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# CORRELATIONS OF THE QUALITY OF THE POLLEN GRAINS WITH THE TEMPORAL SEQUENCE OF POLLEN DISPERSION IN THE DIFFERENT PARTS OF THE INFLORESCENCES

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## Abstract

Free proline concents of the pollen grains of many species have been determined. The pollen grains are differently stained with isatin-reagent and can be divided into the group with "proline-type" pollen and that with "non-proline-type" pollen, respectively. Differences in quality of the pollen grains from different parts of the calathia, panicles and spikes were demonstrated. Better quality pollen were obtained by the first pollen dispersion of the inflorescences.

It was stated that the comparison of pollen collected only from identical pollination regions of the inflorescences can present characteristic genetic data on the selected varieties belonging to one species.

*Key words:* Proline in pollens, staining with isatin, families, genera, species, plant breeding.

## Introduction

It is already known that the proline content of the pollen of many plants is very high. It can be compared with leaves of plants subjected to water deficiency (DASHEK and HARWOOD 1974; BRITIKOV, 1975; AHOKAS, 1978; etc.). Physiological advantage of proline accumulation is known as well (ALARKON et al., 1978; KURSAKOV and RYZHKOV, 1980; PALEG and ASPINALL, 1981; TYANKOVA et al., 1982; PÁLFI and KÖVES, 1984; GULYÁS and PÁLFI,1986). It was demonstrated that mature pollen grains of many species contain excessively high quantity of proline while in material of some other species this compound can be demonstrated only in a very low concentration (PÁLFI and KÖVES, 1984; PÁLFI and MIHALIK, 1985; PÁLFI and GULYÁS, 1985).

In this work the sequence of pollen dispersal in some inflorescences was studied. It was also investigated whether accumulation of proline is correlated with the mode of pollination. Finally data were collected about connections between the evolution of the families and genera and the measure of proline accumulation.

## Materials and Methods

Flowers and pollen collected from the Botanical Garden of the University were fixed and dried at 90 °C.

Isatin-reagent stains the pollen grains with significantly different colours according to proline content (PÁLFI and KÖVES, 1984). The recipe of the reagent: 0.4 ml acetic acid and 0.2 g isatin are added to 20 ml acetone. 2—20 mg pollen grains are mixed with some drops of the reagent on a slide and the staining reaction is performed at 90 °C for 12 minutes. The pollen grains are then dispersed in a drop of paraffin oil and covered. Details of the procedure are published elsewhere (PÁLFI and KÖVES, 1984.). Five microscopic fields each containing 50—100 pollen grains were counted and the per cents of positive isatin reaciton were given as mean values. Proline concentration of pollen estracts was determined by the method of ASPINALL et al. (1973) in three repetitions. The average values were published.

## Results and Discussions

Staining-reaction was considered positive (demonstrating a very high proline content) when the pollen grains stained intensive blue, dark blue or black with the isatin reagent. On black and white photos the positively reacting pollen grains are black while negative reaction appears in different shades of grey (Plate 1.).

On Plate 1. the isatin-reaction of 12 species (belonging to 12 families) is seen. By most species black and grey pollen grains occur mixed. High proline content demonstrated by the isatin-reaction occurs in many species.

Maturing dispersion of pollen grains commences in special parts of the inflorescences. According to this sequence mature pollen of different quality can be collected (Table 1.).

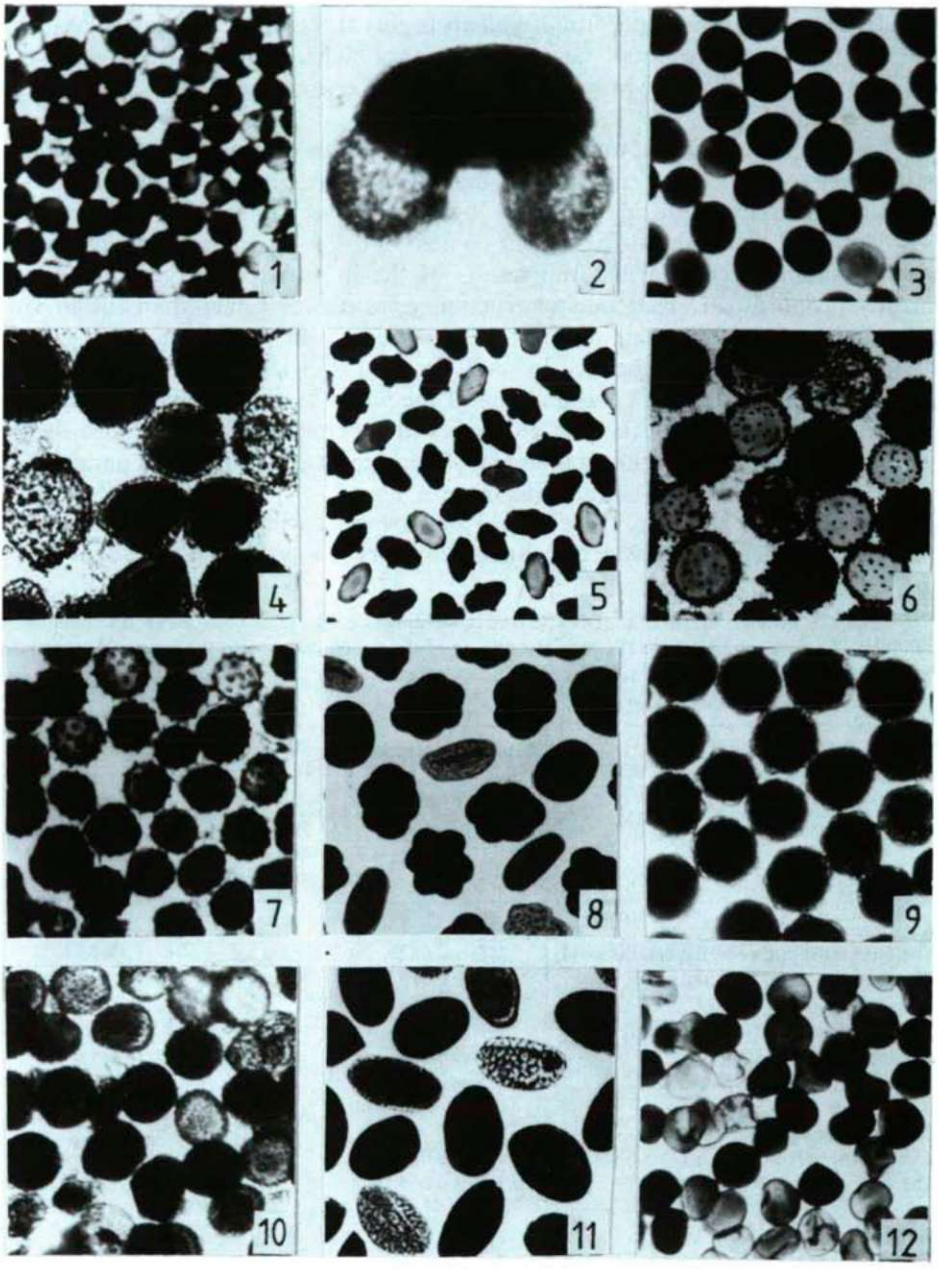
In the case of *Chrysanthemum leucanthemum* the dispersion of the pollens begins at the flowers of the outher circles of the calathium and this part of the inflorescence produces the best pollen quality. Dispersion of pollens advancing centripetally terminates within 3 or 4 days but from the central parts many non-vital grains occur.

Captions

*Plate I.* Mature and normally developed pollen grains stain black with the isatin-reagent. These are the "isatin positive" or "proline type" grains. The pollen grains stained grey contain very few proline, their maturity and quality is of low degree.

Magnification 200—800 x.

1 = *Ranunculus arvensis*; 2 = *Pinus nigra*; 3 = *Phaseolus vulgaris*; 4 = *Lonicera tatarica*; 5 = *Anthriscus caucalis*; 6 = *Hibiscus rosa-sinensis*; 7 = *Chrysanthemum horturum*; 8 = *Glechoma hederaceum*; 9 = *Opuntia vulg.*; 10 = *Iris germanica*; 11 = *Lilium longiflorum*; 12 = *Triticum aestivum*.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In *Sorgum bicolor* dispersion of pollens begins at the tip of the panicle branches and also in this case the initial parts give pollens of higher quality. After 4—5 days at the lowermost parts of the branches the pollen dispersion terminates, giving pollens of low quality.

In the spikes of wheat and of rye the dispersion of pollen begins at the middle part and here the best pollen quality is found. The dispersion of pollen advances to the basis and apex producing pollen of lower and lower quality.

Data of Table 1. show that proline content of the pollen extracts, the per cent of positive staining reaction and the results of the in vitro pollen germination are directly proportional. The staining reaction gives higher values than the in vitro germination. A positive staining reaction is caused by the proline quantity.

In Table 2. data can be seen from 29 species belonging to families of different stage of evolution. Proline concentration of the pollen extract is very high (1.0—2.3 per cent of dry matter) in the first 18 species (numbered with 1 to 18). Also the per cent positive staining reaction is high (44—85 per cent) and it increases paralell with the proline content of the extracts (as in table 1.).

In Table 2., however, there are 11 other species (numbered again beginning with 1) the pollen extract which contains only very few proline: 6 to 10 times fewer

*Table 1.* Proline content of the pollen extracts, staining of pollen grains with isatin, and in vitro germination of pollen grains. Correlations of the quality of the pollen grains with the temporal sequence of pollen dispersion in the different parts of the inflorescences.

| Names of the species and part of the inflorescence | Proline content of pollen extracts; % of dry matter | Percentage | |
|---|---|---|---|
| | | of pollen grains stained black (isatin positiv) | of in vitro germination of pollen grains |
| *Chrysanthemum leucanthemum* | | | |
| The outermost circles of the calathium (1) | 1.71 ± 0.082 | 68 ± 5.4 | 64 ± 6.0 |
| The central part of the calathium | 1.27 ± 0.058 | 41 ± 4.0 | 36 ± 3.7 |
| *Sorghum bicolor* | | | |
| The top of the panicle (1) | 2.15 ± 0.102 | 88 ± 7.1 | 78 ± 6.8 |
| The lower part of the panicle (2) | 1.58 ± 0.073 | 57 ± 4.5 | 51 ± 4.6 |
| *Triticum vulgare* | | | |
| The central part of the ear (1) | 1.73 ± 0.076 | 76 ± 5.2 | 72 ± 6.5 |
| The external part of the ear (2) | 1.57 ± 0.065 | 55 ± 4.3 | 53 ± 5.0 |
| *Secale cereale* | | | |
| The central part of the ear (1) | 1.70 ± 0.081 | 67 ± 5.8 | 64 ± 5.9 |
| The external part of the ear (2) | 1.19 ± 0.053 | 38 ± 2.9 | 35 ± 3.1 |

(1) = Beginning of the pollination;
(2) = Termination of the pollination.

than in the former group. The staining reaction in these 11 species is negative due to the low proline content.

On the basis of this and previous works (PÁLFI and KÖVES, 1984; PÁLFI and GULYÁS 1985; GULYÁS and PÁLFI, 1986) flowering plants were classified into two types: 1. to the "proline type" belong species in which the proline content of the

*Table* 2. Due to their extremely high proline content the pollen grains stain black with the isatin reagent (species 1—18); these are the "proline type" species. In the other species mature pollens due to their very low proline content no one grain stains black; these are the "non-proline-type" species (species 1—11).

| Families | Species | Proline content of pollen extracts; of dry matter % | Pollen grains stained black (isatin positive) % |
|---|---|---|---|
| *Ranunculaceae* | 1. *Paeonia arborea* | 1.28 ± 0.062 | 45 ± 4.1 |
| *Ranunculaceae* | 2. *P. officinalis* | 1.43 + 0.069 | 53 + 5.2 |
| *Fabaceae* | 3. *Lathyrus tuberosus* | 1.56 ± 0.071 | 59 ± 5.5 |
| *Fabaceae* | 4. *L. sativus* | 1.34 ± 0.064 | 50 ± 4.6 |
| *Cucurbitaceae* | 5. *Cucumis sativus* | 1.39 ± 0.060 | 53 ± 5.0 |
| *Solanaceae* | 6. *Solanum luteum* | 1.82 ± 0.083 | 80 ± 7.7 |
| *Solanaceae* | 7. *S. dulcamara* | 1.77 ± 0.081 | 78 ± 7.1 |
| *Solanaceae* | 8. *S. nigrum* | 1.66 ± 0.075 | 65 ± 5.8 |
| *Compositae* | 9.*Senecio vernalis* | 1.49 ± 0.064 | 57 ± 5.1 |
| *Compositae* | 10. *S. vulgaris* | 1.67 ± 0.078 | 68 ± 5.7 |
| *Fagaceae* | 11. *Qercus robur* | 1.26 ± 0.052 | 44 ± 4.2 |
| *Iridaceae* | 12. *Iris germanica* | 1.38 ± 0.062 | 52 ± 4.5 |
| *Iridaceae* | 13. *I. pumila* | 1.25 ± 0.053 | 44 ± 4.0 |
| *Liliaceae* | 14. *Colchicum autumnale* | 2.30 ± 0.110 | 85 ± 7.6 |
| *Graminae* | 15. *Lolium perenne* | 1.46 ± 0.065 | 54 ± 4.3 |
| *Graminae* | 16. *L. multiflorum* | 1.68 ± 0.080 | 67 ± 6.2 |
| *Graminae* | 17. *Glyceria maxima* | 1.50 ± 0.068 | 58 ± 5.2 |
| *Graminae* | 18. *Hordeum vulgare* | 1.91 ± 0.093 | 84 ± 7.3 |
| *Compositae* | 1. *Helianthus annuus* | 0.21 ± 0.010 | — |
| *Polygonaceae* | 2. *Rumex undulatus* | 0.22 ± 0.011 | — |
| *Polygonaceae* | 3. *R. crispus* | 0.20 ± 0.009 | — |
| *Cucurbitaceae* | 4. *Cucurbita maxima* | 0.18 ± 0.009 | — |
| *Cucurbitaeae* | 5. *C. pepo* | 0.21 ± 0.009 | — |
| *Liliaceae* | 6. *Tulipa gesneriana* | 0.20 ± 0.009 | — |
| *Liliaceae* | 7. *T. germanica* | 0.22 ± 0.010 | — |
| *Gramineae* | 8. *Dactylis glomerata* | 0.16 ± 0.007 | — |
| *Gramineae* | 9. *Festuca falcata* | 0.20 ± 0.009 | — |
| *Gramineae* | 10. *F. pratensis* | 0.21 ± 0.008 | — |
| *Gramineae* | 11. *Agropyron repens* | 0.22 ± 0.010 | — |

mature pollen is higher than 1.0 per cent of dry matter. 2. The mature pollen of the "non-proline-type" species contain less than 1.0 per cent proline. In 4 families (*Cucurbitaceae, Compositae,Liliaceae* and *Gramineae*) both the proline-type and non-proline-type species occur. The proline type therefore is not characteristic of a family. All species belonging to the same genus are either of proline-type or of non-proline-type (1. and. 2. *Paeonia*; 3. and 4, *Lathyrus*; 6., 7. and 8. *Solanum*; etc. as well as 2. and 3. *Rumex*; 4. and 5. *Cucurbita*; 6. and 7. *Tulipa*; 9. and 10 *Festuca*).

In Table 2. the families are arranged roughly according to their evolutionary progress. It can be seen that proline-type pollens occur in the families which are primitive (*Ranunculaceae*) moderately developed (*Solanaceae*), more advanced (*Fagaceae*) as well as in the most advanced monocotyledonous families. There is not relation between proline-type and phylogenetic development. In Table 2. entomophilous, anemophilous and autogameous species were compared: the proline-type is not correlated with the mode of pollination.

On examining the pollen quality of selected varieties belonging to one species the comparison of pollen grains collected only from the identical pollination regions of the inflorescences can give reliable genetic data. In all the three kinds of inflorescences studied best quality pollen grains were spread by the first pollen ripening region (calathium, panicle, spikes). Plant breeder experts may come to a conclusion that fertilization with pollen grains from the first pollination regions of the inflorescences ought to provide significant seed vigour, i.e. a considerable biological advantage. It is necessary to study the pollination sequence and quality of a number of plants to benefit from this advantage.

A plant belonging to the "proline-type" group may have a significant role in plant breeding if one takes into consideration that all the most important agricultural species selected long ago do belong to this group: e.g. wheat, maize, barley, rye, rice, potatoes, paprika, lucerne, clover and almost all fruit trees.

## References

AHOKAS, H. (1978): Cytoplasmic male sterility of barley.II. Physiology and anther cytology. — Hereditas. *89*, 7—21.

ALARKON, N.A., LARIONOVA, E.G., and MININA, T.K. (1978): Amino acid of pollen of *Pinus sibirica*. — Fiziol. Rastenij. *26*, 186—189.

ASPINALL, D., SINGH, T.N. and PALEG, L.G. (1973): Stress metabolism. V. Abcisic acid and nitrogen metabolism in barley and *Lolium temulentum* L. — Austral. J. biol. Sci. *26*, 319—327.

BRITIKOV, E.A. (1975): Biologicheskaya roly prolina. Izd. "Nauka" Moskow.

DASHEK, W.V. and HARWOOD, H.I. (1974): Proline, hydroxyproline and lily pollen tube elongation. — Ann. Bot. *38*, 947—959.

DASHEK, W.V. and MILLS, R.R. (1980): Azetidin-2-carboxylic acid and lily pollen tube elongation. — Ann. Bot. *45*, 1—12.

GULYÁS, S., and PÁLFI, G. (1986): Determination of pollen fertility in insect-pollinated plant species by a proline-isatin staining method.— Fiziol. Rastenij. *33*, 610—614.

KURSAKOV, A.G. and RYZHKOV, S.D. (1980): Soderjanie svobodnih aminokislot v raznoimenno zaryajennih frakciyah pilyci chernoj smorodini.— Fiziol. Rastenij. *27*, 735—739.

PALEG, L.G. and ASPINALL. D. (1981): The physiology and biochemistry of drought resisitance in plants.— Academic Press. Sydney, New York, London. 206—231.

PÁLFI, G. and GULYÁS, S. (1985): Complementary data to the viability test of pollens. — Növénytermelés (Hung. res. eng.) *34*, 351—358.

PÁLFI, G. and KÖVES, E. (1984): Determination of vitality of pollen on the basis of its amino acid content. — Biochem. Physiol. Pflanzen. *179*, 237—240.

PÁLFI, G. and MIHALIK, E. (1985): Proline staining as a new method for determining the vitality of pollen grains in wind and insect pollinated plants. — Acta Bot. Acad.Sci.Hung. *31*, 315—321.

TYANKOVA, L., TRIFONOV, A. and KUSMANOVA, R. (1985): A spectroscopic (ATR) approach to the behaviour of proline- and sucrose-treated biomembranes towards dehydratation and rewatering. — Biochem. Physiol. Pflanzen. *177*, 509—514.