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A comparative lowered temperature response in root apex cellular growth of *Festuca pratensis* and ×*Festulolium braunii*

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Abstract

Root apex development was studied in two closely related perennial grasses differing in winter hardiness: highly resistant meadow fescue (*Festuca pratensis* Huds.) and less hardy ×*Festulolium braunii* [(K. Richter) A. Camus]. Cytomorphometric assessment was carried out to determine cellular growth modulations in the root apical meristem (RAM) comprised of cell proliferation and transition domains. At 20°C in hydroponics, root apex growth in *F. pratensis* 'Dotnuva I' and ×*Fl. braunii* 'Punia DS' was rather similar, accounting for about 0.9 mm RAM length after 30 days. Further, upon hardening at temperature +8/+2°C (14/14 days) the mean RAM length in ×*Fl. braunii* exceeded that of *F. pratensis* by 42%, reaching 1.7 and 1.2 mm, respectively (×*Flb*>*Fp* at $P < 0.01$). Such differences were mostly due to the apparent expansion of the transition domain of the RAM in the hybrid. Also, a specific acclimation feature in the RAM of ×*Fl. braunii* was revealed, manifesting itself as a sharp RAM cell width increment (×*Flb*>*Fp* at $P < 0.01$). We assume that different RAM growth modulation under lowered temperature results in setting up a different pattern of plant growth in autumn / early winter period, namely, ×*Fl. braunii* expands its RAM and keeps growing, while *F. pratensis* declines cellular growth in the RAM and halts its vegetation.

For the first time, we describe the RAM growth in ×*Fl. braunii* and *F. pratensis* in detail which could serve as a layout for new methodologies in growth biology studies of perennial grasses.

Key words: cell growth, hardening, perennial grasses, root apical meristem.

Introduction

Herbaceous perennial plants have an annual cycle of development which consists of intensive growth (summer), cold acclimation or hardening (autumn), growth cessation (winter) and de-hardening (early spring). The upper parts, stems and leaves, desiccate before growth cessation, but the lower parts, basal nodes and roots, undergo significant physiological changes and are maintained throughout the winter.

The survival of plants under conditions of seasonality therefore depends on their capability to modulate their growth and to acquire appropriate changes prior to wintering. At the stage of hardening, plants are affected by lowered temperatures which trigger changes in their growth in general and lead to responsive alterations at the cellular and molecular level (Janská et al., 2010; Wingler, 2015; Wingler, Hennessy, 2017; Beine-Golovchuk et al., 2018). Studies in plant physiology have shown that plant roots are especially sensitive to environmental cues, and that their response strategy greatly depends on the root apex which serves as an initial site for signal perception and response coordination (Baskin, 2013).

Roots grow constantly by their apical part that is usually not longer than 1–2 cm and is marked by pronounced cell proliferation and elongation. Ivanov and Dubrovsky (2013) defined three distinct zones in the root apex of maize: the root apical meristem (RAM), an elongation zone and a differentiation zone. Meristem cell divisions provide new cells and remain in the RAM for a few days, and then the cells progressively enter the zone of fast elongation and exit it in 5 to 15 h after they extend tenfold to twentyfold in length. The RAM was found not entirely uniform and structurally consisting of two domains: a cell proliferation domain and a transition domain. The cell proliferation domain is defined as that part of the RAM where probability of cell division is

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high, while the transition domain is referred to as a site where probability of cell division is low (proposed by Ivanov and Dubrovsky, 2013). Other authors use the term "transition zone" instead of "transition domain" and describe it as a place in the root apex where postmitotic cells maintain their length:width ratio ≤2 (Baluška et al., 1994; 2001). For the sake of consistency, in this paper we will use the term "transition domain of the RAM". Cells in the root apex, and in the transition domain of the RAM in particular, are known as a sensor site where plants directly or indirectly are sensing environmental changes of water supply, temperature, ionic concentration, mechanic stimulation and where the plant response is manifested by changes in root cell growth and tissue morphogenesis pattern (Baluška et al., 2004; 2010; Potocka et al., 2011; Baskin, 2013). Also, root architecture development greatly depends on exogenous supply of nitrogen and phosphorus (Zhang et al., 2007; Niu et al., 2013). As the root apex grows, RAM cells subsequently enter the elongation zone, and the phase of fast elongation is also important in response to the abiotic stress factors listed above (Yang et al., 2017). Keeping this in mind, it is expected that under lowered temperature plant root growth in general, and cellular growth in particular, should manifest changes in cell linear parameters as well as shifts in the longitudinal zonation pattern of the tissue.

This study is aimed at comparing changes in the root apex cellular growth upon hardening at temperature +8/+2°C in two related perennial grasses differing in winter hardiness: highly resistant *Festuca pratensis* and less hardy ×*Festulolium braunii* (Lemežienė et al., 2004). Lithuanian cultivars 'Dotnuva I' and 'Punia DS' are used for such a comparative study of lowered temperature response, where meadow fescue 'Dotnuva I' (colchicine induced tetraploid from the original diploid cultivar) is a parental component of 'Punia DS' hybrid (Nekrošas et al., 1995).

Materials and methods

The seeds of meadow fescue (*Festuca pratensis* Huds.) 'Dotnuva I' (2n = 4x = 28, colchicine induced tetraploid from the original diploid cultivar) and ×*Festulolium. braunii* [(K. Richter) A. Camus] 'Punia DS' (2n = 4x = 28) were germinated and planted by single plants for the experiment in hydroponics. A 16 h light (day) and 8 h dark (night) photoperiod was used, illumination applied at 120 µmol m^{-2} s^{-1} photon flux density. The growth medium was as described by Šimkūnas et al. (2007). The following growth temperature was used: +20°C between the 1st and the 30th day, +8°C between the 31st and the 44th day (for 14 days), and +2°C between the 45th and the 58th day (for 14 days). Root apices were collected at end of growth at +20°C, on the 31st day, and at the end of growth at +2°, on the 58th day; plants were at 2–5 tiller developmental stage. Root apices were fixed in FAS fixative (formaline:glacial acetic acid:ethyl alcohol at the ratio 90:5:5), washed and stored in 70% ethyl alcohol.

Permanent slides for cytological analyses were prepared from the microtomic root sections. The apices of 5 mm were washed in ethyl alcohol series 75, 96 and 100 % (for 2 h each) and impregnated with paraffin (containing 5% beeswax) application using chloroform as solvent by a standard method. The series of median longitudinal sections of 7 µm were made by Mod 1130/ Biocut (Reichert-Jung, Austria). Schiff-periodic acid reaction was used for tissue staining and permanent slides were prepared after dehydration by alcohol, followed by xylol application and mounting in Canada balsam. For analysis, central sections of apices were selected, cell length and width measured in the cortex exodermis file and in one of mesodermis files (skipping the first file adjacent to exodermis and taking measurements of the next file), the position of each cell was recorded by its longitudinal row number in the file counting shootward from the quiescence centre. For each of the experimental variant, *F. pratensis* 'Dotnuva I' at +20°C and after +8/+2°C treatment, and ×*Fl. braunii* 'Punia DS' at +20°C and after +8/+2°C, species were sampled by five plants in three replicates and three roots tips were analysed from each plant taking cellular measurements in two central sections. The images were analysed using the software *SigmaScan Pro 5* (Jandel Scientific Corp., USA).

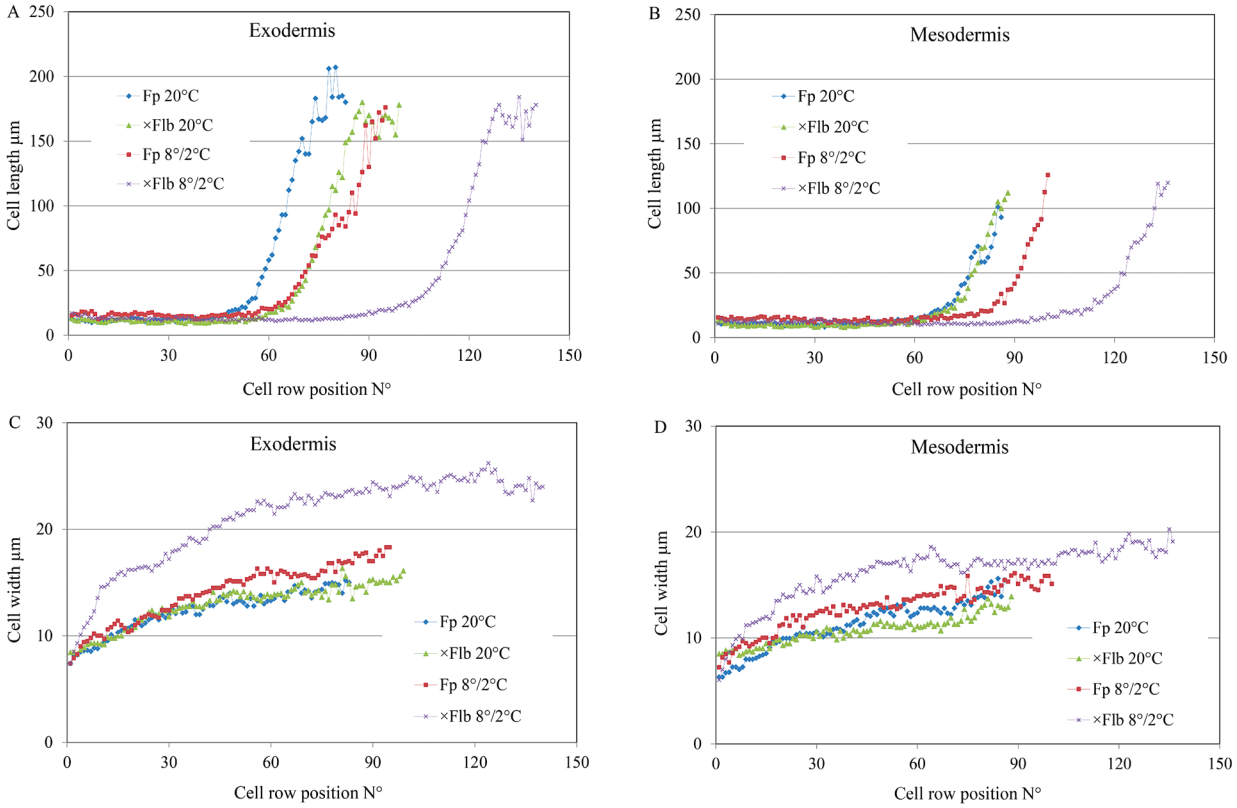
All data are presented as statistical means calculated using the program *STATISTICA 5.5A*. The curves were produced representing relationship between linear cell measurements (y axis) and cell position by longitudinal row number in exodermis and mesodermis tissue (x axis). Differences at the level of $P < 0.05$ or lower are considered significant. Correlation and regression analysis was applied for assignment of cells to one of the two root apical meristem (RAM) parts, the cell proliferation or the transition domain (Ivanov, Dubrovsky, 2013).

Results

The root apical meristem (RAM) is comprised of non-homogeneous cell population where different cells are characterized by specific rate of division and linear / radial growth, which makes the basis for their further development into specific tissue and structure. The quiescence centre (QC) of the RAM in *F. pratensis* 'Dotnuva I' and ×*Fl. braunii* 'Punia DS' was found to be comprised of a few cells similar to the maize root meristem (Ivanov, Dubrovsky, 2013). A symmetrical pattern of the tissue files in relation to the root axis was observed in the longitudinal sections of the root apices. Eight cell files were found in the RAM cortex tissue, and this number was consistent for both species and did not differ at +20°C and upon +8/+2°C hardening. We analysed 5 mm root apices by assessing cell length and width in two RAM cortex tissue files: exodermis, which is an outermost cortex cell file, and the 2nd file of mesodermis (skipping the 1st mesodermis file adjacent to exodermis).

RAM cell length and width are presented in Figure 1A and C for exodermis, and in Figure 1B and D for mesodermis. In each section of Figure 1 two curves relate to 30 days' vegetative growth at 20°C, and two other curves represent cellular growth data upon the hardening at +8/+2°C (14/14 days) for *F. pratensis* 'Dotnuva I' and ×*Fl. braunii* 'Punia DS', respectively.

Vegetative growth at +20°C. The mean RAM cortex cell length in the root apices of *F. pratensis* 'Dotnuva I' and ×*Fl. braunii* 'Punia DS' was fairly similar, ranging from about 10 µm at the lowest cell rows towards QC up to maximum 200 and 100 µm at the highest cell rows shootward for exodermis and mesodermis, respectively (Fig. 1A, B). However, dynamics of the cell length



Note. The value of ±SE ≤ 10% of the mean was defined in this experiment; Fp – *F. prantesis*, ×Flb – ×*Fl. braunii*.

Figure 1. Root apical meristem (RAM) cell length (A, B) and cell width (C, D) of *Festuca pratensis* 'Dotnuva I' and *Festulolium braunii* 'Punia DS' grown at +20°C and upon +8/+2°C (14/14 days) hardening

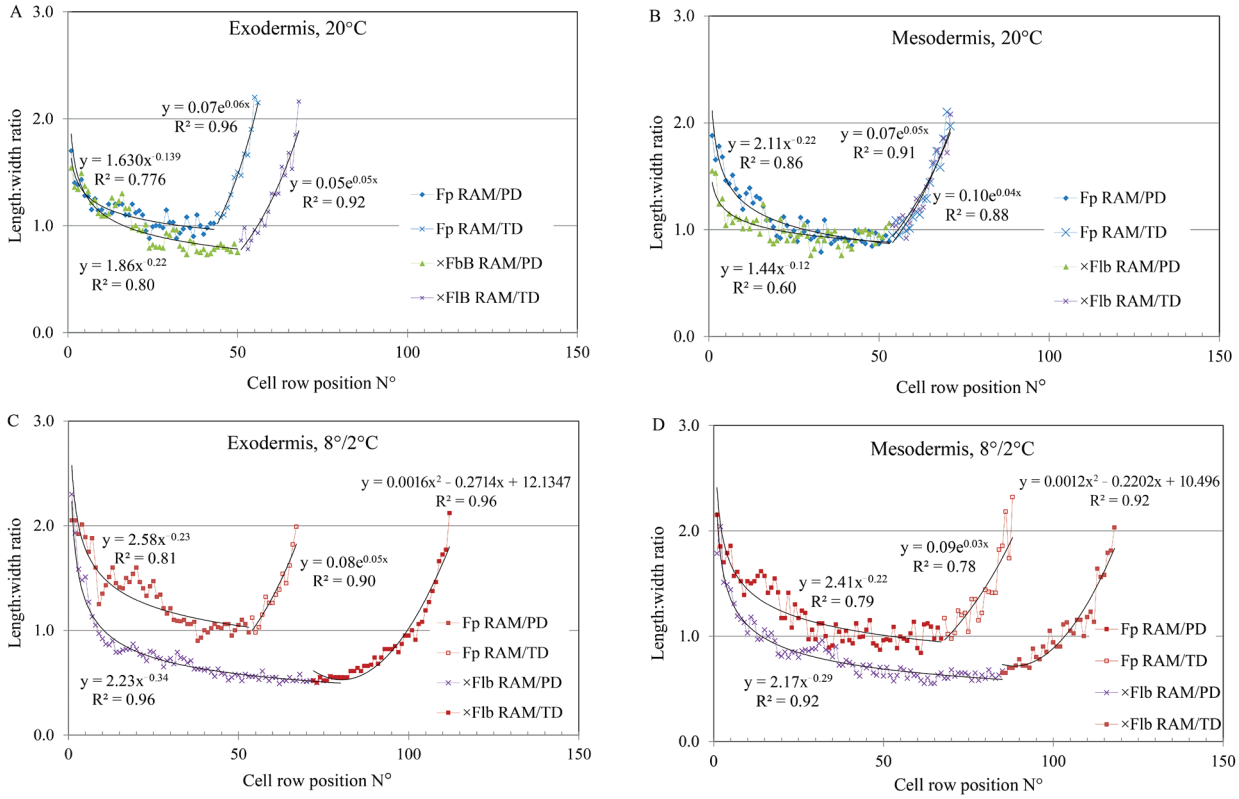
increment in relation to cell row position was different. It is notable that in the ×*Fl. braunii* the beginning of the cell length increment for exodermic cells was markedly shifted shootward as compared to that in *F. pratensis* ($P < 0.01$) (Fig. 1A). In mesodermis, length increment dynamics fell behind that in exodermis. For example, in *F. pratensis* a clear length gain was apparent in the zone between the 50th and 60th row in exodermis, while only by the 70th row in mesodermis (Fig. 1A, B). At +20°C growth, the mean RAM cortex cell width of *F. pratensis* 'Dotnuva I' and ×*Fl. braunii* 'Punia DS' was similar, ranging from about 8 to 15 µm at the cell row positions between 0 (QC) to 90th, respectively (Fig. 1C, D).

In our study we aimed to clearly distinguish the RAM zone versus the elongation zone. We viewed the RAM as a sum of its two parts, the cell proliferation domain and the transition domain. For this purpose, we carried out a quantitative analysis of the cell length and width data and established the curves, giving the length:width ratio in function of cell row position in the file.

As shown in Figure 2, the first part of these curves is declining, which means that the relative gain in cell length is less than that of the width. This represents cell growth dynamics in the cell proliferation domain of the RAM characterized by extensive mitotic activity. Further on, the cell division rate slows down and cells start to expand in length, therefore cell length gradually compensates and finally exceeds cell width, which results in the increase of the length:width ratio. We assumed that the ascending part of the curves in Figure 2 characterizes the cell population in the transition domain of the RAM where cells are present prior to turning into the phase of fast elongation. We considered the value of the length:width ratio ≤2 as a marginal point of the transition domain of the RAM, and the curves above this are assigned to the elongation zone (not studied).

By applying this approach, the RAM zonation pattern in *F. pratensis* 'Dotnuva I' and ×*Fl. braunii* 'Punia DS' grown at +20°C growth regime was found fairly similar, except that the RAM of ×*Fl. braunii* had higher number of cell rows and the transition domain of the RAM was slightly shifted shootward in comparison to that in *F. pratensis* as assessed in cortex exodermis (Fig. 2A). In more detail, in *F. pratensis* 'Dotnuva I' grown at +20°C cell rows assigned to the cell proliferation domain of the RAM were between the 1st and the 43rd row in cortex exodermis and between the 1st to the 53rd row in mesodermis, and those assigned to the transition domain were between the 44th–56th and the 54th–71st row, respectively (Fig. 2A, B). The quantitative data analysis of ×*Fl. braunii* 'Punia DS' grown at +20°C temperature shows that cell rows assigned to the cell proliferation of the RAM were between the 1st and the 50th row in cortex exodermis and between the 1st and the 53rd row in mesodermis, and those assigned to the transition domain were between the 51st–68th and the 54th–71st row, respectively (Fig. 2A, B).

Hardening at +8/+2°C. The assessment of RAM cell parameters revealed clear differences between *F. pratensis* 'Dotnuva I' and ×*Fl. braunii* 'Punia DS' in root growth response to hardening at +8/+2°C (14/14 days) as shown by the curves in Figure 1. Upon +8/+2°C hardening, the RAM cell length increment shootward was lagging behind that at +20°C, and the length gain plateau in ×*Fl. braunii* was significantly wider than that in *F. pratensis* ($P < 0.01$) (Fig. 1A, B). In addition,



Note. The value of ±SE ≤ 10% of the mean was defined in this experiment; RAM/PD – proliferation domain of the RAM, RAM/TD – transition domain of the RAM; Fp – *F. prantesis*, ×Flb – ×*Fl. braunii*

Figure 2. Cell length:width ratio in the root apical meristem (RAM) of *Festuca pratensis* 'Dotnuva I' and *Festulolium braunii* 'Punia DS' grown at +20°C (A, B) and upon +8/+2°C (14/14 days) hardening (C, D)

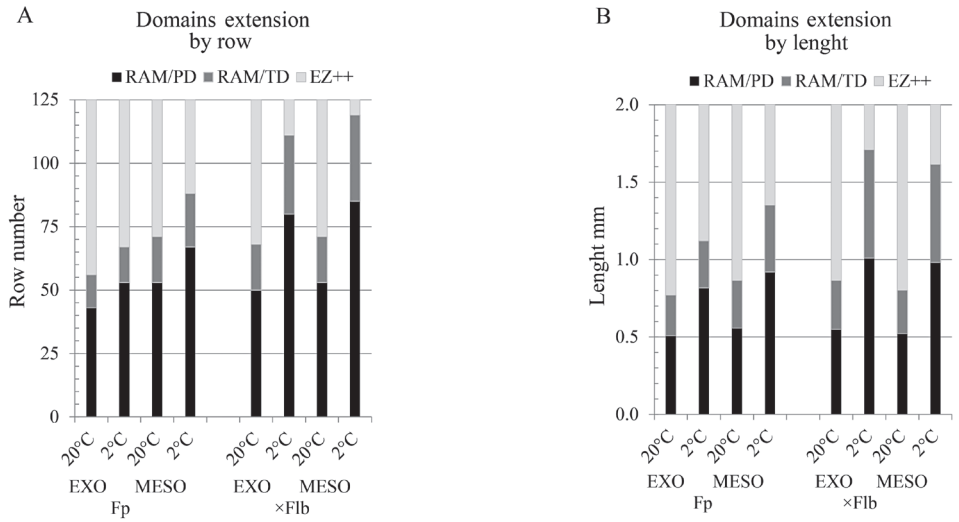
a sharp cell width increment was observed in ×*Fl. braunii* 'Punia DS' at lowered temperature. In the root apices grown at +20°C the maximum RAM cell width was no more than 15 µm while for the ones sampled upon +8/+2°C hardening it was 25 µm (exodermis) and 20 µm (mesodermis) ($P < 0.05$) (Fig. 1C, D).

Also, clear differences were revealed in the RAM longitudinal zonation pattern upon +8/+2°C hardening. The two species' response is shown by the curves in Figure 2C and D. In ×*Fl. braunii* 'the RAM expanded by a higher number of cell rows and the transition domain was evidently shifted further shootward as compared to that of *F. pratensis* ($P < 0.01$). The quantitative data analysis shows that in *F. pratensis* 'Dotnuva I' cell rows assigned to the cell proliferation domain of the RAM were between the 1st and the 53rd row in cortex exodermis and between the 1st and the 67th row in mesodermis, and those assigned to the transition domain were between the 54th–67th and the 68th–88th row, respectively (Fig. 2C, D). For ×*Fl. braunii* 'Punia DS' treated by +8/+2°C hardening, cell rows assigned to the cell proliferation domain of the RAM ranged between the 1st and the 80th row in cortex exodermis and between the 1st and the 85th row in mesodermis, and those assigned to the transition domain were between the 81st–111th and the 86th–119th row, respectively (Fig. 2C, D).

In more detail, cytomorphometric values of cells in the cell proliferation domain of the RAM are compared in Table. In ×*Fl. braunii* 'Punia DS' after hardening, the number of cell rows in the cell proliferation domain has increased by 60% (both in RAM exodermis and mesodermis files), while in *F. pratensis* 'Dotnuva I' such increment was only by 23% and 26% (×*Flb*>*Fp* at $P < 0.01$). The gain in cell length and width was observed both in *F. pratensis* and ×*Fl. braunii*; however, the pattern was different. In ×*Fl. braunii*, the cell length increment of 14% and 17%, and the cell width increment of 64% and 51% (in RAM exodermis and mesodermis) were recorded, whereas for *F. pratensis* these changes were 30% (for both) and 14% and 17%, respectively. Thus, explicit gain in the cell width was characteristic of ×*Fl.braunii* 'Punia DS' in the cell proliferation domain of the RAM upon hardening at +8/+2°C (Table and Fig. 1C, D).

Table. Cytomorphological parameters of cells in the cell proliferation domain of the root apical meristem (RAM) of *Festuca pratensis* 'Dotnuva I' and the ×*Festulolium braunii* 'Punia DS'

Treatment	Exodermis			Mesodermis		
	cell rows	length ±SE µm	width ±SE µm	cell rows	length ±SE µm	width ±SE µm
F. pratensis, +20°C	1–43	11.6 ± 0.1	10.8 ± 0.2	1–53	10.3 ± 0.1	9.9 ± 0.2
+8/+2°C (14/14 days) hardening	1–53	15.1 ± 0.2	12.3 ± 0.3	1–67	13.4 ± 0.2	11.6 ± 0.2
×*Fl. braunii*, +20°C	1–50	10.8 ± 0.1	11.4 ± 0.3	1–53	9.7 ± 0.2	9.9 ± 0.1
+8/+2°C (14/14 days) hardening	1–80	12.3 ± 0.1	18.7 ± 0.4	1–85	11.3 ± 0.1	14.9 ± 0.3



Note. A – by cell row in the tissue file, B – by length, mm; RAM/PD – proliferation domain of the RAM, RAM/TD – transition domain of the RAM; EZ – elongation zone of the root apex (not studied); EXO – exodermis of the RAM cortex, MESO – mesodermis of the RAM cortex; Fp – *Festuca prantesis*, ×Flb – ×*Festulolium braunii*.

Figure 3. Changes in root apical meristem (RAM) longitudinal zonation pattern of *Festuca pratensis* 'Dotnuva I' and *Festulolium braunii* 'Punia DS' upon hardening at +8/+2°C (14/14 days)

Hardening temperature affected the RAM tissue longitudinal zonation pattern and these alterations were particularly apparent in the transition domain of the RAM. Changes in the tissue zonation pattern are summarized in Figure 3 in two ways: by cell row number in the tissue file (an indicator of a cell division event) and the domain length.

The mean number of cell rows in the transition domain of the RAM of ×*Fl. braunii* 'Punia DS' increased from 18 (in both tissue at +20°C) to 31 and 34 (for exodermis and mesodermis upon +8/+2°C), which accounts for a domain expansion by ~80%, while in *F. pratensis* 'Dotnuva I' such gain was marked only by 1 and 3 rows making only ~13% gain (×*Flb*>*Fp* at $P < 0.01$) (Fig. 3A). Thus, the cellular morphometric analysis clearly demonstrated that lowered temperature in *F. pratensis* and ×*Fl. braunii* affects both RAM linear / radial cell growth and longitudinal tissue zonation pattern, and there are pronounced differences between the two perennial grasses studied. Two different strategies of the root apex growth upon +8/+2°C (14/14 days) hardening were distinguished. The total mean RAM length (sum of the cell proliferation and transition domains) expanded in ×*Fl. braunii* 'Punia DS' by about two times (Fig. 3B), from 0.8 and 0.9 mm (exodermis and mesodermis) to 1.6 and 1.7 mm (~100% gain), while in *F. pratensis* 'Dotnuva I' this change was only from 0.8 and 0.9 mm to 1.1 and 1.3 mm (~40% gain), respectively (×*Flb*>*Fp* at $P < 0.01$). As mentioned above, differences in responsive changes at the level of RAM growth were especially evident within the transition domain (Fig. 3A, B).

Our experimental results obtained from the assessment of RAM cell linear / radial growth and tissue zonation pattern in ×*Fl. braunii* 'Punia DS' and *F. pratensis* 'Dotnuva I' show clear differences in root apex cellular development upon +8/+2°C (14/14 days) hardening. This was markedly apparent in the explicit enhancement of the RAM in *Fl. braunii* along with the sharp cell width increment in comparison to that in *F. pratensis* (×*Flb*>*Fp* at $P < 0.01$).

Discussion

Winter hardiness of perennial grasses within *Lolium* and *Festuca* species have been one of the hottest topics for decades with many data obtained from field trials, which have been pointing out peculiarities of the species and complexity of the traits investigated (Paplauskienė et al., 1999; Østrem et al., 2013; Seppänen et al., 2013). A deep insight, in particular, was achieved in disclosing the background of the low temperature acclimation of *F. pratensis* (Darginavičenė et al., 2008; Rudi et al., 2010; Sandve et al., 2011; Rapacz et al., 2014). Nonetheless, keeping in mind the scope of the knowledge available, stress physiology and genetic research at the level of rhizosphere in perennial grasses has been long lagging behind those of model crops (reviewed in *Arabidopsis* by Wachsman et al., 2015).

In our study we investigated the RAM cellular growth and the longitudinal tissue zonation pattern in two related perennial grasses differing in their winter hardiness: highly resistant *F. pratensis* and less hardy ×*Fl. braunii*, using Lithuanian cultivars 'Dotnuva I' and 'Punia DS', where *F. pratensis* 'Dotnuva I' (used as colchicine induced tetraploid from the original diploid cultivar) together with *L. multiflorum* 'Muljam' represent parental components of 'Punia DS' hybrid (Nekrošas et al., 1995). It is notable that hybridization between *F. pratensis* × *L. multiflorum* has been widely exploited since the early seventies and made the basis for commercial ×*Fl. braunii* cultivars developed by different groups of European breeders (Kopecky et al., 2006; Nekrošas et al., 2007). Over the ten years' field assessment revealed that *Festulolium* hybrids are highly productive; however, they evidently experience more damage under wintering conditions than their parental *F. pratensis* species (Lemežienė et al., 2004).

Acclimation of plants is regarded as a modification of structure and metabolism to alleviate stress factors. Theoretically, it is expected that upon perception of a stress signal plants would direct their physiology to alter growth strategy both in their shoots

and roots. We studied the development of the RAM zone which is a "key" site for root growth. In 30 days at vegetative growth temperature of 20°C, the root apex growth in *F. pratensis* and ×*Fl. braunii* was fairly similar, accounting for 0.9 mm RAM length (cf. in maize – 1.6 mm) (Ivanov, Dubrovsky, 2013). Further, upon hardening at +8/+2°C (14/14 days) the RAM length of ×*Fl. braunii* surpassed that in *F. pratensis* by 42%, reaching 1.7 and 1.2 mm, respectively. More precisely, such differences were mostly due to the apparent expansion of the transition domain of the RAM in the hybrid. The transition domain of the RAM is regarded as a kind of a command centre where cells receive environmental sensory information from the root tip and instruct the motoric responses of cells in the subapical elongation zone (Baluška, Mancuso, 2013). To perform their coordinative function, cells of the transition domain were shown to experience cytoskeletal rearrangements, endocytic vesicle recycling, as well as electric activities (Baluška et al., 2004; Baluška, Mancuso, 2013). Among other things, while discussing root growth acclimation Baskin (2013) pointed out expansion responses in the length of the RAM zone, also highlighting the importance of the meristem and the elongation zone boundaries as a key regulatory site.

Our study revealed a specific feature of ×*Fl. braunii* 'Punia DS' expressed as a sharp root cell width increment in the RAM cortex upon plant hardening at +8/+2°C. It is evident that the gain in cell width leads to the enlargement in the root diameter. The importance of a larger root diameter for plant adaptation was demonstrated in some Poaceae, where root thickness was found to correlate with enhanced drought tolerance, in *Oryza sativa* (Jeong et al., 2013), and root longevity, in *Bouteloua gracilis* (Gill et al., 2002). In addition, some authors assume that root thickening in the cold represents a useful means to conserve heat (Yang et al., 2017). Finally, the comparison of annual and perennial species has shown that thicker roots reflect a physiological adaptation to survive in environments where competition is strong (Roumet et al., 2006).

In grassland farming, perennial grasses offer significant advantages over annuals because of their prolonged photosynthetic activity, greater root biomass, and deeper rooting (Marshall et al., 2016). On the other hand, perennials must be equipped with a remarkable system of environmental plasticity to be able to withstand seasonal multifactorial changes, as well as some extremities. In our previous experiments it was demonstrated that ×*Fl. braunii* hybrid combines two parental traits – high growth rate from *L. multiflorum* and a tendency to direct assimilates into their roots from *F. pratensis* – which determine their larger root mass and better chances of recovery (Šimkūnas, Pašakinskienė, 2003 a; b; Šimkūnas et al., 2007).

Our new experimental findings at the cellular level revealed a distinct RAM growth modulation at lowered temperature, and the highest impact was observed in the transition domain of the RAM. We can state that while *F. pratensis* upon +8/+2°C hardening regards this signal as being critical and halts cell proliferation in the RAM, the ×*Fl. braunii* discounts this change as extreme and life threatening, continues to keep up cell division activity and markedly expands the growing apex of its root. As the RAM, and its transition domain in particular, has been regarded a sensor site or even some kind of plant 'brain' in stress signal perception and response coordination (Baluška et al., 2010; Baluška, Mancuso 2013; Baskin, 2013), our findings could serve as a layout for new methodologies and theoretical approaches in elucidating the growth strategy of perennial grasses under environmental conditions of annual seasonality.

In general, it can be considered that ×*Fl. braunii* hybrids have the advantage of prolonged seasonal growth which provides for higher biomass yield in the late season compared to parental *F. pratensis*. On the other hand, our study shows that at lower temperatures, +8/+2°C, they stay physiologically active in their root growth, which means they have not become hardened enough and cannot withstand well the sudden lowering of temperature. At this point, they also may suffer from other physical stress factors, including the hard-frozen soil, which explains their lower winter survival score (Lemežienė et al., 2004).

Root developmental processes and responses are not so easy to analyse, phenotyping of so-called "hidden half" of plants requires innovative experimental approaches. The latest studies in uncovering the genetic regulation of root anatomy and architecture are reviewed in Wachsman et al. (2015). Currently, new root phenotyping technologies offer broad range of tools based on automated scanning, thermal infrared imaging, fluorescence imaging and some other approaches (Fiorani, Schurr, 2013; Downie et al., 2014; Marshall et al., 2016). Fluorescent molecular probes in gene expression profiling (Benitez-Alfonso et al., 2013) when applied in perennial grasses may also contribute significantly to obtaining new integrative data on rhizogenesis modulations while surviving climatic constraints.

Conclusions

We carried out cytomorphometric assessment of the root apex in meadow fescue (*Festuca pratensis* Huds.) 'Dotnuva I' (2n=4x=28, colchicine induced tetraploid from the original diploid cultivar) and ×*Festulolium braunii* [(K. Richter) A. Camus] 'Punia DS' (2n=4x=28), under two temperature regimes in hydroponics: (i) vegetative growth at +20°C for 30 days followed by (ii) hardening at +8/+2°C for 14/14 days. Our findings from the comparison of the root apical meristem (RAM) growth in two species are as follows:

1. At +20°C, the root apex growth in *F. pratensis* and ×*Fl. braunii* was found fairly similar, accounting for about 0.9 mm RAM length; also the linear / radial cellular parameters in the RAM, cell length and width, were found comparably near.

2. Upon +8/+2°C (14/14 days) hardening, mean RAM length expanded in ×*Fl. braunii* by about two times up to 1.6 and 1.7 mm (~100% gain, in RAM exodermis and mesodermis), while in *F. pratensis* it reached only 1.1 and 1.3 mm (~40% gain), respectively (×*Flb*>*Fp* at $P < 0.01$); responsive differences at the level of RAM growth were especially evident within the transition domain of the RAM.

3. Upon +8/+2°C hardening, a specific acclimation feature in the RAM of ×*Fl. braunii* was revealed, manifesting itself as a sharp RAM cell width increment (×*Flb*>*Fp* at $P < 0.01$); this was clearly apparent in the proliferation domain of the RAM where the cell width increment of 64% and 51% (in RAM exodermis and mesodermis) was recorded for ×*Fl. braunii* in comparison to 14% and 17% gain for *F. pratensis*, respectively.

4. We assume that different RAM growth modulation under lowered temperature results in setting up a different pattern of plant growth in autumn / early winter period, namely, ×*Fl. braunii* expands its RAM and keeps growing, while *F. pratensis* declines cellular growth in the RAM and halts its vegetation.

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Festuca pratensis ir ×*Festulolium braunii* šaknies apikalinės mersitemos ląstelinio augimo palyginimas paveikus žema temperatūra

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Santrauka

Tirtos dvi artimai giminingos daugiamečių žolių rūšys, skirtingai išgyvenančios žiemos laikotarpį – gerai žiemojantis tikrasis eraičinas (*Festuca pratensis* Huds.) ir mažiau ištverminga Brauno eraičinsvidrė (×*Festulolium braunii* [(K. Richter) A. Camus]). Citomorfometriškai įvertintas jų šaknies apikalinės meristemos augimas, kurioje išskirtos dvi sritys: proliferacijos ir pereinamoji. Auginant hidroponikoje 20° C temperatūroje, *F. pratensis* 'Dotnuva I' ir ×*Fl. braunii* 'Punia DS' augalų šaknies meristemos augimas buvo panašus – praėjus 30 dienų jos ilgis siekė vidutiniškai 0,9 mm. Vėliau, paveikus +8/+2° C (14/14 dienų) grūdinimo temperatūra, ×*Fl. braunii* augalų šaknų meristema 42 % viršijo *F. pratensis* meristemą ir siekė atitinkamai vidutiniškai 1,7 ir 1,2 mm. Šį skirtumą iš esmės lėmė ryškus hibridinių augalų šaknies meristemos pereinamosios srities pailgėjimas (×*Flb*>*Fp*, $P < 0,01$). Taip pat nustatytas specifinis ×*Fl. braunii* šaknies meristemos prisitaikomasis pokytis, pasireiškiantis ląstelių pločio padidėjimu (×*Flb*>*Fp*, $P < 0,01$). Galima teigti, kad toks skirtingas šaknų meristemos vystymasis esant žemai temperatūrai lemia nevienodą augalų augimą rudens arba ankstyvos žiemos laikotarpiu: ×*Fl. braunii* augaluose šaknies meristema aktyvai plėtojaisi ir augalai toliau auga, o *F. pratensis* šaknies meristemos prieaugis stabdomas ir augalų vegetacija sustoja.
Tyrimas įgalino pirmą kartą išsamiai aprašyti ×*Fl. braunii* ir *F. pratensis* meristemos vystymąsi ląsteliniame lygmenyje; tai galėtų būti metodiškai naudinga toliau tyrinėjant daugiamečių žolių augimo biologiją.

Reikšminiai žodžiai: daugiametės žolės, grūdinimas, ląstelinis augimas, šaknies apikalinė meristema.