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PROLINE-TYPE POLLENS AND THEIR VITALITY IN THE *ROSACEAE* AND THE SPECIES OF OTHER FAMILIES

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Abstract

In several plant species the free proline content of the vital pollens is between 1 and 2%. These are the proline-type pollens. The vitality percentage decreases to the same ratio as the ratio to which the proline content of the pollens is lower than 2.5%. The isatin reagent applied by us stains the pollen grains rather varying colours, depending on whether they possess sufficient proline content for the development of vitality, or not. Studies were performed on the pollens of 54 species belonging to 22 families of the *Angiospermae*. From these the pollens of 49 species were proline-typed, and the pollens of 5 species contained proline in an amount lower than 0.03%. The latter are the non-proline-type pollens. The 18 species of the Rosaceae family were all uniformly proline-typed. Our staining was positive in the case of 41 entomogame and 8 anemogame species, thus the mode of pollination does not influence the new determination of vitality. The isatin staining can only be applied in the case of proline-type species for the determination of the vitality of the pollens. This staining, however, needs to be studied separately for each species.

Key words: pollen, proline-isatin reaction, insect- and wind-pollination, angiospermal families.

Introduction

The water content of the pollen grains is quite low compared to other plant parts, according to species it varies between 20–40% of the fresh-matter (STANLEY and LINSKENS, 1974; BRITIKOV, 1975; ALARKON et al. 1978). At the same time, the water content of the mesophyte, the soft-stalked shoots and the leaves ranges from 80 to 95% of the fresh-matter. If the water amount in the leaves decreases to a level which is normal in the pollens, lethal water-insufficiency comes forth. It has been determined several times that in the case of the leaves of several soft-stalked plant species, the extremely high concentration of a specifically protein-amino-acid, the proline, is attained on the effect of the gradually developing water-deficit (ASPINALL et al. 1973; BATES et al. 1973; STEWART et al. 1977; TYMMS and GAFF, 1979; CHAUHAN et al. 1980; PÁLFI et al. 1983; 1984). Furthermore it has been proved that similarly to the leaves of plants suffering from water deficit, the proline concentration in the pollens of great number of plant species is also high (TUPY, 1963; DASHEK and HARWOOD, 1974; LINSKENS, 1974; DASHEK and MILLS, 1981; ZHANG and CROES, 1983). Considering their water and proline-contents, the pollens can therefore be regarded as cells showing water deficit. According to our opinion the degree of

vitality, germinative ability and fertility, resp., is directly proportional to the amount of accumulated proline in the species concentrating proline in their pollens. Several authors have published the physiological advantages of proline accumulation (ASPINALL et al. 1973; DASHEK and MILLS, 1981; TYANKOVA et al. 1982; ZHANG et al. 1982; ELTHON and STEWART, 1984). It has been determined that the high level of proline concentration increases the tolerance of water deficit in the plants and takes part in the protein synthesis following dehydration. According to ZHANG and CROES (1983) the proline accumulated in the pollens also promotes the protection against too high, or rather low temperatures.

KURSAKOV and RYZHKOV (1980) have determined that the proline functions in the development of the fertility of the pollens and its higher amount also significantly increases the rapidity of pollen germination as well as the elongation of the sac, respectively.

It has been demonstrated by TUPY (1963), LINSKENS (1974), MASCARENHAS (1975), AHOKAS (1978), DASHEK and MILLS (1981) and ZHANG and CROES (1983) that the proline of the pollen has important role also in the energetic transformations as well as in the interaction with the style. Besides, it is the effective activator of the Krebs-cycle and regulates the water balance and the normal functioning of certain enzymes as well. The authors have established that correlation can be found between the proline content of the pollens and the vitality as well as fertility, resp., in the case of numerous species of the flora.

PÁLFI et al. (1981), G. PÁLFI and ZS. PÁLFI (1982) have prepared amino acid extracts from the pollen masses of 18 kinds of inbred maize lines and have measured their proline content. According to their findings, the pollens belonging to that inbred line where the proline concentration is higher possess higher vitality. Even from the various rye species, the in vitro germinative percentage was higher for the species in which the proline concentration was also higher (PÁLFI and KÖVES, 1984). Authors determined that from the pollens of culture types belonging to the same species the vitality of those are of higher degree which species contain more proline. This fact has also been supported by the data of the in vitro germinations of the pollens on artificial, agar fostering soil, as well as of the proline concentrations in the amino acid extracts of the pollen masses.

Our new, rapid staining technique based on the proline content of the pollens has been described earlier, by which the approximate degree of vitality can be determined (ZS. PÁLFI and G. PÁLFI, 1982; PÁLFI and KÖVES, 1984). The isatin-reagent worked out experimentally by us stains the vital pollen grains dark blue or black. The non-staining pollens do not possess vitality. The aim of this paper was to study the vitality of the pollens of the *Rosaceae* family's most important fruit-bearing species on the basis of the proline content, using our isatin reagent. The proline concentration of the amino acid extracts prepared from the pollen masses was also determined. The isatinic vitality staining of a few wind-pollinated species was performed as well. Furthermore, it was also studied whether the vitality determination based on the proline content of the pollens can be adapted to every flowering plant species.

Materials and methods

The fruit-tree species belonging to the Rosaceae family and their certain cultivars, as well as the wind-pollinated species are listed in the Tables. The pollens in sacs were prepared in laboratory. The pollens were fixed and dried at 90 °C. The isatin staining indicating vitality can be performed with both living, or fixed pollens. The isatinic staining of the collected and dried pollens can be carried out either immediately or 1–2 years later.

The new composition of our isatin reagent is as follows: 0.6 ml of acidum aceticum is added to 20 ml of acetone in which 0.2 g of isatin is dissolved (stored in refrigerator it remains reactive for 3–4 weeks). Upon staining, 2–10 mg of pollen mass is placed on the slide and mixed well with 2 drops of isatin-reagent until the acetone evaporates. One-one drop of isatin is added twice more to the pollen mass, and the dissolvent is again evaporated by stirring. Then it is placed into the exsiccator heated up to 90 °C, where the stain is left to react for 12 minutes. Following this the preparation is taken out and left to cool. Then the slide is cleaned around the pollen mass with a slightly damp piece of cotton-wool. A drop of paraffine oil is dosed on the adhered pollens and the pollen grains are dispersed with a glass-stick. The pollens stained per grain to different colours are then covered by a glass cover and the colours are studied under microscope. Statistical evaluation is made of the yellow and light brown, as well as separately of the dark blue and black pollen grains, resp., so an approximate percental result can be obtained of the vitality degree. The colours of the preparations stained with isatin last differently according to species for 2–10 days, and even for 3 months in case of certain species (e.g. *Zea mays*).

The free proline content in the amino acid extracts prepared from the pollen masses (20–50 mg) was measured with the method of ASPINALL et al. (1973), as well as BATES et al. (1973).

Results and discussion

The vital pollen grains stain the following colours with isatin reagent, on the basis of the increasing order of their proline concentrations: greenish-blue, blue, dark blue, or black. The non-vital pollen grains keep their original yellow colour, or are stained light brown. In the black and white photographs, however, such colour deviations can not be seen — only the various shades of black and grey (Figs. of plate I. and II.). In the photos the black indicates the vital pollen grains and the shades of grey the non-vital ones.

The figures of Plate I. show the pollens of 6 species of the Rosaceae family following isatin staining. Owing to the insect circulation, however, there are also many pollens of foreign species. It can be determined that the majority of the pollen grains stained black, i.e. they are vital (PÁLFI and KÖVES, 1984). All studied species of the family are in general insect-, or self-pollinated. Regarding the figures of Plate I. the magnifications considerably differ from each other, nevertheless, the size and shape of the pollen grains of the various species are quite similar.

To clarify the proline accumulation and vitality degree, resp., of the pollens of the Rosaceae family, the pollens of a total of 18 species were collected. The obtained results can be seen on Table I.

According to the data of Table I. the amino acid extract of the total studied species of the *Rosaceae* family contains more than 1% proline. It is clear from the Table that the proline concentration in the pollen mass extracts varies in conformity with the percental result of the positive staining with isatin in the case of the various

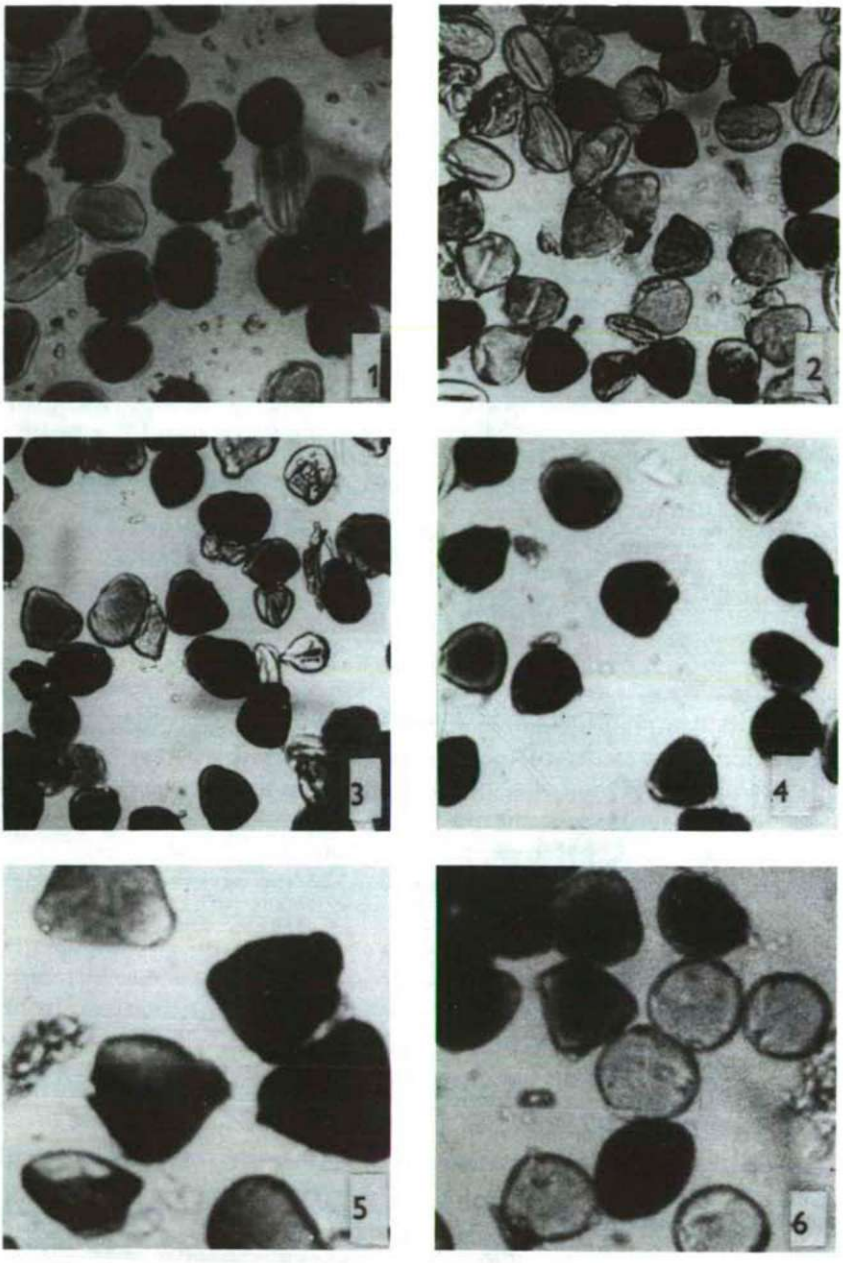

Plate I. The vital pollen grains stain black with isatin reagent. Insect-pollinated species — *Rosaceae* family. Magnification: 100-200 x

1 = *Rosa canina* L. ; 2 = *Pirus communis* L. cv. Keefer;
3 = *Malus pumila* L. cv. Jonathan; 4 = *Armeniaca vulgaris* LAM.
5 = *Persica vulgaris* MILL. cv. Madeline poujet; 6 = *Cerasus vulgaris* MILL. cv. Pándy.

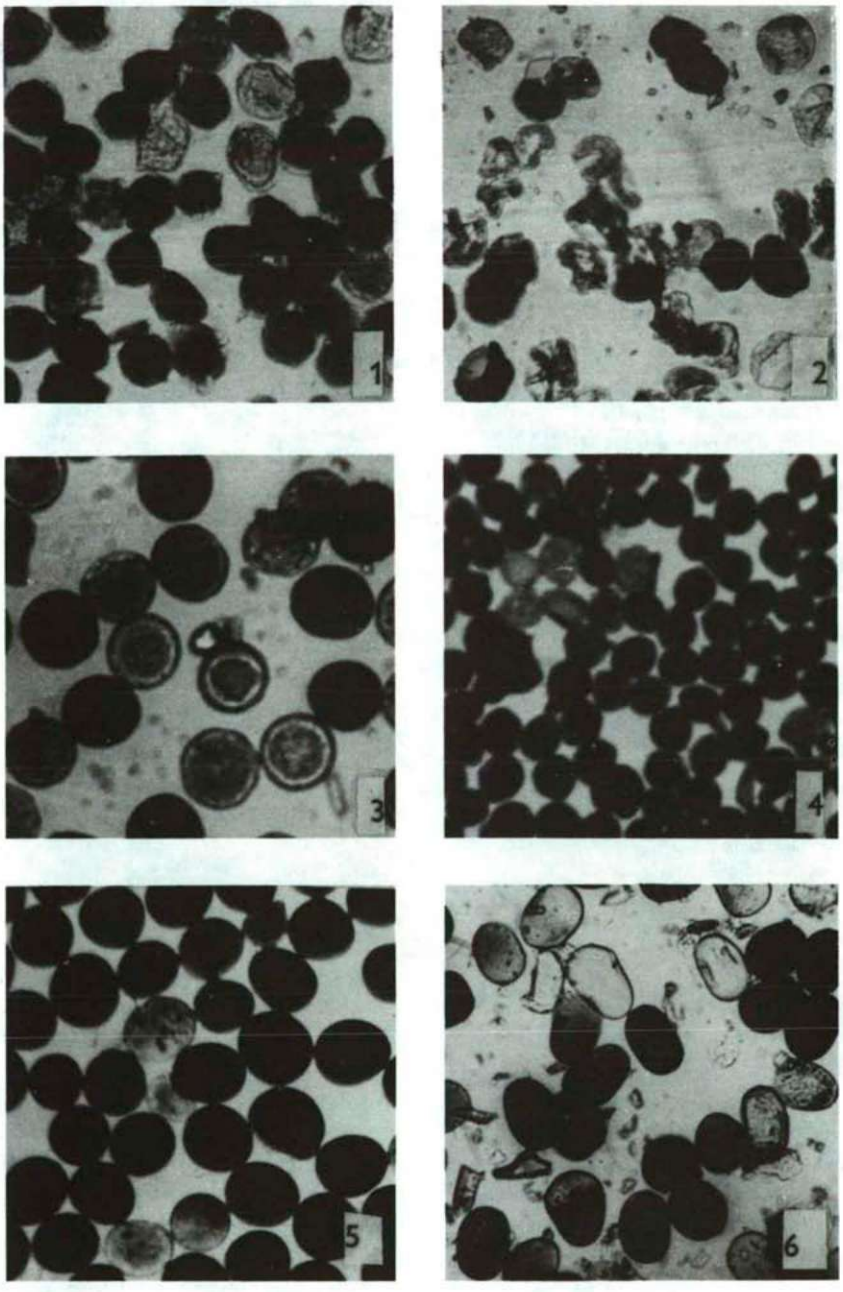

Plate II. Wind-pollinated species. The vital pollen grains are black. Magnification: 100 x

1 = *Fagaceae — Quercus robur* L.; 2 = *Salicaceae -Populus tremula* L.
3 = *Juglandaceae — Juglans regia* L.; 4 = *Betulaceae — Corylus avelana* L.
5 = *Gramineae — Zea mays* L.; 6 = *Secale cereale* L.

Table 1. Reaction to isatin staining of the pollens of 18 species belonging to the *Rosaceae* family. The pollen grains staining black and dark blue are isatin positive (vital). The proline concentration of the amino acid extracts prepared from the pollen masses is given in the percentage of the dry-matter.

Species	Proline concentration of extracts %	Positive staining with isatin (Vitality; %)
1. *Spiraea media* FR. SCHM.	1.84	81
2. *Exochorda korolkowii* LAVALL	1.66	73
3. *Cotoneaster horizontalis* DENCE	1.73	75
4. *Chaenomeles japonica* (THUNB) LINDL.	2.12	91
5. *Pyrus achras* GÄRTN.	1.18	46
6. *P. communis* cv. „Keefer"	1.26	50
7. *Malus pumila* cv. „Jonathan"	1.65	70
8. *M. floribunda* HOUTTE	1.47	57
9. *Crataegus oxyacantha* L.	1.63	72
10. *C. monogyna* JACQ.	1.61	70
11. *Pyracantha coccinea* ROEMER	1.36	53
12. *Rosa canina* L.	2.25	93
13. *R. polyantha* CARR cv. „Golden showers"	1.08	44
14. *Cerasus vulgaris* MILL. cv. „Pándy"	1.38	56
15. *Cerasus avium* MÖNCH. cv. „Germersdorfi"	1.46	58
16. *Armeniaca vulgaris* LAM. cv. „Mammut"	1.77	76
17. *Persica vulgaris* MILL. cv. „Madeline poujet"	1.45	53
18. *Prunus domestica* L. cv. „Olaszkék"	1.82	79

(Average deviation being below ± 5 per cent; n = 4 and 5)

species, as it has already been determined by others as well as ourselves (TUPY, 1963; STANLEY and LINSKENS, 1974; BRITIKOV, 1975; G. PÁLFI and ZS. PÁLFI, 1982; PÁLFI and KÖVES, 1984).

It was further studied to what extent this extremely great proline accumulation of the pollens has spreaded among the angiospermal, flowering plants. To partially answer this question the proline content and isatin staining of the pollens of further 23 species belonging to 16 families were investigated (Table 2).

It can be established from Table 2. that the proline concentration of all 23 species is between 1 and 2% (counted for dry matter). It can also be observed that the positive isatinic staining, i.e. the vitality percentage also changes in accordance with the proline concentrations of the amino acid extracts prepared from the pollen masses.

Table 2. Isatin positive staining (vitality %) of the pollens of 23 insect-pollinated species belonging to 16 families. Proline percentage(dry-matter).

Families	Species	Concentration of the proline; %	Positive staining (Vitality; %)
Paeoniaceae	1. *Paeonia officinalis* L.	1.43	56
Ranunculaceae	2. *Ranunculus acris* L.	1.75	76
Grossulariaceae	3. *Ribes rubrum* L.	1.18	45
	4. *R. aureum* PURSH.	1.26	51
Caesalpiniaceae	5. *Cercis siliquastrum* L.	1.55	60
Papilionaceae	6. *Caragana sophorae* LAM.	1.26	49
Hippocastanaceae	7. *Aesculus hippocastanum* L.	1.88	82
Caprifoliaceae	8. *Viburnum lantana* L.	1.34	53
	9. *Lonicera tatarica* L.	1.53	61
Malvaceae	10. *Lavatera thuringiaca* L.	1.42	56
	11. *Abutilon theophrasti* MEDIK	1.69	74
Euphorbiaceae	12. *Euphorbia cyparissias* L.	1.81	80
Labiatae	13. *Lamium purpureum* L.	1.06	43
Papaveraceae	14. *Chelidonium majus* L.	2.17	92
Cruciferae	15. *Lepidium draba* L.	1.24	46
	16. *Arabis procurrens* L.	1.63	71
Compositae	17. *Bellis perennis* L.	1.50	60
	18. *Senecio vernalis* W. et K.	1.68	73
Cactaceae	19. *Opuntia vulgaris* MILL.	2.06	88
	20. *Cereus peruvianus* L.	1.73	75
Primulaceae	21. *Primula veris* HUDS.	2.15	90
	22. *P. acaulis* L.	2.18	90
Iridaceae	23. *Iris germanica* L.	1.37	54

(Average deviation being below ± 5 per cent; n = 4 and 5)

The pollens of the 41 species belonging to the 17 families published so far are in general insect-pollinated ones. Now 6 figures of isatin staining are demonstrated prepared of the pollens of mainly wind-pollinated species (Figs. of Plate II).

The figures of Plate II. give evidence that the proline accumulation of the pollen grains (black grains) also has prevalence in the case of the wind-pollinated plants. It follows from this that the demonstration of the vitality of the pollen grains with the rapid isatinic technique can also be applied for the wind-pollinated species. Naturally, further study and results of several species are required for final decision.

The proline concentrations and positive isatin staining of the extracts prepared from the pollen masses were determined in the case of 8 wind-pollinated species belonging to 5 families (Table 3).

Table 3. shows that all proline concentrations in the studied 8 kinds of wind-pollinated species are between 1 and 2.5%, similarly to those of the insect-pollinated species. In accordance with the changes in the proline concentrat-

Table 3. Isatin positive staining and proline concentration of the pollens of wind-pollinated species. The 8 species belong to 5 families.

Families	Species	Concentration of the proline; %	Positive staining (Vitality; %)
Betulaceae	1. *Corylus avelana* L.	2.28	92
	2. *Betula pendula* ROTH	1.33	50
	3. *Alnus glutinosa* L.	1.27	46
Fagaceae	4. *Quercus robur* L.	1.81	80
Juglandaceae	5. *Juglans regia* L.	1.52	59
Salicaceae	6. *Populus tremula* L.	1.45	56
Gramineae	7. *Secale cereale* L. (cv.Lovászpatonai)	1.36	52
	8. *Zea mays* L. (Inbred linie V216)	2.34	94

(Average deviation being below ± 5 per cent; n = 4 and 5)

ions, the percental result of the positive isatin staining changes here, too. Thus, during the course of our experiments carried out so far, the isatinic rapid staining technique processed by us, indicating the vitality of the pollen grains, can also be applied in the case of the wind-pollinated species. The proline concentration of the vital pollens was above 1% here, too, therefore, according to our naming the pollens of these species are also „proline-typed". 5 species have been found among the pollens of the 54 species studied so far, which do not possess proline-typed pollens. Accordingly, not every pollen of the angiospermal species of the flowering plants is proline-typed (Table 4).

Table 4. Vital pollens of species in which the proline concentration of the amino acid extracts does not reach 1.0% of the dry-matter; non „proline-type pollens". The 5 kinds of insect-pollinated species belonging to 3 families produce non-proline-type pollens. The proline concentration of these is below 0.03%, therefore they do not stain wih isatin reagent.

Families	Species	Concentration of the proline; %	Positive staining (Vitality; %)
Begoniaceae	1. *Begonia semperflorens* LK. et OTTO	0.026	–
Cucurbitaceae	2. *Cucurbita pepo* L.	0.022	–
	3. *Cucurbita maxima* DUCH	0.025	–
	4. *Cucurbita moschata* DUCH	0.018	–
Compositae	5. *Helianthus annuus* L.	0.021	–

Table 4. comprises 5 such insect-pollinated species that have proline concentrations not reaching 1%, moreover, being below 0.03%. These are the real, „non-proline-type species". In the case of such low proline content, naturally the positive isatin staining of the pollen grains cannot be attained (although, as studied by us, the pollens possess a significant degree of vitality). It can be seen that in regard to the pollen, the non-proline-type species constitute only approximately 10% of the total species studied by us so far. Therefore, there are essentially more proline-type species in nature.

It has already been established by numerous authors (TUPY, 1963; CHUVASHINA and MELYNIKOV, 1964; STANLEY and LINSKENS, 1974; MASCARENHAS, 1975; AHOKAS, 1978) that the proline concentration of the most highly vital pollens falls to 2.5% of the dry matter (if the species is proline typed).

It can unambiguously be determined from Tables 1–3. that the percental ratio of the positively stained pollens decreases to the same degree as the proline concentration is less than 2.5%. Namely, in the case of plant species or types where the total pollen grains are vital, the proline concentration reaches the value of 2.5%.

Considering that the protein and free amino acid composition of the pollens of the different species varies, the isatinic staining of the vital pollen grains may appear by the different shades of the dark colours. Accordingly, for the purpose of determining the degree of vitality, the isatinic staining of every species should be studied separately.

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