ACTA BIOL. SZEGED. 33. PP. 25—34 (1987)

CONCENTRATION OF PROLINE IN POLLENS: A NEW TAXONOMICAL INDEX

G. PÁLFI[1], S. GULYÁS[2] AND I. SZÖLLŐSI[2]

[1]*Department of Plant Physiology, Attila József University*
H—6701 Szeged, P.O.B. 654. Hungary
[2]*Department of Botany, Attila József University*
H—6701 Szeged, P.O.B. 657. Hungary

(Received: December 8. 1986)

Abstract

The free poline of the pollen of 86 plant species have been investigated. It was found that in 66 species the proline content was very high, more than 1.0 per cent of dry matter (proline-type pollens). The proline content of 20 species was lower than 0.3 per cent (non proline-type pollens). The high proline accumulation is not connected with phylogenetical development. Proline-type pollen occurred among gymnosperms as well as among dicotyledonous and monocotyledonous angiosperms. Extent of accumulation of proline in pollen showed no correlation with the mode of pollination; proline-type pollen occurred among entomogameous, anemogameous and autogameous species alike.

Mature pollen contained in general 1.0 — 2.5 per cent proline which could be quickly demonstrated with our method based on a colour reaction with isatin. Proline accumulation is not uniform within families; 6 families were found which had proline-type and non-prolyne-type species as well. Species of the same genera, however, were alike in this respect: they were all of the proline-type or all of the non-proline-type. Therefore the isatin reaction of the pollen could be considered as a chemotaxonomical index.

Key words: Angiosperms, Dicotyledons, Gymnosperms, Istain reaction, *Monocotyledons,* Pollens.

Introduction

It is already known that free proline accumulates in extreme high concentrations in the pollen of many species; its concentration is higher than that of the other protein amino acids altogether (TUPÝ, 1963; AHOKAS, 1978; HESLOP-HARRISON, 1979; DASHEK and MILLS, 1981; PÁLFI et al. 1981).

DASHEK and HARWOOD (1974), RAI and STOSKOPF (1974), MASCARENHAS (1975), ALARKON et al. (1978), DASHEK and MILLS (1980) established that proline has an important role in the energetic transformations of the pollens interacting with the stigma. At the same time proline is an effective activator of the Krebs cycle and it regulates the water balance and the function of several enzymes. Other authors also publicated that proline has an important role in the synthesis of the glycoproteins rich in hydroxy-proline which glycoproteins are necessary for the elongation of the cell walls and pollen tubes (TUPÝ, 1963; DASHEK et al. 1971; BRITIKOV, 1975; KURSAKOV and RYZHKOV, 1980; DASHEK and MILLS, 1981).

Proline increases vitality through its positive effect on drought-resistance and cold-resistance as well (PÁLFI and JUHÁSZ 1970; YAMADA and KONO 1976; TYANKOVA, 1980; TYMMS and GAFF, 1979; LEWITT, 1980; PALEG and ASPINALL, 1981; SIMINOVITCH and CLOUTIER, 1981; THEBUD and SANTARIUS 1981; van SWAAIJ and JAKOBSEN, 1985).

In this paper authors tried to answer the question: which proline concentrations occur in the mature pollen grains by the most species.

Investigating the pollen of many species authors tried to establish the occurrence of high proline accumulation among the plants, whether it occurs in the more ancient families or in the more advanced ones. The most important task was to elucidate to a certain degree whether high proline accumulation could be used as a chemotaxonomical index.

PÁLFI (1982) and PÁLFI and KÖVES (1984) already published a new rapid staining method with isatin with the aid of which proline content of individual pollen grains can be determined. In this paper proline concentrations of the extracts of pollens are compared with per cents of positivity of the new isatin staining reaction.

Materials and Methods

Names of families and species investigated can be seen in the Tables. The pollen were fixed and dried at 90 °C on the day of harvesting. After this kind of conservation the staining with isatin can be performed immediately or even after 1 or 2 years; proline concentration of the fixed pollen does not change (EPPENDORFER and RILLE 1973; PÁLFI et al. 1974).

The new formule of our isatin reagent is as follows: to 20 ml acetone 0.4 ml glacial acetic acid is added and in the mixture 0.20 g isatin is solved. The staining is performed on slides detailed description of the method is already published (PÁLFI 1982; PÁLFI and KÖVES, 1984; PÁLFI and GULYÁS 1985). For the evaluation of the staining light microscope was used, magnification 100 — 300 x according to the size of the pollens. Proline content of the amino acid extracts was determined according to ASPINALL et al. (1973) in 4 repetitions; the mean values are here reported. In cases where the deviation of individual values from the mean was more than ± 5 per cent the measurings were repeated.

Result and discussion

Mature pollen grains were stained with the isatin reagent intensive blue, dark blue or black due to their high proline content; this is the positive isatin reaction. Pollen with low proline concentration retain their original colour or they are stained light brown (negative isatin reaction). On the black and white microphotos pollen with high proline content are black while that of low proline content show different shades of grey (Plate I. and II.). On the photos occur mostly black and grey pollens (containing much and few proline respectively) together. The photos show only a part of the field of sight. Mostly such fields were counted on which 120 — 150 pollen grains could be seen, the staining values of 5 fields were averaged and the per cent of

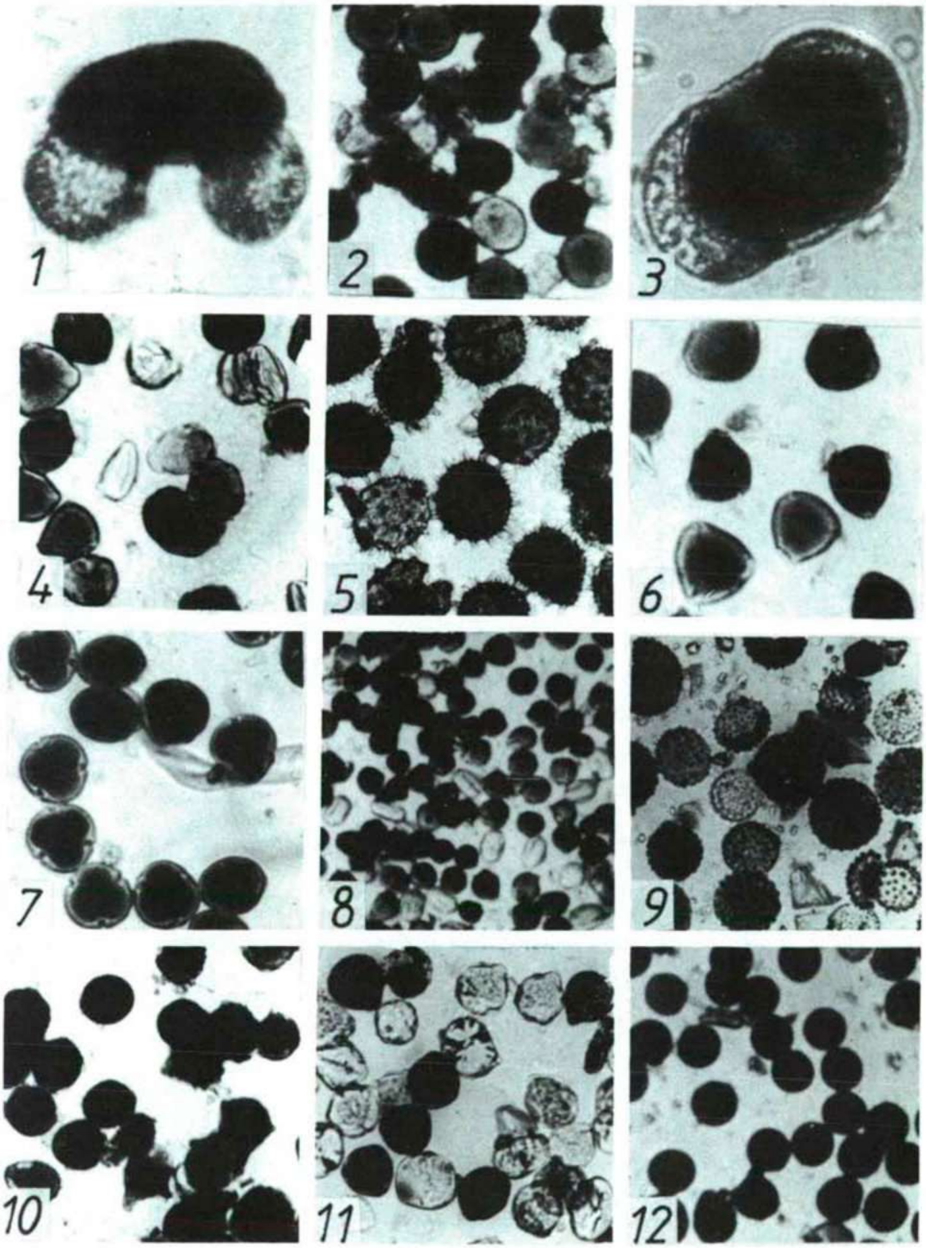

Plate 1. Pollen grains of many species stain dark blue or black (on the photo black) with the isatin reagent due to their high proline concentration. The grey pollens contain very few proline. The magnification is different at the different species; generally 100 — 200 x, at *Pinus* 600 x. 1 = *Pinus nigra;* 2 = *Ranunculus acer;* 3 = *Pinus silvestris;* 4 = *Malus pumila;* 5 = *Hibiscus rosa-sinensis;* 6 = *Armeniaca vulgaris;* 7 = *Tilia cordata;* 8 = *Rosa canina;* 9 = *Abutilon theophrasti;* 10 = *Solanum melongena;* 11 = *Solanum tuberosum;* 12 = *Datura inoxia.*

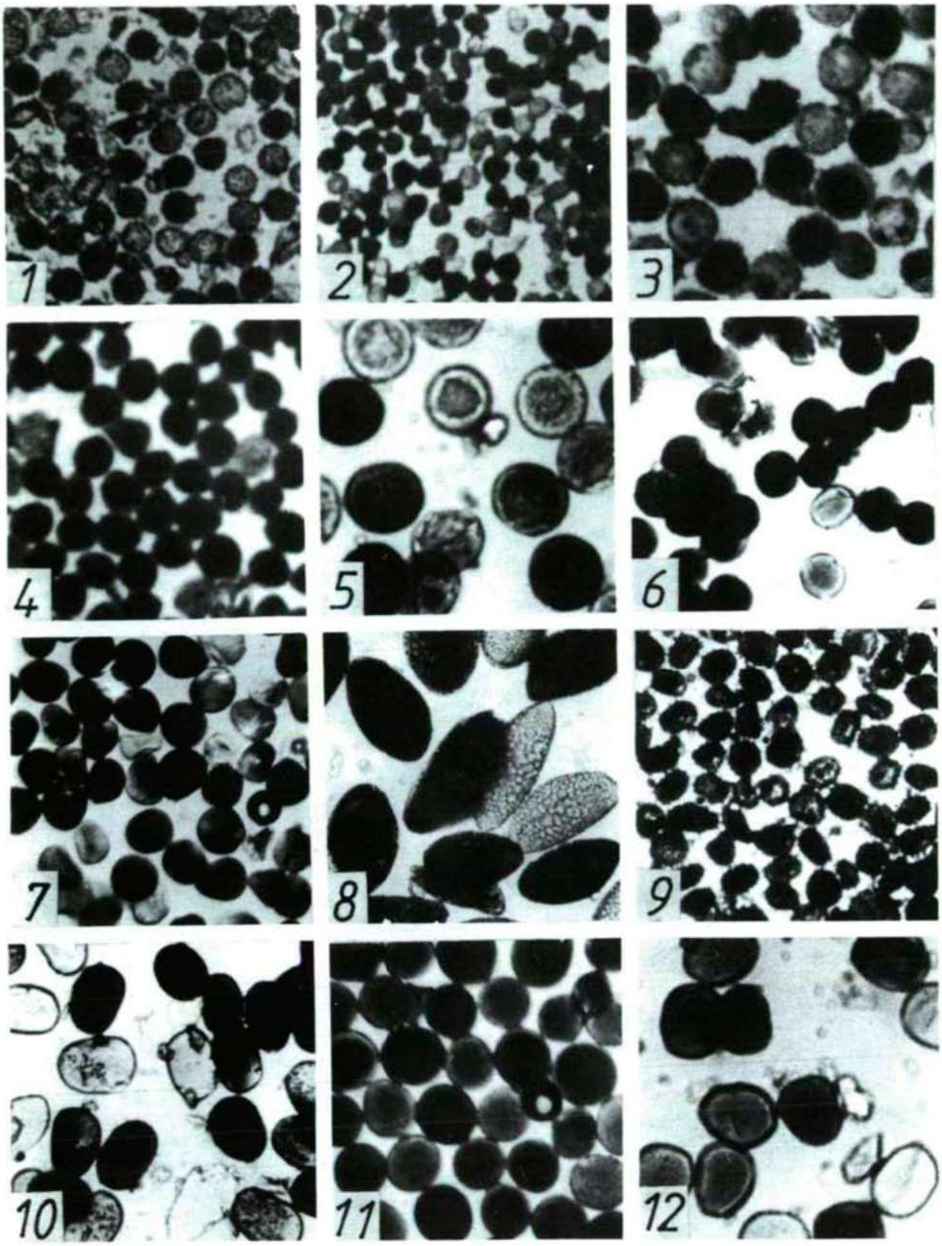

Plate 2. Pollen grains stained black are of extremely high proline concentration while the grey ones contain only an insignificant amount of proline. Magnification generally 100—200 x; by *Juglans* (5) and *Hemerocallis* (8) 500 x, by *Secale* 300 x.
1 = *Dahlia variabilis;* 2 = *Papaver rhoeas;* 3 = *Chrysanthemum hortorum;* 4 = *Corylus avellana;* 5 = *Juglans regia;* 6 = *Salix babylonica;* 7 = *Triticum aestivum;* 8 = *Hemerocallis fulva;* 9 = *Colchicum autumnale;* 10 = *Secale cereale;* 11 = *Zea mays;* 12 = *Hordeum vulgare.*

positive staining calculated. Besides valuating the colours of the isatin reaction the proline concentration of the pollen extracts was also reported (Table 1.).

Serial numbers of the 45 species in Table 1. approximately correspond to their state of development i.e. to their phylogenetic sequence. The species belong to 20 families. In Table 1. entomogameous, anemogameous and autogameous species together occur. It can be seen that proline concentration of the pollen extracts was higher than 1.0 per cent by all of the 45 species and even higher than 2.0 per cent by 9 species. Per cents of the pollen grains showing positive isatin reaction varied between 30 and 95.

Table 1. shows that the proline concentration of the pollen extracts is directly and proportionally correlated with the per cents of the positive isatin reaction.

Table 2. shows results about the most developed plants: the monocotyledons.

In Table 2. data of proline concentration and of positive isatin reaction of 16 entomogameous, anemogameous and autogameous species belonging to 3 families are reported. Proline concentration of the pollen extracts varies between 1.11 and 2.38 per cent. It can be seen that the lowest per cent of isatin reaction was given by the species which showed the lowest proline concentration (No. 7.: *Lilium regale*). And the species having the highest proline concentration shows the highest per cent of positive isatin reaction (No. 16: *Zea mays*). There are no essential differences between the dicotyledonous and the monocotyledonous species although they represent two entirely different levels of development.

In Table 3. species are reported in which proline concentration of the mature disseminating pollens is entirely different as compared with the afore-mentioned species. Data of 16 species belonging to 10 families (dicotyledons and monocotyledons together) are reported here. The proline concentration of their pollen extracts is very low: 0.12 — 0.28 per cent of dry matter. At such a low proline concentration the isation reaction is negative: no dark blue or black staining of pollen grains occur. In the case of these 16 species our isatin reaction unsuitable for the determination of proline concentration. Among these species there occur species of the lower and higher level of phylogenesis as well.

Data based on the proline concentration reported here and in earlier papers (PÁLFI 1982; PÁLFI and KÖVES, 1984) a concentration value was sought with the aid of which the species could be divided into two groups: 1. proline-type" species where proline concentration of the mature pollens reaches 1.0 per cent of dry matter and 2. non-proline-type" species where proline concentration in the mature pollens is much more lower than 1.0 per cent (mostly ten times lower than at the species of the first group).

Pollens of the most inferior flowering plants, the gymnosperms were also investigated (Table 4.).

As reported in Table 4. among the 9 species belonging to 3 families there are 5 of the proline type and 4 of the non-proline-type. Accordingly, these 2 types of proline accumulation occurred already in the most ancient flowering plants: the grade of proline accumulation does not indicate neither a primitive nor an advanced state.

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		percent	
Ranunculaceae	1. *Ranunculus acer*	1.54	62
Nymphaeaceae	2. *Nymphaea alba*	1.29	45
Rosaceae	3. *Rosa canina*	2.23	91
	4. *R. polyantha*	1.87	81
	5. *Pyrus communis*	1.28	44
	6. *P. achras*	1.24	43
	7. *Malus pumila*	1.38	54
	8. *M. floribunda*	1.15	35
Grossulariaceae	9. *Ribes aureum*	1.37	56
	10. *R. rubrum*	1.19	38
Fabaceae	11. *Trifolium repens*	1.36	52
	12. *T. pratense*	1.17	38
	13. *Robinia pseudo-acacia*	1.47	53
	14. *R. hispida*	1.36	52
Eleagnaceae	15. *Elaeagnus angustifolia*	1.56	64
Hippocastanaceae	16. *Aesculus hippocastanum*	1.82	80
Caprifoliaceae	17. *Sambucus nigra*	1.18	35
Tiliaceae	18. *Tilia cordata*	2.10	88
	19. *T. platyphyllos*	2.22	94
Malvaceae	20. *Abutilon theophrasti*	1.41	54
	21. *Hibiscus rosa-sinensis*	1.12	33
Solanaceae	22. *Solanum tuberosum*	1.37	53
	23. *S. melongena*	1.54	62
	24. *S. nigrum*	1.50	59
	25. *Datura inoxia*	1.68	69
	26. *D. arborea*	1.57	63
Papaveraceae	27. *Papaver somniferum*	1.46	56
	28. *P. rhoeas*	2.37	93
Cruciferae	29. *Lepidium draba*	1.36	55
Cucurbitaceae	30. *Cucumis sativus*	1.42	53
Compositae	31. *Dahlia variabilis*	1.46	57
	32. *Chrysanthemum hortorum*	1.42	50
	33. *C. corymbosum*	1.39	47
	34. *C. leucanthemum*	1.56	64
Primulaceae	35. *Primula veris*	2.20	92
	36. *P. acaulis*	2.16	90
Betulaceae	37. *Corylus avellana*	2.24	95
	38. *Betule pendula*	1.08	30
Fagaceae	39. *Quercus robur*	1.59	63
Juglandaceae	40. *Juglans regia*	1.10	30
Salicaceae	41. *Populus tremula*	1.12	31
	42. *P. alba*	1.26	45
	43. *Salix babylonica*	2.18	91
	44. *S. cinerea*	1.69	68
	45. *S. smithiana*	2.05	87

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

Table 1. Proline concentration in per cent of dry matter in pollen extracts of dicotyledonus species and per cent of positive isatin reaction of the pollen grains. Among the 52 species belonging to 20 families there are entomogameous,anemogameous and autogameous species. Pollens of the proline-type".

Table 2. Proline concentration in pollen extracts and positive isatin reaction of the pollen grains of monocotyledonous species. Pollens of the proline-type".

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		percent	
Liliaceae	1. *Colchicum autumnale*	2.18	89
	2. *Hemerocallis fulva*	1.39	52
	3. *Allium schoenoprasum*	1.42	55
	4. *Allium cepa*	1.88	80
	5. *Lilium candidum*	1.14	41
	6. *L. martagon*	1.27	50
	7. *L. regale*	1.11	39
	8. *Muscari comosum*	1.63	71
Iridaceae	9. *Iris pumila*	1.26	45
	10. *I. germanica*	1.32	50
Gramineae	11. *Secale cereale*	1.17	38
	12. *Triticum aestivum*	1.51	59
	13. *T. durum*	1.47	54
	14. *Hordeum vulgare*	1.67	73
	15. *Oryza sativa*	1.93	82
	16. *Zea mays*	2.38	92

Evaluating the data of the Tables 1.—4. it turns out that from the 86 species investigated 66 are of the proline-type and 20 species of the non-proline-type — according to authors' data covering species from 30 families.

Generally 2.0—2.5 per cent proline concentration is the highest.

These data also show that the extent of proline accumulation has no correlation with the family boundaries. Six families were demonstrated in which proline-type pollens as well as non-proline-type pollens occurred *(Fabaceae, Malvaceae, Cruciferae, Cucurbitaceae, Compositae, Liliaceae)*.

In contrast to this, investigated species belonging to the same genus were all of the proline-type or all the non-proline-type. Therefore, when species assigned to the same genus would be different as regards their proline type, a revaluation of their taxonomical situation is indicated — proline type being a genus charasteristic.
Following genera are of the same proline type: by the gymnosperms (Table 4.) all of the 5 *Pinus* species are of the proline-type. At the same time the 3 *Juniperus* species are of the non-proline-type. In the angiospermous dicotyledons (Table 1.) all investigated species of the genera *Rosa, Pyrus, Malus, Trifolium, Robinia, Tilia,*

Solanum etc. are of the proline-type.In the most advanced class (monocotyledons; Table 2.) there are also genera the species of which are all of the proline-type *(Allium, Iris, Lilium)*. In the table of the non-proline-type species (Table 3.) there are also species belonging to the same genus (the dicotyledonous *Cucurbita* and the monocotyledonous *Tulipa*). Consequently, the isatin reaction of the pollens could be properly used for the chemotaxonomic characterization of genera.

Table 3. Dicotyledonous and monocotyledonous species having pollen with such a low concentration of proline that they are not stained with the isatin reagent. Pollens of non-proline-type"

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		percent	
Fabaceae	1. *Coronilla varia*	0.20	—
Malvaceae	2. *Lavathera thuringiaca*	0.27	—
Labiatae	3. *Ajuga genevensis*	0.28	—
	4. *Lamium purpureum*	0.22	—
	5. *Glechoma hederacum*	0.18	—
Cruciferae	6. *Brassica napus*	0.26	—
	7. *Arabis procurrens*	0.15	—
Tamaricaceae	8. *Tamarix tetranda*	0.12	—
Begoniaceae	9. *Begonia semperflorens*	0.23	—
Cucurbitaceae	10. *Cucurbita pepo*	0.17	—
	11. *C. ficifolia*	0.21	—
	12. *C. maxima*	0.20	—
Compositae	13. *Helianthus annuus*	0.23	—
Liliaceae	14. *Tulipa germanica* (gelb)	0.18	—
	15. *T. gesneriana* (rot)	0.23	—
Amaryllidaceae	16. *Narcissus pseudo-narcissus*	*0.28*	—

Among the 86 species in the Tables 1.—4. occur autogameous, entomogameous and anemogameous species; mode of pollination is not connected with the proline concentration of the pollens.

It was demonstrated by KURSAKOV and RYZHKOV (1980) in the case of *Ribes nigrum* and PÁLFI and KÖVES (1984) in the case of *Zea mays* that adding free proline to the substrate of the in vitro germination experiments elongation of the pollen tubes increased for 100 per cent as compared with the control without proline. This indicates that the presence of a great quantity of proline is really advantageous during the germination of the pollens (LEWITT, 1980; PALEG and ASPINALL, 1981; ELTHON and STEWART, 1984). Notwithstanding, all 2 plants species in these experiments are of the proline-type *(Ribes nigrum and Zea mays)*.

Table 4. Proline concentration in pollen extract and isatin reaction of pollen grains of gymnospermous species. Proline concentration in the pollens of 4 species is very low and therefore these do not give positive reaction with isatin.

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		percent	
Taxaceae	1. *Taxus baccata*	0.06	—
Abietaceae	2. *Pinus silvestris*	1.28	42
	3. *P. nigra*	1.45	58
	4. *P. strobus*	1.17	39
	5. *P. mugo*	1.23	41
	6. *P. griffithii*	1.14	36
Cupressaceae	7. *Juniperus communis*	0.19	—
	8. *J. virginiana*	0.13	—
	9. *J. chinensis*	0.16	—

References

AHOKAS, H. (1978): Cytoplasmic male sterility of barley. II. Physiology and anther cytology of msml. — Hereditas. *89,* 1-21.

ALARKON, N.A., LARIONOVA, E.G. and MININA, T.K. (1978): Amino acid of pollen of *Pinus sibirica.* — Fiziol. Rast. *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 rol prolina. — Izd. Nauka". Moskva.

DASHEK, W.V., HARWOOD, H.I. and ROSEN, W.G. (1971): The significance of a wall bound hydroxyproline-rich glycopeptide in lily pollen tube elongation. — In: HESLOP-HARRISON, J. (Ed.) Pollen development and physiology. Butterworths, London.

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.

DASHEK, W.V. and MILLS, R.R. (1981): Proline metabolism by germinating Lilium longiflorum. I. Labelling of cytoplasmic wall and culture medium molecule. — Acta Soc. Bot. Pol. *50,* 51-66.

ELTHON, T.E. and STEWART, C.R. (1984): Effect of proline analog L-Thiazolodine-4-carboxylic acid on proline metabolism. — Plant Physiol. *74,* 213-218.

EPPENDORFER, W.H. and RILLE, S.W. (1973): Decomposition in presence of nitrat of amino acids especially tyrosine, during acid hydrolysis of plant material and standard amino acid solution. — Plant and Soil. *38,* 215-218.

HESLOP-HARRISON, J. (1979): Aspects of the structure, cytochemistry and germination of the pollen of rye *(Secale cereale).* — Ann. Bot. *44,* Suppl. 1.

KURSAKOV, A.G. and RYZHKOV, S.D. (1980): The content of free amino acids in oppositely charged fractions of black currant pollen. — Fiziol. Raszt. *27,* 735-739.

LEVITT, J. (1980): Responses of plants to environmental stresses. II. Water, radiation, salt and other stresses. — Acad. Press. New York, London, Toronto, Sydney, San Francisco.

MASCARENHAS, J.P. (1975): The biochemistry of angiosperm pollen development. — Ann. Bot. *41*, 259-314.

PALEG, L.G. and ASPINALL, D. (1981): The physiology and biochemistry of drought resistance in plants. — Acad. Press. Sydney, New York, London, Toronto, San Francisco.

PÁLFI, G. and JUHÁSZ, J. (1970): Increase of proline level in water deficient leaves as a reaction to saline or cold root media. — Acta Agron. Acad. Sci. Hung. *19*, 79-88.

PÁLFI, G., KÖVES, E. and BITÓ, M. (1974): The role of amino acids during water stress in species accumulating proline. — Fyton. *32*, 121-127.

PÁLFI, G., PINTÉR, L. and PÁLFI, Zs. (1981): The proline content and fertility of the pollen of inbred maize lines. — Acta Bot. Acad. Sci. Hung. *27*, 179-187.

PÁLFI, G. and PÁLFI, Zs. (1982): A rapid method for the determination of fertility of maize pollen with the proline-isatin reaction. — Maydica. *27*, 107-111.

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 GULYÁS, S. (1986): Complementary data to the vitality test of pollens. — Növénytermelés (Hung. res. eng.). *34*, 351-358.

RAI, R.K. and STOSKOPF, N.C. (1974): Amino acids comparisons in male sterile wheat derived from Triticum timopheevi ZHUK. Cytoplasm and its fertile counterpart. — Theor. Appl. Genet. *44*, 124-128.

SIMINOVITCH, D. and CLOUTIER, Y. (1981): 24-Hour induction of freezing and drought resistance in plumules of winter rye seedlings by desiccation at room temp. in the dark. — Plant Physiol. Suppl. *67*, 345.

THEBUD, R. and SANTARIUS, K.A. (1981): Effect of freezing on isolated plant mitochondria. — Planta. *152*, 424-427.

TUPÝ, J. (1963): Free amino acids in apple pollen from the point of view of its fertility. — Biol. Plantarum. *5*, 154-159.

TYANKOVA, L. (1980): Soderzhanie prolina, svobodnih aminokislot, obshchego i belkovogo azota i sahar kak funkcijah prodolzhitelnosti i stepeni podsuhaniya otdelennih listev tabaka. — Fiziol. Rast. *27*, 806-813.

TYMMS, M.J. and GAFF, D.F. (1979): Proline accumulation during water stress in resurrection plants. — J. Exp. Bot. *30*, 165-168.

VAN SWAAIJ, A.C., JACOBSEN, E. and FEENSTRA, W.J. (1985): Effect of cold hardening, wilting and exogenously applied proline on leaf proline content and frost tolerance of several genotypes of *Solanum*. — Physiol. Plant. *64*, 230-236.

YAMADA, N. and KONO, Y. (1976): Studies on the developmental physiology in rice pollen. — Proc. Crop. Sci. Soc. Tokyo. *45*, 279-287.