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THE PROLINE TEST — A METHOD TO THE DEMONSTRATION OF THE TOLERANCE OF WATERDEFICIENCY AND OF FROST — AND TO THE QUALIFICATION OF POLLENS

G. PÁLFI[1], S. GULYÁS[2], E.S. RAJKI[3] AND L. CSEUZ[3]

[1]*Department of Plant Physiology and* [2]*Department of Botany, Attila József University, H-6701, Szeged, P.O.B. 654, Hungary*
[3]*Cereal Research Institute, H—6701, Szeged, P.O.B. 391, Hungary*

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Abstract

Based on the proline test a new method was developed by authors for the investigation of drought resistance and frost tolerance of herbaceous cultivated plants and for the estimation of the quality of their pollens. It was established that among the selected varieties of a cultivated plant the drought resistance is higher in these varieties which accumulate more free proline in their isolated leaves exposed to lethal water deficiency (live-wilting) in light during 3 days. The proline test was performed and the grade of drought tolerance was estimated on 7 sorghum hybrids, 3 maize hybrids and on 2 wheat varieties. In frost the cells of the leaves loose very much water and similarly to the soil dryness a significant water deficiency emerges. To protect themselves against this water deficiency the leaves accumulate large quantity of proline. Drought resistance is estimated in the blooming phase while the frost tolerance is investigated on young, 2—4 weeks old shoots. Ripe Pollens of many plants have extremely high (2.0 per cent) proline concentration. The quality of these pollens is proportional with their proline content. Authors' new isatin reagent colours the pollen grains deep blue or black when their proline content is high (these pollens have a very high quality). Most cultivated plants accumulate, high quantity of proline (pollens of "proline type"): the fruit trees of the *Rosaceae* family, the fodder and food plants of the *Papilionaceae*, the cultivated species of the *Solanaceae*, our most important timber woods, and naturally, our cereals.
Key words: mezophyta, families, species, varieties, stress-amino acid = proline

Introduction

A number of authors published that high water deficiency induces accumulation of free proline in the leaves and shoots of herbaceous plants and this significant physiological advantages are assured (TYMMS and GAFF, 1979; CHAUHAN et al., 1980; LEVITT, 1980; TYANKOVA, 1980; PALEG and ASPINALL, 1981).

In different species different quantities of proline accumulate in consequence of gradually obtained and similarly high water deficiency. From the level of proline accumulation no conclusion can be drown to the drought resistance of species (SINGH et al., 1972; PÁLFI et al., 1974; 1975; WALDREN and TEARE, 1974). However, inside a species those varieties, inbred lines and their hybrids are the more drought resistant in which more proline accumulates as the result of a lethal water deficiency. According to VAN DE DIJK (1981) the same level of "outer deficiency" (soil dryness) produces different level of "inner water deficiency" in different

varieties of the same species. This source of error can be eliminated through the equally gradual approach of water deficiency (PINTÉR et al., 1978, 1979; PÁLFI and PINTÉR, 1980; PÁLFI et al., 1983). Investigating 46 species (PÁLFI et al., 1975) it was established that the determination of drought tolerance by proline test can be performed not only on intact plants grown in the field but also on isolated herbaceous shoots and on isolated leaves in 3—4 days (PÁLFY et al., 1974, 1975). During the gradually obtaining of water deficiency ("live-wilting") it is necessary to apply artificial illumination, and adequate temperature and relative humidity as in the case of isolated shoots of alfalfa, clover, wheat or in the case of isolated leaves of maize, tobacco and paprika (PÁLFI et al., 1978; PINTÉR et al., 1979; PÁLFI and PINTÉR, 1980; PÁLFI et al., 1983). By these investigations it was also established that the collection of leaves is the more advisable at blooming time because in this phase accumulate the largest quantity of proline and so — higher degrees of difference among the varieties can be obtained. According to several authors the cold resistance of the plants is realised through diffusion of water from the cells into the intercellulars and departure from there by transpiration through the cuticules. If water frozes inside the cells, the ice crystals disrupt the finer structures, the organells and membranes; this is the frost-killing (HEBER et al., 1971; DRAPER, 1972; THEBUD and SANTARIUS, 1981).

PAQUIN and PELLETIER (1981), PERUANSKIY and STACHENKO (1981) published that among varieties (e.g. selected varieties of autumnal wheat or rye) the most resistant is that which endures better a strong water deficiency, i.e. which accumulates more proline. During repetition of minor cooling and frost, the plants become hardened to frost. Moreover it is well known that in the case of autumnal cereals the several weeks long cold and frost (Yarovization) is absolutely necessary for crop production.

SIMINOVITCH and CLOUTIER (1981) demonstrated on autumnal rye that the variety which has higher drought tolerance, i.e. accumulates more proline, endures better strong frosts.

Sometimes at the blooming time of the cereals and fruit — trees occure dryness in soil or hot spells and also low humidity in the air, and cold snaps with frost as well. Plants accumulate high quantity of proline as a defence againts these extremities of climate. Similarly the pollens of the cultivated plants accumulate much proline even at favourable weather conditions (TUPY, 1963; STANLEY and LINSKENS, 1974; BRITIKOV, 1975).

DASHEK and HARWOOD (1974), ALARKON et al.(1978), KURSAKOV and RYZHKOV (1980), ZHANG et al. (1982) demonstrated that during the germination of the pollen grains the high proline content defends them against unfavourable weather and therefore proline concentration in the pollens is taken as a quality indicator.

Materials and methods

The most important test of drought resistance is the "livewilting", i.e. gradually realized lethal water deficiency in the isolated leaves. In the case of wheat, maize and sorghum leaves collected at blooming time from the same level of the shoots — were spreading on filter paper and fixed by cell tapes at the two end and covered air tight with colourless transparent plastic sheets. They were incubated for 3—4 days in a climate chamber or on a stand at constant illumination of 5000 lux. Under the cover a temperature of 24—28 °C was maintained. In the first 24 hours the relative humidity was 90 per cent, on the second day it was reduced to 80 per cent and on the third day to 60 per cent. Twice in a day the setting up was ventilated for 15 minutes by opening the plastic cover. In the last 12 hours the lethal water deficiency in the leaves must be attained in each varieties (this means an equal level of "inner waterdeficiency"). (Fig. 1—4).

Immediately after the live-wilting process all plant organs were chopped, fixed at 90 °C and desiccated for 8 hours (air dry material). The material was then pulverized in an electric desintegrator and closed in airtight dark containers till performing the analyses.

For determination of cold resistance and frost tolerance the 2—4 weeks old bean, paprika, wheat and rye plants grown in cultivating pots were exposed to 0 °C or -2 °C for 3—4 days. Thereafter the shoots were cut up, fixed, dryed and pulverized and their proline content was determined. The variety which accumulates more proline, is the more frost resistant one (CLOUTIER and SIMINOVITCH, 1981; PAQUIN and PELLETIER, 1981; PERUANSKIY and STACHENKO, 1981).

An other frost resistance test was performed as well. Young shoots grown at optimal conditions were cut off and incubated at a low temperature, required by the variety for 2—4 days under illumination. The proline content of the fixed and dryed shoots was determined (PÁLFI and GULYÁS, 1986).

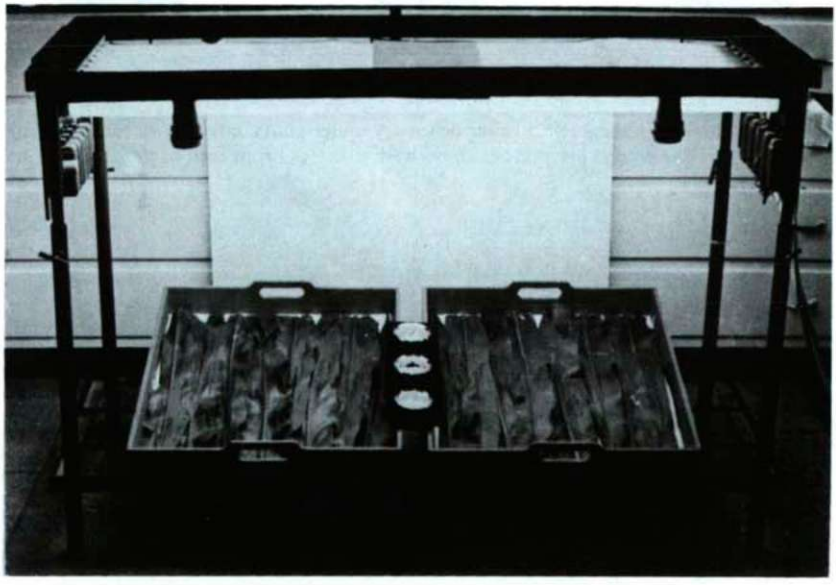

Fig. 1. Gradually obtained lethal water deficiency (livewilting) for 3 days in isolated leaves of 16 inbred maize varieties. The leaves were isolated at he blooming phase and put on plates covered with transparent plastic sheets. They were illuminated with 5000 lux at 24—28 °C at 90 per cent relative humidity which was later reduced to 80 per cent and at last to 60 per cent.

CLOUTIER and SIMINOVITCH (1982) in 9 sorts of autumnal wheat attained by a desiccation stress of young plants the same grade of frost tolerance than by cold hardening at +2 °C for 4 weeks. Autors of this paper (1981) established besides in wheat also in rye that the proline levels in young shoots provoked by live-wilting are indicators of frost tolerance as well.

For determination of proline, the method of ASPINALL et al. (1973) and of BATES et al. (1973) can be used.

The simple and exact new method of proline determination elaborated by the authors of this paper is as follows:

Two hundred mg of the pulverized airdry plant material was mixed with 1 g quartz sand and extracted three times with altogether 20 ml ethanol of 30 per cent concentration each time the mixtures being centrifuged and the supernatants decanted and united forming the amino acid extract. From this 0.05 or 0.10 ml was put on in small portions on the start line on the chromatographic paper from a

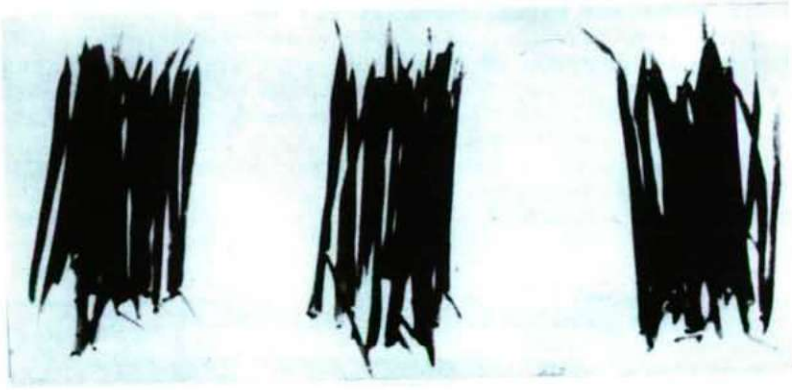

Fig. 2. Gradually obtained lethal water deficiency under plastic cover in wheat leaves isolated at the blooming period. The second leaves from above were isolated. From each of the 3 wheat varieties 20 leaves were taken.

Fig. 3. Provocation of water deficiency in isolated budding alfalfa shoots by mannitol solution of high osmotic pressure. The pot at the left contains water (control). The following pots contain gradually hightening mannitol solutions: 0.4 M; 0.6 M; and 0.8 M respectively. It can be seen thet by increasing the mannitol concentration the shoots are wilting accordingly.

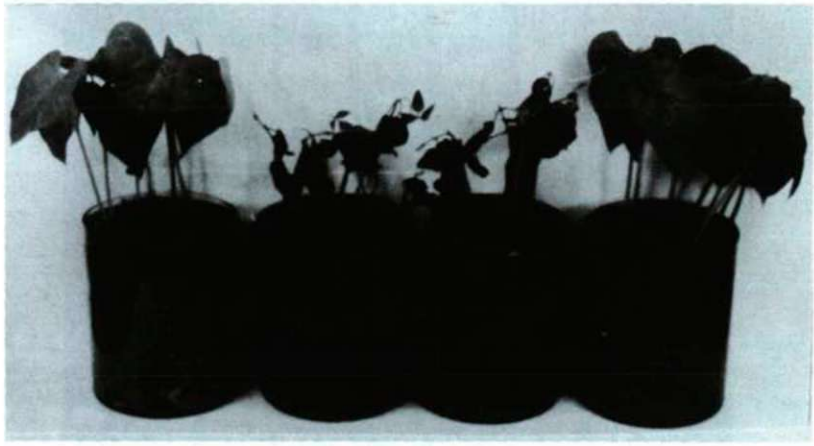

Fig. 4. High water deficiency provoked by "chilled medium" in young bean plants. The water content in the soil of the pots was optimal (70 per cent of the water capacity). The first and the last pots were incubated at 20 °C for 3 days while the two in the centre at 0 °C respectively. The cold stress resulted in heavy wilting.

micropipette and the spots dryed with hot air stream. Each extract was applied three times side by side (3 replications). On the same paper the concentration series of proline standard was applied in 7 stripes (2, 4, 6, 8, 10, 12, and 14 μg from a solution of 1 μg in 0.01 ml). (μg = microgramme).

For one-dimensional developing a phenol-water mixture (4:1 vol.) was used. One night (16—18 hours) is enough for developing (Fig. 5.). The developed paper was taken out and dryed in warm air stream. The dryed paper was immerged into the isatin reagent or drawn 5—6 times through it. Placing the immerged paper on double layer of filter paper it was put in a 90 °C warm exsiccator for 20 minutes; during this time the colours were developed. (Composition of the isatin reagent: to 200 ml acetone 5 ml concentrated acetic acid and 2 g isatin is added). From the paper the superfluous yellow isatin was washed out in running tap water (12—15 minutes) and the paper was blotted between filter papers. Proline has the highest Rf value, it appears as well separated deed blue spot at the top of the paper. The blue spots were cut out with scissors, cut up into small pieces and eluted in a 25 ml Erlenmeyer flask with 5.0 ml mixture of phenol-water (4:1). The flasks put on a plate were incubated in the dark for 15—20 minutes meanwhile they were shaken 4—5 times together with the plate. When the solution is blue and the paper stripes are coulourless, the light absorption was determined at 620 nm in cuvettes of 1 cm in diameter.

From the extinctions of the proline standards a graph was designed from which the proline concentrations of the amino acid extracts can be read off. Thereafter the 0.05 or 0.10 ml developed extract is converted into 18 ml (this quantity remained from the original 20 ml solvent). The obtained value refers to 200 mg dry material. If this value is multiplied by 5 and divided by 1000 the result is expressed in mg proline in 1 g dry material.

In the case of two-dimensional paperchromatography in the first dimension with buthanol-acetic acid-water (3:1:1) is developed at -4 °C — -6 °C. The solvent for the second dimension is phenol-water (4:1), this development is performed at room temperature. The amino acid spots are detected with ninhydrinereagent and fixed with a copper solution.) (Ninhydrine reagent: to 192 ml aceton 1.0 ml concentrated acetic acid, 7 ml distilled water and 1.0 g ninhydrine were added. The paper should be immerged 3—4 times. Copper solution for fixation: to 80 ml methanol and 120 ml isopropanol, 0.8 ml 10 per cent nitric acid and 4.0 ml saturated solution of copper nitrate were added. This is a solution for immersion).

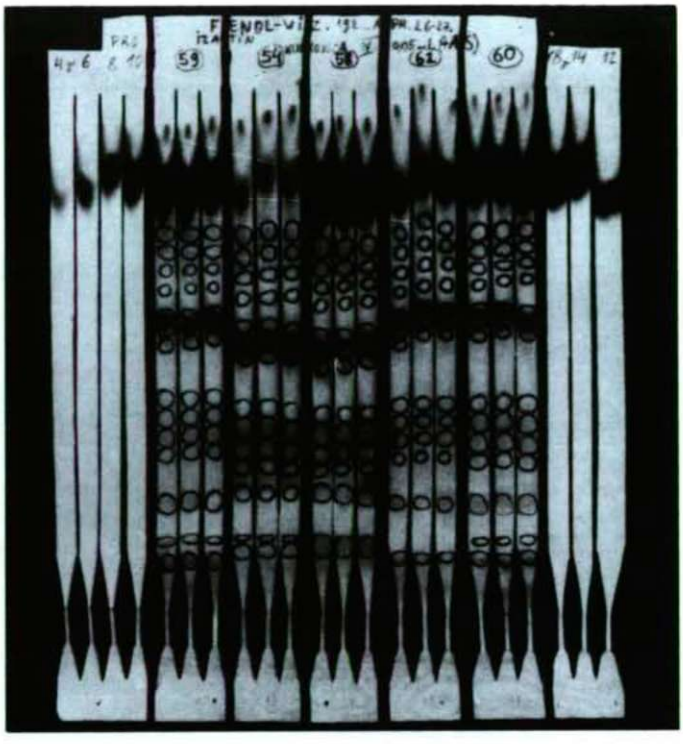

Fig. 5. Unidimensional ascending paper chromatogram of amino acid extracts of inbred maize lines. The five inbred lines show total different proline storages by similar live-wilting. The largest spots on the figure above are that of proline. Each extract developed in 3 parallels. The proline standards can be seen on the outer stripes. The stripes are 1 cm wide while the chromatogram paper is 30 cm high.

From the anemogameous plants (maize and rye) a large quantity of ripe pollens can be collected in a short time. In the case of this species the proline content of the pollens can be determined with the method described above starting from 50, 100 or 200 mg at 90 °C fixed and then dryed and homogenized material. In the autogameous and entomogameous species only small quantities of ripe pollens can be obtained at one occasion. For these plants a new staining method was elaborated with the aid of which the proline concentration in the pollen grains can be estimated on the basis of the colour of the pollen. The staining reagent used is: 20 ml acetone containing 0.4 ml acetic acid and 0.2 g isatin. The staining is performed on the slides-placed pollens. They were mixed with 3—4 drops of the reagent for 5—6 minutes, the colour thereafter was developed at 90 °C for 20 minutes. The preparate was covered with paraffin oil. Detailed description of the method was published elsewhere (PÁLFI and KÖVES, 1984; PÁLFI and GULYÁS, 1985; GULYÁS and PÁLFI 1986; PÁLFI et al., 1987).

Results and discussion

On the two-dimensional paperchromatograms treated with ninhydrine proline shows a relatively weak yellow colour while the other amino acids show intensive purple, blue, violet, brownish or greyish colours (Fig.6.). Fixed with the copper solution nearly all amino acids become red and the colour of proline and asparagine fades out.

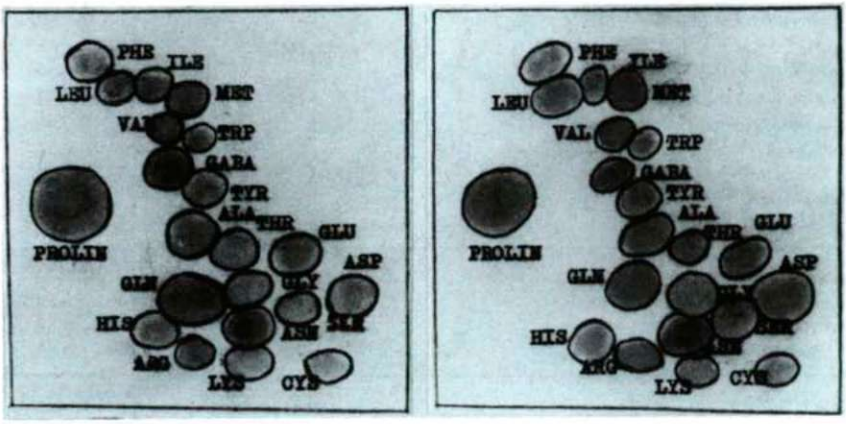

Fig. 6. Twodimensional ascending paper chromatograms of amino acid extracts from wheat and rye leaves. To lethal water deficiency exposed isolated wheat leaves (on the left) and rye leaves (on the right) show significantly higher amino acid content but the largest spot belongs to proline.

On Figure 6. not only the proline spots of the extracts of live-wilted wheat and rye leaves are considerably large but also the other amino acids show relatively large and intensive spots. In the isolated, live-wilted leaves lethal water deficiency induces the accumulation most of the amino acids as well. Intensive accumulation of amino acids, especially that of the amino acid amides occur not only as a result of water deficiency but also as the consequence of one-sided overfeeding with nitrogen or phosphorus or potassium — or the consequence of the absence of a nutritive or of an infectious plant disease. A general accumulation of the amino acids is not specific for water deficiency. The exceedingly high accumulation of proline is absolutely characteristic of water deficiency (PÁLFI and JUHÁSZ, 1971; SINGH et al. 1972; ASPINALL et al., 1973; PÁLFI et al., 1974, 1975; LEVITT, 1980).

Proline concentrations in the leaves of sorghum and maize hybrids and wheat varieties after induced lethal water deficiency (i.e. live-wilting) are seen in Table 1.

Table 1. shows that the levels of proline synthesis and accumulation in the hybrids and varieties of the same species are not the same and sometimes are very different even in the same growing period, in the same level of leaves and even when

Table 1. Proline concentration in isolated leaves of sorghum and maize hybrids and wheat varieties after 3 days long wilting at illumination leading to lethal water deficiency. The leaves were collected in blooming phase. The proline concentration of the non live-wilted and with water optimally supplied leaves never exceeded 0.3 per cent of the dry material.

No.	Species, hybrids and varieties	Proline concentration of the extracts	
		in mg/l g dry matter	per cent of the dry matter
	Sorgum vulgare PERS. hybrids		
1.	Napsugár	5.52	0.55
2.	Remény	5.70	0.57
3.	NK x 7902	4.83	0.48
4.	Alföldi — 1	5.08	0.51
5.	NK x 3221	3.87	0.39
6.	Hybar — 456	3.58	0.36
7.	GKI — 1	4.46	0.45
	Zea mays L. hybrids		
1.	SzeTC 255	2.17	0.22
2.	SzeMSC 267	3.92	0.39
3.	KSC 360	3.24	0.32
	Triticum aestivum, varieties		
1.	Lonja	14.68	1.47
2.	Gk Ságvári	19. 25	1.92

(Average deviation being below ± 5 per cent; n = 3)

the same method was applied for the induction of water deficiency. Among the sorghum hybrids the highest proline concentration was 59.2 per cent larger than that of the smallest one. Among the maize hybrids this difference is higher: 80.6 per cent. The difference in this respect between the two wheat varieties is 31.1 per cent. According to the proline concentration data in Table 1. the investigated hybrids and varieties can be arranged in a series of drought tolerance grades.

Authors investigated with the proline test 23 inbred maize lines. Four of them was found exceedingly drought tolerant (PINTÉR et al., 1978, 1979; PÁLFI and PINTÉR, 1980; PÁLFI et al., 1983). Crossing two such inbred maize lines hybrids with a higher drought tolerance than known up to now could be obtained.

The inbred maize line which accumulates the highest amount of proline concentration during live-wilting produce low yield. But it has an inbred relative with high yield and low drought tolerance. Crossing the two related lines, 64 individuals of the descendants were investigated. The grains of the 5 individuals showing the highest proline concentration during live-wilting were sown next year and 64 individuals were again investigated.

Evaluated statistically the results, it can be established that in the second year the proline content of the leaves and the grade of drought resistance were significantly higher.

Individuals were found the leaves of which contained 7.0 mg proline in 1 g dry material at lethal water deficiency provoked by live-wilting. Such a high value in maize never have been found before (PINTÉR et al., 1978, 1979; PÁLFI and PINTÉR, 1980; PÁLFI et al., 1983).

Cold and frost tolerance

Detetmination of frost tolerance is different from the determination of drought tolerance because in the former case not isolated leaves of adult plants were investigated but young shoots of 2—4 weeks old. Proline accumulation in wheat, rye bean and paprika varieties in consequence of cold is evaluated in Table 2.

It can be seen in Table 2. that the free proline content of the shoots is very low and the differences between the varieties and hybrids are not significant. This proline content can be risen 8—18 times (800—1800 per cent) as a result of cold or frost.

It is interesting that the cold induced proline concentration increases is significantly different between the two varieties of the investigated species. The difference is 35.5 per cent at the wheat, 40.7 per cent at the rye, 33.8 per cent at the bean and 36.2 per cent at the paprika. With these values the colt and frost tolerance of the varieties can be well characterized.

CLOUTIER and SIMINOVITCH (1981) established significant differences between young wheat and rye plants at frost. According to them the increase of proline content is direct proportional to the grade of frost tolerance.

PERUANSKIY and STACENKO (1981) demonstrated also significant differences between frozen young wheat shoots of different varieties. PAQUIN and PELLETIER (1981) established that the proline level in the leaves and roots of wheat varieties increase with their frost tolerance — but only till the falling of the leaves. The higher the frost tolerance the greater is the proline accumulation.

This shows also that photosynthesis has some significance in the formation of frost tolerance.

VAN SWAAIJ et al. (1985) demonstrated on ten potato varieties that the grade of cold tolerance is higher in varieties which accumulate higher quantity of proline caused by cold. They proved also that through externally given proline — the proline content of the shoots can be increased and with this the grade of cold tolerance as well.

YOU-LIANG and STEFONKUS (1983) observed on protoplasts isolated from rye leaves that proline influences the behaviour of non-acclimatized protoplasts. This proves — partly at least — the role of proline in the defence against cold.

According to the above mentioned facts it can be determined with the aid of

Table 2. Proline accumulation in young shoots of two varieties of wheat, rye, bean and paprika, provoked by chilling at —2 °C and 0 °C for 3 days at illumination.

Species, varieties	Temperature of the soil	Proline concentration, in mg/l g dry matter
Triticum aestivum L. varieties		
Lonja	20 C°	0.34
GK Ságvári	20 C°	0.33
Lonja	–2 C°	4.11
GK Ságvári	–2 C°	5.57
Secale cereale L. varieties		
Lovászpatonai	20 C°	0.43
Verhnigskaja	20 C°	0.42
Lovászpatonai	–2 C°	4.08
Verhnigskaja	–2 C°	5.74
Phaseolus vulgaris L. varieties		
A — 328	20 C°	0.16
B — 432	20 C°	0.15
A — 328	0 C°	2.97
B — 432	0 C°	2.22
Capsicum annuum L. varieties		
A — 38—43	20 C°	0.33
B — 27—57	20 C°	0.37
A — 38—43	0 C°	4.25
B — 27—57	0 C°	3.12

(Average deviation being below ± 5 per cent; n = 3)

proline test which selected variety of plant species has higher cold and frost tolerance.

Proline content can be a quality indicator of pollens in many plants.

Investigating the isatin stained pollens in light microscope: intensive blue, deep blue and black pollen grains can be seen ("positive reaction with isatin"). These colours appear when proline concentration of the pollens is very high (2 per cent). The high proline content is correlated with high vitality because proline defends the pollens against high temperature and dryness of air as well as against cold and frost

(BRITIKOV, 1975; AHOKAS, 1978; ALARKON et al., 1978; DASHEK and MILLS, 1981; ZHANG and CROES, 1983).

Such high quality pollens appear black on the black and white photos (Fig.7).

The light greenish blue or light blue (transparent) coloured grains has low proline content and this indicates low quality. These colours are not considered as "positive isatin reaction". Many grains retain their original yellow colour in spite of staining with isatin or they show light red or brownish colours. These grains contain proline in traces only, they are of the lowest quality (Fig.7).

In Table 3. the proline concentration and the per cent values of positive isatin staining of the pollens of 36 species belonging to 12 differently developed families are shown.

It can be established that positive isatin reaction grows proportionally (from 32 to 92 per cent) to the proline concentration of the pollen extracts. The isatin reagent produces deep blue or black colours only in those grains, which contain high quantity of proline (2.0 per cent).

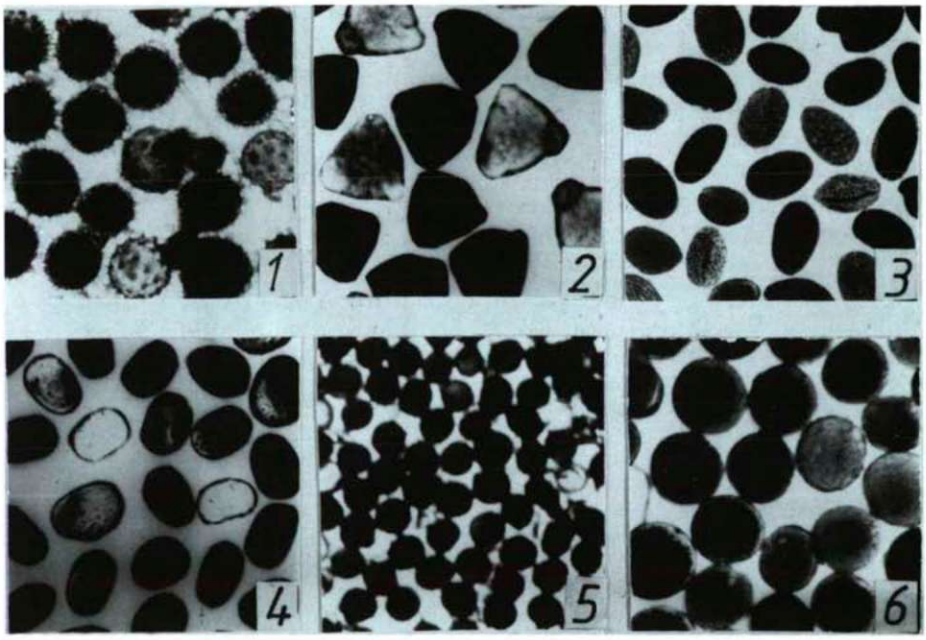

Fig. 7. Estimation of the quality of pollen grains with isatin reagent. The proline content of the deep blue and black coloured (on the picture all black) pollen grains is very high: these are pollens of excellent quality. The pollen grains coloured yellow, light brown and greenish (on the pictures grey) contain few proline, therefore they represent lower quality.

Magnified: 100—400 times.

1 = *Hibiscus rosa sinensis*; 2 = *Persica vulgaris*; 3 = *Lilium candidum*;
4 = *Secale cereale*; 5 = *Triticum aestivum*; 6 = *Zea mays*.

Table 3. Proline concentration in the dry material of the pollens and the percentage of isatin positive grains (5x100 grains were counted) of 36 monocotyledonous and dicotyledonous species. Isatine positive are the grains coloured deep blue or black. The species belong to 12 families; among them there are autogameous, anemogameous, and entomogameous too.

Families	Species	Proline concentration of the extracts	Positive reaction with isatin
		per cent	
Rosaceae	1. *Armeniaca vulgaris*	1.37	53
	2. *Pyrus communis*	1.28	44
	3. *Malus pumila*	1.39	54
	4. *Cerasus vulgaris*	1.43	57
	5. *Persica vulgaris*	1.45	55
	6. *Prunus domestica*	1.66	64
Fabaceae	7. *Trifoliun repens*	1.35	51
	8. *Medicago sativa*	1.34	50
	9. *Robinia hispida*	1.38	53
	10. *Vicia faba*	1.32	47
	11. *Pisum sativum*	1.54	61
	12. *Phaseolus vulgaris*	1.33	50
Solanaceae	13. *Solanum tuberosum*	1.38	54
	14. *S. melongena*	1.52	62
	15. *Capsicum annuum*	1.60	64
	16. *Lycopersicon escul.*	1.37	57
	17. *Nicotiana tabacum*	1.42	56
Papaveraceae	18. *Papaver somniferum*	1.45	57
Cucurbitaceae	19. *Cucumis sativus*	1.53	63
Compositae	20. *Dahlia variabilis*	1.36	52
Betulaceae	21. *Betula pendula*	1.16	35
	22. *Corylus avellana*	2.20	92
Fagaceae	23. *Fagus silvatica*	1.63	66
	24. *Quercus robur*	1.56	61
Juglandaceae	25. *Juglans regia*	1.15	32
Salicaceae	26. *Salix cinerea*	1.62	66
	27. *Populus alba*	1.25	45
Liliaceae	28. *Lilium candidum*	1.18	39
	29. *Allium cepa*	1.76	77
Gramineae	30. *Lolium perenne*	1.84	80
	31. *Secale cereale*	1.32	47
	32. *Triticum eastivum*	1.53	64
	33. *Hordeum vulgare*	1.45	56
	34. *Sorgum vulgare*	1.71	73
	35. *Zea mays*	2.22	91
	36. *Oryza sativa*	1.63	65

(Average deviation being below ±5 and ±9 %; n=3 and 8)

The grains with positive isatin reaction represent the highest quality as already is described by several authors (TUPY, 1963; STANLEY and LINSKENS, 1974; BRITIKOV, 1975; ALARKON et al., 1978; DASHEK and MILLS, 1981).

KURSAKOV and RYZHKOV, 1980, ZHANG and CROES (1983) and PÁLFI and KÖVES (1984) demonstrated that germination of pollens and elongation of their tubes in agar medium can be increased by 50 per cent by externally added proline.

According to these authors high concentration of proline stabilizes water economy and respiration, promotes germination and the elongation of tube and has a role in the synthesis of the proteins of the tube wall. Proline is at the same time the protein amino acid which has the highest solubility in water and it is not toxic to the plants even in high concentration (PÁLFI and KÖVES,1984). At the same time proline is the most stabile amino acid as well (PÁLFI and GULYÁS, 1985; GULYÁS and PÁLFI 1986).

Investigating many families it was established, however, that not every plant species has pollens with high quantity of proline; in spite of their excellent germination they contain only 0.10 — 0.25 per cent proline. These pollens are named as "non-proline-type".

Such non proline type pollens has *Coronilla varia, Brassica napus, Begonia semperflorens, Cucurbita pepo, C. maxima, Helianthus annuus, Dactylis glomerata, Bromus inermis, Poa pratensis, Holcus lanatus, Festuca pratensis* and *F. vaginata.*

Ripe pollens of most of the important cultivated plants react positive with isatin reagent due to their high proline content, they are of the "proline type". To this type belong all fruit-trees of the *Rosaceae* family (Table 3.), the most important food and forage plants of the *Papilionaceae*, the food species of the *Solanaceae*, our timber woods and all the cereals.

In plant breeding the crossing of varieties having pollens of high proline content, new varieties can be obtained which has pollens with significantly higher proline content. Several authors suppose that ability to proline accumulation is a hereditary character (BRITIKOV, 1975; AHOKAS, 1978; ALARKON et al., 1978).

Authors of this paper established also that in inflorescences (e.g. spike, panicle, calathium) ripening of the pollens begins at the different parts of the inflorescence and at the first place of dispersion are produced the grains of best quality (PÁLFI et al., 1987).

In the spikes of wheat, barley and rye the ripening of the pollens begins in the middle, in maize and rice on the upper parts of the panicle and at *Compositae* on the outher circles of the calathium. It is therefore important that comparing pollens the samples were taken from the same parts of the inflorescence.

Proline has the most important role in the defense against water deficiency and frost. Proline has the same function also in influencing the quality of pollens.

Proline can be named therefore as a "stress amino acid".

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