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ENHANCEMENT OF THE DEGREE OF DROUGHT-RESISTANCE IN VARIETIES BY CROSSING AND SELECTION ON THE BASIS OF PROLINE-CONTENT

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Abstract

It has been determined that from the cultivated varieties (inbred lines and hybrids) belonging to one species, that has the highest level of drought-resistance which accumulates the largest amount of free proline in the isolated leaves, as the consequence of the lethal water deficiency developing gradually within three days. From 36 types of inbred corn lines the isolated leaves of the corn line "Le 60" showed the highest proline concentration developing on the effect of lethal — thus the same internal — water deficiency, provoked by live-wilting. An artificial population was established by the three way crossing (TC) of this line and two related ones, and for a period of two years proline selection was carried out individually on the offspring plants. Studies on the 64 individuals of the „S_o" and „S_1" generations demonstrated that the drought-resistance of the inbred lines can be increased significantly by crossing and individual selection, on the basis of the degree of proline accumulation.
Key words: *Zea mays*, drought-resistance, proline-content

Introduction

Several researchers have found that on the effect of strong water deficiency the degree of drought-resistance of certain varieties of soft-stalked plant species is directly proportional to the proline concentration synthetized and accumulated in the leaves, if the same level of „internal water deficiency" is produced in the plants (BLUM and EBERCON, 1976; BRITIKOV, 1975; GOAS, 1966; HUBAC and GUERRIER, 1972; LEWITT, 1972; MALI and MEHTA, 1977; SASHIDHAR et al. 1977; SINGH et al. 1972; SRINIVASA, 1977; VAN DE DIJK, 1981).

FLOWERS et al. (1977) as well as STEWART (1971, 1972) interpret proline accumulation as the compensation of the osmosis potential, and with the fact that proline is the only amino acid which does not inhibit the activity of the enzymes even in a rather high concentration.

According to GÖRING and THIEN (1979) the increase in the proline concentration of leaves produces the longer staying of proteins in solution and strores such reducing energy which after the ceasing of waters tress appears in the form of NADH-H^+ during the course of the re-development of proline into glutamic acid.

PÁLFI et al. (1975) studied the proline accumulation characteristics of 46 soft-stalked, mainly cultivated plant species belonging to 14 plant families — their studies being related to the degree of water deficiency. Authors determined that on the effect of the same highleveled, lethal water deficiency even the species belonging to one family synthetize and accumulate completely differing amounts of proline. On the effect of water deficiency, therefore, the degree of proline accumulation is firstly a characteristic of species. Thus, the level of proline content and the degree of drought-resistance are only correlated in the case of cultivated types belonging to the same species, and in the case of subspecies and varieties, respectively.

PINTÉR et al. (1978, 1979, 1981), PÁLFI (1969), PÁLFI and JUHÁSZ (1971), PÁLFI et al. (1973, 1978), PÁLFI and PINTÉR (1980) studied the drought-resistance of paprika, sunflower, maize, lupine and rye with the help of the proline test. They studied 36 inbred corn lines and 12 hybrid corns. The inbred line having the highest proline concentration was crossed with two related corn lines and thus, an artificial population; then the "S_0" and "S_1" generations of this were established. The aim of the present study is to examine the drought-resistance of the two generations of the artificial population with the help of live-wilting and proline test, resp. By this means it can also be cleared whether the degree of drought-resistance of the inbred lines can be enhanced with the new method, that is, with crossing and selection on the basis of proline concentration.

Materials and methods

From the studied 36 inbred corn lines the "Le 60" line gave the highest proline concentration — 6.25 mg in 1 g dry-matter — on the effect of lethal water deficiency. "Three way cross", i.e. "TC" with (Le 60 x Le 24) x Le 28 was carried out with this line. The latter two lines are related to the "Le 60", having similar agronomical characteristics to it, and and weaker in respect of drought-resistance and the degree of proline accumulation. The proline accumulation caused by lethal water deficiency was 2.10 mg in the case of "Le 24" and 2.46 mg in the case of "Le 28" in 1 g dry-matter.

As in the previous experiments (PÁLFI et al. 1978; PÁLFI and PINTÉR, 1980; PINTÉR et al. 1978, 1979, 1981), for live-wilting the first leaf above the completely developed carpellary inflorescence was cut form each plant in the present study, too. From the "S_0" generation of the above-mentioned three way crossing, one leaf from each of a total of 64 plant individuals was cut off (taking a group of 16 plants four times according to the time of flowering). Using the method of live-wilting sublethal, then lethal water deficiency was produced gradually within 3 days — under constant light (5000 lx), at 24 C, in the isolated leaf samples. The moisture of the substance of the leaves exposed with their reverse sides upwards and fixed with transparent scotch tape (Fig. 1) was regulated, so that the water loss of the leaves was 20—25% within 24 h, 45—50% within 48 h (sublethal), and 65—75% within 72 h (lethal). By this means the "internal water deficiency" of each leaf after the three days was completely equal, that is, of lethal level. Then the leaves were separately cut into little dried pieces within 4—5 h, at 80°C. (air-dried material) and ground to dust. The proline determinations of the amino acid extracts were carried out with spectrophotometry according to the method of TROLL

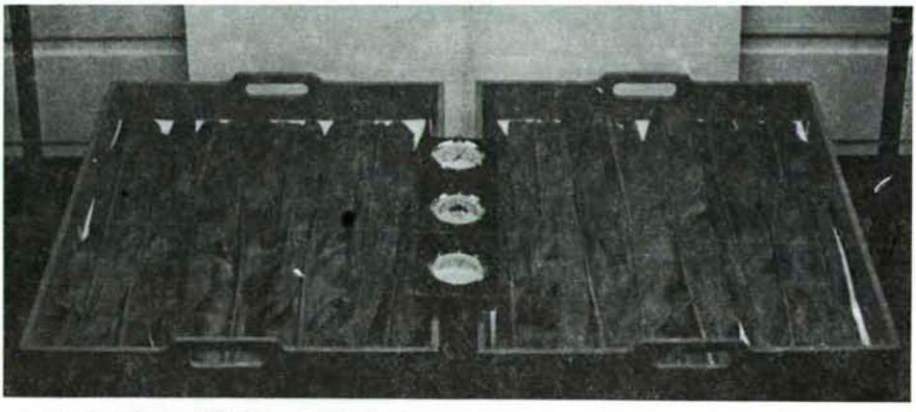

Fig. 1. The lethal water deficiency of the isolated corn leaves was gradually produced by the method of live-wilting. The leaves were placed in the trays close to each other so that they were situated with their shoulders on the opposite sides alternately, and with their reverse sides being upwards. The photosynthesis was functioned by illumination. The moisture of the air was daily set to 90, 80, and 60% during the three days. On the third day the lethal water deficiency, i.e. the equal level of "internal water deficiency" was observable in the case of every studied leaf.

and LINDSLEY (1955). The method of CHINARD (1952) was also applied for controlling. The demonstration of free proline was repeated four times, and if any of the repetitions varied from the average result by ±3% the analysis of the whole group of 16 plants was repeated.

From the 64 plant individuals of the "S_0" generation studied by leaf-analysis, crops of those 6 plants were separated where the cut off one-one leaf showed the highest proline concentration on the effect of lethal water deficiency provoked by live-wilting.

In the next vegetation year only the seeds of these 6 crops were sowed, and the plants developping from the seeds formed the "S_1" generation.

At the time of complete development of the carpellary inflorescence of the "S_1" generation plants 1—1 leaf specimen was taken again from 64 plant individuals and after the development of lethal water deficiency provoked by live-wilting the proline measurements were carried out again on the dried and crushed material. Then the results obtained in the case of the "S_0" generation were compared with those of "S_1".

Apart from the proline determinations, the quality analysis of certain amino acids and the measurements of the free protein-building total amino acids were also performed. The applied methods have already been reported earlier (PÁLFI and JUHÁSZ, 1971; PÁLFI et al. 1973, 1978; PÁLFI and PINTÉR, 1980).

Results and discussion

It has been determined that no quality changes appeared in the isolated leaves of the 64 plants of the artificial corn population on the effect of strong water deficiency in regard of the free amino acids. However, significant differences were demonstrated in the total amino acid concentration of the live-wilted leaves. Nevertheless, it became evident that the differences did not show relationship with the degree of proline accumulation.

From the amino acids and their amides the highest values were given by the concentration of asparagine and not proline. Furthermore, the amount of glutamine was also significant and was in many cases found to be accumulated in a higher amount than proline. Taking into consideration that no correlation was found between the degree of concentration of proline and other amino acids, and amides, resp. only the proline amounts are reported in relation to drought-resistance (Table 1).

Adding separately the proline concentration of the leaves of the 64 corn individuals according to the two generations (first and second year), it can be seen from the Table 1. that a significantly larger amount was obtained in the case of the „S_1" generation than in the „S_0".

Taking the average of the amounts yearly, according to the 64 plants, a proline concentration of 2.82 mg in the case of the „S_0" generation, and of 3.21 mg in the case of the „S_1" generation was demonstrable.

Taking the proline average of the „S_0" generation as 100%, the growth of the „S_1" generation was 13.83%.

It could be determined therefore that the proline concentration and degree of drought-resistance, resp. could be enhanced in the isolated leaves of the studied artificial corn population plants, by the means of selection according to the proline concentration caused by strong (lethal) water deficiency.

It was worthwhile of studying that, as a matter of fact, what significant changes took place between the plants of the „S_0" and „S_1" generations shown in Table 1, as the consequence of the selection carried out on the basis of the proline concentrations (on the effect of the provoked strong water deficiency). For comparison the proline concentrations of the 64 corn plants were divided into qualification categories per mg. The intervals between the highest and lowest proline concentrations received in the 36 inbred corn lines studied by us so far were taken as a base in forming the categories, independently of whether individuals occurred or not in the different categories in our present experiment (PÁLFI et al. 1978, 1980; PINTÉR et al. 1978, 1979).

No.	Proline concentration, in mg/1 g dry-matter		No.	Proline concentration, in mg/1 g dry-matter	
	S_0	S_1		S_0	S_1
1.	2.50	3.42	33.	2.59	2.08
2.	2.25	1.47	34.	3.05	2.53
3.	1.91	3.24	35.	2.81	2.57
4.	3.50	1.93	36.	2.54	2.83
5.	4.19	2.29	37.	2.45	1.96
6.	3.35	2.34	38.	2.59	5.58
7.	3.04	3.56	39.	4.14	2.10
8.	3.08	3.60	40.	2.95	3.14
9.	3.56	2.34	41.	4.24	2.21
10.	2.21	3.26	42.	4.12	2.35
11.	2.59	2.20	43.	2.84	2.89
12.	3.02	2.74	44.	2.12	2.66
13.	1.69	1.35	45.	4.03	2.43
14.	2.12	1.84	46.	2.75	1.89
15.	2.12	3.15	47.	2.18	5.10
16.	3.06	3.58	48.	3.07	4.12
17.	2.09	2.65	49.	2.70	5.10
18.	2.09	4.18	50.	4.64	5.24
19.	1.84	3.70	51.	3.56	1.75
20.	2.15	4.14	52.	3.53	2.65
21.	1.83	5.13	53.	3.51	4.10
22.	2.11	6.21	54.	2.93	2.16
23.	2.28	3.53	55.	3.15	3.38
24.	2.58	1.30	56.	1.94	2.12
25.	2.56	3.15	57.	2.54	2.10
26.	2.14	2.38	58.	5.63	6.88
27.	1.70	1.26	59.	2.97	4.45
28.	1.73	4.45	60.	2.59	5.16
29.	2.46	4.18	61.	2.61	4.59
30.	2.75	2.47	62.	2.07	3.60
31.	3.58	3.10	63.	2.93	4.06
32.	3.63	3.10	64.	3.15	4.10
	81.71	97.24		98.92	107.88

From 1 to 64, total: $S_0 = 180.63$ $S_1 = 205.12$
Average of the 64 leaves: $S_0 = 2.82$ $S_1 = 3.21$

The proline growth average of the generation compared: 13.83%

Table 1. Free proline concentration of leaves from 64 individuals of the "S_0" and "S_1" generations of the artificial population of the related, inbred corn lines, on the effect of strong (lethal) water deficiency.
From the individual plants No. 1 to 64 the number of proline mg-s totalized was 180.63 mg in case of "S_0"; and 205.12 mg in that of "S_1". From this the average of the 64 leaves was 2.82 mg in "S_0" and 3.21 mg in "S_1". The proline growth average of the "S_1". generation was 13.83% compared to the "S_0".

The category "rather high" appears three times between the 4 and 7 mg because in the case of our studies in the first year 4.3 mg was the highest proline concentration, and in the following years proline amounts above 5 and 6 mg were also obtained in the case of other lines. During the course of our experiments so far, however, no line has reached a proline level above 7.0 mg as yet.

The qualification category of rather low proline amount, i.e. under 1.0 mg can be seen in Table 2, since in the 36 inbred corn lines studied so far, such a low proline concentration has been observed in 4 lines.

It can be determined from Table 2 that the groups formed according to proline

Groups according to proline concentration, in mg/1 g dry matter	No. of individuals divided into groups		Qualification on the basis of proline amount
	S_0	S_1	
6 and 7 mg between	—	2	„Rather high"
5 and 6 mg between	1	6	„Rather high"
4 and 5 mg between	6	10	„Rather high"
3 and 4 mg between	16	15	„High"
2 and 3 mg between	34	22	„Moderate"
1 and 2 mg between	7	9	„Low"
0.5 and 1 mg between	—	—	„Rather low"
Total	64	64	individuals

Table 2. The isoated leaves of the 64 individuals of the "S_0" and "S_1" generations from the artificial corn population were divided into qualification categories on the basis of their proline concentration, what was provoked by the gradually developing lethal water deficienc of the leaves. Groups (categories) according to proline concentration in mg/l g dry matter. Numbe- of individuals divided into groups. Qualification according to the amount of proline: "Rare her high", "High", "Moderate", "Low", "Rather Low". A total of 64 individuals; betwetn 0.5—1 mg—1—2 mg—6—7 mg.

amounts show significant variations in the "rather high" category. 7 plants could be divided into this category in case of the "S_0" generation, and 18 in that of the "S_1". generation. The "S_0" and "S_1" generations did not show essential changes in the "high" and "low" categories. In contrast to this, there was a significant decrease in the amount of plants divided into the "moderate" category from the "S_1" generation, compared to the "S_0". Furthermore, the number of plants which could be divided into the "moderate" category in the case of the "S_1" generation (12) showed such a decrease, compared to which the number of plants from the same generation which could be divided into the "rather high" group showed an increase of almost the same amount (11). It can be concluded from this that the significant amount of plants from the "S_0" generation belonging to the „moderate" category immediately entered the

"rather high" group after proline selection; overstepping the group qualified as "high". Significant progress was obtained, therefore, by using the method of selection on the basis of proline.

VAN DE DIJK (1981) pointed out that it is not enough to produce an „external water deficiency" of the same level in the substance of the plant varieties to be studied, since this would cause various degrees of "internal water deficiency" in the plants having different grought-resistance. The consequence of this would be that due to the higher level of "internal water deficiency" of the less drought-resistant variety a higher amount of free proline would be synthetized and accumulated than in the case of the variety with higher drought-resistance (the internal water deficiency of which would be lower at this stage). A method was elaborated by the author with which the same level of "internal water deficiency" could be produced in the case of the plant variety to be studied.

With our live-wilting method (PÁLFI, 1969; PÁLFI and JUHÁSZ, 1971; PÁLFI et al. 1973, 1978; PÁLFI and PINTÉR, 1980; PINTÉR et al. 1978, 1979) the water deficiency of the isolated leaves developed gradually, reaching the sublethal state within 2 days, and by the third day the leaves of each studied plant reached the lethal level, by which time the „internal water deficiency" had no physiological significance any more.

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