2].

The company produces F<sub>1</sub> hybrid seeds of *Platycodon* (SPD 2008) by cross-pollination between two parental lines. The most productive and desirable hybrid seed can be obtained from the female parent when there is a perfect coincidence of stigma receptiveness and pollen viability. The ability of the stigma to support germination of viable, compatible pollen is called stigma receptivity [3]. The effective pollination period is one of the most important factors determining successful fertilization. Successful fertilization is determined by the longevity of the ovule and minus time lag between pollination and fertilization, providing that this value does not exceed the length of the stigmatic receptivity [4]. Sanzol and Herrero [5] reviewed that the effective pollination period is affected by three main events during the reproductive process such as stigma receptivity, pollen tube kinetics, and ovule longevity. It is the critical stage in the maturation of a flower, pollination success at different stages in the flower life cycle, the interference between male and female functions, and the rate of competition via improper pollen transferring [6]. The proteins present at the pollen, stigma and extra cellular matrix of pistil play an important role in pollen-pistil communication. The best receptive stage of the stigmatic surface is most critical for fertilization of ovule for seed setting. Because pollen tube grows on the stigmatic surface and pollen tube grows along the style of the flower [7]. Obviously, day and time of pollination plays a crucial role in hybrid seed production [8].

Thus, a comprehensive knowledge on floral biology of parent lines and their environmental relations are very important for successful production of F<sub>1</sub> hybrid seeds. Unavailability of information regarding best receptive stage and receptive time of stigma is a limitation and it has led to reduced seed yield. Average yield obtained under the current pollination practice at the existing growth stage is 42 seeds/ pod. Therefore, the present study was aimed at identifying the best receptive stage and receptive time of the day of *Platycodon grandiflorus* to improve seed quality and yield under tropical greenhouse conditions.

## Materials and methods

### *Location and climatic condition*

The experiment was carried out under the tropical greenhouse condition at Boralanda in Sri Lanka. This area belongs to the agro-ecological zone of the Upcountry Intermediate Zone (IU<sub>30</sub>) (Latitude: 6°49'44"N, Longitude: 80°53'39"E). During the experiment period, the average

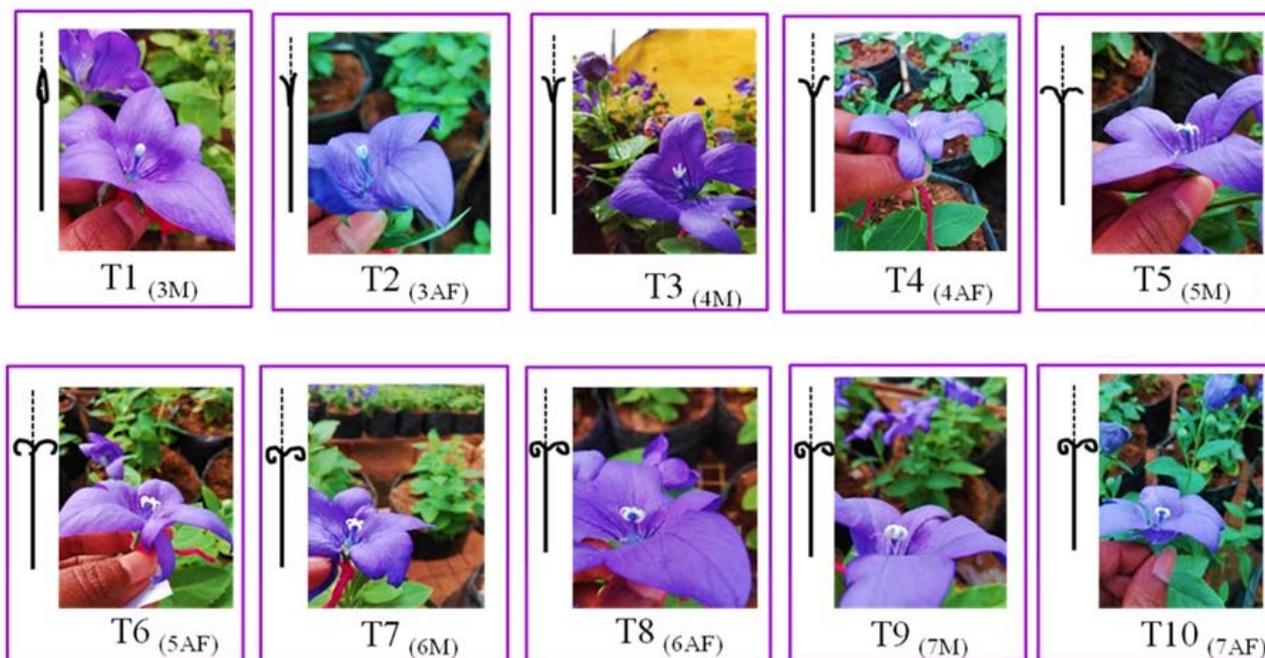
temperature and relative humidity of environment were 19-22 °C and 75-85% at night and 27-30 °C and 60-70% during the day time, respectively.

#### **Treatments**

There are distinct stages in the stigmatic surface of *Platycodon* flower, from blooming to wilting. The temperature of the environment at morning and afternoon was also variable. Therefore, the combinations of pollination date after emasculation and pollination time of the day were used as treatments (T1<sub>(3M)</sub> - Artificial pollination in the morning (8.45 a.m. - 9.00 a.m. time period) after 03 days of emasculation; T2<sub>(3AF)</sub>- Artificial pollination in the afternoon (1.30 p.m. - 1.45 p.m. time period) after 03 days of emasculation; T3<sub>(4M)</sub>- Artificial pollination in the morning after 04 days of emasculation; T4<sub>(4AF)</sub>- Artificial pollination in the afternoon after 04 days of emasculation; T5<sub>(5M)</sub>- Artificial pollination in the morning after 05 days of emasculation; T6<sub>(5AF)</sub>- Artificial pollination in the afternoon after 05 days of emasculation; T7<sub>(6M)</sub>- Artificial pollination in the morning after 06 days of emasculation; T8<sub>(6AF)</sub> - Artificial pollination in the afternoon after 06 days of emasculation; T9<sub>(7M)</sub>- Artificial pollination in the morning after 07 days of emasculation; T10<sub>(7AF)</sub>- Artificial pollination in the afternoon after 07 days of emasculation.

#### **Experimental design and procedure**

2012/99 (Female) and 2026/99 (Male) *Platycodon grandiflorus* varieties were selected to the experiment as parent lines to produce the F<sub>1</sub> hybrid seeds of SPD 2008 variety. Those plants were 3 1/2 months old (middle stage of the flowering period). Uniform sized flower buds without any external defects, were selected randomly for emasculation. Pollen was collected day before pollination from mature flowers of *Platycodon* male line and kept in the refrigerator. Before pollination, the stored pollen was exposed to room condition for about 5 minutes to remove the cold condition. The same amount of pollen was applied on each stigma using a camel hair brush. The ten treatments were replicated 15 times.



**Plate 1:** Cross section and the appearance of the *Platycodon* stigmatic surface of respective treatment

Treatments were started three days after emasculation and experiment was conducted up to the seventh day after emasculation. The stigma life-span of *Platycodon grandiflorus* was about 9 days. However, according to Wei *et al.* [9] the optimal time for pollination is 4-6 days after the

petals opening with the stigma splitting lightly or significantly. Therefore, the time duration to the experiment was selected as 3-7 days after the emasculation under tropical greenhouse condition. The research was conducted in Complete Randomized Design (CRD).

Self-pollinating flowers were used as the control treatment. Fifteen replicates were used for the control, too. It was separated from other plants by using polythene so that wind could not transfer pollen from one flower to another.

During the experiment period, after care practices were done according to recommendations. Plants reached reproductive growth about 21 days after transplanting and took another 20 days to reach full bloom.

#### ***Data collection and analysis***

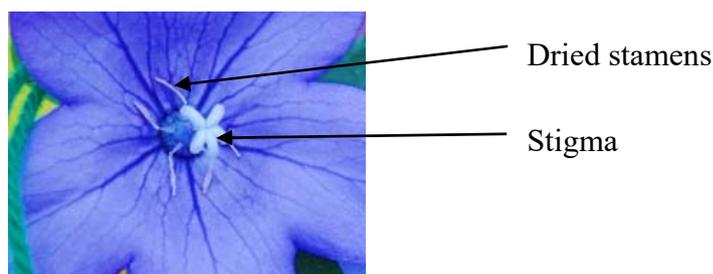
The number of quality seeds per pod was counted manually and the dried weight of quality seeds per pod was measured using the digital analytical balance. Then mean seed number per pod and mean seed weight per pod were calculated for each treatment. After harvesting of pods, 100 seeds were selected randomly from each treatment and the germination percentage for each treatment was checked.

Data were analyzed using Excel and Statistical Analytical Software (SAS) packages. The analysis of variance by GLM procedures with Duncan's Multiple Range Test was used to determine the significance of the treatments at  $p < 0.05$  level.

### **Results and discussion**

#### ***Mean number of quality seeds per pod***

There was no seed setting in the control treatment (self-pollination). Ji-ri *et al.* [10] reported that the anther opening and pollination condition during the blossom period of *Platycodon* shown that the different growth period of pistil and stamen in the same flower caused to the low seed setting rate by self-pollination. In addition, Wei *et al.*[9] reviewed that the natural fructification rate of self-flower-pollination was 4.8% attributing to the difference in maturing period of pistil and stamen, as well as the short pollen life-span. Accordingly, pre-maturation of anthers and/ or drying of stamens (Plate 2) may have led to the seedless pods of control treatment where there was hardly any chance of transferring pollen from another flower.



**Plate 2:** Dried stamens of the *Platycodon* flower

This study found that there was a significant effect of treatments (combination effect of pollination date after emasculation and the pollination time of the day) on the mean number of quality seeds per pod. There was a significant difference ( $p < 0.05$ ) between T1<sub>(3M)</sub> and other treatments. There was no a significant difference ( $p < 0.05$ ) between T2<sub>(3AF)</sub> and T3<sub>(4M)</sub> treatments. And also, both of these were significantly different from other treatments. There was a significant difference ( $p < 0.05$ ) between T4<sub>(4AF)</sub> and other treatments. There was no a significant difference among T5<sub>(5M)</sub>, T6<sub>(5AF)</sub>, T7<sub>(6M)</sub>, T8<sub>(6AF)</sub>, T9<sub>(7M)</sub> and T10<sub>(7AF)</sub> treatments but yielded the highest mean number of quality seeds per pod. Pollination between 5-7 days of emasculation had no significant difference in seed yield. The late maturity nature of ovules may have led to high yield in flowers pollinated at later stage but

low yield in flowers pollinated at early stage. Therefore, the effective pollination period of *Platycodon* may be 5-7 days after emasculation.

**Table 1.** Mean number of quality seeds per pod

Treatment	Number of pods	Mean number of seeds per pod
T1 <sub>(3M)</sub>	10	25 <sup>D</sup>
T2 <sub>(3AF)</sub>	12	32 <sup>C</sup>
T3 <sub>(4M)</sub>	13	31 <sup>C</sup>
T4 <sub>(4AF)</sub>	14	40 <sup>B</sup>
T5 <sub>(5M)</sub>	15	69 <sup>A</sup>
T6 <sub>(5AF)</sub>	15	81 <sup>A</sup>
T7 <sub>(6M)</sub>	15	72 <sup>A</sup>
T8 <sub>(6AF)</sub>	14	80 <sup>A</sup>
T9 <sub>(7M)</sub>	15	79 <sup>A</sup>
T10 <sub>(7AF)</sub>	15	79 <sup>A</sup>

*Values are presented as mean of replicates and values in a column with different superscript letters are significantly different ( $p < 0.05$ )*

The results showed that the pollination in the afternoon was significantly better than the pollination in the morning between 3-4 days after emasculation in terms of yield. It was observed that the opening of stigma in the afternoon was better than the opening of stigma in the morning. Therefore, it may have affected positively on seed setting during 3-4 days after emasculation. But there was no significant difference of the seed yield between 5-7 (‘effective pollination period’) days of emasculation with respect to pollination time of the day.

The results from mean comparison revealed that, the highest mean seed number (81) per pod was shown by T6<sub>(5AF)</sub> (Artificial pollination in the afternoon after 05 days of emasculation), while the lowest mean seed number per pod (19) was shown by T1<sub>(3M)</sub> (Artificial pollination in the morning after 03 days of emasculation). The opening of stigma in earlier days was apparently much lower than that of latter days (Plate 3) possibly causing lower pollen retention on the stigma of flowers pollinated early. Therefore, it may have led to the lower yield of T1 than T6 treatment.



**Plate 3:** Shape of the stigma of T1 and T6 flowers

### Mean seed weight

There was no a significant difference ( $p < 0.05$ ) between T1<sub>(3M)</sub> and T2<sub>(3AF)</sub> treatments. And also, both of these were significantly different from other treatments. There was a significant difference ( $p < 0.05$ ) between T3<sub>(4M)</sub> and other treatments. There was no significant difference among T4<sub>(4AF)</sub>, T5<sub>(5M)</sub>, T6<sub>(5AF)</sub>, T7<sub>(6M)</sub>, T8<sub>(6AF)</sub>, T9<sub>(7M)</sub> and T10<sub>(7AF)</sub> treatments. The results from mean comparison revealed that the highest mean seed weight (2.58 mg) was shown by T3<sub>(4M)</sub> (Artificial pollination in the morning after 04 days of emasculation), while the lowest mean seed weight (0.43 mg) was shown by T5<sub>(5M)</sub> (Artificial pollination in the morning after 05 days of emasculation). Li and Hua [11], reported that the weight of a thousand seeds of *Platycodon* was 0.9735 - 1.0930 g. T1<sub>(3M)</sub>, T2<sub>(3AF)</sub> and T3<sub>(4M)</sub> treatments had shown somewhat higher mean seed weight values than other treatments and standard value. Therefore, those treatments were not fit for the production of standard quality seeds of *Platycodon*. When considered about rest of treatments, T4<sub>(4AF)</sub>, T6<sub>(5AF)</sub>, T8<sub>(6AF)</sub> and T10<sub>(7AF)</sub> treatments had shown somewhat closer mean seed weights to the standard value. Pollination of flowers in the afternoon, between 4-7 days of emasculation, produced seeds with standard quality.

**Table 3.** Mean seed weight

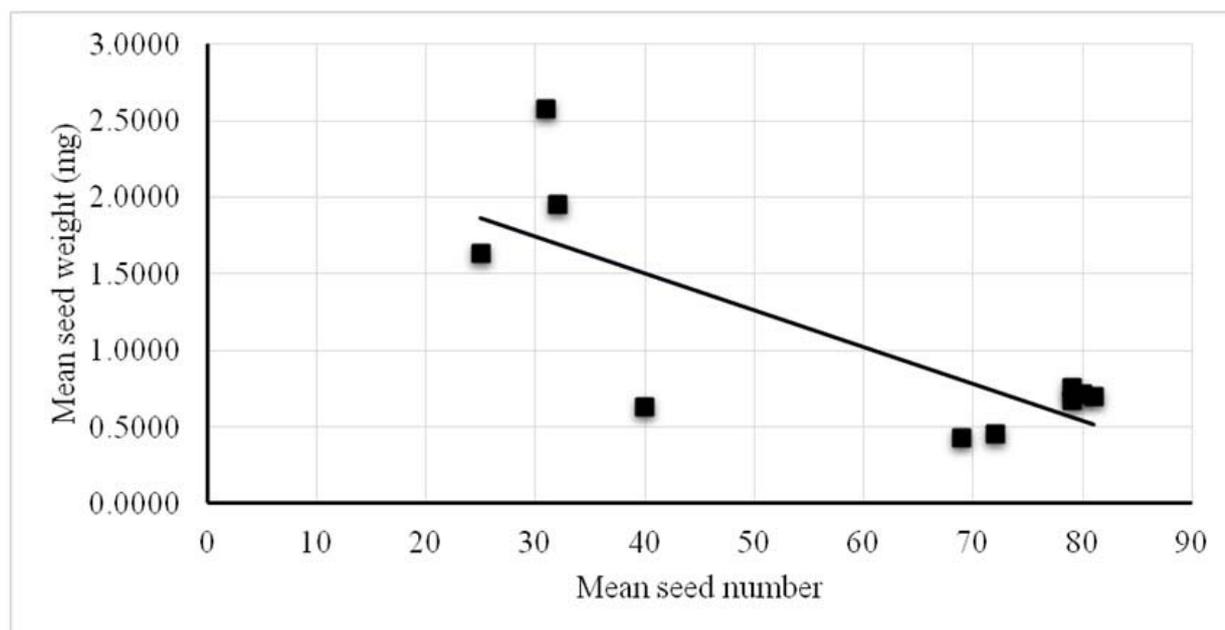
Treatment	Number of pods	Mean seed weight (mg)
T1 <sub>(3M)</sub>	10	1.6321 ± 0.2726 <sup>B</sup>
T2 <sub>(3AF)</sub>	12	1.9565 ± 0.1225 <sup>B</sup>
T3 <sub>(4M)</sub>	13	2.5781 ± 0.3400 <sup>A</sup>
T4 <sub>(4AF)</sub>	14	0.6301 ± 0.0629 <sup>C</sup>
T5 <sub>(5M)</sub>	15	0.4323 ± 0.0597 <sup>C</sup>
T6 <sub>(5AF)</sub>	15	0.6987 ± 0.0447 <sup>C</sup>
T7 <sub>(6M)</sub>	15	0.4532 ± 0.0661 <sup>C</sup>
T8 <sub>(6AF)</sub>	14	0.7156 ± 0.0469 <sup>C</sup>
T9 <sub>(7M)</sub>	15	0.6740 ± 0.0464 <sup>C</sup>
T10 <sub>(7AF)</sub>	15	0.7558 ± 0.0471 <sup>C</sup>

*Values are presented as mean ± SE of replicates and values in a column with different superscript letters are significantly different ( $p < 0.05$ )*

### Correlation between the mean seed weight and the mean number of quality seeds per pod

This study found that the correlation between the mean seed weight and the mean number of quality seeds per pod was -0.765. It showed that there was a strong, negative (inverse) relationship between those factors (Figure 2).

The mean seed weight decreased with the increasing mean number of quality seeds per pod. Meng *et al.* [12], reported that there was an inverse relationship between seed weight and seed number of *Sinopodophyllum hexandrum*. The seed weight-number trade-off changes because of the mechanisms to enhance resource acquisitions during the fruit developments. Therefore, the limitation of resources has led to the inverse relationship between the weight and the seed number of fruits. Seed number and size are often negatively correlated indicating that intrinsic resource level may limit investment in total seed mass of individual fruit [13]. Therefore, the limitation of resources may have led to the production of smaller seeds in pods.



**Fig. 2.** The correlation between the mean seed weight and the mean number of quality seeds per pod

#### Germination percentages of harvested seeds of each treatment

The highest germination percentage (85%) was shown by T6(5AF) (Artificial pollination in the afternoon after 05 days of emasculation), while the lowest germination percentage (32%) was given by T3(4M) (Artificial pollination in the morning after 04 days of emasculation). Li and Hua [11] reported that the germination rate of *Platycodon* seeds was 61.0%-87.0%. Therefore, pollination between 5-7 days after emasculation assures accepted percentage of germination when they are tested for their seed germination.

**Table 4.** Germination percentage of hundred seeds of each treatment

Treatment	Germination percentage (%)
T1(3M)	34
T2(3AF)	32
T3(4M)	50
T4(4AF)	57
T5(5M)	80
T6(5AF)	85
T7(6M)	66
T8(6AF)	72
T9(7M)	68
T10(7AF)	70

#### Conclusion

Pollinating *Platycodon grandiflorus* flowers either in the morning or in the afternoon, 5-7 days after emasculation gives nearly a two-times higher yield of quality seeds than that of contemporary pollination practices under tropical greenhouse conditions.

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