BIOLOGIJA. 2007. Vol. 18. No. 1. P. 64–68

Root growth characteristics of *Festuca*, *Lolium* and *Festulolium* in relation to stress tolerance

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Linear root growth of meadow fescue (*Festuca pratensis* Huds.), Italian ryegrass (*Lolium multiflorum* Lam.) and their hybrid *Festulolium braunii* was studied under a hardening temperature of +4 °C. It was defined, that the root growth rate of *Festuca pratensis* was considerably lower than that of *Lolium multiflorum* ($v_{F.} << v_{L.}$). The cytological growth analysis suggests that the main reason for the lower root growth rate of *Festuca pratensis*, compared to *Lolium multiflorum*, was a low cell production rate ($V_{F.} < V_{Fl.} < V_{L.}$). *Festulolium braunii* took the intermediate but closer to *Lolium multiflorum* position within these parameters. An exceptionally short root elongation zone *L* of *Festuca pratensis* was the result of a low cell production rate. We suggest that the low cell production rate *V* as a consequence of low meristem activity is a cytological expression of growth slowdown and a stress state into which plants transit and in such a way express stress tolerance. Thus, a short root elongation zone *L* can be a marker of a stress state and cold resistance.

Key words: *Festuca pratensis, Lolium multiflorum, Festulolium braunii,* root linear growth, cell production rate, stress tolerance, stress state, rounded cells

INTRODUCTION

According to J. P. Grime's plant strategy classification, three groups are defined: competitors, ruderals and stress-tolerators [1]. The adaptation of stress-tolerators to unfavourable factors (including low temperatures) is physiologically expressed by a relatively low growth rate, by a lower energy consumption for growth and small respiration expenditure [1, 2]. These exceptionally stress-tolerant characteristics are the expression of the stress state into which plants transit under an intensively active stressor [3]. In this qualitatively new stress state, the new genetic programs get into action, specific biochemical processes occur, the growth-blockage appears, thus determining the plant cold resistance [3–6]. Plants of other strategies (competitors and ruderals) do not drift into the stress state and therefore do not acquire the above-mentioned characteristics, and under unfavourable conditions they die.

Italian ryegrass (*Lolium multiflorum* Lam.) is an annual cold-unresistant summer plant, meadow fescue (*Festuca pratensis* Huds.) being very close to Italian ryegrass is a perennial, significantly more cold-resistant winter plant [7]. This complex of characteristics shows that *Festuca pratensis* is much more stress-tolerant than *Lolium multiflorum.* The plants of the genera *Festuca* and *Lolium* are phylogeneticaly very close – their natural hybrids exist [8]. Besides, their relationship is confirmed by the chromosome conjugation tendency in *Lolium* and *Festuca* hybrids and other genetic traits [9]. Despite the relationship of *F. pratensis*, *L. multiflorum* and their hybrid *Festulolium braunii* ((K. Richt.) A. Camus), these plants are characterized by different cold resistance and show different ecological "strategies". Therefore, by investigating the above-mentioned plants it is possible to define which growth characteristics determine an integral plant characteristic – stress tolerance.

While studying plant adaptation to low temperatures it was observed that roots often remain in an active growth state, they use the energy resources for that purpose and thus are less cold-resistant compared to other vegetative plant organs [10, 11]. It is likely that at a hardening temperature of +4 °C the root growth of cold-resistant *Festuca pratensis* should be slowed down as compared to that of *Lolium multiflorum* and *Festulolium braunii.* The aim of this study was to define the root growth characteristics that determine stress tolerance and their cytological mechanism.

MATERIALS AND METHODS

The research was carried out at the Laboratory of Genetics and Physiology of the Lithuanian Institute of Agriculture.

The following varieties were used in this study: *L. multiflorum* 'Macho' (2n = 4x = 28), *F. pratensis* 'Dotnuva I' tetraploid analogue (2n = 4x = 28), and *Fl. braunii* 'Punia' (2n = 4x = 28). The plants were grown for 35 days under +20 °C and 43 days under the hardening temperature of +4 °C.

The plants were grown in a climate chamber by the hydroponics method under the following growth conditions: photoperiod 11 h, illumination 7000 lx. The composition of the medium was as follows: macroelement salts 4 mM $Ca(NO_3)_2 \times 4\ H_2O$, 0.5 mM NH_4NO_3, 0.185 mM $(NH_4)_2SO_4$, 1 mM KH_2PO_4, 3.5 mM KNO_3, 2 mM $MgSO_4 \times 7\ H_2O$; microelement salts – 9,1 μM $MnSO_4 \times H_2O$, 0.3 μM $CuSO_4 \times 5\ H_2O$, 0,8 mM $ZnSO_4 \times 7\ H_2O$, 30 μM NaCl, 0.1 μM $NaMoO_4 \times 2\ H_2O$, 10 μM H_3BO_3; iron source 26.7 mM FeNaEDTA. To maintain the stability of the media, it was changed weekly.

Upon growing the plants at +4 °C for 35 days, marks of ink were put in the root differentiation zone (15 mm from the root apical tip) on adventitious roots of similar length (~4 cm). After 9 days, when the mark had moved away from the previous position (increment $\Delta L_{inc.}$), samples were taken – the root was cut at the point of the mark. Five samples were taken from different plants. From the root increment $\Delta L_{inc.}$ and meristem, longitudinal incisions were cytological preparations were made. The following measurements were done: the length of root increment (ΔL_{inc}, mm), the length of cells in root cortex increment (l, μm), cell number in root cortex increment (ΔN_{inc}, cells), the length of the root elongation zone (L, μm). The following values were calculated: linear root growth rate $v = \Delta L_{inc.}/\Delta t$ (μm · day^{-1}), cell production rate $V = \Delta L_{inc.}/(l \cdot \Delta t)$ (cells · day^{-1}), relative cell elongation rate $k = \Delta L/(L \cdot \Delta t)$ (day^{-1}). The standard deviation was calculated to show the variations of the above-mentioned values.

RESULTS

Root linear growth rate and major linear parameters. At +4 °C linear root growth rate (and the length of root increment ΔL_{inc}) of *F. pratensis* ($v_{F.}$) is significantly lower than that of *L. multiflorum* or *Fl. braunii* ($v_{F.} << v_{L.}$; $v_{F.} << v_{Fl.}$). The linear growth rate of *Fl. braunii* occupies an intermediate position, but is closer to *L. multiflorum* ($v_{F.} < v_{Fl.} < v_{L.}$) (Table, Fig. 1).

The mean length of mature cells (l) in the root cortex increment did not differ appreciably for all species. It ranged between 190 and 240 μm (Table). Whereas, the number of cells in the root cortex increment ($\Delta N_{inc.}$) for *F. pratensis* was about 4.3 times smaller than for *L. multiflorum* (Table). *Fl. braunii* took an intermediate position, but was closer to *L. multiflorum* in this respect.

The root elongation zone (L) of *F. pratensis* was 3.5 times shorter than that of *L. perenne* ($L_{F.} << L_{L.}$). *Fl. braunii* occupied an intermediate position, but was closer to *L. multiflorum* ($L_{F.} << L_{Fl.}$) (Table).

Cytological analysis of root growth rate. To explain the difference in the linear growth rate of the plants, it is necessary to analize their growth process on the cell level (how many cells are produced by a meristem per time unit, how big mature cell are, etc.). Since the plants were grown in constant environmental conditions, root growth was stationary, i. e. the length of the meristem, elongation zone and the number of cells in these zones remained constant. Furthermore, the number of cells transiting from the meristem into the elongation zone and from there into the differentiation zone was equal and constant. That is why the growth analysis on the cell level is conducted for the stationary root growth.

The linear growth rate (v) of stationary growing roots is equal to the product of cell production rate V and average mature cell length l [12]:

$$v = V \cdot l. \quad (1)$$

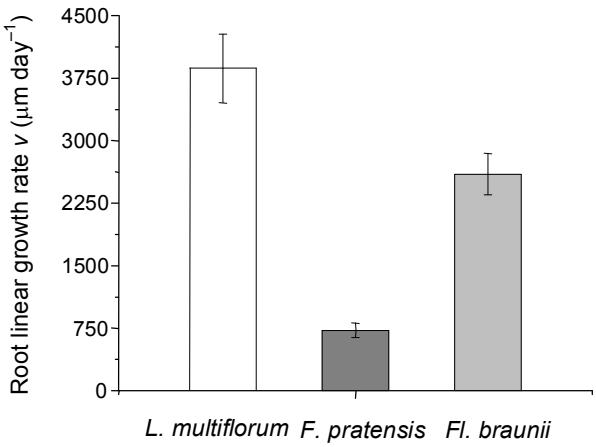

Fig. 1. Root linear growth rate of *Lolium multiflorum*, *Festuca pratensis* and *Festulolium braunii*

Table. **Primary root growth characteristics of *Lolium multiflorum*, *Festuca pratensis* and *Festulolium braunii***

Parameters	Species		
	L. multiflorum	*F. pratensis*	*Fl. braunii*
$\Delta L_{inc.}$ (mm)	34.80 ± 3.70	6.53 ± 0.78	23.40 ± 2.23
$\Delta N_{inc.}$ (cells)	146.16 ± 33.84	33.93 ± 10.26	96.03 ± 38.25
l (μm)	238.20 ± 11.70	192.16 ± 14.96	243.67 ± 6.40
L (μm)	5340.60 ± 570.70	1565.01 ± 200.02	4315.13 ± 683.40

$\Delta L_{inc.}$ – the length of root increment, $\Delta N_{inc.}$ – the number of cells in the root cortex increment, l – average cell length in the root cortex increment, L – the length of root elongation zone

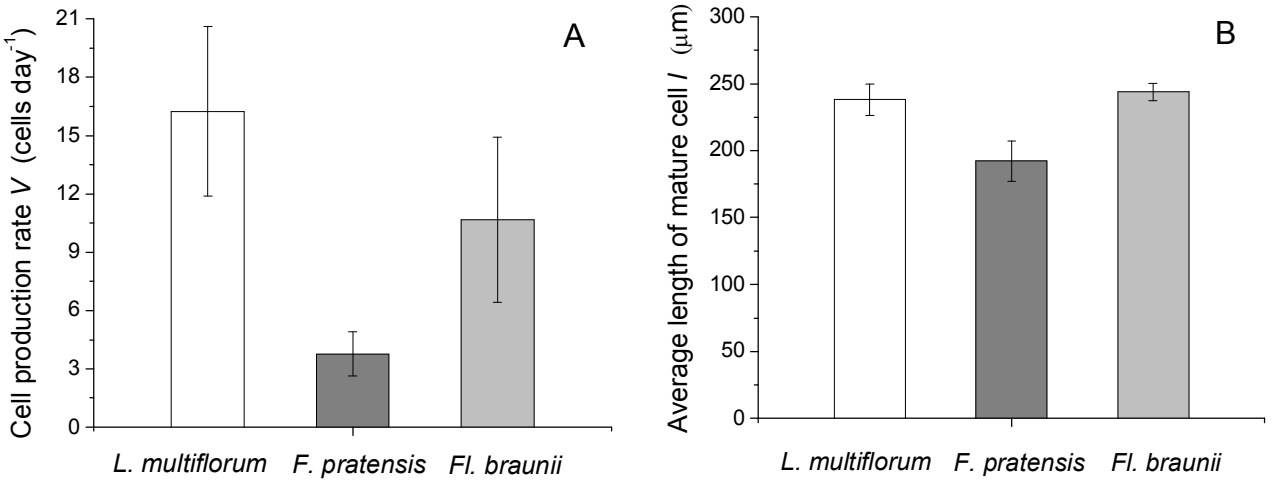

Fig. 2. Components determining root linear growth rate: cell production rate (A), average length of elongated root cortex cell (B)

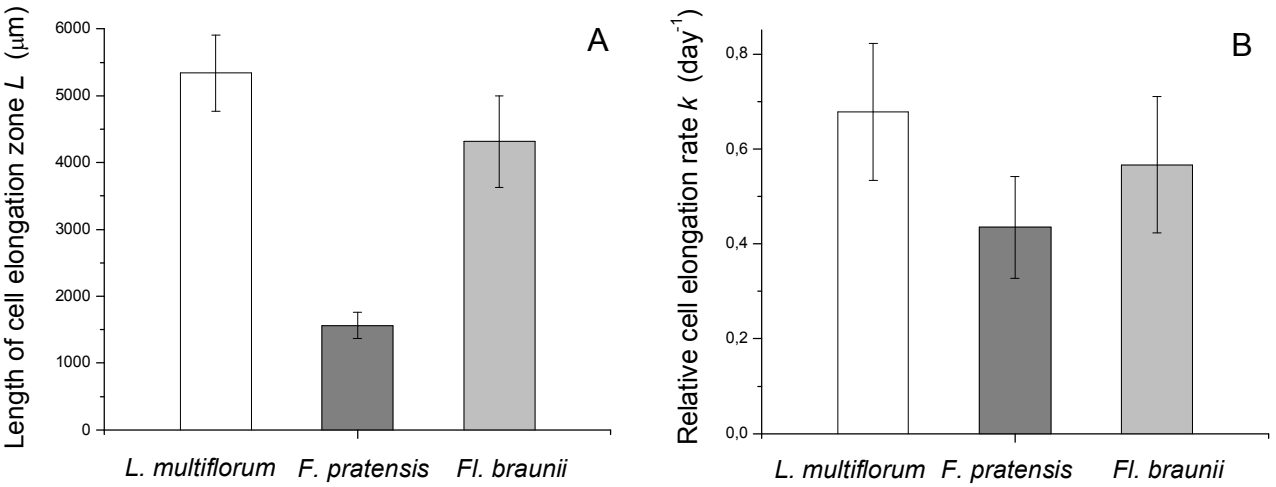

Fig. 3. Root elongation characteristics: the length of root elongation zone (A), relative root elongation rate (B)

At a temperature of +4 °C, the root cell production rate of *F. pratensis* is significantly lower than that of *L. multiflorum* ($V_{F.} << V_{L.}$), and *Fl. braunii* occupies an intermediate but closer to *L. multiflorum* position according to this parameter (Fig. 2 A). At the same time, the differences in the length of average elongated cells for all plants studied were inconsiderable (Fig. 2 B).

Following the above root linear growth rate formula ($v = V \cdot l$) and its values (V, l), we make the conclusion that the major reason for a lower linear root growth rate ($v_{F.} < v_{L.}$; $v_{F.} < v_{Fl.}$) of *F. pratensis* compared to the other species studied is a low cell production rate ($V_{F.} < V_{L.}$; $V_{F.} < V_{Fl.}$). This phenomenon is far less influenced by the shorter length of the mature cells ($l_{F.} < l_{L.}$; $l_{F.} < l_{Fl.}$). *Fl. braunii* grows more slowly than *L. multiflorum* ($v_{Fl.} < v_{L.}$) only due to the slower cell production rate ($V_{Fl.} < V_{L.}$) because the size of the mature cells is almost the same ($l_{Fl.} \approx l_{L.}$). The linear root growth rate of *Fl. braunii* is intermediate ($v_{F.} < v_{Fl.} < v_{L.}$) because the cell production rate is intermediate ($V_{F.} < V_{Fl.} < V_{L.}$).

The length of the elongation zone (L) is one of the parameters that characterize root growth and is easily observable. It is the part of the root between the meristem and the differantiation zone, where root hairs start. As was described earlier, at a temperature of +4 °C the root elongation zone of *F. pratensis* was 3.5 times shorter than that of *L. multiflorum*. *Fl. braunii* occupied an intermediate position, but was closer to *L. multiflorum* (Fig. 3 A).

It has been theoretically proven that the length of the elongation zone (L) of a stationary grown root may be expressed by the equation [3]

$$L = \frac{V \cdot l}{k}. \qquad (2)$$

At a temperature of +4 °C the length of mature cells (l) is similar or approximately the same, and the difference in the relative cell elongation rate (k) is not considerable in the species studied (Fig. 3 B). Consequently, the difference of the elongation zone length (formula 2) is mostly influenced by the cell production rate V. The length of the elongation zone of *F. pratensis* is much shorter than that of *L. multiflorum* and *Fl. braunii* because of a considerably lower cell production rate, i. e. a smaller number of cells transits into the elon-

gation zone. Consequently, the length of the elongation zone *L* can serve as a diagnostic characteristic for the assessment of cell production rate without cytological analysis for the species.

Thus, the analysis illustrates that the main factor defining the root linear growth rate of the species studied is the cell production rate *V*. Specifically, this growth component determines that *F. pratensis* linear growth rate at a temperature of +4 °C is considerably slower than that of *L. multiflorum*. The cell production rate *V* influences the elongation zone length *L*, which is related to root growth.

Cell shape tests. The typical shape of the elongated root cortex cells in longitudinal incisions is close to a rectangle in which cell length is 3 times as large as the width. However, longitudinal incisions of the root increment of *F. pratensis* show that cortex cells of some samples clearly deviated from its typical shape, i. e. they were shortened, widened and with rounded contours. The ratio of rounded cells in different root cortex files is illustrated in Fig. 4.

The smallest number of deformed cells was found in the middle files (4th, 5th). The greatest number of rounded cells was found close to the epiblem (2nd and 3rd files) as well as closest to the central stele (7th and 8th files) (Fig. 4).

DISCUSSION

There are specialised types of plants in nature, and their differences are mostly characterised by growth rate and use of assimilators [1, 2]. Exceptionally, stress-tolerators are capable of transiting into a higher stress-resistant state in which the plant growth becomes slow [3]. The roots linear growth rate *v* of the highly cold-resistant plant *F. pratensis* at the +4 °C hardening temperature is much lower than that of *L. multiflorum* and *Fl. braunii*. This suggests that *F. pratensis* is capable of consuming small energy resourses for growth, accumulating them for stress-tolerant processes (renewal, accumulation of cryoprotectors). The intensive root growth of *L. multiflorum* and *Fl. braunii*, not retaining resources for adaptation and not transiting into the stress state, is an expression of the other life strategies, opposite to stress-tolerant ones [1, 2, 13].

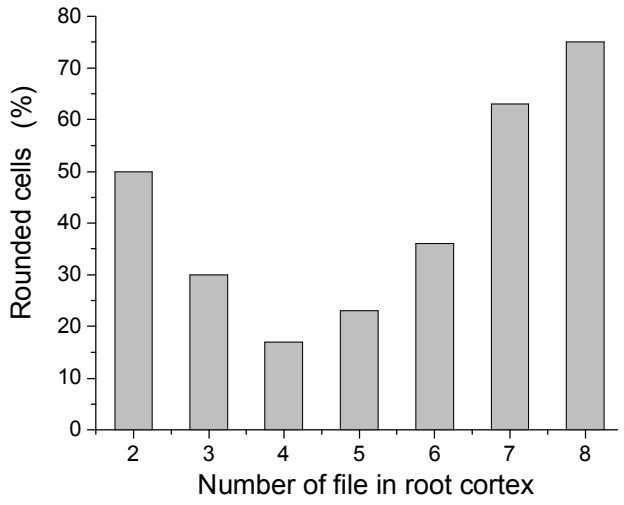

Fig. 4. Percentage of rounded cells in root cortex files of *Festuca pratensis*. The row number of cortex cells is counted from the root epiblem. The first file was not measured due to incision defects

Cytomorphological tests allow us to find the reasoning for adaptive stress-tolerant characteristics. The slow root growth of *F. pratensis* is determined only by the meristem: due to the slower root cell production rate *V* fewer cells are produced. According to theoretical data, slow cell division is conditioned by a more considerable cell cycle G_1-phase prolongation [14]. That determines the increased number of cells being in the G_1-phase and forming the roots quiescent center. It is likely that this center, as an accumulation of resistant G_1 cells, covers the bigger part of the meristem and thus determines its resistance [15]. Moreover, it has been defined that the short elongation zone *L* of *F. pratensis* is the result of a small cell production (it means that the smaller number of cells transit into the elongation zone). Consequently, a short elongation zone may be a marker of the resistance state.

The low linear root growth rate *v* and root cell production rate *V* are the indicators of cold resistance**.** This is confirmed by our study: the highly cold-resistant *F. pratensis* had better adaptive characteristics, while in the unresistant *L. multiflorum* they were worse and in *Fl. braunii* intermediate.

In *F. pratensis* we noticed widened, rounded root cortex cells which were not identified in *L. multiflorum* and *Fl. braunii*. As mentioned above, stress-tolerators have the ability to transit into a new stationary state – stress, thus acquiring resistance. The process of transiting from one stationary state into another is possible only via instability [4, 16]. So it is hypothetically possible that the unusual shape of cells may be an expression of instability because of the changes to a new state under the low-temperature stressor. This additionally proves stress to be a qualitatively new state of resistance in *F. pratensis*.

CONCLUSIONS

1. At a temperature of +4 °C, the root linear growth rate of *F. pratensis* is much more lower than that of *L. multiflorum* and *Fl. braunii*. This suggests that *F. pratensis* consumes less energy resources for growth than the other species studied, and it is characteristic of a stress state and stress-tolerant strategy.

2. The slower root growth rate *v* of *F. pratensis* is determined by a meristem, i. e. a considerably lower meristem cell production rate *V*. The low root cell production rate of *F. pratensis* shows the changed meristem which is adapted to a low temperature stressor.

3. A considerably shorter elongation zone (*L*) in *F. pratensis* than in the other species studied is the

result of a low cell production rate (the smaller number of cells transit into the elongation zone). Thus, the length of the elongation zone *L* is a macroscopic value and a visual sign characterizing the root cold-resistant state.

4. Widened and rounded root cortex cells were found in individual *F. pratensis* plants.

5. *Fl. braunii*, which is more cold-resistant than *L. multiflorum*, has better root linear growth characteristics in terms of cold resistance. According to root linear growth rate, cell production rate and elongation zone length, *Fl. braunii* occupies an intermediate position but is closer to *L. multiflorum*.

Received 21 May 2006
Accepted 25 January 2007

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FESTUCA, LOLIUM IR *FESTULOLIUM* ŠAKNŲ AUGIMO SAVYBIŲ RYŠYS SU TOLERANTIŠKUMU STRESORIUI

Santrauka

Ištirtas gausiažiedės svidrės (*Lolium multiflorum* Lam.), tikrojo eraičino (*Festuca pratensis* Huds.) ir jų hibrido – Brauno eraičinsvidrės (*Festulolium braunii* Richt. Camus) – šaknų linijinis augimas +4 °C grūdinimo temperatūroje. Nustatyta, kad tikrojo eraičino šaknų augimo greitis yra kur kas mažesnis negu gausiažiedės svidrės ($v_e << v_{sv}$). Citologinė augimo analizė rodo, kad mažesnį tikrojo eraičino linijinį šaknų augimo greitį, lyginant su svidre, lemia mažas ląstelių dalijimosi greitis ($V_e < V_{ers} < V_s$). Pagal šiuos rodiklius hibridinė Brauno eraičinsvidrė užima tarpinę, kiek artimesnę svidrei, padėtį. Pastebėta, kad išskirtinai trumpa tikrojo eraičino šaknų tįsimo zona *L* yra mažo ląstelių dalijimosi greičio pasekmė. Taigi mažas ląstelių dalijimosi greitis *V*, kaip meristemos veiklos pasekmė, yra augimo stabdymo, stresinės būklės ir tokiu būdu pasireiškiančio tolerantiškumo stresoriui citologinė išraiška, o trumpa šaknų tįsimo zona gali būti stresinės būklės ir atsparumo rodiklis.

Raktažodžiai: *Festuca pratensis, Lolium multiflorum, Festulolium braunii*, šaknų linijinis augimas, ląstelių dalijimosi greitis, tolerantiškumas stresoriui, streso būklė, suapvalėjusios ląstelės