STOMATAL CELL LENGTH AND PLOIDY LEVEL IN FOUR TAXA BELONGING TO THE *PHLEUM* SECT. *PHLEUM*

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Stomatal cell length was examined in four closely related taxa of sect. *Phleum* in the genus *Phleum* (*P. nodosum*, *P. pratense* and in two cytotypes of *P. commutatum*). It was found that the polyploid taxa (*P. pratense*, 2n = 6x = 42 and *P. commutatum*, 2n = 4x = 28) have longer stomatal cells than their diploid relatives (*P. nodosum*, 2n = 2x = 14 and *P. commutatum*, 2n = 2x = 14). In these two pairs of taxa, stomatal cell length can be a rapid and useful indirect ploidy level indicator and can assist in their identification. Material taken from live and dried specimens of a given taxon did not differ in stomatal cell length, meaning that different cytotypes can be identified from herbarium material as well.

Key words: *Phleum*, grasses, stomata, ploidy level.

INTRODUCTION

Root tip cell chromosome counting is time-consuming and not suited for routine screening of ploidy level. For this purpose, the stomatal cell length can be a rapid and reliable indirect ploidy indicator. The length of stomatal guard cells is correlated with the ploidy level in a range of natural and commercial cytotypes/varieties of different plant species (Speckman et al., 1965; Przywara et al., 1988; Cohen and Yao, 1996; Mishra, 1997).

Grasses are a particularly promising group of plants in which stomatal features (stomatal frequency, stomatal guard cell length and stomatal index) generally can be used to determine of ploidy level. These plants often show significant intraspecific and intrageneric chromosome number variability, and rapid identification of their different cytotypes is of great importance in taxonomy, preliminary selection and breeding. For this reason the correlations between stomatal features and ploidy level in grasses have been examined many times (Speckman et al., 1965; Miskin and Rasmusson, 1970; Heichel, 1971; Teare et al., 1971; Liang et al., 1975; Sapra et al., 1975; Tan and Dunn, 1973, 1975; Lea et al., 1977a,b; Santen and Casler, 1986; Borrino and Powell, 1988). In the majority of results published so far, guard cell length was measured manually using an ocular micrometer on a microscope, and the number of stomata measured per plant was small (6–25). To rationalize this kind of study, measurements should be automated at least in part, to make it easier to obtain and compare a larger number of measurements.

Here we attempted to determine stomatal guard cell length in four taxa from the section *Phleum* in the genus *Phleum*. The examined taxa (*P. nodosum*, 2n = 2x = 14, *P. pratense*, 2n = 6x = 42, and *P. commutatum*, 2n = 2x = 14, 2n = 4x = 28) represent three different ploidy levels and are closely related

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TABLE 1. Provenance and chromosome number of the *Phleum* samples studied

Taxon	Locality	Chromosome number (2n)	Number of plants
Phleum commutatum Gaud.	[1] Austria, Alps - Nationalpark Hohe Tauern	14 {a}	5
	[2] Sweden, Åre vicinity	14 {a}	5
	[3] Poland, Tatra Mts.	14 {b}	5
	[4] Sweden, Åre vicinity	28 {a}	5
	[5] Scotland, Aonach Beag	24 {a}	5
Phleum nodosum L.	[6] Poland, Cracow vicinity	14 {b}	5
	[7] Sweden, Stockholm vicinity	14 {c}	5
Phleum pratense L.	[8] Poland, Cracow vicinity	42 {b}	5
	[9] Sweden, Stockholm vicinity	42 {c}	5

Chromosome numbers established in: {a} – Joachimiak and Kula (1996); {b} – Joachimiak and Kula (1993); {c} – this study.

TABLE 2. Stomatal guard cell length in representatives of four *Phleum* taxa from different populations

P. commutatum 2n = 2x		*P. commutatum* 2n = 4x		*P. nodosum* 2n = 2x		*P. pratense* 2n = 6x	
No	ACL	No	ACL	No	ACL	No	ACL
[1]	24.5±2.4	[5]	39.8±3.5	[6]	29.0±3.7	[8]	35.9±3.9
				(*)	29.2±2.9	(*)	36.0±3.7
[2]	22.5±2.3	[4]	29.2±2.9	[7]	22.1±2.2	[9]	27.1±2.6
[3]	25.3±2.4						
Mean:	24.1±2.4	Mean:	34.5±3.2	Mean:	25.5±2.9	Mean:	31.5±3.4

No – population number (see Tab. 1); ACL – average stomatal guard cell length in μm; ± – standard deviation; (*) – herbarium material.

(Joachimiak and Kula, 1997). There is a lack of good morphological features to enable differentiation of *P. nodosum* (=*P. bertolonii* DC) from *P. pratense* and between the diploid and tetraploid forms of *P. commutatum*, frequently occurring side by side in Europe. In particular, morphological identification of different cytotypes within the *P. pratense* group (*P. pratense* and *P. nodosum*) is of great importance for taxonomy and for practical purposes. *P. pratense* is largely used as a pasture species and *P. nodosum* is used as a turf grass, and both species are used in various breeding programs in Europe and other parts of the world. However, no morphological characters previously studied (plant height, flag leaf length and width, inflorescence diameter, glume length, awn length, cilia length and number) are reliable in distinguishing different ploidy levels (for review: Cenci et al., 1984). So far, representatives of these taxa can be reliably identified only on the basis of differences in chromosome number (Nordenskiöld, 1945; Joachimiak and Kula, 1993, 1996) or cytometric DNA estimations. The amounts of 2C DNA in different races of *P. pratense* and *P. nodosum* are well correlated with their ploidy level (Alan Steward, personal communication).

We also checked whether stomatal length measurements can be facilitated by automated computer procedures and whether stomatal cell length can be used to identify herbarium specimens.

MATERIALS AND METHODS

The research material consisted of fragments of upper epidermis from fully developed leaves. For stomatal measurements the third leaf below the panicle was sampled from each analyzed plant. The majority of plants had been collected from various natural populations in Europe and transferred to an experimental field. Two years later, leaves were cut from them for further analysis. In the case of *P. pratense* and *P. nodosum*, fragments of epidermis from herbarium specimens were examined also. All the *Phleum* specimens were cytologically examined and identified (Tab. 1).

Each piece of upper epidermis was removed with forceps from the central part of the leaf and

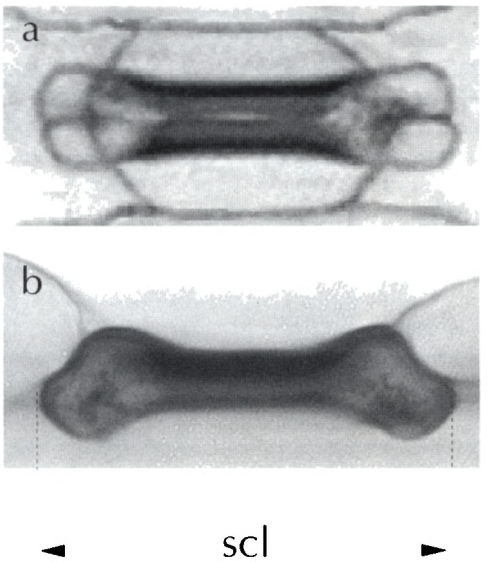

Fig. 1. *Phleum* stomatal apparatus and measurement of its length. (**a**) Vertical view, (**b**) Lateral view. scl – stomatal guard cell length.

placed in commercial chlorine bleach for 30 min, then rinsed with distilled water and stored in 70% ethanol. Leaves from herbarium specimens were soaked in distilled water for 24 h before the epidermis fragments were removed. The material was stained with a 1% solution of safranin in alcohol for 5 min, rinsed in 70% alcohol and distilled water, counterstained with an acidic solution of alcyan blue for 10 min, and mounted in a drop of water on a microscope slide. The preparations were analyzed under an Eclipse E800 microscope (Nikon Corp., Japan) equipped with a Panasonic video camera. Image grabbing and stomatal guard cell length measurements were done with Lucia G software (Laboratory Imaging Ltd.). During the measurements, special attention was paid to measure the total length of the guard cells, together with those parts which can be poorly visible in preparations because of slipping under epidermal cells (Fig. 1). In each specimen the lengths of at least 200 guard cells were measured. By using a number of leaves for each examined collection, at least 1000 measurements were obtained.

RESULTS AND DISCUSSION

Each of the examined taxa showed a strictly defined range of stomatal guard cell length variability (Tab. 2). The ranges overlap partially, thus stomatal length alone cannot be an absolute criterion for taxonomic identification of particular specimens within the section *Phleum*. In spite of this, measurement of stomatal length can be very useful for proper taxonomic identification of morphologically indistinguishable specimens belonging to closely related pairs of taxa (*P. nodosum* / *P. pratense* or diploid/tetraploid *P. commutatum*), because both polyploid forms (*P. pratense*, 2n = 6x = 42 and *P. commutatum*, 2n = 4x = 28) have definitely longer stomatal cells than their diploid relatives (*P. nodosum*, 2n = 2x = 14 and *P. commutatum*, 2n = 2x = 14) (Tabs. 2, 3).

TABLE 3. *Phleum pratense* / *nodosum* (6x/2x) and *P. commutatum* (2x/4x). Results of two-tailed Student's test for mean stomatal cell lengths. Compared means are statistically significant if P<0.0001. AU – Austria, PL – Poland, SW – Sweden; ** significant, (–) not significant

P. pratense / *P. nodosum* (6x/2x)		*P. nodosum* PL	*P. nodosum* SW	P. pratense SW
P. pratense	PL	**	**	**
	SW	(–)	**	
P. nodosum	SW	**		
P. commutatum (2x/4x)		4x SW	2x AU	2x PL
2x	SW	**	(–)	(–)
2x	PL	**	(–)	
2x	AU	**		

The stomata of *P. nodosum* and *P. pratense* originating from Sweden are generally smaller than those of plants from other areas (Tab. 2, and unpubl. results). The observed differences between Polish and Swedish representatives of these taxa are statistically significant (Tab. 3). Intraspecific differences in guard cell length were previously observed among various cultivars of *Triticum aestivum* (Teare et al., 1971) and *Bromus inermis* (Tan and Dunn, 1975).

Another question is the assessment of stomatal cell length in *P. commutatum* from Scotland. Specimens of this taxon were obtained from seeds collected from a natural population on Aonach Beag, received from the Royal Botanic Gardens, Kew Seed Bank, England. In previous research (Joachimiak and Kula, 1996) it was stated that plants grown from these seeds were hypotetraploid with somatic chromosome number 2n = 24. The mean length of their stomata was about 10 μm more (mean = 39.8 μm) than in Swedish representatives of this tetraploid taxon (mean = 29.2 μm). Such a difference can suggest the possible influence of aneuploid changes on the stomatal cell length, but may well be that the

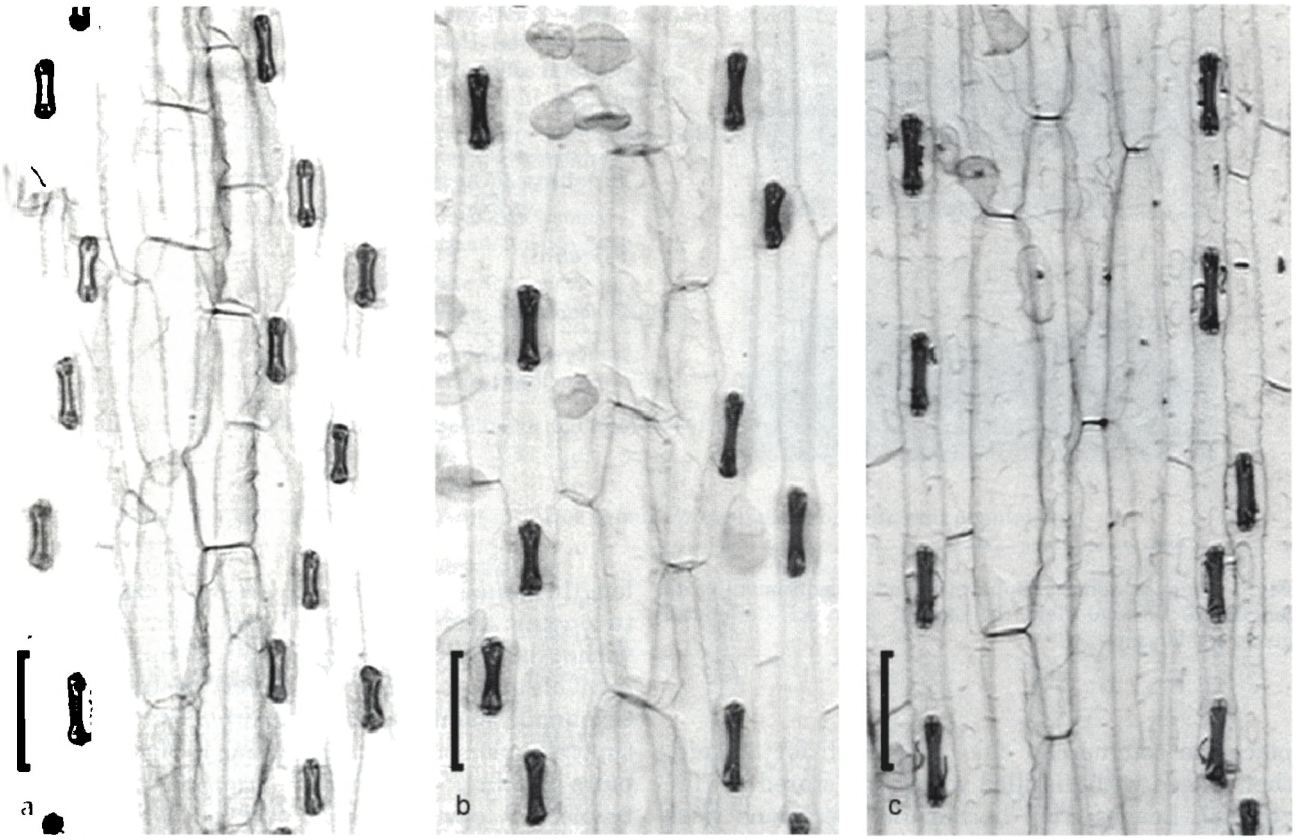

Fig. 2. Stomatal length and distribution in leaf epidermis of Polish material [6, 8]. (**a**) *P. nodosum*, (**b, c**) *P. pratense*. a, b – from living specimens, c – from herbarium specimens. [] – origin of *Phleum* samples (see Table 1). Bar = 50 μm.

P. commutatum from that area is of different origin and is a separate taxon within the *P. alpinum* group (in which the diploid and tetraploid *P. commutatum* as well as *P. alpinum* subsp. *rhaeticum* are included). It is even more probable because previous authors have suggested different (auto- or allopolyploid) origins of the tetraploid races of *P. commutatum* from different areas (Gregor and Sansome, 1930; Müntzing, 1935; Nordenskiöld, 1945; for review: Joachimiak and Kula, 1993, 1996).

The adopted procedure yielded epidermis fragments and stained stomata from both live and dried specimens. Neither stomata morphology nor stomata staining ability underwent significant changes due to storage in a herbarium. The mean stomatal guard cell length and the range of their variability were very similar between the two types of specimens (Tab. 2; Fig. 2b,c). This way of identifying cytotypes in herbarium material provides an extra tool in taxonomic research.

These results indicate that stomata length measurements can be useful not only for diploid and polyploid conspecifics (*P. commutatum*) but also for species with different ploidy level if they are very closely related to each other (Fig. 2a,b). This was the case of *P. nodosum* and *P. pratense*, which are considered two separate species, however closely related. Previous studies found at least four genomes of *P. nodosum* in the karyotype of the hexaploid *P. pratense* (Joachimiak and Kula, 1993; Cai and Bullen, 1994). Measurements of stomatal guard cell length are especially useful for identifying different cytotypes of plants growing in mixed populations or in geographically near localities (Tabs. 2, 3).

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