Euphytica 14 (1965): 225–230

THE LENGTH OF STOMATA AS AN INDICATOR FOR POLYPLOIDY IN RYE-GRASSES

G. J. SPECKMANN, J. POST Jr and H. DIJKSTRA
Foundation for Agricultural Plant Breeding,
Wageningen, The Netherlands

With 4 figures
Received 2 Apr. 1965

Abstract

The suitability of stomata length as a criterion in the distinction between diploid and tetraploid rye-grass plants was tested.

From the data it appears that diploid and tetraploid plants can be separated with a large degree of certainty if the selection is based on the stomata length.

Introduction

Soon after it was found that unlimited numbers of autotetraploid plants could be produced in a simple way by means of colchicine, the large-scale production of tetraploid populations was started. The determination of the number of chromosomes by means of cytological research is very time-consuming and requires much experience, which is felt to be a drawback. Therefore attempts have been made to find other methods of ploidy determination. The length of stomata which generally increases with chromosome number was one of the characteristics which was studied in this connection.

At plant breeding stations in some other countries the difference in stomata length is used as a selection criterion in breeding polyploid rye-grasses. In the experiment described in this paper the suitability of this characteristic for the Dutch polyploid grass material was tested.

Literature

Differences in stomata length between diploids and autotetraploids were found by RANDOLPH *et al* (10) in maize, by COOPER (3) in lucerne, by FRANDSEN (6) in clover, lucerne and *Brassica* species, and by LEVAN (8) in clover.

SCHWANITZ (12) showed that in several genera the range of stomata length within the ploidy levels is too wide to base a reliable distinction between di- en tetraploid plants on this characteristic.

EVANS (5) discovered reliable differences in stomata length among plants of a different ploidy level in clover and lucerne and she proved that, based on this characteristic, a satisfactory distinction can be made between diploid and tetraploid plant parts in C_0 material.

In the meantime there were other characteristics found in these crops which proved to be suitable as a selection criterion in ploidy breeding. In beet a connection was found between the number of plastids in the guard cells of the stomata and the number of chromosomes (DUDLEY, 4; BUTTERFASS, 1). FUNKE (7) found a connection between

the number of germinal pores in the pollen and ploidy in clover. In grasses there is no connection between these characteristics and ploidy.

MATERIAL AND METHOD

The plant material used in this experiment was derived from diploid commercial seed and tetraploid seed which was under trial at the Institute for Research on Varieties of Field Crops at Wageningen. The length of the stomata was determined in the epidermis of young leaves which had reached a corresponding stage of development (figs. 1 and 2). The method of preparing the epidermis was taken from the publication by CLARKE (2). The method was adapted to application in serial work by some symplifying modifications.

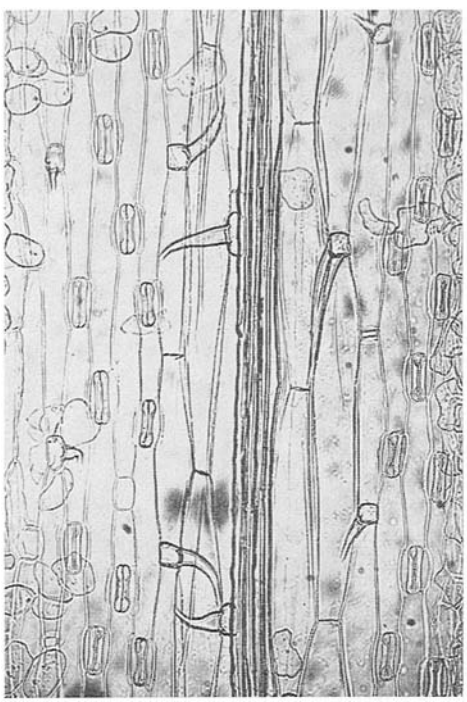

FIG. 1. Stomata of Lolium perenne, diploid (magn. ca. 200 ×)

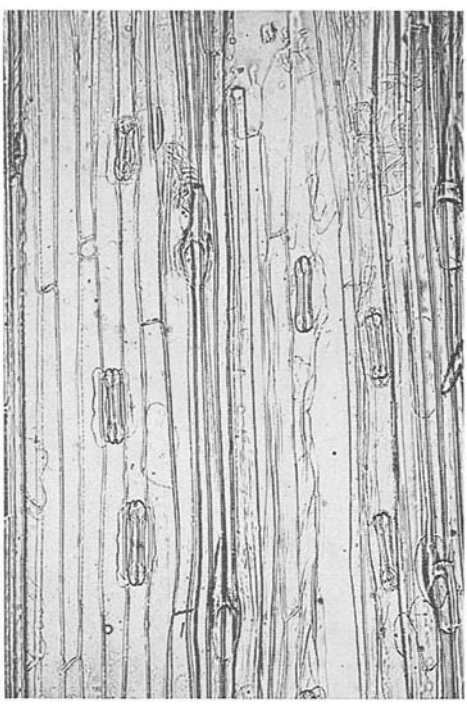

FIG. 2. Stomata of Lolium perenne, tetraploid (magn. ca. 200 ×)

Pieces of leaves are boiled for 10 minutes in 70% alcohol until the chlorophyl is largely extracted and afterwards boiled for another 10 minutes in 90% lactic acid to make the tissue transparent. After having been for 10 minutes in the lactic acid at room temperature, it is ready for analysis. If necessary the material can now be kept for some weeks in tap water. The material is placed on a slide and covered with a cover-slip.

Measurements were made by means of an ocular-micrometer (magnification 250 times). The measurements made were approximated to the nearest whole micro unit; 1 micro unit = 5 μ. The results were mathematically converted to determine the variation of stomata length within the ploidy levels and the reliability of differences in length between diploid and tetraploid plants.

RESULTS

1. *The variation of stomata length within single plants*

In order to determine the variation within the plant, 28 plants of *Lolium perenne* (14 diploids and 14 tetraploids) were analysed (table 1). Five leaves were taken from each plant, and the length of five stomata was determined per leaf.

The average of the measurements in the diploids was found to be 7.27 micro units (= approximately 36 μ) with a range of 5.8–9.4. The average stomata length in the tetraploid material was 9.72 units (approximately 48 μ) with a range of 8.0–12.8.

Ten plants of both types of *Lolium multiflorum* were examined. In the diploid material the average stomata length was 8.74 micro units (= approximately 43 μ); range 7.0–11.8; in the tetraploid material 11.60 micro units (=approximately 58μ), range 9.2–13.6.

The variation analysis showed no significant differences between the leaves of single plants. In comparing the stomata length in the upper halves of the leaves to that of the lower halves, and the costal stomata rows to the marginal costal rows, no differences within the leaves were found. Nor could differences in stomata length be found in fully developed leaves of different ages.

2. *The variation of stomata length among plants of the same ploidy level and difference between diploid and tetraploid plants of Lolium perenne and Lolium multiflorum*

To determine the variation in stomata legnth of plants of the same ploidy level the results of the measurements were considered on plant basis. These results can be found in tables 1 and 2.

TABLE 1. THE MEAN STOMATA LENGTH OF DIPLOID AND TETRAPLOID *Lolium perenne* PLANTS, MEASURED ON 5 LEAVES PER PLANT (5 STOMATA PER LEAF)

Diploid			Tetraploid		
Plant Nr	Nr of measurements	Average length	Plant Nr	Nr of measurements	Average length
1	25	6.52	1	25	9.72
2	25	7.68	2	25	9.10
3	25	7.72	3	25	9.48
4	25	7.20	4	25	10.00
5	25	6.64	5	25	8.96
6	25	7.80	6	25	11.66
7	25	7.28	7	25	9.92
8	25	7.28	8	25	10.28
9	25	7.08	9	25	9.64
10	25	7.08	10	25	9.28
11	25	7.72	11	25	10.20
12	25	7.12	12	25	10.04
13	25	7.48	13	25	10.04
14	25	7.12	14	25	9.08
	350	7.27		350	9.72

l.s.d. (P = 0.05) = 0.74
(P = 0.01) = 0.88

From table 1 it appears that significant differences can be found among plants of *Lolium perenne* both in the diploid and in the tetraploid group. In all cases the differences in stomata length between diploid and tetraploid plants proved to be significant.

TABLE 2. THE MEAN STOMATA LENGTH OF DIPLOID AND TETRAPLOID *Lolium multiflorum* PLANTS' MEASURED ON 5 LEAVES PER PLANT (5 STOMATA PER LEAF).

Plant Nr	Diploid		Plant Nr	Tetraploid	
	Nr of measurements	Average length		Nr of measurements	Average length
1	25	8.64	1	25	10.76
2	25	9.08	2	25	10.08
3	25	8.00	3	25	10.68
4	25	7.52	4	25	11.04
5	25	8.36	5	25	10.84
6	25	7.68	6	25	10.92
7	25	8.76	7	25	10.36
8	25	9.28	8	25	12.48
9	25	9.84	9	25	12.80
10	25	10.24	10	25	11.60
	250	8.74		250	11.60

l.s.d. ($P = 0.05$) = 0.69
($P = 0.01$) = 0.82

As appears from table 2 also in *Lolium multiflorum* of a certain ploidy level the stomata length sometimes differs significantly from plant to plant.

The variation in *Lolium multiflorum* is considerably greater than in *Lolium perenne*. In some cases the differences between diploid and tetraploid plants are not reliable.

APPLICATION OF STOMATA MEASUREMENTS IN DETECTING TETRAPLOIDS

For the practical use of differences in stomata length for the determination of polyploid plants, the question has to be answered how many measurements are necessary to reach a satisfactory result. In the following graphs (figs 3 and 4) the results of 10 stomata measurements per plant are given of *Lolium perenne* and *Lolium multiflorum*. The measurements were made on 20 plants both of the diploid and the tetraploid type.

As appears from the graphs the tetraploid plants can be separated from the diploid ones with a large degree of certainty by determining the average stomata length of 10 measurements per plant. Because of the great variation there is an overlapping in the *Lolium multiflorum* material. This results in an error of about 18%.

DISCUSSION

The results of this experiment justify the expectation that the stomata length in rye-grass can serve as a selection criterion in separating tetraploid from diploid plants.

Starting from a threshold value, to be derived from diploid standard material, it will be possible to recognize the tetraploid plant parts in C_0 material or to distinguish

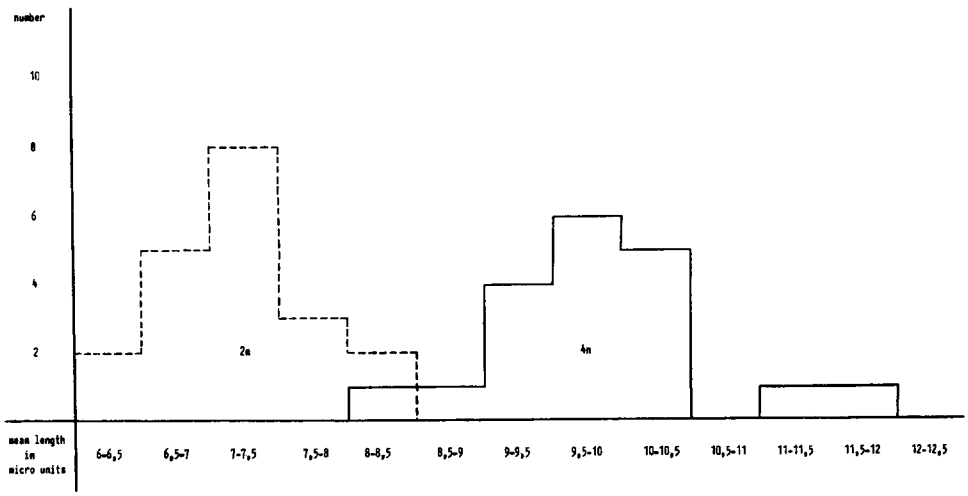

FIG. 3. Lolium perenne 2n and 4n; mean lengths of the stomata in micro units.

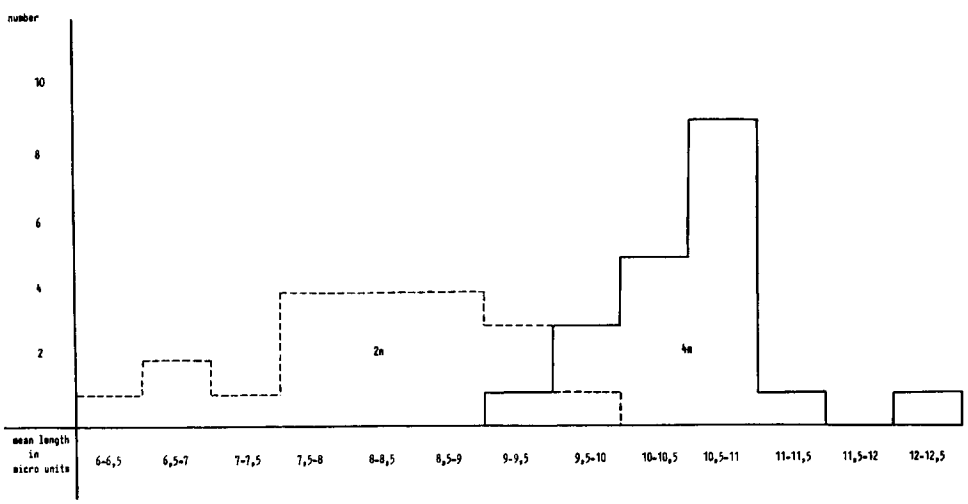

FIG. 4. Lolium multiflorum 2n and 4n; mean lengths of the stomata in micro units.

tetraploid plants from the diploids in the C_1. To prevent selection towards coarse-leaf types, this selection should not be based on extreme values.

Though the material available at the Foundation for Agricultural Plant Breeding was not sufficient to draw definite conclusions, preliminary research on alloploid C_0 plants gave the impression that the determination of the stomata lenght can also be used as a selection basis in species crossing programmes. By treating the plant with colchicine during the vegetative stage mixoploidy occurs to an even larger extent than after seed treatment. In many cases the polyploid sectors in the C_0 are eliminated by

diplontic selection. Polyploid sectors in the plant may be detected by determining the stomata length in C_0 plants.

SAMENVATTING

Stomatalengte als criterium voor het opsporen van polyploïde raaigrassen

De bruikbaarheid van stomatalengte als criterium bij het onderscheiden van di- en tetraploïde raaigrasplanten werd onderzocht.

Uit de gegevens blijkt, dat met selectie op basis van stomatalengte diploïde en tetraploïde planten met grote zekerheid gescheiden kunnen worden.

REFERENCES

1. BUTTERFASS, T., Die praktische Ermittlung des Polyploidiegrads von Zuckerrüben durch Zählen der Schliesszellen Chloroplasten. Der Züchter **28** (1958): 309–314.
2. CLARKE, J., Preparation of leaf epidermis for topographic study, Stain Technology **35** (1960): 35–39.
3. COOPER, D. C., Artificial induction of polyploidy in Alfalfa. American Journal of Botany **26** (1939): 65–76.
4. DUDLEY, J., Number of chloroplasts in the guard cells of inbred lines of tetraploid and diploid sugar beets. Agronomy Journal **50** (1958): 169–170.
5. EVANS, A., The production and identification of polyploids in red clover, white clover and lucerne. The New Phytologist **54** (1955): 149–162.
6. FRANDSEN, K. J., Iagttagelser over polyploide Former af nogle Kulturplanter. Tidskrift for Planteavl **49** (1945): 445–496.
7. FUNKE, C., Eine Schnellmethode zur Selektion polyploider Pflanzen. Die Naturwissenschaften **43** (1956): 66.
8. LEVAN, A., Framställning av tetraploid rödklöver. Sveriges Utsädesföreningens Tidskrift **50** (1940): 115–123.
9. MOCHIZUKI, A., and M. SUEOKA, Genetic studies on the number of plastids in stomata – I. Effects of autopolyploidy in sugar beets. Cytologia (Tokyo) **20** (1955): 358–366.
10. RANDOLPH, L. F., E. C. ABBE and J. EINSET, Comparison of shoot apex and leaf development and structure in diploid and tetraploid maize. Journal of Agricultural Research **69** (1944): 47–76.
11. RANDOLPH, E. C. and J. EINSET, Development pattern of the leaf blade in 2n and 4 n Zea maize as related to the structure of the growing point. American Journal of Botany **26** (1939): 26–45.
12. SCHWANITZ, F. Einige kritische Bemerkungen zur Methode der Bestimmung der Polyploidie durch Messung der Pollen und Spaltöffnungsgrösse. Der Züchter **22** (1952): 273–275.