

12th Australasian Plant Breeding Conference
15th-20th September 2002
Hyatt Regency Perth

Assignment of copyright

Abstract No : 2P20

Title of the paper : Pollen viability and stigma receptivity in Sturt's desert pea
(*Swainsona formosa*)

Authors: Z. ZULKARNAIN, A. TAJI and N. PRAKASH

Submission of this paper to the 12th Australasian Plant Breeding conference implies that

- all authors agree to its publication
- authors have obtained permission to use material from prior published sources and have properly credited these sources
- all authors agree that the work may not be used in any way the implies that the Australasian Plant Breeding Association or the organisers of the 12 Australasian Plant Breeding Conference endorses any product or procedure.

I agree that the Australasian Plant Breeding Association holds the copyright to this paper published in 'Plant Breeding for the 11th Millennium. Proceedings of the 12th Australasian Plant Breeding Conference'. However authors will retain proprietary rights other than copyright, such as patent rights, and the right to reproduce their papers in other publications with the acknowledgement that they first appeared in the Proceedings of the 12th Australasian Plant Breeding Conference. You may also use data from your conference paper in other publications.

I acknowledge that on acceptance of the paper the author must attend the conference as a paying delegate and present the paper/poster.

Signed on behalf of all authors by the corresponding author

Name: Z. ZULKARNAIN Signature:  Date: 29th April 2002

Please return this form to your symposium manager when you submit the hard copy of your paper.

Thank you

Pollen viability and stigma receptivity in Sturt's desert pea

Z. Zulkarnain^{A)}, A. Taji^{A)} and N. Prakash^{B)}

^{A)}School of Rural Science and Agriculture, ^{B)}School of Environmental Sciences and Natural Resources Management, University of New England, Armidale NSW 2351, Australia

Abstract. The work reported here was aimed at determining the duration of pollen viability and stigma receptivity in *Swainsona formosa* (Sturt's desert pea, an Australian native legume), as an initial step in conventional breeding via artificial crossing by hand pollination. The pollen viability was determined for pollen shed from anthers and retained inside the keel under glasshouse conditions (temperature 25 - 32°C, light intensity ranging from 650 - 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and photoperiod 10 - 14 hours) and for pollen harvested from anthers and stored under cold (4°C) and total darkness conditions. The receptivity of stigma was determined using stigma on intact plants under glasshouse conditions. The stigmatic surface was exposed and rubbed with fingertips. Pollen from anthers which had just begun shedding was applied to the stigmatic surface one day prior to anther dehiscence, on the day of anther dehiscence and daily thereafter until 8 days after anther dehiscence. The result indicated that pollen grains stored at 4°C remained fertile up to 21 days after storage (more than 50% of pollen germination was achieved on test solution). The pollen fertility in pollen grain retained inside the flower in the glasshouse declined within 5 days after shedding. The stigma receptivity reached a maximum 2 - 3 days after anther dehiscence and dropped significantly by day 6. The work reported here provides an insight into the correct timing for hand pollination in Sturt's desert pea.

Additional keywords: *Swainsona formosa*, cut flower, legume, plant breeding.

Introduction

Sturt's Desert Pea (*Swainsona formosa*), an Australian native legume, is of economic interest for its use as an ornamental crop. Its potential as a cut flower plant was first recognised a number of years ago and a large market has been unearthed in Japan.

The plant has large (up to 100 mm long) flag-shaped red flowers, consisting of an upper standard petal with a shiny black boss, and a lower keel containing the sexual organs (Mitchell and Wilcox 1994). Other colour variations, such as white or pink with or without a distinctive boss, have been found. A market survey in Japan in 1989 revealed that flowers with lighter colours were in greater demand than flowers with stronger colours (Barth and Bennel 1989). The flowers consist of clusters (umbels) of up to six florets in a whorl at the end of short upright stalks (Williams and Taji 1987) and the peduncle may measure up to 20 cm long (Jusaitis 1994). Vase life of *S. formosa* flowers can reach 3 weeks in winter, although it may shorten to 7 - 10 days in summer. At a constant temperature of 23°C, most scapes remain attractive for 10 - 14 days (Kirby 1996).

S. formosa produces a large amount of pollen grains, which may stain the petals when the anthers dehisce, reducing flower quality. Post-harvest self-pollination may also occur during transportation, resulting in quick flower degeneration and reduction in vase life. Therefore, in order to support the commercialisation of *S. formosa*, the breeding program must focus on improvement of flower quality, breeding flowers with more attractive colours, and less or no pollen grains, and lengthening vase life. We studied pollen viability and stigma receptivity as an initial step in the conventional breeding of this species. Some basic aspects have been identified and the results are discussed.

Materials and methods

Pollen viability

This study consisted of two trials: pollen grains remained on plant (within the keel) in the glasshouse and pollen grains were harvested and kept at a low temperature (4°C) in total darkness. In the first trial, pollen grains were taken from 2 – 3 umbels of 10 glasshouse-grown plants on the day of anther dehiscence (day 0) through 10 days after anther dehiscence (day 10), at daily intervals.

In the second trial, pollen grains were harvested from 2 – 3 umbels from 10 different glasshouse-grown plants on the day of anther dehiscence, collected and mixed in a vial, and immediately refrigerated. The viability of pollen was assessed on the day of harvesting and after up to 28 days of storage.

The germination of pollen was carried out on a modified BK medium (Brewbaker and Kwack 1963) containing 720 ppm $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 200 ppm $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 200 ppm KNO_3 , 20 ppm H_3BO_3 and 10% sucrose. Pollen was spread evenly on the surface of 2 drops of this medium, on microscope slides. The slides were placed in closed Petri dishes on a layer of moistened filter paper. The dishes were kept at room temperature, and pollen germination was assessed after 30 minutes.

Each trial was arranged in a Completely Randomized Design, with 5 replicates. There were 10 fields of vision (observations) on each replicate. The percentage of pollen tube formation was determined by counting the number of pollen forming tubes, divided by all pollen grains, in one field of vision. Data of one replicate were derived from mean values of 10 observations.

Stigma receptivity

This work sought to ascertain the time at which the stigma of *S. formosa* was most receptive, so that the optimal time for hand pollination (carried out in the glasshouse for breeding purposes) could be determined.

Flower emasculation was undertaken 1 - 2 days prior to anther dehiscence (for practical reasons this was the earliest that floral buds could be manipulated without causing damage). The stigma was rubbed several times with fingertips to remove the stigmatic cuticle and expose the stigmatic surface. The stigmatic surface was pollinated with newly released pollen grains from the same plant, or a different plant, at the time indicated by treatment, i.e. one day before anther dehiscence, on the day of anther dehiscence, one day after anther dehiscence until 8 days after anther dehiscence at daily intervals. Three to five umbels (each consisting of 5 – 7 florets) on each plant were selected as flower samples. Three plants from each treatment were selected as samples.

Results and discussion

Pollen viability

The viability of pollen grains was high at the time of collection (more than 50% pollen germinated in BK medium). If pollen grains were left within the keel of flowers on plants in the glasshouse, the viability was still reasonably high (> 50%) up to 4 days after anther dehiscence. The viability dropped dramatically on day 5, when the percentage of germination was less than 40%, and a further decrease in viability (less than 20% pollen germinated) was found at 10 days after anther dehiscence (Figure 1). However, if pollen grains were kept at low temperature (4°C) in total darkness, the viability remained high (> 50%) for up to 21 days of storage (Figure 2). Prolonged storage (more than 21 days) was found to reduce pollen viability.

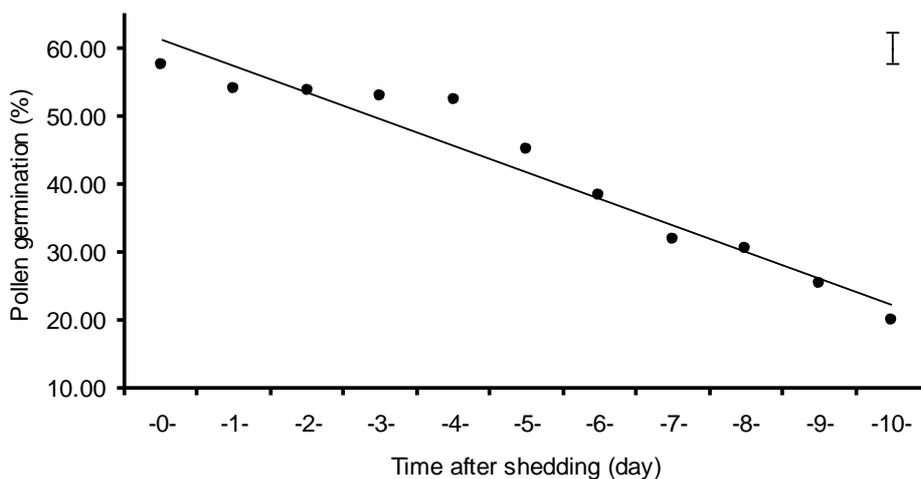


Figure 1. Germination rate of Sturt's desert pea pollen grains from day 0 through to day 10 (after shedding), when pollen remained within the keel of flowers on plants in the glasshouse (bar represents $SE \pm 2.17$).

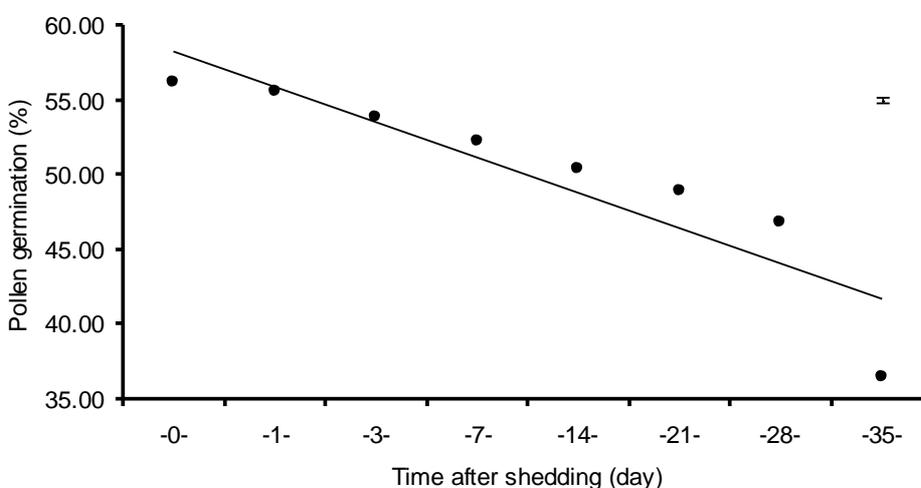


Figure 2. Germination rate of Sturt's desert pea pollen grains from day 0 through to day 35 after shedding, when kept at 4°C in total darkness (bar represents $SE \pm 0.23$).

Stigma receptivity

The results indicated that one day before anther dehiscence the stigma is already receptive (Figure 3). The receptiveness reached its peak on day 2 and 3 after anther dehiscence, indicated by 100% pod formation, and gradually decreased from day 4. On day 8 after anther dehiscence, the petal started to degenerate and the stigma receptiveness decreased significantly. On day 9, the stigma completely lost its receptiveness.

Judging from the fairly high percentage of pod formation (57.72%) it was assumed that the stigma had already been receptive several days before anther dehiscence. However, Jusaitis (1994) claimed that the most convenient time for emasculating and performing hand pollination was when the petals were still closed but could easily be opened using fingers. This stage of flower development was approximately one day before anther dehiscence. Therefore, the earliest time for

practically emasculating and carrying out hand pollination in the present trial was one day before anther dehiscence. If the emasculations were carried out earlier, it would have damaged the floral parts, particularly petals, because the flower was still tightly closed and hard to open.

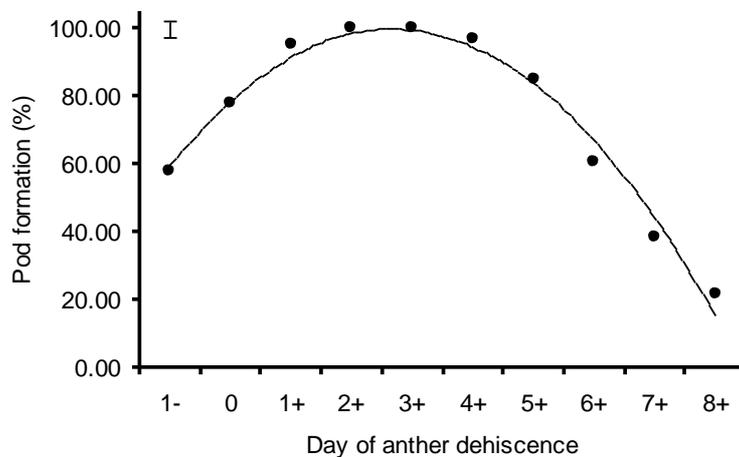


Figure 3. Stigma receptiveness in relation to the timing of anther dehiscence in emasculated glasshouse-grown Sturt's desert pea flowers (bar represents $SE \pm 2.35$).

Conclusions

These results indicate that hand pollination in Sturt's desert pea could be done at one day before anther dehiscence, but would optimally be undertaken at 1-5 days after anther dehiscence, at which time the stigma was most receptive. When there were no receptive stigma readily available for pollination, the pollen grains might be left within keel in the glasshouse but just for a short period of no more than 4 days. Alternatively, the pollen grains could be stored at low temperature (4°C) in total darkness, under these conditions pollen viability could remain high for up to 28 days.

References

- Barth GE and Bennel M (1989) 'Market Development of Sturt's Desert Pea (*Clianthus formosus*) for The Cut Flower Market in Japan.' South Australia Department of Agriculture, Adelaide.
- Brewbaker JL and Kwack BH (1963) The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany* **50**, 859-865.
- Jusaitis M (1994) Floral development and breeding system of *Swainsona formosa* (Leguminosae). *HortScience* **29**, 117-119.
- Kirby GC (1996) Sturt's Desert Pea as cut flower crop. In '4th National Workshop for Australian Flower'. Perth, Australia pp. 204-209
- Mitchell AA and Wilcox DG (1994) 'Arid Shrubland Plants of Western Australia.' (University of Western Australia Press: Nedlands, Australia).
- Williams RR and Taji A (1987) Propagation of Sturt's Desert Pea by seed, cutting and tissue culture. *Australian Horticulture* **85**, 47-53.

POLLEN VIABILITY AND STIGMA RECEPTIVITY IN SWAINSONA FORMOSA

Z. ZULKARNAIN, A. TAJI and N. PRAKASH

University of New England, Armidale NSW 2351 Australia
zzulkarn@metz.une.edu.au

Introduction

Sturt's desert pea, an Australian native legume, is of economic interest for its use as an ornamental crop. Its potential as a cut flower plant had been recognised for many years ago and proved to be enormous by the fact that it was in demand by the cut flower market in Japan (Barth and Bennel 1989).

The plant has large flag-shaped red flowers consisting of an upper standard petal with a shiny black boss and, a lower keel. Other colour variations were found such as white or pink with or without a distinctive boss (Figure 1). In order to support its commercialisation the breeding program plays an important role in the improvement of flower quality such as more attractive colours, less or no pollen grains, as well as longer vase-life. We studied the pollination as an initial step of conventional breeding of this species. Some basic aspects have been identified and the results are discussed.



Figure 1. White-flowered Sturt's desert pea.

Materials and methods

The viability of pollen grains was assessed in pollen remained within the keel in the glasshouse and those kept in low temperature (4°C) and total darkness. In the first, pollen grains were taken from the plants on the day of anther dehiscence through 10 days afterwards. In the second, pollen grains were harvested on the day of anther dehiscence and immediately stored in fridge, the viability of pollen was assessed up to 28 days of storage.

Pollen grains were spread evenly on the surface of BK medium (Brewbaker and Kwack 1963) on a microscope slide. The slide was placed in a closed Petri dish on a layer of moistened filter paper. The dish was left at room temperature and pollen germination was assessed after 30 minutes.

For stigma receptivity assessment, flowers were emasculated 1 - 2 days before anther dehiscence. The stigma was pollinated with newly released pollen grains from the same plant or a different plant at a time indicated by treatment, i.e. one day before anther dehiscence until 8 days afterwards in daily intervals. The pod formation was used as an indicator of stigma receptivity.

Results

The viability of pollen grains was relatively high at the time of collection. If pollen grains were left within the keel in the glasshouse, the viability was still reasonably high (> 50% germination) up to 4 days after anther dehiscence. The viability dropped significantly on day 5 when the percentage of germination was less than 40%. Further decrease of viability (less than 20%) was found on day 10 after anther dehiscence (Figure 2). However, if pollen grains were kept at low temperature (4°C) and total darkness, the viability remained high (>50%) for up to 21 days of storage (Figure 3). Prolonged storage at low temperature for more than 21 days was found to reduce the viability of the pollen.

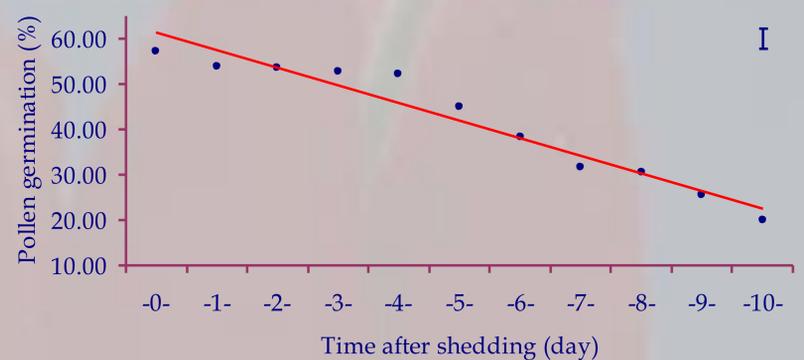


Figure 2. Germination rate of Sturt's desert pea pollen grains from day 0 through to day 10 after shedding when remained within the keel in glasshouse (bar represent SE ± 2.17).

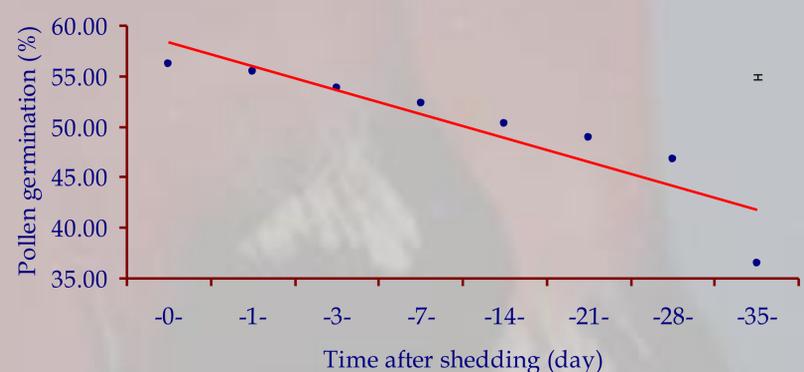


Figure 3. Germination rate of Sturt's desert pea pollen grains from day 0 through to day 35 after shedding when kept under 4°C and total darkness (bar represent SE ± 0.23).

It was found that one day before anther dehiscence the stigma has already been receptive (Figure 4). The receptiveness reached its peak on the days 2 and 3 after anther dehiscence as indicated by 100% pod formation, and began to decrease on day 4. On day 8 after anther dehiscence the petals started to degenerate and the stigma receptiveness decreased significantly and on day 9 the stigma completely lost its receptiveness.

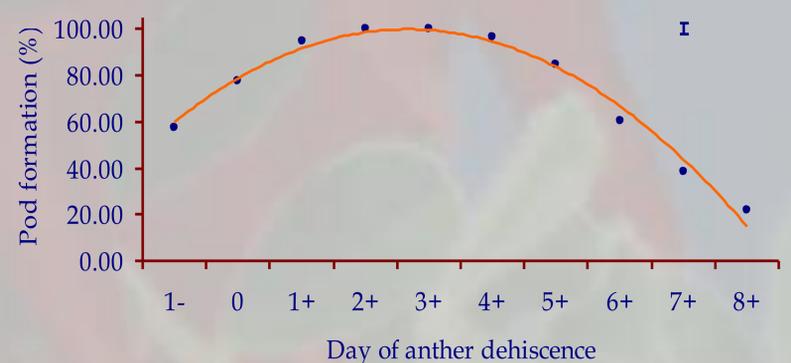


Figure 4. Stigma receptiveness in relation to the timing of anther dehiscence in glasshouse-grown Sturt's desert pea (bar represents SE ± 2.35).

Conclusions

The results indicate that hand pollination in Sturt's desert pea could be done at one day before anther dehiscence, but the best time when the stigma was most receptive was on days 1 to 5 after anther dehiscence. When there was no receptive stigma readily available for pollination, the pollen grains might be left within the keel in the glasshouse but just for a short period of no more than 4 days. Alternatively, the pollen grains could be stored under low temperature (4°C) in total darkness, under which conditions pollen viability could remain high for up to 21 days.

References

- Barth GE, Bennel M (1989) 'Market Development of Sturt's Desert Pea (*Clanthus formosus*) for The Cut Flower Market in Japan.' South Australia Department of Agriculture, Adelaide.
- Brewbaker JL, Kwack BH (1963) The essential role of calcium ion in pollen germination and pollen tube growth. American Journal of Botany 50, 859-865.