COMPARATIVE STUDY ON THE PHYSIOLOGY OF NATURAL POLYPLOIDS

I. The influence of various hydrogen ion concentrations upon the growth and productivity of Puccinellia distans 2n=42, and P. limosa 2n=28.

BY

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INTRODUCTION

Results of cytogeographical research call our attention to contradictions between the physiological characteristics of natural and artificial polyploids. As opposed to the dynamic, resistant, large-sized, abundantly productive natural polyploids, adaptable to extremes in life conditions (see the literature cited ap. FELFÖLDY, 1948), the diminished vitality of the artificially induced polyploid, with its disturbed propagation and often smaller yield than the diploids, has been a great disappointment to the practical specialists working with colchicine (Cf. GYÖRFFY, 1941, NOGGLE, 1946). Research work was hence at first obliged to turn to the natural polyploids, to discover the secret of their better quality, so as to be able to use our experiences to induce artificial polyploids.

The following series of experiments was also conceived with this idea in mind. At the same time the solution of certain plant georgraphy problems could be expected from it, which we wished to make use of for experimental ecology.

In Hungary three species of the genus *Puccinellia* are to be found (JÁVORKA, 1937:34), besides the endemic and rare *P. Peisonis* BECK, the *P. distans* (JACQ.) PARL. and *P. limosa* SCHUR. Some authors mention the latter as a ssp. of *P. distans*, called *P. distans* (JACQ.) PARL. *ssp. limosa* (SCHUR.) JÁV. On the basis of chromosome investigations and their morphological characteristics we can consider them separate species.

Three different chromosome races are given in the literature under the name of *Puccinellia distans*:2n=14,42 AVDULOV, 1931, and 2n=28 STÄHLIN, 1929, TARNAVSCHI, 1939. PÓLYA (1947) mentions a 2n=28 *ssp. limosa* in plants from the Hortobágy. We found 2n=42 for *P. distans* at Tihany and for *P. limosa* 2n=28 at Hortobágy among our experimental plants.

EXPERIMENTAL PROCEDURE

Among the plants examined *P. distans*, with 2n=42 chomosomes, derived from the alkali-soil meadow of the Tihany Külső-tó. We planted it in our experimental garden in May, 1947. Mr. L. PÓLYA brought the *P. limosa* from the Hortobágy in June, 1947, and it was also planted in our garden. The plants produced a crop there and the seeds from it were planted out of doors in the autumn of 1947, and from there the plants were transferred on April 17th, 1948, to water-culture vessels. Their initial weight:

Puccinellia distans 2n=42:2.26±0.17
P.limosa 2n=28: 2.31±0.08

We used 1 l glass jars as water-culture vessels, painted first with black, then with white Trinat enamel, and washed out with diluted hydrochloric acid and distilled water.

In pH experiments we may expect exact results only if we can assure constant hydrogen ion concentrations in the vessels for several weeks. This can be provided in water or sand cultures only by constantly flowing, always changing fluids (PIRSCHLE, 1939:584), which, however, we were obliged to disregard for technical reasons. But as the life processes of the roots of the plants change the pH of the nutrient solution, it was necessary to control it constantly and change it in case of need.

Composition of the initial solution:

0.2 g potassium dihydrophosphate,
0.1 g sodium chloride,
0.1 g magnesium sulphate cryst.
0.2 g potassium nitrate,
0.4 g sodium nitrate,
3 drops 5% ferric chloride,
1 ml A—Z solution according to HOAGLAND.

To obtain the desired pH values the following quantities of acids or alkalis were necessary:

To 1 liter solution:

n-HCl	pH 4:	6.09 ml
,,	pH 5:	5.45 ,,
,,	pH 6:	4.54 ,,
n-NaOH	pH 7:	1.00 ,,
,,	pH 8:	3.59 ,,
,,	pH 9:	6.00 ,,

The pH was established in each case to an exactude of ±0.1. The nutrient solutions were mixed from the initial solutions in an 11 liter bucket to assure homogeneity. The pH was measured electro-metrically by quinhydrone electrode with calomel cell, with a SZONNTÁGH valve potentiometer. The solutions were controlled daily and if a difference of 0.5 was found in a vessel, the solutions in all of them were changed.

Dates:

Beginning:	April	16, 1948.
I. change	,,	23
II. ,,	,,	30
III. ,,	May	7
IV. ,,	,,	12
V. ,,	,,	15
VI. ,,	,,	18
Flowering began	,,	20
VII. change	,,	21
Harvested	,,	25

The experiment lasted for 40 days, during which the grasses, with the exception of those with pH 4 solutions, grew into stalks and blossomed. The tetraploid *P. limosa* began flowering a few days earlier, indeed at the end of the experiment it began to lose its anthers, which is a sign that blossoming is over.

There were 10 vessels in each series, each containing 2 plants, so the measurements and analyses were calculated from the average of 20 plants, except when some specimens died.

On harvesting them we measured the length of the shoot the fresch weight, then killed them is a drying chamber, one hour at 120° C, and dried them to constant weight at 90—10° C, to establish dry weight and dry content. The powdered samples were kept in paper bags in an exsiccator filled with $CaCl_2$ until analysed. The total nitrogen was determined by the KJELDAHL semi-micro method in a PARNASS-WAGNER apparatus. For calcium determination the dry matter was reduced to ash in a platinum crucible in an electric oven, the ash dissolved in hydrochloric acid and the Ca in it determined by MURER's (1937) method.

The measurements and analyses proved that the optimal pH-values for the plants could be determined only by taking several characteristics into consideration. The results of the measurements must also be evaluated statistically, so that the real optimum can be distinguished from the apparent. We made the statistical calculations with the following formulae:

$$M = \frac{\Sigma x}{n}, \text{ where}$$

M=the mean value, x=the members of the series observed or measured, n=the number of members.

$$\sigma = \sqrt{\frac{\Sigma(d^2)}{n-1}}, \text{ where}$$

σ =the standard deviation, d=the deviation of the different members from the mean value, without regard to plus or minus, n=number of members.

$$m = \frac{\sigma}{n}, \text{ of which k, the significant difference:}$$

$$k = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}}.$$

If $k > 2$ there is probably a statistical difference between the two mean values, if $k > 5$ then there is certainly a significant difference between them.

EXPERIMENTAL RESULTS

Length of shoot

The values in respect to length of shoot are summarized in Table I. Analysis of variance showed that the differences in lengths of the shoots are very significant in the case of the tetraploid *P. limosa.* The optimum at pH 9 is probable (the k values are: 8.58, 3.26, 5.00, 3.21, 3.96). In the case of pH 5—8 there is no difference statistically ($k=$

0.12—1.00). The hexaploid species is much more uniform; with 5—9 pH values there is no difference in the length of the shoot. *P. distans* (2n=42) was in all cases taller than P. limosa (2n=28). (Figure 1)

TABLE I.

Length of shoot (cm).

	Puccinellia limosa 2n = 28				*Puccinellia distans* 2n = 42		
pH	cm	σ	m	pH	cm	σ	m
4	32.8	11.06	3.68	4	38.0	6.22	2.07
5	60.2	3.83	1.27	5	64.8	13.82	4.60
6	59.0	2.82	0.94	6	67.0	2.61	0.87
7	60.5	3.56	1.18	7	65.0	5.32	1.77
8	60.0	3.28	1.02	8	69.0	7.54	2.51
9	65.0	2.28	0.76	9	65.4	2.49	0.83

	k						k				
pH	5	6	7	8	9	pH	5	6	7	8	9
4	7.04	6.91	7.17	7.1	8.5	4	5.3	12.9	9.9	9.5	12.0
5	—	0.7	0.1	0.1	3.2	5	—	0.4	0.04	0.8	0.1
6	—	—	1.0	0.7	5.0	6	—	—	1.0	0.7	1.3
7	—	—	—	0.3	3.2	7	—	—	—	1.3	0.2
8	—	—	—	—	3.9	8	—	—	—	—	0.1

FRESH WEIGHT

The data in Table II show two optima for fresh weight in the tetraploid species (pH 5 and 9). The optimum at pH 5 cannot be confirmed statistically (σ=1.82, k=0.43—0.95) but the optimum at pH 9 is

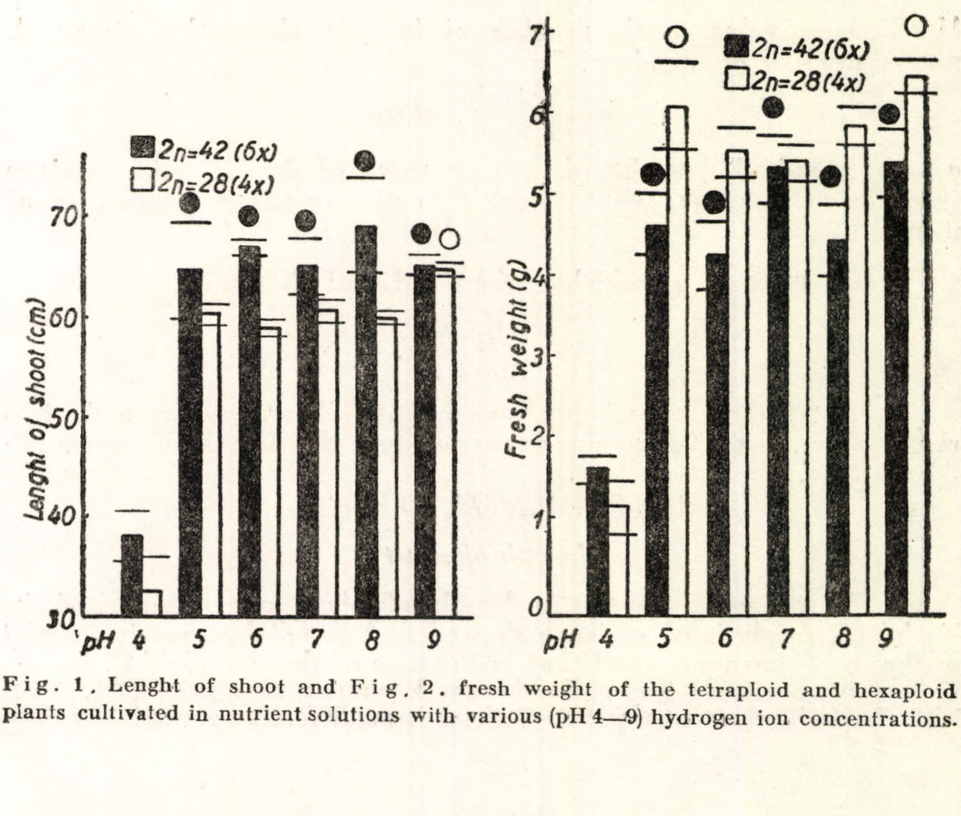

Fig. 1. Lenght of shoot and Fig. 2. fresh weight of the tetraploid and hexaploid plants cultivated in nutrient solutions with various (pH 4—9) hydrogen ion concentrations.

very probable (σ=0.67, k=13.6, 0.63 — as related to pH 5 — 2.61, 3.29, 2.19). In the hexaploid species we find cases, as in the length of shoot, where there is no significant difference (k=0.21—1.37) between the values of plants raised in the pH 5—9 nutrient solutions. It is interesting that from the standpoint of fresh weight the tetraploid species surpasses the hexaploid, which can be explained by the greater bushiness of *P. limosa.*

TABLE II.

Fresh weight (g).

Puccinellia limosa 2n = 28				*Puccinellia distans* 2n = 42			
pH	g	σ	m	pH	g	σ	m
4	1.16	1.03	0.34	4	1.62	0.51	0.17
5	6.10	1.82	0.60	5	4.68	1.20	0.40
6	5.51	1.03	0.34	6	4.24	1.40	0.46
7	5.48	0.76	0.25	7	5.26	1.44	0.48
8	5.82	0.76	0.25	8	4.44	1.44	0.48
9	6.50	0.67	0.22	9	5.28	1.39	0.46

	k						k				
pH	5	6	7	8	9	pH	5	6	7	8	9
4	7.2	9.0	10.2	11.4	13.6	4	3.3	2.7	3.8	2.9	3.8
5	—	0.8	0.9	0.4	0.6	5	—	0.3	0.5	0.2	0.5
6	—	—	0.7	0.7	2.6	6	—	—	0.8	1.7	0.9
7	—	—	—	1.0	3.2	7	—	—	—	0.7	0.5
8	—	—	—	—	2.1	8	—	—	—	—	0.7

DRY WEIGHT AND DRY CONTENT IN %

The weight of dry matter is given in Table III and Figure 3 and the dry content of fresh weight (%) in Table IV and Figure 4. The values in dry weight change similarly in both species, for in both of them the optimum pH falls at 7—8—9. But the dry weight of the tetraploid species is considerably greater than that of the hexaploid. In *P. limosa* (2n =28) an apparent optimum can also be observed at a pH 5 value (Figure 3).

TABLE III.

Weight of dry matter (g).

Puccinellia limosa 2n = 28				*Puccinellia distans* 2n = 42			
pH	g	σ	m	pH	g	σ	m
4	0.28	0.18	0.06	4	0.33	0.10	0.03
5	1.40	0.47	0.16	5	1.00	0.20	0.06
6	1.34	0.34	0.11	6	0.96	0.19	0.06
7	1.76	0.40	0.13	7	1.20	0.18	0.06
8	1.78	0.29	0.09	8	1.16	0.22	0.07
9	1.62	0.24	0.08	9	1.28	0.32	0.10

	k						k				
pH	5	6	7	8	9	pH	5	6	7	8	9
4	6.5	5.5	10.5	15.0	13.4	4	10.0	9.4	12.9	10.9	9.1
5	—	0.3	1.8	2.1	1.2	5	—	0.4	2.3	1.7	2.4
6	—	—	2.4	3.1	2.1	6	—	—	2.8	2.1	2.7
7	—	—	—	0.1	0.9	7	—	—	—	0.4	0.6
8	—	—	—	—	1.3	8	—	—	—	—	0.9

In respect to dry matter content the species with 2n=28 chromosomes had a remarkably low value at pH 9, so that the optimum occurs at pH 7 and 8. In *P. distans* we find the optimum at pH 8 and 9 (Figure 4).

TABLE IV.

Dry content as % of fresh weight.

	Puccinellia limosa 2n = 28				*Puccinellia distans* 2n = 42		
pH	%	σ	m	pH	%	σ	m
4	15.2	3.63	1.21	4	19.4	5.58	1.86
5	23.4	4.43	1.47	5	21.8	2.18	0.72
6	26.1	2.60	0.86	6	23.5	3.05	1.01
7	30.0	3.16	1.05	7	21.9	0.95	0.31
8	29.5	4.64	1.55	8	25.7	2.08	0.69
9	25.3	3.54	1.18	9	24.5	2.26	0.75

	k						k				
pH	5	6	7	8	9	pH	5	6	7	8	9
4	4.3	6.1	9.2	7.2	6.0	4	1.2	1.9	1.5	3.1	2.5
5	—	1.5	4.4	2.8	1.0	5	—	1.3	0.1	3.9	2.5
6	—	—	2.8	1.9	0.5	6	—	—	1.5	1.8	0.8
7	—	—	—	0.2	2.9	7	—	—	—	5.0	3.2
8	—	—	—	—	2.1	8	—	—	—	—	1.1

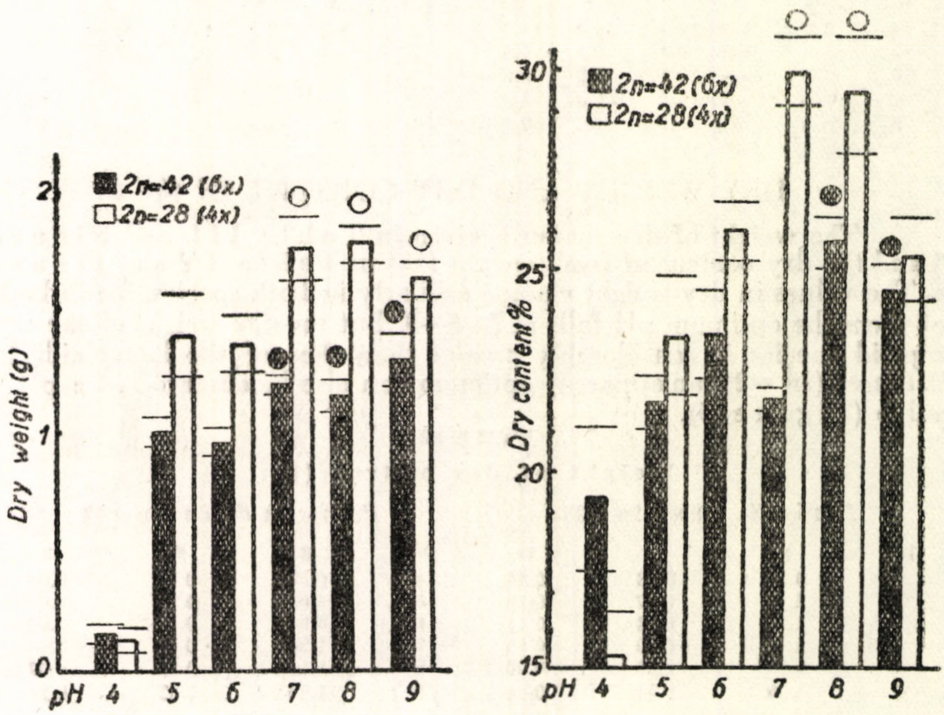

Fig. 3. Dry weight, Fig. 4. dry content and their standard deviations, in the tetraploid and hexaploid plants.

ASH CONTENT

The percentual ash content of the dry matter is summarized in Table V. In the tetraploid species an optimum can be seen (pH 9, the values of which, however, refer only to statistical probability: k=2.20, 4.12, 1.96, 2.30. In the other data there is no such difference (k=0.46—

1.06). In the hexaploid species we found the optimum at the usual places (pH 7—8—9). (Figure 5)

TABLE V.

Ash content of dry matter (%).

	Puccinellia limosa $2n = 28$				*Puccinellia distans* $2n = 42$		
pH	%	σ	m	pH	%	σ	m
5	8.07	1.22	0.43	5	9.27	1.15	0.47
6	8.35	0.65	0.23	6	8.65	0.44	0.22
7	7.60	0.55	0.20	7	6.86	0.86	0.32
8	7.59	0.42	0.14	8	7.49	0.83	0.37
9	6.99	0.61	0.25	9	7.51	1.06	0.35

		k					k		
pH	6	7	8	9	pH	6	7	8	9
5	0.5	1.0	1.0	2.2	5	1.1	4.3	3.0	4.8
6	—	25.0	29.2	4.1	6	—	4.7	2.6	2.7
7	—	—	0.4	1.9	7	—	—	1.3	1.3
8	—	—	—	2.3	8	—	—	—	0.04

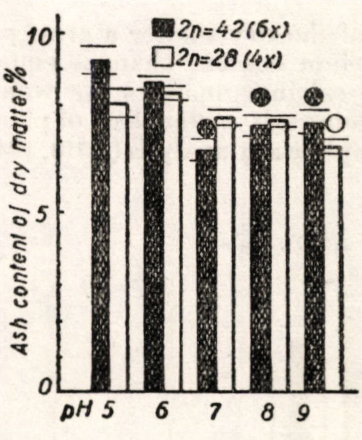

Fig. 5. Ash content of dry matter.

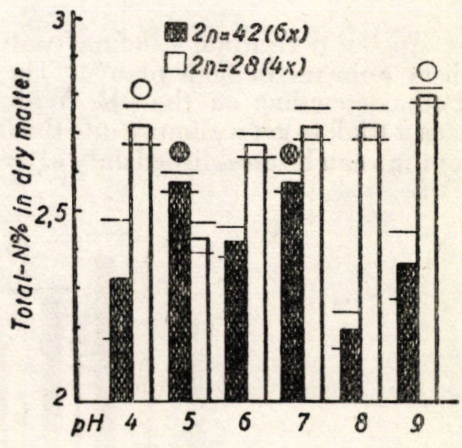

Fig. 6. Total nitrogen content in % of dry matter.

TOTAL NITROGEN

The total nitrogen content of the dry matter of the two species is very interesting. In *P. limosa* we find the most nitrogen at values of pH 4 and pH 9, and in P. distans at pH 5 and 7. The low values of *P. distans* at pH 8 and 9 are very remarkable and may be connected with its ash content. Both in the case of ash and fresh weight pH 7 can be seen to be the apparent optimum (Figure 6).

TABLE VI.

Total nitrogen content of dry matter (%).

	Puccinellia limosa 2n = 28				*Puccinellia distans* 2n = 42		
pH	%	σ	m	pH	%	σ	m
4	2.72	0.21	0.05	4	2.33	0.54	0.16
5	2.43	0.14	0.04	5	2.57	0.11	0.03
6	2.67	0.11	0.03	6	2.43	0.14	0.04
7	2.72	0.12	0.04	7	2.57	0.06	0.02
8	2.72	0.14	0.04	8	2.19	0.15	0.05
9	2.80	0.05	0.01	9	2.36	0.27	0.09

	k					k				
pH	5	6	7	8	9	pH	5	6	7	8	9
4	4.1	0.7	0.0	0.0	1.4	4	15.0	0.5	15.0	8.2	0.1
5	—	4.4	5.3	4.6	7.8	5	—	2.5	0.0	7.0	2.2
6	—	—	0.9	0.9	3.6	6	—	—	2.9	3.8	7.0
7	—	—	—	0.0	2.2	7	—	—	—	7.9	2.3
8	—	—	—	—	1.7	8	—	—	—	—	17.0

CALCIUM

In the percentual calcium content of the dry matter a great parallelism appears in both plants. The calcium content changes equally in both, depending on the pH. With the calcium analyses we wanted only as a trial, to get a glimpse into the inorganic salt metabolism of plants; the optima can be established only after complete ash analysis (ILJIN, 1948),

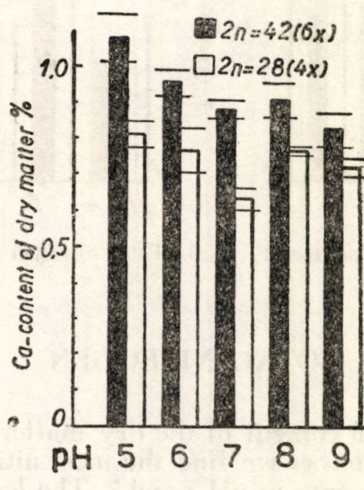

Fig. 7.

which we were obliged to omit because our material was used up. As we see in Figure 7, the hexaploid species exceeds the tetraploids in respect to Ca content.

TABLE VII.

Calcium content as % of dry matter.

	Puccinellia limosa 2n = 28				*Puccinellia distans* 2n = 42		
pH	%	σ	m	pH	%	σ	m
5	0.81	0.12	0.04	5	1.07	0.18	0.07
6	0.76	0.25	0.08	6	0.96	0.08	0.04
7	0.64	0.09	0.03	7	0.89	0.07	0.03
8	0.77	0.04	0.01	8	0.91	0.11	0.05
9	0.72	0.05	0.02	9	0.83	0.14	0.05

DISCUSSION

If we want to establish the effect of the hydrogen ion concentration in the nutrient solution of the two species under investigation, we must sum up the optima of the characteristics observed. The simplest method for doing this can be seen in Figure 8. In Figures 1—6 we marked with small circles the statistically certain or probable optima. I transferred these circles to Figure 8: Black centers=*P. distans*, 2n=42; white centers=*P. limosa* 2n=28. From this drawing conclusions can be drawn:

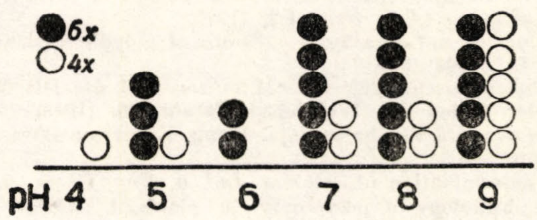

Fig. 8. The optima of the characteristics observed. (See text p. 131.)

The ecological amplitude of the hexaploid, i. e. higher polyploid P. *distans* is wider: it exists equally well at pH 7 to 9. Its optimum at the lower pH value is indistinct, it cannot be proved statistically. Conversely, the ecological amplitude of the tetrapoid *P. limosa* is narrow. Its optimum at pH 9 is very definite. It is worthy of notice that its curve thus has two peaks; we do not find an optimum at pH 6. The two optima, very distant from one another (pH 4—5 and 9) have very likely some connection with the isoelectric point of the plasma (BUCHNIČEK, 1946).

It is very interesting that though *P. distans* is a taller plant, *P. limosa* produces more, whether in fresh weight, dry weight or dry content. The nitrogen and protein contents of the tetraploid are greater than that of the hexaploid, which, however, may be due to different stages of blossoming in the flowering specimens. *P. limosa* had already begun to lose its anthers by the end of the experiment, which is an indication of an advanced stage of flowering and of the seeds' being older.

There is no great difference in ash content between the different pH series, nor between the two species. Besides the length of shoot, its calcium content was a characteristic in which the hexaploid exceeded the tetraploid.

The ecological rôle of the two species is also comprehensible from their behaviour respect to pH. *P. limosa* is a plant typical in an alkali meadow of variable humidity (pH=9.30—8.25, UJVÁROSI, 1937:173). *P. distans* has a much more extensive dispersal, corresponding to its greater degree of polyploidy, forming associations on the muddy bottom of the ditches in the Tihany Külső-tó (pH 8.7) but it can be found in the same place in the *Agrostis alba-Juncus Gerardi* association (pH 6.8) in alkali strata, and even beside the artificial brook of the Nagyerdő park at Debrecen (SOÓ, 1948:49, FELFÖLDY, 1949:2).

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СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ФИЗИОЛОГИИ ЕСТЕСТВЕННЫХ ПОЛИПЛОИДОВ

Автор: ЛАЙОШ ФЕЛЬФЭЛДИ

РЕЗЮМЕ

Мы выращивали Puccinellia limosa Schur, 2п = 28 и P. distans (Jacq.) Parl. 2п = 42, растения равного возраста, в питательных растворах разного pH. (4—9). Сорок дней спустя, в течение которых мы 7 раз сменяли питательный раствор, чтобы получить постоянный pH, мы измеряли: длину стебля, свежий вес растений, сухой вес, содержание неорганического остатка, золы, общего содержания азота и кальция неорганического остатка. В результате этих опытов установлено:

1. При равных условиях гексаплоид Puccinellia distans выше роста, чем P. limosa.

2. Содержание кальция выше у гексаплоидного вида, чем у тетраплоидного вида.

3. С другой стороны тетраплоид P. limosa уступает по отношению свежего веса, сухого веса, сухого остатка и белка.

4. Имеющая 42 хромосомов P. distans распологает экологической амплитудой гораздо шире относительно pH питательного раствора, чем имеющая 28 хромосомов P. limosa. Это и можно было ожидать на основе цитогеографических данных.

5. Выяснив потребность в pH, становится понятным и экологическая роль этих видов.

6. На основе вышесказанного очевидно, что эти растения, различные с точек зрения морфологических и цитологических, различаются и физиологическими качествами. Таким образом их можно считать независимыми видами.