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Variation in the Longevity of Pollen Grains in some of Poppy Species

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Abstract: Initial investigations have revealed the existence of 9 species from this genus in the Ardebil province of Iran. Plants of this genus have a rather vast spread in the province and the lack of prior investigations with regard to the longevity of their pollen grains has prompted this study. In this research, initially, the plant species were identified and their growth areas in the Province were determined. Subsequently, the longevity of pollen grains from each species was determined with the use of Tetrazolium Bromide solution. The number of live and dead pollen grains in a count of 500 were determined at one hour intervals. The grain count was continued until the percentage of live grains was dropped to a zero value. With regard to each species, the type of correlation between the percentage of pollen grain longevity and time was determined. Using the calculated equations, it was hence possible to predict, with a minimum error, pollen grain longevity for different time intervals. The results of this research has revealed that the highest live pollen percentage and longevity belongs to *Papaver oriental* L. *var. parviflora Bush.* (99.96% and 70 h) and the lowest value belong to *Papaver gaubae Cullen* and *Rech.f.* (zero percent and zero h). In addition it was revealed that a close relationship exist between the environmental factors and the longevity of pollen grains. This type of relationship also exists among populations within each species. In other words, pollen grain longevity in various populations is variously under the effect of environmental factors in each species.

Key words: Poppy, papaver species, pollen viability

INTRODUCTION

The province of Ardebil is located in the North West of Iran with a geographic specification of from 37° 45'-39° 42' North Latitude and from 47° 2'-48° 55' East Longitude. This province with an area of 17953 km^2 comprises 1.09% of the country's total area (Azimi-Motem, 2001). A difference in altitude of about 4800 m exists in the province between the lowest point with an altitude of 20 m in the banks of the Araxes river and the highest point at the top of Sabalan mountain with an altitude of 4811 m. This region is exposed to 5 climatic fronts during summer and 6 climatic fronts during winter months (Talaie *et al*., 2005). The existing climatic and ecological variation has resulted in the growth and establishment of various plant species including a number of poppy species in the region.

The genus *papaver* belongs to class of Dialypetales, 0rder of Parietales, Suborder of Rhoedales and the Family of Papaveraceae. This genus consists of about 100 species in the world of which 40 species can be found in Iran. The species of this genus are herbaceous and among its major characteristics is the existence of a capsule in the form of a sphere, a club, egg shaped, or in the form of an inverted pyramid and occasionally in cylindrical shape. The capsule has a number of small pores at its top which are formed beneath the stigma (Ghahreman, 2006). There is a need to study poppy plants from various viewpoints in the light of its importance in the pharmaceutical industry. One important aspect is the longevity of their pollen grains. By determining the variation in the longevity of pollen grains in the course of time it would be possible to compare the ability of pollen grains to fertilize the ovule in different species. In addition it would be possible to determine the length of time for the viability of pollen grains in each species.

Our search of the literature has indicated that with regard to the use of above mentioned methods for the study of various species in the *Papaver* genus, not much data is available. Cohen *et al.* (1989) have studied pollen longevity, pollen tube growth, germination and storage in papaya and have concluded that there is a positive correlation between pollen viability and percent germination. Carapetian (1994) has studied the viability of

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pollen grains in safflower (*Carthamus tinctorius* L.) using Tetrazolium Bromide and has concluded that pollen viability in this species is over 60%. Ettore *et al*. (1997) has determined the maximum longevity of pollen grains in six species of angiosperms having two different modes of pollination to be 72 h.

MATERIALS AND METHODS

This research has been carried out in 3 phases as follows:

Field study: Various regions of *papaver* growth have been identified in the province and samples for herbarium has been collected and identified. In this phase of research, information such as collecting site, collecting date, elevation from sea level and geographic location has also been recorded.

Laboratory investigations: For the preparation of tetrazolium bromide solution, 2.65 g gelatin was dissolved in 50 mL hot distilled water and 28.5 g sucrose was added. While the solution is still hot, 45 mg tetrazolium powder was gradually added and stirred (Carapetian, 1994). Anthers were obtained from each species before their opening such that pollen grains were not contaminated. Each anther was carefully opened and pollen grains were transferred onto a glass slide and one drop of tetrazolium bromide solution was added. One hour later, a total of 500 grains were scored as viable (dark purple) or inviable (colorless) (Fig. 1). This scoring process was repeated in time intervals both in the field and laboratory until the percentage of live pollen grains was diminished to one or zero percent. Slides were photographed under the microscope as necessary.

Data analysis: The collected data was stored in computer files and was subsequently analyzed with Excel. For each species, percent pollen viability was scored against time in hours. Regression analysis was carried out to obtain the regression line for pollen grain viability. Various statistical methods are used for the study of relationship between variables in which the dependent variable is estimated by the use of one or more independent variables. In the regression method, large volume of data take part in the calculations such that the error factor is diminished (Rezaie, 2004). In this study, in order to determine the relationship between percent viability of pollen grains in the course of time, the regression method with two variables was used. In this method, the values of one dependent variable (Y' = percent pollen viability) is

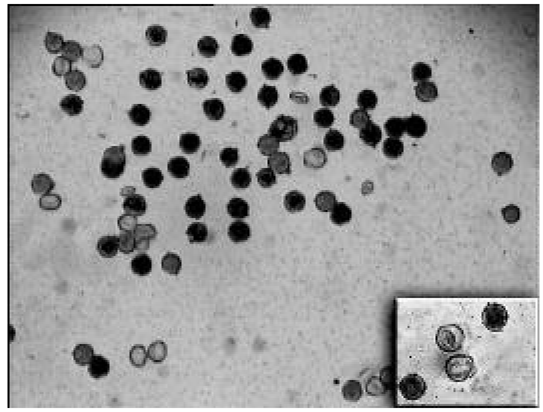

Fig. 1: Photograph showing viable (dark color) and inviable (colorless) pollen grains of Papaver faugus. Scale is 110 um

estimated through the values of the independent variable (X_i = time period) using the following formula.

$$Y' = b_0 (X_i) + b_1 \quad (1)$$

In the above formula, Y' is the estimation of Y, b_0 is the slope (regression coefficient) and b_1 is the Y intercept (regression constant). However, the relationship between X_i as an independent variable (time period) and Y as the dependent variable (percent viability) may not be linear as the above formula explains. This relationship may be logarithmic, exponential, polynomial etc. It is then often more satisfactory to work with nonlinear relationships between X_i and Y. The simplest curve to use as a regression model if a straight line will not suffice is a parabola. Its equation can be written in the form:

$$Y' = b_0 (X_i^2) + b_1 (X_i) + b_2 \quad (2)$$

These methods can be extended to polynomial curves of higher degree; also the logarithmic equation of the reqression is:

$$Y' = b_0 \, Ln(X_i) + b_1 \quad (3)$$

In this study, a major purpose of the statistical analysis has been to find out the best relationship between the two variables as it would predict the percent pollen viability in each species with a minimum error. The criterion for the best fit of the regression line is that the X and Y values have the minimal distance from the regression line. The utilized computer software such as

Spss and Excel have performed the needed calculations for adjusted R squares and have selected the best regression lines for pollen grain viability in each species under investigation.

RESULTS

Field studies have revealed that plants from nine species of the Papaver genus can be found in Ardebil province. Repeated counting and calculations of percent pollen viability in the course of time has made it possible to draw the correlation diagrams (Fig. 2). The different type of variation in pollen viability indicates the existing differences in the rate of downfall in percent viability in the course of time in various species. Based on these differences, the formulas used for the calculations of correlations is also different. This relationship is polynomial in the 5 species of *P. oriental* L., *P. argemone* L., *P. rhoeas* L., *P. fugax var. fugax Poir.* and *P. fugax var. platydiscus Poir.*, Fig. 2a, straight line relationship in the 3 species of *P. arenarium* L., *P. glaucum Boiss* and *P. lasiotrix*. Figure 2b and it is logarithmic in *P. dubium*. Fig. 2c and Table 1.

P. oriental has the highest percent of initial pollen viability and in the course of time, it shows the best persistence among the species under study and it grows in the humid environments of mountainous Sabalan (south and southwest of Ardebil city). On the other hand, *P. gaubae* having no live pollen grain grows in the southern parts of the province and in the banks of Ghzel Oozoon river which is located in the semi-dry to cold dry regions (Table 2).

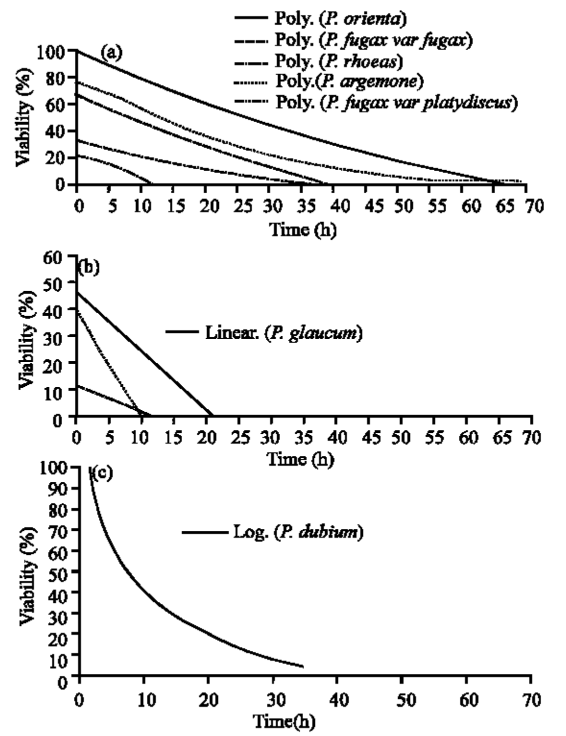

Fig. 2: Regression lines showing the percent pollen viability with passing time in various poppy species (a) polynomial, (b) linear and (c) logarithmic relationship

Table 1: Regression equation and coefficient of correlation between pollen grain viability and time in poppy species

Regression equation	Coefficient of correlation	Species name
$y' = 0.0092x^2 - 2.082x + 98.699$	$R^2 = 0.995$	*Papaver oriental* L.
$y' = -30.3 \text{ Ln}(x) + 111.0$	$R^2 = 0.951$	*Papaver dubium* L.
$y' = 0.019x^2 - 2.388x + 76.21$	$R^2 = 0.957$	*Papaver argemone* L.
$y' = 0.010x^2 - 2.098x + 66.16$	$R^2 = 0.971$	*Papaver fugax var fugax Poir.*
$y' = -2.171x + 45.33$	$R^2 = 0.973$	*Papaver glaucum Boiss.*
$y' = -3.914x + 39.28$	$R^2 = 0.859$	*Papaver arenarium M.B.*
$y' = 0.0125x^2 - 1.298x + 32.17$	$R^2 = 0.977$	*Papaver fugax var platydiscus Poir.*
$y' = -0.039x^2 - 1.190x + 21.49$	$R^2 = 0.963$	*Papaver rhoeas* L.
$y' = -0.974x + 10.79$	$R^2 = 0.978$	*Papaver lasiotrix Fedde*

Table 2: The maximum percent and duration of pollen grain viability and environmental conditions of *Papaver* species in the Ardebil province, Iran

Species name	Maximum pollen viability (%)	Maximum duration for viable pollen (h)	Elevation from sea level (m)	Environment based on corrected Demarten
L.*Papaver oriental*	99.96	70	2500-2800	Humid, extreme cold
Papaver lasiotrix Fedde.	95.63	46	990-1600	Dry cold
Papaver dubium L.	80.23	66	90-2000	Semi-dry, extreme cold, dry cold and Mediterranean extreme cold
Papaver argemone L.	61.43	42	1000-1150	Dry cold
Papaver fugax var fugax Poir.	46.42	20	2500-2620	Humid, extreme cold
Papaver glaucum Boiss..	40.3	12	900-953	Semi-dry extreme cold to dry cold
Papaver arenarium M.B.	34.71	40	1383	Mediterranean extreme cold
Papaver fugax var platydiscus Poir.	25.72	17	2100-2800	Mediterranean extreme cold to semi-dry extreme cold
Papaver rhoeas L.	11.3	7	1064	Semi-dry cold
Papaver gaubae Cullen and Rech.f..	0	0	948	Semi-dry cold to dry cold

DISCUSSION

Considering the genetic and environmental effects on the viability of pollen grains, it would be possible to evaluate and discuss in various aspects the similarities and differences observed among different species, subspecies and populations. One of these aspects is the relationship and closeness among species. Results of our investigation has indicated that despite the differences in the environmental conditions of *P. oriental* and *P. lasiotrix*, these two species are rather similar with respect to percent pollen viability and this similarity may be due to the close relationship among them in terms of phylogeny. Another aspect to be considered for pollen viability is the environmental factor. Grass pollen loses viability rapidly under natural conditions. Depending upon species, the duration of pollen viability can range from a few hours to one day (Barnabas, 1985). In this respect, Augustin and Mark (2005) also Parantainen and Pulkkinen (2002) have shown that with an increase in relative humidity and reduction in temperature, the percent pollen viability and longevity of pollen grains is increased. This observation is clearly seen among the populations within a single species. This type of changes in the percent pollen viability of *P. dubium* can be seen among the two climatic conditions of Moghan valley with a dry and cold climate having an elevation of less than 100 m on one hand and the slopes of Sabalan mountain with an elevation of more than 1000 m having a Mediterranean and extreme cold climate such that the difference among the pollen viability in these two climates is significant with a longer longevity in Sabalan as compared with Moghan. In addition, the observed difference in percent viability among the two subspecies of *P. fugax* in 2 different environments in spite of their close relationship is another proof that pollen viability is drastically affected by the environmental conditions. The percent pollen viability in *P. gaubae* has been observed to be zero and this may be due to the fact that the pollen surface is totally covered with sporopolinin and no pores could be observed on the pollen surface for germination. The longest pollen viability of 70 h belongs to *P. oriental*. The observed reduction in percent pollen viability has been slow in some of the species and fast in some other. In *P. lasiotrix* the initial pollen viability of 95.63% is quickly reduced to zero within 46 h whereas in *P. oriental* it takes 70 h for the viability of 99.96% to drop to a zero level. In some species e.g. *P.glaucum* having an initial pollen viability of 40.3% the reduction of viability is quicker than other species such as *P. arenarium* having a lower initial viability of 34.71%. This trend in pollen viability is probably due to the effects of environmental factors such as humidity and temperature. For example *P. glaucum* in the Moghan region is faced with semi-dry extreme cold to dry cold environment whereas *P. arenarium* grows around the city of Meshkin Shahr which is a mountainous and cold region. Luna *et al.* (2001) reported that corn pollen exposed to hot and dry field conditions (San Jose del Valle, Nayarit, Mexico) decreased to 20% viability in 1 h and was 100% nonviable within 2 h. Pollen viability also was reported to be greatly reduced by high temperatures (Johnson and Herrero, 1981). Schoper *et al.* (1986) showed that high temperature stress on the tassel at the time of anthesis cause a large reduction in pollen viability. However, pollen viability is not affected by plant water deficits (Hall *et al.*, 1982; Westgate and Boyer, 1986). There is general agreement among investigators that functional life of pollen is longer at relatively low temperatures and high RH (Johri and Vasil, 1961).

CONCLUSION

Therefore, the pronounced effect of varying environmental conditions on the percent and longevity of pollen viability should be accepted. In addition, it is important to allow priority for the investigations of pollen viability and longevity in each of the species with an exclusion of the environmental stresses.

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