Aspects relating to seed production in *Gloriosa superba* L.

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Open- and hand pollination, ovule morphology, seed structure and germination of *Gloriosa superba* L. are discussed as possible factors influencing reproduction. The protogynous flowers in the frondose raceme are psychophilous and ornithophilous with a stigma receptivity period of at least four days. Development of the female gametophyte conforms to the Polygonum type. The inner integument acts as an endothelium due to the absence of the nucellus on the lateral sides of the embryo sac, where the nucellus has degenerated. A well-developed funicular obturator directs pollen tubes to a nucellar epistase where a partial self-incompatibility reaction appears to occur. A hypostase on the chalazal side contains transfer cells connecting the vascular tissue with the embryo sac. Seeds are tegmic, surrounded by a sarcotesta showing adaptations for endozoochory. Seed production was slightly better after cross-pollination, and also when 48 hour-old pollen was used, especially when pollination was done in the second flower phase. When the three factors were combined and applied to the proximal flowers in the inflorescence, seed production was significantly improved.

Keywords: Seed production, ovule development, sarcotestal seed, zoochory, seed germination.

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Introduction

Gloriosa superba L. is a typical cryptophyte with ephemeral, climbing stems and a perennial subterranean hypopodial tuber (Le Roux & Robbertse 1994a). This genus is classified under the family Colchicaceae (Tribus: Iphigenicae) and occurs along the warmer east coast regions of southern Africa through central Africa to Ethiopia and is also found in eastern Asia (Dahlgren & Clifford 1982).

Colchicine is one of the 19-tropolene alkaloids extracted from the seed and tubers of *Gloriosa* (Dvorackova *et al.* 1984). This highly poisonous alkaloid is used in western orthodox medicine for the relief of pain and inflammation in the treatment of arthritis and gout (Hutchings & Terblanche 1989). Chemical analysis showed that the amount of colchicine in *Gloriosa* seed is ten times that of the tubers (Sarin *et al.* 1974). Corms of *Colchicum autumnale* and *Iphigenia* have previously been utilised for colchicine extractions but the increase in demand for the alkaloid necessitated the search for an alternative plant source (Sarin *et al.* 1974). *Gloriosa* seed is of potential economical importance as a source for colchicine extraction.

A local *Gloriosa* grower reported that seed production in this species is disappointingly low. Preliminary artificial pollination experiments done over a period of two years at the University of Pretoria confirmed seed set was low and variable, and thus warranted investigation.

Some aspects of the reproductive biology, for example anther dehiscence, stigma receptivity, seed set and pollination mechanism, have been reviewed (Narain 1976). Artificial pollination by Narain (1976) showed that *G. superba* had a seed set of 90% after self- and cross-pollination. The cited author unfortunately used no statistical tests to prove the significance of figures obtained. A study on anther ontogeny and pollen viability showed, except for rare cases of male sterility, the pollen quality of *G. superba* to be high and not contributing to low seed set (Le Roux *et al.* 1994b). A paper on the transmitting tract of *G. superba* relating to the ultrastructure, pistil exudate and pollen tube growth showed that a partially pre-zygotic self-incompatibility mechanism could contribute to low seed yield (Le Roux *et al.* 1996).

This paper is part of an extensive study on the reproductive biology of *Gloriosa superba*. We report on ovule morphology, seed set after open- and hand-pollination, seed and ovule structure, as well as germination of *G. superba*, as possible factors affecting seed production.

Materials and Methods

All experimental procedures were performed on plants cultivated at the University of Pretoria (24°45'S 28°14'E) as described by Le Roux & Robbertse (1994a). These plants were obtained from a local grower and will be referred to as the 'ennobled type', since they differ morphologically from the local 'wild type'.

The open pollinated 'wild type' collection of *G. superba*, consisting of 40 plants growing at the Rietondale experimental farm near Pretoria, was utilised for observations on pollinators and seed set. At the end of the flowering season plant height was measured while the total number of seeds per plant and the average number of seeds per fruit were also recorded.

Pollination.

In the 'ennobled type' hand pollinations was done in the following four floral phases:

PHASE I - perigone green and closed, just before anthesis, anthers non-dehisced.

PHASE II - three days after Phase I, perigone yellow/orange, in an inverted vertical position, anthers non-dehisced. Stigmas became receptive and stayed receptive for at least four days.

PHASE III - one day after Phase II, flowers similar in appearance to phase II but anthers dehisced.

PHASE IV - two days after Phase III, perigone darker in colour, anthers withered.

The above-named floral phases were artificially self- and cross-pollinated with fresh, 24 and 48 hour-old pollen for determining seed set. Pollen from freshly dehisced anthers was stored and aged in sterile vacu-test tubes on silica gel crystals at 25°C. For each treatment 4 replicates of 10 flowers each were used (total of 960 flowers). The response variable distribution (average number of seeds per fruit) deviates from the expected and non-countant variance of the treatments. The Poisson distribution applies to data in the form of counts, or averages of counts, and therefore these data on seed set were analysed using the generalised linear model with the Poisson

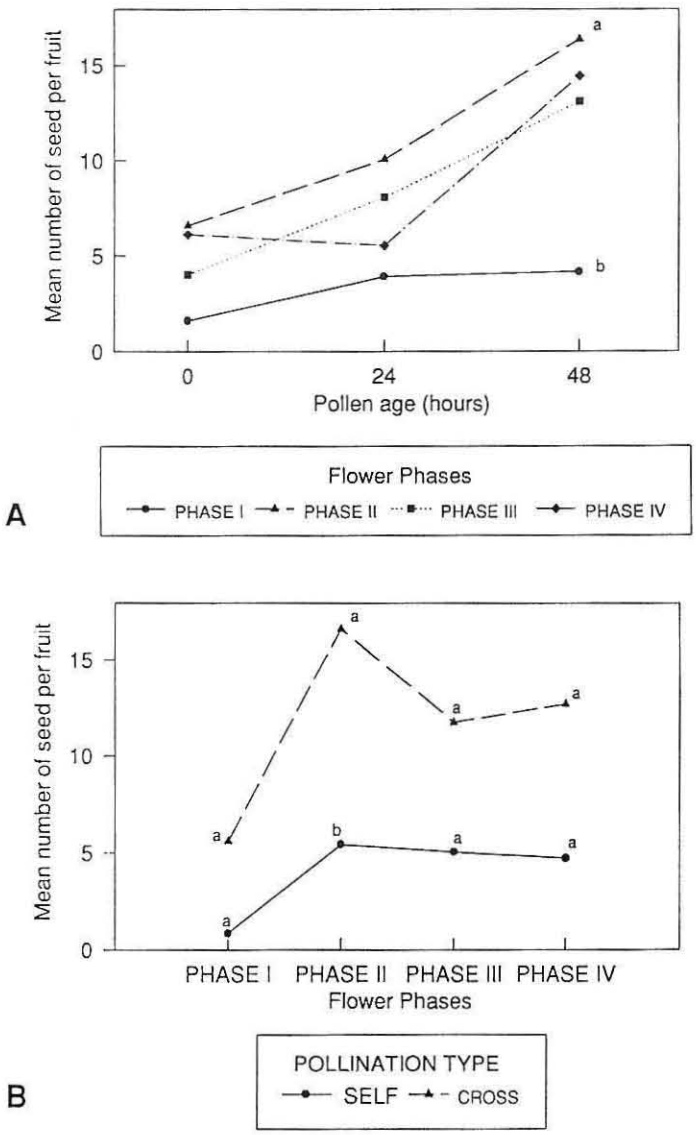

Figure 1 Seed production in *Gloriosa superba* L. in the 'ennobled type' showing:
A. The effect of pollen ageing on seed set in different floral phases. LSD of Bonferoni - t_v $\alpha/2p = 2.7054(5\%)$.
B. The effect of self- and cross-pollination on seed set in different floral phases. LSD of Bonferoni - t_v $\alpha/2p = 2.5554$ (5%).

distribution. To determine the least significant differences the Bonferroni constant was used applying the following formula: $KBV_B = t_v^{\alpha/2p} [S^2_{x1} + S^2_{x2}]^{1/2}$.

Ovule morphology and seed structure

Buds, flowers and seed were collected and fixed in 5% (w/v) glutaraldehyde solution buffered with 0.075M sodium phosphate (pH 7.4). A solution of 0.5% caffeine (Mueller & Greenwood 1978) was added to the glutaraldehyde to stabilise phenolic compounds. The material was embedded in glycol methacrylate (GMA) and 2–4 µm thick sections were stained with Periodic acid/ Schiff's reagent (PAS) and toluidine blue (O'Brien & McCully 1981) and studied with a Nikon Optiphot light microscope. Some material was fixed in FAA (formalin - alcohol - acetic acid) and later preserved in 70% alcohol. The material was dehydrated in a series of ethyl alcohol for wax embedding and sectioned at 8–10 µm, stained with safranine O/ fast green (Johansen 1940).

Some of the ovules were cleared in Herr's fluid (Herr 1971) for whole mounts and viewed with Nomarski interference optics. For scanning electron microscopy (SEM) seeds were fixed with 2.5% glutaraldehyde in 0.075M phosphate buffer and post-fixed with 0.25% aqueous osmium tetroxide. Samples were critically point dried with CO_2, vacuum coated with gold and studied with a Jeol 840 SEM operated at 8kV.

Seed germination

Freshly collected seeds were stored in paper bags for the required dormancy period of four months as described by Le Roux and Robbertse (1994a). Seed with the sarcotesta intact or removed was sterilised in 1% hypochlorite subjected to a water jet vacuum pump for five minutes. Seeds were then rinsed four times in distilled water, placed in petri-dishes on wet filter paper and incubated at 20°C, 25°C, 30°C and 35°C respectively for 34 days, with a 12h:12h alternatively light:dark cycle. For each treatment 10 replicates of ten seeds were used, and cumulative germination percentages were determined at regular intervals. Petri dishes were randomly placed in the germination cabinet and the seed was watered twice a week during the incubation period.

Results

Morphology and pollination

Plants produced from tubers planted in the beginning of December started flowering after approximately 5 weeks, and continued flowering for a period of ± 7 weeks. Seeds were harvested about six weeks after pollination.

Pedicelled flowers of *G. superba* are borne singly, forming a frondose raceme (Weberling, 1992). Anthesis is acropetal and all the flowers develop from displaced axillary buds, giving the impression that flower stalks are not associated with the subtending euphyls. The ovary is superior, tricarpellate and trilocullate with exile placentation and contains about 30 ovules per locule in the 'ennobled type'. The anatropous ovules form a double row on the placenta and are lined up with their micropylar ends pointing away from the placenta towards the stylar end.

In the 'ennobled type', seed set occurred after hand pollination in all four investigated floral phases (Figure 1A and B). Although there was mostly no statistically significant difference in seed set between flowers pollinated in the different phases there was, however, a definite trend in favour of flowers pollinated in phases II, III and IV. Flowers pollinated in Phase I produced virtually no seed, while the highest average seed set of 16.43 per fruit was observed in Phase II (Figure 1A). Both aged and fresh pollen used during pollination proved to be fit for fertilisation and viable seeds were produced by both. Although pollen age had no statistically significant effect on seed set, pollen aged for 48 hours produced more seed than fresh or 24-hour-old pollen (Figure 1A). Cross-pollinated flowers produced an average of 11.67 seeds per fruit at a comparison with 4.025 seeds per fruit in self-pollinated ones, an almost threefold improvement (Figure 1B).

The fully-grown 'wild type' plants reached a maximum height of 65cm and produced approximately 20 flowers per plant in comparison with the 15 flowers per plant of the 'ennobled type', which reached a height of up to 150cm. 'Wild type' plants that reached a height of approximately 60cm produced a total average

Table 1 Seed set results as recorded for the open pollinated *Gloriosa* 'wild type' population at Rietondale

Plant height (cm)	Average total seed set per plant	Average number of seed per fruit
60–65	258 ±214.7	17.5 ±7.06
45–50	128 ±92	22.2 ±7.75
35–40	30 ±21.2	16.2 ±10.1

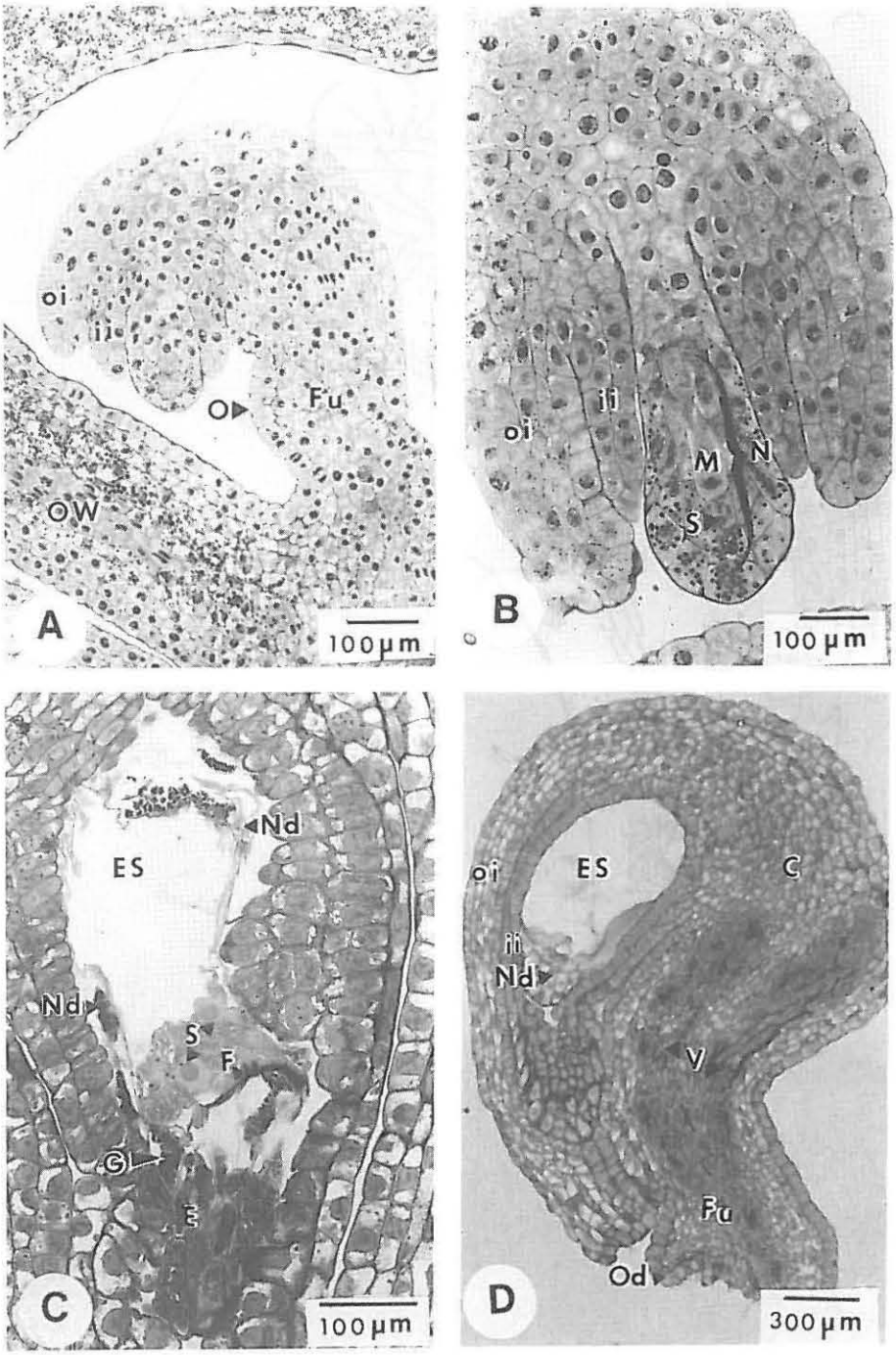

Figure 2 Ovules of *Gloriosa superba* in different developmental stages.
A. A young anatropous ovule showing obturator (O) development from proliferating funicular tissue. (Fu - funiculus, oi - outer integument, ii - inner integument, ow - ovary wall). **B**. Ovule in spore mother cell (M) stage showing nucellus (N) tissue with a few subdermal (S) nucellar cells possibly of parietal origin. (oi - outer integument, ii - inner integument). **C.** Ovule with mature embryo sac (ES) showing an epistase (E) impregnated with starch grains (G) and the filliform apparatus (F) associated with a synergid (S). (ND - degenerating nucellus). **D**. Ovule after fertilization showing the expanding embryo sac (ES) shifting away from the chalaza (C) to a semi-campylotropous position. (Fu - funiculus, oi - outer integument, ii - inner integument, V - vascular tissue, ND - degenerating nucellus, Od - degenerating obturator.

of 258 seeds per plant with an average of 17,5 seeds per fruit. (Table 1). Smaller plants (45cm–50cm) produced a total average of 128 seeds while the smallest plants had an even lower yield (Table 1). The average number of seeds per fruit in both 'ennobled' and 'wild type' plants was low, when seed counts from the entire inflorescence per plant were made. Fruit developing from the first flowers in the inflorescence, however, predominantly produced more seed per fruit than flowers developing later in the season.

In the open pollinated *Gloriosa* 'wild type' population, flowers were mostly visited by day-flying Lepidopterans (of which *Papilio demodocus* was the most common), *Apis mellifera* (honey bees), ants and sugar birds. Nectar is produced by nectaries situated at the perigone base. Bees and ants utilised nectar but were seldom observed near the anthers. The butterflies that were attracted by the flowers had a wingspan of approximately 6 cm, and were able to touch both the stamens and stigma with their lower wing surface during a visit. Sugar birds were mostly covered with pollen while foraging for nectar. It is, therefore,

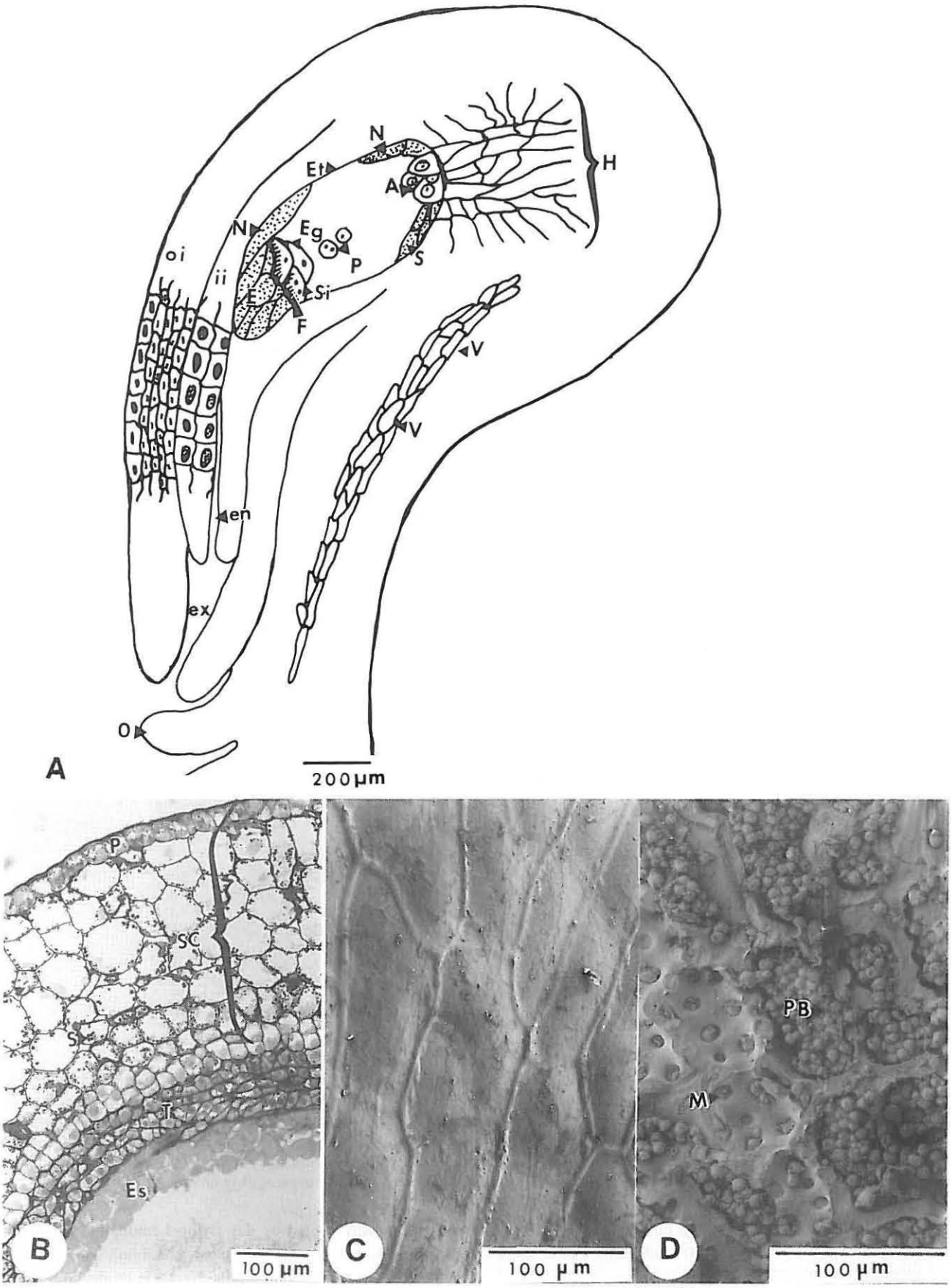

Figure 3 **A**. Semi-diagramatic presentation of *Gloriosa superba* ovule just proir to fertilization in longitudional section. (A - antipodal cells, Eg - egg cell, Si - sinergid, F - filliform apparatus, N-nucellus, P - polar nuclei, ex - exostome, en - endostome, V - vascular tissue, S- starch grains, H - hypostase, E - epistase, O - obturator, Et - endothelium). **B**. Section of the seed coat showing the sarcotesta (SC), tegmen (T), endosperm (Es). (S - starch grains, P - highly pigmented epidermis. **C.** SEM micrograph of the outer epidermal surface of sarcotesta characterized by anticlinal cell wall ridges. **D**. SEM micrograph of split mature seed showing endosperm cells packed with aleurone like protein bodies (PB) and cell walls perforated by plasmodesmata (M).

possible that butterflies and birds could successfully pollinate these flowers.

Ovule and seed structure

The anatropous ovule develops an obturator from proliferating funicular tissue which covers the micropylar region but degenerates soon after fertilisation. (Figures 2A and 3A). Obturator cells are cytoplasm dense and have thickened, outer epidermal cell walls.

At the time of the spore mother cell stage the outer and inner integuments have grown half-way along the nucellus (Figure 2B). The formation of a parietal cell was not observed, although a few subdermal nucellar cells were observed. The chalazal megaspore of the linear tetrad develops into a 8-nuclear, female gametophyte. The embryo sac (ES) conforms to the Polygonum type (Figure 3A). A well developed filiform apparatus giving a strong PAS positive reaction was observed (Figure 2C). The two polar nuclei remained separate until just prior to fertilisation (Figure 3A). More than one nucleolus was occasionally observed in the synergids and central cell. The three antipodal cells are two- to three nucleate and degenerate slowly after fertilisation and can still be clearly observed in the presence of the zygote.

In ovules with mature ES, the central peripheral nucellus has already started degenerating, but a nucellular cap with cells reminiscent of an epistase is left covering the ES and plugging the micropylar area (Figure 2C). Nucellus cells surrounding the antipodal cells and in close association with the hypostase were also detected (Figure 3A). These irregularly shaped nucellar cells have a cytoplasm rich in starch grains (PAS positive). The micropyle consists of an exostome formed by the 4–5 layered outer integument, and an endostome formed by a 3 layered inner–integument. The inner epidermal cells of the latter integument also act as an endothelium on the lateral sides of the ES where the nucellus cells have degenerated. These cells are radially elongated and contain prominent nuclei.

The chalazal tissue facing the antipodal end of the ES differentiates into a hypostase characterised by thickened cell walls that show a clear fluorescence with aniline blue, indicating the presence of callose. Just after fertilisation the ES expands rapidly while the cells on the antiraphal side and/or the inner integument cells on the raphal side become meristematic, causing the ES to be shifted away from the chalaza into a semi-campylotropous position (Figure 2D). Due to the extended hypostasal area, there is a considerable distance between the end of the vascular tissue as a result of the displacement of the ES. The hypostasal cells become enlarged, their cytoplasm stains densely, and peculiar granular substances appear in the cell wall area, reminiscent of typical transfer cells.

After pollination, pollen tubes took 4–5 days to reach and fertilise the ovules. Zygotes surrounded by endosperm were present 6 days after fertilisation, while seeds required another six weeks for development before harvesting. The nuclear endosperm consists of numerous nuclei, lining the embryo sac. The mature seeds are endospermous. Endosperm cells are packed with aleurone-like, protein bodies and cell walls are perforated by numerous plasmodesmata (Figure 3D). At approximately 9 days after pollination, ovaries contained many shrivelled ovules. The ES of these ovules were normal but fertilisation apparently never occurred. In rare cases, ES with a disturbed polarity were found. In these ES nuclei clumped at the micropylar end and did not conform to the normal Polygonum type.

Cells of the outer integument divide anticlinally and periclinally, giving rise to the pigmented sarcotesta consisting of 8–9 layers of cells containing PAS positive starch grains (Figure 3B). The dark orange epidermal layer of the sarcotesta is more densely pigmented than the lighter subdermal layers. In SEM view, the outer epidermal surface of the sarcotesta is characterised by raised cell wall outlines (Figure 3C). Cells of the three-layered inner integument (tegmen) divide mostly anticlinally and become thicker walled to function as a mechanical layer in the seed coat. A well-developed vascular raphal bundle is embedded in the fleshy sarcotesta causing a distinct chalazal scar of the more resistant tegmen. A hilum can clearly be seen on the outer surface of the sarcotesta (Figure 4A). Removal of the sarcotesta shows the hilum situated next to the micropyle (Figure 4B). The orientation of the linear embryo towards the raphe of

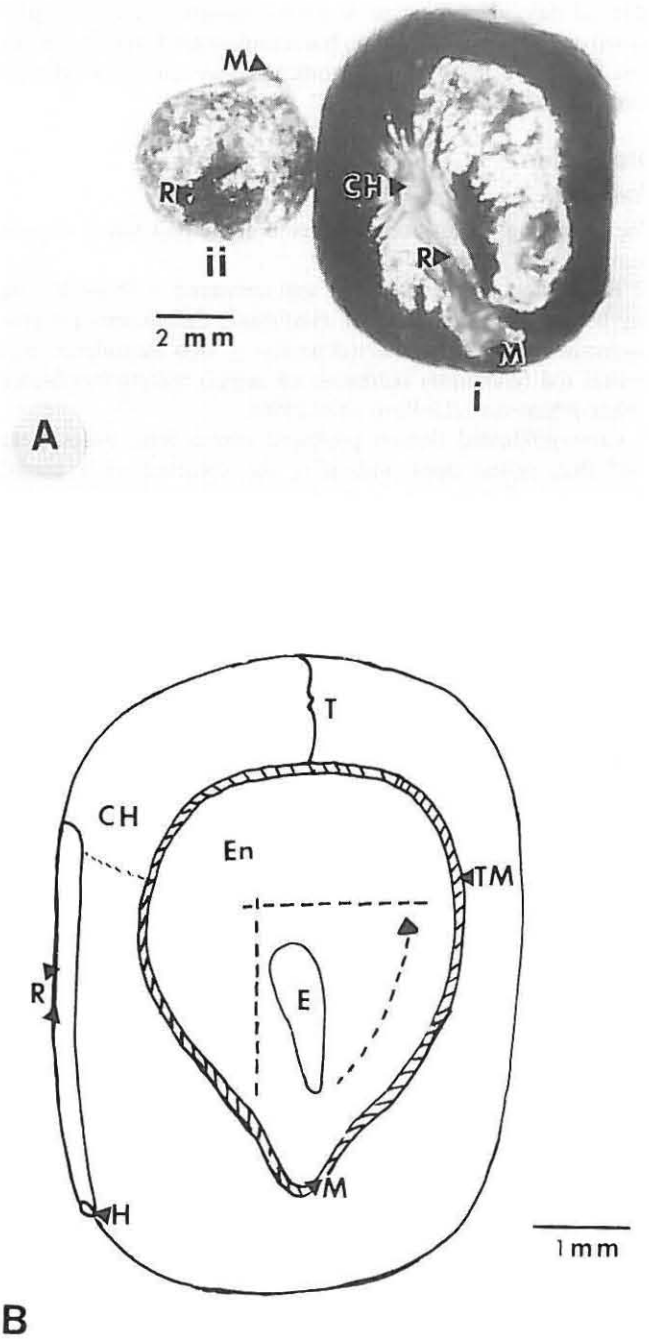

Figure 4 **A**. i. Raphal view of mature seed with the clearly visible chalaza (CH), raphe (R) and micropyle (M). ii. The seed after sarcotestal removal showing the micropyle (M) and raphe (R). **B**. Longitudional section of mature seed showing the different embryo positions. (H - hilum, R - raphe, T - testa, TM - tegmen, En - endosperm, M - micropyle, CH - chalaza). The arrow between the axis indicates possible embryo positions.

the seed varies from directly parallel (typically anatropous seed) to a position at right angles towards the raphe (Figure 4B).

Seed germination

Seeds with the sarcotesta intact never germinated and were severely contaminated by fungi. When the sarcotesta was removed, seed started germinating 13 days after imbibition at 20°C or 25°C, showing a germination percentage of 21.5% (±4.9). However, only 1% of the seeds germinated at 30°C and none at 35°C. (Figure 5). Germination was irregular in the entire temperature range, showing a maximum germination of 97% (±1.4) for seeds incubated at 20°C and 25°C after an incubation period of 31 days. At 30°C only 79% of the seeds germinated after 34 days. Germination at higher temperature (30°C) promoted primary root elongation but inhibited the formation of the first vegetative leaf. This phenomenon was not observed with seeds germinated at 20°C or 25°C.

Discussion

Pollination

The following trends were observed from pollination experiments:

1. The highest average seed set was measured in Phase II (one day before anther dehiscence), confirming that flowers are protogynous. Stigmas also reacted positively with auramine O and neutral red (chemicals indicative of stigma receptivity) before anther dehiscence. (Le Roux *et al* 1996).
2. Cross-pollinated flowers produced almost three times more seed than selfed ones, indicating the presence of a partial self-incompatibility system. Studies on pollen tube growth (Le Roux *et al.* 1996) revealed that pollen tubes of self-pollinated ovules occasionally penetrated and bundled in the nucellar cap at the micropyle followed by heavy callose deposition near the synergid. This is indicative of self-incompatibility.
3. Pollen aged for 48 hours gave the best seed set results. This observation is substantiated by *in vitro* pollen germination studies where pollen aged for 48 hours germinated best and also tolerated temperature extremes and different sucrose concentrations better than fresh pollen (Le Roux *et al.* 1994b). Longer viability of pollen could be an adaptation for enhancing cross pollination by natural pollinators.
4. It was clearly observed that proximal flowers in the inflorescence largely produced more seed per fruit than other flowers developing later in the season in the same inflorescence.

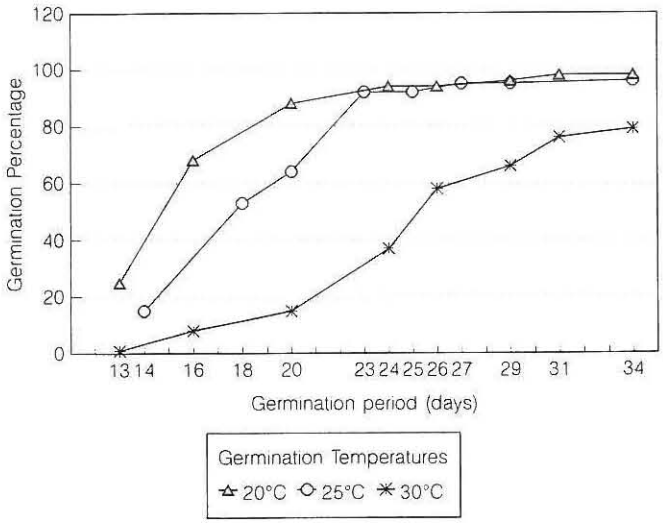

Figure 5 The effect of temperature on seed germination during an incubation period of 34 days.

This phenomenon could explain low average seed set per fruit. More experimental work will have to be done to determine if higher seed yields could be obtained by supplying additional nutrients and/or by decapitating inflorescence apices.

Even though seed does set after selfing, the *Gloriosa* stigma is situated sufficiently above the anthers to prevent self-pollination (see Figure 1A in Le Roux *et al.* 1994b). The perianth is bright red and yellow, colours that could be a further adaptation for cross-pollination by insects or birds. Narain (1976) suggested that these plants are wind and bee pollinated but our findings suggest that Lepidopterans (psycophily) with a wide wingspan or birds (ornithophily) would seem to be more efficient in pollinating flowers while utilising nectar. This is in accordance with Lindstrom's (1926) suggestions that only a large-sized butterfly or moth would be at all effective in reaching the stigma with any parts of its body or wings. The hypopeltate anther attachment displays the sticky, yellow pollen grains in such a way that it is an easy target for a suitable pollinator (Le Roux *et al.* 1994b). The probability for wind pollination in *Gloriosa* with flowers well adapted for insect and bird pollination and sticky pollen seems to be insignificant.

Ovule and seed structure

Simultaneous development of megaspores up to the binuclear stage occurred in *G. superba* (Afzelius 1918) and was also reported in *Androcymbium* sp. (Cave 1967), *Disporum* sp. (Sugihara *et al.* 1969) and *Iphigenia indica* (Sulbha 1954). According to Afzelius (1918) the fusion of polar nuclei of *Gloriosa* took place at an earlier stage which contradicts our finding that the polar nuclei of the 'ennobled type' were not yet fused just prior to fertilisation.

In *G. superba* a well developed hypostase with transfer cells connecting the vascular tissue with the ES, possibly plays a role in the transport of nutrients between the embryo sac and maternal tissue (Gunning & Pate 1969). The variation in the orientation of the linear embryo could be a result of a loosely implanted embryo in the early nuclear endosperm (Figure 4B).

Only a limited number of ovules developed into seed. Many ovules were not fertilised, which could be ascribed to the partial incompatibility system discussed earlier. In rare cases, disturbed polarity of the ES, as suggested by the aberrant distribution of ES nuclei probably also contributed to abortion of ovules. This phenomenon was also described in *Trillium camschatcense* where deviations in the number of nuclear divisions at the chalazal ends and delay in the number of nuclear divisions at the chalazal and micropylar end were noticed (Nauwmova 1978). The development of the female gametophyte in abnormal *G. superba* ovules needs to be reinvestigated.

The attractively coloured sarcotesta, developing from the cells of the outer-integument, is a clear adaptation for endo-zoochory. The thick walled tegmen most probably provides protection against the gut or crop of animals, most probably birds, responsible for seed dispersal. The seed is furthermore equipped with an aleurone-rich endosperm to ensure survival in unfavourable conditions.

Chandra and Tarar (1988) reported that seed germination of *G. superba* under laboratory conditions was very poor and treated seeds with Gamma rays, ethyl methane sulphonate and diethyl sulphate. A maximum seed germination of 29.5% was obtained after Gamma radiation, while treatment with other mutagens was less successful. Evidence is supplied in this paper that by removing the sarcotesta a higher germination percentage (97%) can be obtained. The sarcotesta of the mature seed still contains starch and other nutritional substances and removal of the sarcotesta could limit contamination by micro-organisms. The desiccated (untreated air dry) seeds have a dormancy period of about 4

months (Le Roux & Robbertse 1994a), germinate best after ± 13 days at 20°C or 25°C and are sensitive to higher temperatures. The difference in timing of germination could be ascribed to physiological diversity in seed produced by the same plant. For example, some seed coats could be more impervious to water or O_2 (Ting 1982), thus causing a delay in germination. In spite of irregular germination patterns, a very high percentage of seed germinated after a period of 34 days in this study.

Except for rare cases of disturbed polarity in the ES, accompanied by an aberrant distribution of ES nuclei, ovule and seed ontogeny are normal and mostly do not contribute to low seed set. Although there were no dominating factors affecting seed production, a combination of manipulatory procedures did increase seed yield: seed production improved after cross pollination and also when 48 hour-old pollen was applied to the proximal flowers in the inflorescence during the second flower phase. We also found that terminal flowers on the inflorescence usually did not set fruit and if these outer fruit did set, only a few seeds were produced. More experimental work is essential to determine if higher seed yields could be obtained by supplying additional nutrients and/or by decapitating inflorescence apices.

References

AFZELIUS, K. 1918. Zur Entwicklungsgeschichte der Gattung *Gloriosa superba*. *Acta. Hortic. Bergiani* 6: 3–12.

CAVE, M.S. 1967. The megagametophyte of Androcymbium. *Phytomorph.* 17: 233–239.

CHANDRA, N. & TARAR, J.L. 1988. Effect of mutagens on seed germination in *Gloriosa superba* L *Indian J.Bot.* 11(1): 11–16.

DALHGREN, R.T.M. & CLIFFORD, H.T. 1982. The monocotyledons: a comparative study. Academic press, London.

DVORACOVA, S., SEDEMERA, P., POTESILOVA, H., SANTAVY, F. & SIMANEK, V. 1984. Alkaloids of *Gloriosa superba* L. *Collect. Czech. Chem* 49(6): 1536–1542.

GUNNING, B.E.S. & PATE, J.S. 1969. 'Transfer cells' - plant cells with wall ingrowths, specialised in relation to short distance transport of solutes: Their occurrence, structure and development. *Protoplasma* 68: 107–133.

HERR, J.M. 1971. A new clearing squash technique for the study of ovule development in Angiosperms. *Amer. J. Bot.* 58: 785–790.

HUTCHING, A. & TERBLANCHE, S.E. 1989. Observations on the use of some known and suspected toxic Lilliflorae in Zulu and Xhosa medicine. *S. Afr. Med . J.* 75:62–69.

JOHANSEN, D.A. 1940. Plant microtechnique. New York. Mcgraw-Hill.

LE ROUX, L.G., ROBBERTSE, P.J. 1994a. Tuber ontogeny, morphology and vegetative reproduction of *Gloriosa superba* L. 1994. *S. Afr. J. Bot.* 60(6): 321–324.

LE ROUX, L.G., ROBBERTSE, P.J. & COETZER, L.A. 1994b. Studies on the anthers and pollen of *Gloriosa superba* L. *J. S. Afr. Hort. Sci.* 4(2): 27–32.

LE ROUX, L.G., ROBBERTSE, P.J. & VAN DER MERWE, C.F. 1996. The transmitting tract in *Gloriosa superba* L. Ultrastructure, pistil exudate and pollen tube growth. *S. Afr. J. Bot.* 62(4): 204–208.

LINDSTROM, E.W. 1926. An unusual adaptation for cross-pollination. *J. Herid.* 17: 232–234.

MUELLER, W.C. & GREENWOOD, A.D. 1978. The ultrastructure of phenolic-storing cells fixed with caffeine. *J. Exper. Bot.* 29(110): 757–764.

NARAIN, P. 1976. Studies on the pollination mechanism and breeding system of *Gloriosa*. *Ind. J. Hortic.* 33(2): 194–199.

NARAIN, P. 1977. Morphological studies on some species and cultivars of *Gloriosa*. *New. Bot.* 4 (14): 75–84.

NAUWMOVA, T. N. 1978. Peculiarity in macrogametogenesis and post-fertilisation development of *Trillium camschatcense* Kev-Gawl. *Bull. Soc. Bot. Fr.* 125: 183–186.

O' BRIEN, T.P. & McCULLY, M.E. 1981. The study of plant structure: principles and selected methods. Termacarphi, Melbourne.

SARIN, Y.K., JAMWAL, P.S., GUPTA, B.K. & ATAL, C.K. 1974. Colchicine from the seeds of *Gloriosa superba* L. *Cur. Sci.* 43:87.

SUGIHARA, Y., YOSHIMURA, H. & UMEZU, M. 1969. Embryo sac development in *Disporum*. *Phytomorph.* 19: 112–114.

SULBHA, K. 1954. Embryo sac of *Scilla*. *Curr. Sci.* 23: 89–90.

TILTON, V.R 1980a. The nucellar epidermis and micropyle of *Ornithogalum caudatum* (Liliaceae), with review of these structures in other taxa. *Can. J. Bot.* 58: 2059–2066.

TING, I.P. 1982. Plant Physiology. Addison-Wesley Publishing Company. Massachusetts, Menlo Park, California.

VAIKOS, N.P. & PAI, R.M. 1986. The floral anatomy of *Gloriosa superba* L. and *Tricyrtis pilosa* Wall. (Liliaceae). *Proc. Indian Acad Sci.* (Plant Sci.) 96(3): 233–239.

WEBERLING, F. 1992. Morphology and Inflorescences. Cambridge University Press. Cambridge. New York.