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DETERMINATION OF THE DROUGHT-RESISTANCE OF INBRED MAIZE LINES WITH PROLINE TEST

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Abstract

In our experiment we have started from the understanding that from among the cultivated sorts belonging to the same plant species that is more drought-resistant the leaves of which accumulate more free proline. Water deficiency in the isolated leaves was artificially induced.

1) The proline concentration in the isolated leaves of sixteen sorts of inbred maize lines, owing to water deficiency, ranged from 1 to 6.1 mg. Considering that the difference between the two extreme values is 610 per cent, it is to be established that the degrees of drought-resistance are well-differenciated by proline test. It is shown by our results that more drought-resistant hybrids can be got by cross-fertilizing with each other or with other parent partners some inbred lines that have accumulated, as a result of water deficiency, more proline.

2) In case of water deficiency of leaves, the highest concentrations were given not by proline but by asparagine. At any rate, the asparagine and total amino-acid concentrations of maize lines did not change proportionally with proline quantities.

3) In the isolated leaves, in case of seven inbred lines, a non-protein-forming amino acid, the pipecolic acid appeared but at nine lines it did not. This piperidine-alpha-carbonic acid originates from lysin, in case of a disturbance of protein synthesis.

Introduction

A great majority of the agricultural lands of our country (94 per cent) cannot be irrigated. The study of drought-resistance of the cultivated plants is, therefore, a considerable task. It has already been demonstrated by several researchers that in the soft-stalked plants, in the course of the development of a strong water deficiency, most protein-composing amino acids, particularly proline, very considerably accumulate (BARNETT and NAYLOR, 1966; KUDREV and TJANKOVA, 1966; PÁLFI et al., 1978).

It was also published that the proline content, which considerably increased in the leaves, ensured considerable biochemical advantages for drought-resistance (LEWITT, 1972; BLUM and EBERCON, 1976; PINTÉR et al., 1978).

It was established that the concentration of free proline in the leaves of plants has in every case increased in direct ratio to the degree of increase in water deficiency. The considerable increase in proline is exclusively realized as a result of water deficiency (PÁLFI, 1969).

As the considerable proline accumulation in leaves is connected with essential advantages in drought-resistence, we suppose that a higher degree of drought-resistance have the sorts that accumulate more proline as a result of a strong soil dryness at the same level. The measurement of proline can, therefore, be used for evaluating drought-resistance (PÁLFI, 1969; PÁLFI and JUHÁSZ 1971).

The linear connection between the level of proline accumulation and the degree of drought-resistance is, as it came to light, the "hereditary character" of the sorts of their hybrids within one species. It is, therefore, possible to determine the degree of the relative drought-resistance of the cultivated sorts belonging to one species — as compared with one another — with the help of the "proline test" (PÁLFI, 1969; PÁLFI and JUHÁSZ, 1971).

We have worked out experimentally on several plants that, in the course of the loss of water, free proline and total amino acid accumulate not only in the leaves of the "intact" plants from the plough-land but also in the excized but surviving leaves. It is important, at any rate, that the strong water deficiency of the isolated and laid-out leaves should develop during the functioning of photo-synthesis. The process inducing the high-level water deficiency has been named live-wilting.

The proline test, elaborated by us (PÁLFI, 1969; PÁLFI and JUHÁSZ, 1971) was first applied in Australia (SINGH et al., 1972), where — owing to water deficiency of the root medium — the highest proline concentration and, with that, the highest drough—resistance was achieved from among ten barley sorts by the so-called "Bánkuti early". The amount of crop, obtained in free field, under dry conditions, was also the largest with this sort.

The aim of this work is, to let known the results of drought-resistance at the inbred maize lines, applying the proline test. Apart from this, we also investigate into the total amount of Cd-ninhydrine positive amino acids, accumulated as a result of the strong loss of water in the isolated leaves, as well as the asparagine concentrations. We study, as well, whether a qualitative difference can be demonstrated in the composition of the free protein- building amino acids of the isolated leaves of the different sorts, as a result of the strong water deficiency.

On the basis of the investigation into 54 soft-stalked plant species, we have ranged the species into two groups: 1) those of "proline type", in the isolated leaves of which the concentration of free proline reaches — as a result of wilting — 10 mg in 1 g dry matter, i.e. 1.0 per cent, or even exceeds that (it may also be 3 to 5 per cent). 2) Those of "non-proline type" are the species in which proline does not reach, as a result of wilting, 10 mg in 1 g dry matter, i.e., it remains below 1.0 per cent (PÁLFI et al., 1978)

Materials and Methods

The preservation and selection of the internationally acknowledged inbred maize lines was carried out in the "Ságvári" Experimental Station of the Cereal Research Institute, Szeged, in agricultural loam. The signs of 16 sorts of inbred maize lines, investigated by us, are given in figures and tables.

The strong loss of water was ensured by the wilting of the isolated leaves, after the first leaves — four of each sort — above the female flowers had been isolated, in the time of 50 per cent female flowering. The total fresh weight of leaf groups was weighed. Then the matter was laid out on trays of known weight and the trays were exposed to illumination for three days in the climate chamber (4000 lux). The temperature of the air medium was regulated between 24—26 °C, the relative vapour content between 60—80 per cent. In the meantime, weighing the leaf groups every 12 hours, we have directed the tempo of their loss of water so that in three days 80—90 per cent of their water contant should be lost gradually by tanspiration (lethal water deficit) The "wilting weights" obtained thus were recorded. Then the leaves, cut into small pieces, were exsicated at 80 °C and their dry matter was weighed, as well. Finally, after being ground, the matter was well-condensed and hermetically closed in small vessels.

For the purpose of amino-acid analyses, from 200 mg of the pulverized leaves an extract was made. Homogenization was carried out with 1.0 g quartz sand and 20.0 ml 40 p.c. ethanol. After purification, the original amino-acid extracts were dry distilled, on water bath. Then the amino--acids were again solved in 40 per cent ethanol, being only one-quarter volume as compared with the original amount, that is to say, the analyses were carried out from 4 times concentrated extracts.

Proline was measured according to Chinar's (1952), the total amino acid according to Rosen's method (1957), as modified eluation-colorimetrically. The demonstration of asparagine and the detailed processes were already described (PÁLFI et al., 1978).

The average results of the fourfold repetition of analyses are published. If the difference of a measurement from the mean (deviation) exceeded ±5 per cent, the whole part analysis was repeated.

Results and their evaluation

The leaf samples of the studied 16 sorts of inbred lines were taken and elaborated in two groups of eight. In Table I, we have given the quantitative data of proline, asparagine and total amino acids of the inbred lines ranged into the first group of eight

In Table 1 we can see an "extremely high" proline concentration, produced by the line of sign V 12. In the course of our investigations carried out mainly with hybrids, such a high proline content has not been demonstrated as yet (PINTÉR et al., 1977, 1978; PÁLFI et al., 1978). Expressing our data published in mg/1 g dry matter in the percentage of the dry matter, we obtain 0.61 per cent. It is to be established that even the highest quantity is considerably below 1 per cent proline concentration, which is characteristic of the species of the "proline type".

In Table 1, we have not found any line below 6.1 meg proline content, reaching either 5 or 4 or 3 mg. The next highest proline (2.6 mg) was given by line V 41. And the lowest proline content (1.1 mg) belonged to No. V 5. At the same time, three more lines (V 46, V 14, V 24) can be ranged into the (comparatively low) interval between 1.0 and 2.0 mg. Between 2 and 3 mg, take similarly place 3 lines (V 18, V 17, V 41). These are forming the "medium" proline level.

It appears in Table 1 that, as a result of the strong loss of water of leaves, from among the amino acids and their amides asparagine has reached the highest concentration (maize is no species of proline type!). The amount of asparagine approached in several cases even half of the total amino-acid concentration of Cd-ninhydrine positive (V 5), or it reached (V 41) or even exceeded this (V 12). At five sorts of lines, the asparagine content exceeded even the largest proline quantity, i.e., 6.1 mg. If we compare the asparagine concentrations of the single lines with their own proline quantities, we twice obtain identical concentrations, in cases of the other six sorts of lines, the asparagine content considerably exceeds the proline quantities. At the single lines, however, the proline and asparagine concentrations change, as a result of the loss of water, not parallel, i.e., not in direct ratio.

In case of the total amino-acid contents of Cd — ninhidrine positive ih Table 1, it turned out, as well, that — like in case of asparagine — the larger quantities do not always coincide with the larger and largest proline concentrations. We cannot draw, therefore, any conclusion from these data in respect to the determination of drought-resistance.

Investigating into the qualitative composition of the "protein-composing amino acids", we have established that there is no difference between the lines.

The "non-protein-composing pipecolic acid" was, however, present in the extract of seven lines; in nine of these, on the other hand, it was not present.

About the appearance of pipecolic acid in the leaf tissues it was published by Diener and Decker (1954) that it takes place in the older leaves of peach in case of the infection by "Western X" virus in a considerable quantity. This was later demonstr ated by BOZARTH and DIENER (1963) about other plants, as well, suffering from other virus diseases. YATSU and BOYNTON (1959) demonstrated pipecolic acid as a result of treating strawberry with agents inhibiting protein synthesis and growth. SEHGAL and BOONE (1964) found no pipecolic acid in the leaf of normal strawberry plants but they found that in those fertilized with "Multiplier" disease.

Pipecolic acid, consisting of piperidine rings, carboxylyzed in alpha position, was first demonstrated in our country from the leaves of rice plants (PÁLFI, 1967, 1968). Mainly at the flowering of rice, in a gloomy-rainy weather, resp. in the time of a strong fall in temperature. These external conditions predispose rice to diseases. According to the data of the author, pipecolic acid results from lysin in the leaves of infected tobacco, potato and wheat plants (PÁLFI, 1967, 1968; PÁLFI and JUHÁSZ 1969) established in case of the investigated species (wheat, rye, barley, rice, maize) of the Gramineae family that pipecolic acid is the production of a disturbed protein synthesis, in case of damaging external conditions. like mainly an infection, strong loss of water, salty-alkali soil, unfavourable high or low temperature, etc. MCDONALD (1974) investigated normal potato tubers and those infected with leaf-roll viruses and always found pipecolic acid only in infected tubers. In his opinion, pipecolic acid appears as a result of the inhibited growth and the protein synthesis, disturbed by the disease.

In case of maize, the cause of the origin of pipecolic acid is not cleared up, as yet. The artificial (enforced!) self-pollination has, as it is known, an inhibitive effect on protein synthesis, resp., growth. The shoots of the inbred lines reach, namely, generally only 120—140 cm height. It may be supposed that the appearance of pipecolic acid is elicited, at some sorts, by self-pollinations.

We may think as well, that in the time of a strong pollen fall at female flowering a large amount of pollen accumulates generally in the axils of leaves below the tassel. The large mass of pollen with rough surface induces the physical lesion of leaf sheaths, the penetration of the spores of fungi and promote infections in this way. At the same time, the fast disintegrated pollen may be considered as a very good, increasing culture medium. This phenomenon can be regarded as one of the original causes of the infections by "Fusarium" species, inducing the putrefaction of the stalk, which may be connected with the appearance of pipecolic acid.

NAIK and BUSCH (1978) demonstrated that the infection of *Fusarium graminearum* is stimulated by the maize pollen. According to the authors, the dark lesions found in the leaf sheaths are induced by the deposited anthera and pollen. Starting on this track, the authors have proved that the germination of conidia is characteristically increased by pollen dialyzates. At the same time, dextrose and saccharose, as culture media — without any pollen dialysate — have only resulted in an infection in case of using the highest concentration of conidium suspension (an artificial infect-

Table 1. The Cd-ninhydrine positive total amino acid, asparagine (Asn) and proline (Pro) concentration of the isolated leaves of eight sorts of inbred maize lines, in the ratio of mg/l g dry matter- as well as the sum of these, in case of a strong water deficiency. As samples, the first leaves above the female flowers were taken. The average results of the repetitions of analyses are published. The deviations of the single repetitions from the average results (dispersion) do not exceed ±5 per cent. The data published in the mg/l g dry matter divided by ten, the results are obtained in the percentage of the dry matter.

Signs of the inbred maize lines	Cd-ninhydrine positive amino acids	Asparagine (ASN)	Proline (PRO)	Together: ASN+PRO+ +Cd-ninhydrin positive amino acids
	mg/l g dry matter			
V 5	23.1	10.4	1.1	34.6
V 12	27.0	15.2	6.1	48.3
V 18	23,1	8.0	2.5	33.6
V 46	32.7	9.6	2.0	44.3
V 14	15.2	1.6	1.6	18.4
V 17	16.0	3.2	2.2	21.4
V 24	15.2	1.6	1.6	18.4
V 41	24.0	12.0	2.6	38.6

ing agent). At this phenomenon, it is also to be taken into consideration, if the upper leaves of the stalk — as compared with the stalk itself — belong to the standing-up type. In this case, namely, a larger amount of pollen can accumulate in the leaf sheaths, for which the chance of being fertilized can increase, as well.

The quantitative data of proline, asparagine and total amino acids of the eight sorts of maize lines, taken in the second time, are given in Table 2.

It is shown by Table 2 that the asparagine concentrations are generally higher than those of the eight sorts of the earlier ripening inbred sorts in Table I. It can also be established that the degree of the asparagine content is not connected either in direct or in inverse ratio with the proline quantities of sorts.

The Cd-ninhydrine positive, protein-forming, total amino-acid contents of the maize lines, shown in Table 2, are generally also larger than the quantities in Table 1. At the same time, the interval of results is straiter. It is to be seen that there is shown no correlation with the proline concentration of lines either by the total sums of asparagines or by those of total amino acids, amino acids or amids. It is, therefore, necessary to evaluate the proline contents separately.

In Table 2, four lines achieved a proline concentration above 3, resp. 4 mg. Although the highest proline quantity was achieved at a line in Table 1, the inbred lines of Table 2 finally gave, nevertheless, higher total proline results.

In ultimate analysis, from among the 16 lines, published in Tables 1 and 2, the "high-level" drought-resistance, determined on the basis of the proline content (higher than 3 mg proline) was demonstrated in five inbred lines. For making a comparison, we mention that in the last two years we carried out an wilting proline test with 12 sorts of hybrid maize which proved good in public cultivation, as well (PINTÉR et al., 1977, 1978; PÁLFI et al., 1978), and a high-level drought-resistance was only achieved with a single hybrid (KSC 360, improved by JÁNOS NÉMETH).

Table 2. The Cd-ninhydrine positive total amino acid, asparagine and proline concentration of the isolated leaves of eight sorts of inbred maize lines investigated in group 2, as well as the sum of these, in case of a strong water-deficiency. The average results of the repetitions of the single repetitions from the average results (dispersion) do not exceed ±5 per cent.

Signs of the inbred maize lines	Cd-ninhydrine positive amino acids	Asparagine (ASN)	Proline (PRO)	Together: ASN+PRO+ Cd-ninhydrine positive amino acids
	mg/1g dry matter			
V 2	27.0	16.8	1.7	45.5
V 3	21.9	9.2	3.7	34.8
V 4	24.6	3.2	4.8	32.6
V 15	23.1	10.0	4.3	37.4
V 27	25.8	7.2	1.0	34.0
V 28	23.1	10.4	3.6	37.1
V 31	33.5	15.2	2.9	51.6
V 32	30.6	10.4	2.5	43.5

A proline concentration above 4 mg was not shown, at the same time, by any of the hybrids.

Taking into consideration that we have demonstrated in our present experiment among the investigated lines some sorts having a considerably higher drought-resistant faculty, as compared with the hybrids, after further investigations, resp. selection, the breeding of better drought-resistant hybrids can also be realized by the systematic cross-fertilization of the inbred lines of higher proline content.

The recent distribution of the proline test of drought-resistance is also worth studying.

In the United States, BLUM and EBERCON (1976) have established in the course of a strong water deficiency of eight sorts of broomcorn that the quantity of the free proline accumulated in leaves is connected with the degree of drought-resistance and this phenomenon is a phenomenon of "genotypical" regularity.

MALI and MEHTA (1977) investigated the drought-tolerance of two rice sorts, similarly on the basis of the proline accumulation in the course of water-deficiency. They have demonstrated in a medium of −10 bar osmotic pressure, in the more drought-resistant sort a 5.4 - fold increase in the proline concentration but in the sort which was more sensitive to drought-deficiency, only a 1.2-fold concentration. The authors tried to select rice-sorts of lower water-demand for the so-called "dry-cultivating" rice production.

SRINIVASA (1977) established on saft-stalked plants belonging to dicotyledons that the quantity of free proline grew in the leaf of every "genotype", as a result of water stress, proportionately to the degree of drought-tolerance. SASHIDBAR (1977) carried out drought-resistance tests on ten sorts of ground-nuts, on the basis of the proline test. He has demonstrated that, as a result of a strong water deficiency of uniform level, the droughtresistant forms accumulate considerably more proline than those being more sentivive to water-deficiency and that this character of the sorts is "genotypic".

The theory and practical effectuation of the proline test of drought-resistance was first elaborated and published in our country (PÁLFI, 1969; PÁLFI & JUHÁSZ, 1971). The proline test — in fact, by different varieties of inducing water-deficiency — has since then been spreading all over the world. At the same time, it is to be mentioned that the proline test was first applied in the practical plant-improving work, on ten sorts of barley, by SINGH et al (1972), on maize by PINTÉR et al. (1977), and on rye and yellow lupine by PÁLFI et al. (1978).

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