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EFFECT OF SUGAR CONCENTRATION ON POLLINIUM GERMINATION IN SOME MEMBERS OF ASCLEPIADACEAE

TAHIR ALI AND S.I.ALI

Department of Botany,
University of Karachi, Karachi-75270. Pakistan.

Abstract

Effect of sugar concentration on *in-vitro* pollinium germination in 8 members of Asclepiadaceae viz., *Calotropis procera* ssp. *hamiltonii*, *Caralluma edulis*, *Caralluma tuberculata*, *Ceropegia bulbosa*, *Glossonema varians*, *Leptadenia pyrotechnica*, *Pentatropis nivalis* and *Pergularia daemia* was studied. Although pollinia of each taxa germinated in a wide range of sucrose concentration (i.e. 5-40%) but each taxa showed a specific sucrose concentration at which optimal pollinium germination occurred. In *P. nivalis* and *Per. daemia*, coiling and beading of pollen tubes occurred at 20-40% sucrose concentrations while in other taxa at 5-15% concentrations. Optimal sucrose concentration required for *in-vitro* pollinium germination was similar to the average sugar concentration observed in the floral nectar of the studied taxa except in *P. nivalis* where maximum pollinium germination occurred at 10% sugar concentration as compared to 24% sugar concentration in its floral nectar.

Introduction

Pollen grains in the members of the subfamily Asclepiadoideae (Asclepiadaceae) are produced in paired packages known as "Pollinia". These pollinia are removed from the flowers by the pollinating insects. On a subsequent visit to another flower, the pollinating insect may insert the pollinium into a stigmatic chamber where it may remain and bring about pollination (Ali & Ali, 1989). The pollinium normally germinates in a solution of nectar, which is produced by the nectaries located within the stigmatic chamber with a wide range of sugar concentration (Eisikowitch, 1986; Wyatt & Shannon, 1986). The nectar in Asclepiads acts as the natural germinating medium and as a result the variation in its sugar concentration may also affect the pollinium germination. The present report describes the effect of sugar concentration on *in-vitro* pollinium germination in 8 members of the family Asclepiadaceae viz.,*Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali, *Caralluma edulis* (Edgew.) Benth., *Caralluma tuberculata* L., *Ceropegia bulbosa* Roxb., *Glossonema varians* (Stocks) Hook. f., *Leptadenia pyrotechnica* (Forssk.) Decne., *Pentatropis nivalis* (Gmel.) Field and Wood, and *Pergularia daemia* (Forssk.) Chiov.

Materials and Methods

Nectar Sugar Concentration: Mature floral buds (N=25; just prior to opening) were bagged (nylon-nested/mashed) and tagged in the morning (6-7 a.m.) and in the afternoon (5-6 p.m.) for diurnal and nocturnal species, respectively. The nectar was withdrawn with microcapillaries and its sugar concentration immediately assessed with

a hand-held refractometer (Bellingham & Stainly, U.K.), after removing the bags at the time of maximum insect activity for diurnal and at 6-7.30 p.m. for nocturnal taxa.

Pollinium Germination: Pollinium germination was carried out under laboratory conditions by the method of Khatoon & Ali (1983). Sucrose liquid medium was used in order to remain close to the natural conditions for germination since sucrose dominated nectar is a natural germinating medium for pollinium (Wyatt & Shannon, 1986). Sucrose solutions ranging from 5-40% w/v, mixed with 0.003% $Ca(NO_3)_2$, 0.002% $MgSO_4$, 0.001% KNO_3 and 0.001% boric acid were used.

Twenty pollinia (two/flower) from fresh flowers of each species were transferred to each concentration of sucrose solution at room temperature (30-35°C) in a moist chamber. Pollinia of nocturnal species i.e., *P. nivalis* and *Per. daemia* (Ali, 1994), were inoculated between 8-9 p.m., whereas, of diurnal species between 8-9 a.m. *G. varians* is andromonoecious (Ali & Ali, 1996) and the pollinia from both hermaphrodite (perfect) and male (staminate) flowers were inoculated separately in each sugar concentration. After 12 h of inoculation, the percentage of germinated pollinia was calculated. From each germinated pollinium the number of germinated pollen grains was assessed by counting the number of pollen tubes emerged from the pollinium and the length of the longest pollen tube was measured microscopically.

Results

Nectar Sugar Concentration: In *C. procera* ssp. *hamiltonii* sugar concentration in floral nectar ranged from 30-52% with an average of 37%. In *P. nivalis* and *Per. daemia* average nectar sugar concentration was 24% and 17% that ranged from 20-32% and 15.8-18%, respectively. Sugar concentration in rest of the studied species was not determined, due to very small amount of nectar production.

Pollinium Germination: In *C. procera* ssp. *hamiltonii*, 30% sugar concentration was found to be optimal for pollinium germination with maximum number of germinated pollen grains/pollinium and pollen tube length with healthy and longest pollen tube (Fig. 1). In 5-20% sugar concentrations, pollinium germination started within an hour of treatment, whereas in higher sugar concentrations of 25-40% it was delayed upto 2nd hour. At 5% and 30% sugar concentrations, 100% pollinia germinated. At low sugar concentrations of 5-15%, the pollen tubes showed coiling and became beaded, whereas, at higher concentrations of 20-40%, it was normal and at 25% and 30% concentrations healthy and straight pollen tubes were observed.

In hermaphrodite (perfect) flowers of *G. varians*, 15% sugar concentration showed maximum pollinium germination and 20% concentration in male (staminate) flowers (Fig. 1). Similar to *C. procera* ssp. *hamiltonii*, pollinium germination started earlier in lower sugar concentrations with malformation of the pollen tubes.

In *P. nivalis*, best pollinium germination and pollen tube growth was observed at 10% sugar concentration (Fig. 1). Similar to *C. procera* ssp. *hamiltonii*, germination started earlier at lower concentrations as compared to higher ones. All the pollinia germinated at 5, 10 and 25% sugar concentrations, 60% at 15 and 40% concentrations and 80% pollinia at 20 and 30% concentrations. At 5 and 10% sugar concentrations normal germination was observed with coiling and bead formation at 15-40% concen-

trations.

In *Per. daemia* best pollinium germination and pollen tube growth was observed at 15% sugar concentration (Fig.1), with 100% pollinia germination at all the sugar concentrations. Normal pollen tubes were produced from 5-15% sugar concentrations while malformation was observed at 20-40% concentrations.

In *Car. edulis*, *Car. tuberculata* and *Cer. bulbosa* best pollen germination was observed at 20 and 25% sugar concentrations, respectively (Fig. 1). In all the three species 100% pollinia germinated in all the sugar concentrations with slight malformation of pollen tubes at lower concentrations.

In *L. pyrotechnica* best pollinium germination and pollen tube growth was observed at 25% sugar concentration (Fig. 1), with 100% pollinia germination in all the sugar concentrations. Pollinium germination from 5-15% sugar concentrations started within an hour of inoculation with malformation of pollen tubes while at 20-40% concentrations germination was delayed with normal pollen tube growth.

Discussion

Generally, Bee-pollinated plants produce concentrated nectar with an average of about 37% sugar concentration (Pyke & Waser,1981). Thus, the nectar characteristic of *C. procera* appears to match the preference of Bees and provide additional support to the findings of Eisikowitch (1986) and Ali & Ali (1989) that *C. procera* is predominantly pollinated by large Bees i.e., Carpenter Bees. Ali (1994) reported that although *P. nivalis* and *Per. daemia* are nocturnal taxa but both of them are pollinated in the evening by Bees and at night by Moths. The nectar sugar concentration of *Per. daemia* was found to be within the range reported by Cruden *et al.,* (1983) and Percival (1965) for Moth pollinated taxa, whereas, of *P. nivalis* falls within the range reported by Cruden *et al.,* (1983) for small Bee - small Moth and Hawkmoth pollinated taxa . Other studied taxa produced small quantity of nectar and are pollinated by Bees, Wasps, Flies and Beetles (Ali, 1994). The optimal sugar concentration required for *in-vitro* pollinium germination of *C. procera* and *Per. daemia* was more or less similar to the average sugar concentration of their floral nectar. Our results are thus, in conformity with Shannon & Wyatt (1986) and Eisikowitch *et al.,* (1990). However, in *P. nivalis* best pollinium germination was observed at 10% sugar concentration while an average of 24% sugar concentration was found in its floral nectar. One of the plausible reason may be that several plants actively regulate their nectar concentration to accommodate the needs of different pollinators (Stephenson & Thomas, 1979; Willson & Bertin, 1979). Thus, it is possible that similar phenomenon may be operating in *P. nivalis*, in which the nectar concentration produced in the evening matches with the Bee preferences and at night it becomes low due to nectar secretion to favour both Moth-pollination and optimal pollinium germination. Thus, the role of nectar in providing "time niches" for different pollinators, remains to be tested in *P. nivalis*.

Randhawa & Iyer (1964) stated that near similarity in the osmotic concentration of the nutrient medium and that of the pollen is a pre-requisite for germination and the percentage of germination and pollen tube length are directly proportional to osmotic concentration, while bursting is inversely proportional. Besides, the pollen tube abnor-

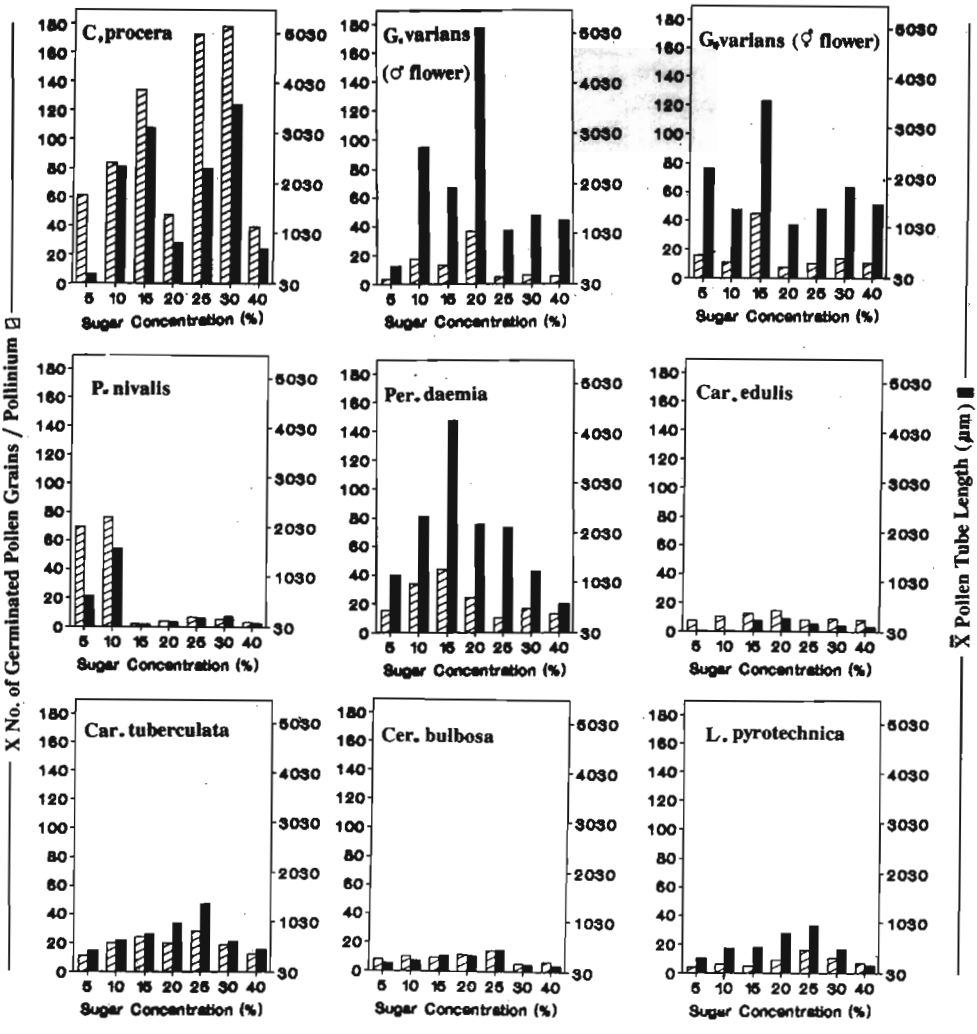

Fig.1. *In vitro* pollinium germination (average number of germinated pollen grains/pollinium and pollen tube length) at different sucrose concentrations.

mality and even bursting in low sugar concentrations (5-15%) in *C. procera, Car. edulis, Car. tuberculata, Cer. bulbosa, G. varians* and *L. pyrotechnica* can also be explained in the view that floral nectar of all these taxa is characterized by high sugar concentration in response to their high sugar concentration demanding pollinators (i.e., Bees, Wasps, Flies and Beetles). Thus, their pollinia seem to be also adapted to germinate in high sugar concentrations rather than low ones that seldom occur in their nectar. Similarly, nectar of *P. nivalis* and *Per. daemia* is dominated by low sugar concentration in response to their low sugar concentration demanding pollinators (i.e., Moths), thus, their pollinia which are adapted to low concentration do not germinate well in

higher sugar concentrations. There is thus need to study the nectar production (i.e., pattern, volume, concentration etc.,) in response to pollinators in these taxa.

It would suggest that sugar concentration greatly affect the *in-vitro* pollinium germination. Although, pollinia of all the 8 taxa studied can germinate in a wide range of sugar concentration but each species requires a specific sugar concentration for optimal pollinium germination.

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