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Short Communication

Comparative systemic analysis of the cellular growth of leaves and roots in controlled conditions

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ABSTRACT

The comparative cytological analysis of the leaf/root growth of *Lolium multiflorum* has been performed. It revealed differences of the mentioned above/under-ground organs that express the whole plant's polarity. To perform accurate and simultaneous growth comparison a climatic-hydroponics system has been implemented. A sharp increase in the epidermis cell length of the leaf meristem has been detected for the first time. It allows the proposal of a new way to demarcate the boundary of the meristem and suggests a lengthed leaf meristem, that is 4 times longer than the root meristem. As the cell cycle duration in leaves and roots is similar, the prolonged leaf meristem and a higher leaf growth rate could be determined by the longer life span of cells in meristem, resulting in more cell cycles. The prolonged meristem provides a significantly higher leaf growth rate, ensuring a functional balance with roots. The elongation zone of the roots is significantly shorter than in leaves, which is caused by the larger relative root elongation rate and the slower meristemic root growth rate. The novelty formulated, i.e., the prolonged leaf meristem, opens theoretical perspectives in longitudinal zonation, in finding molecular markers and provides practical significance for the biology of productivity.

1. Introduction

The above-ground and under-ground parts of a plant are different in their growth characteristics. It is established that the processes of organ growth, i.e. the cell division and elongation in roots are more intensive then those in shoots: the duration of the cell cycle in root meristems is significantly less than that in the shoot growth point, and the relative elongation growth rate in roots is higher than in shoots (Ivanov, 1983; Silk, 1984). These cytological peculiarities of root growth are associated with the dense soil medium and provide the ability to push through the soil to reach the nutrient solution. At the same time, the more rapid linear growth of the above-ground stems is related with the longer duration of cell division and cell elongation, which determines the greater number of cell cycles and, consequently, the larger cell production (Ivanov, 1983, 2011). These growth differences of the above/under-ground organs express the polarity of the whole plant, which imparts the ability of different plant parts to adapt themselves to the environment with entirely different characteristics (humidity, illumination, density, etc.) (Cove et al., 1999; Avramova et al., 2015).

A leaf, as an above-ground lateral organ, descending from the peripheral zone of the shoot apical meristem is characterized by determined (limited) growth (Fiorani, 2001; Tsukaya, 2005). It has certain growth characteristics possessed by other organs of the above-ground part. For example, it is known, that the relative elongation rate of leaves, as well as of stems, is relatively low compared with the elongation of roots, and in the case of Dicots, the cell division occurs together with elongation, as the latter is slow (Dale, 1982; Ivanov, 1983). Meanwhile, leaves as above-ground organs, are characterized by rapid linear growth (in the case of Dicots – exponential growth), a result of the long duration of cell division during elongation. This results in a significantly higher number of cell cycles and a higher cell production (Dale, 1982; Maksymowych, 1990). On the contrary to Dicots, the leaves of Monocots (incl. *Poaceae*) have a strict separation of growth zones (i.e. division and elongation-only zones) and grow with one-dimensional axis (Fiorani et al., 2000; Avramova et al., 2015). Besides, both *Poaceae* leaves and roots are characterized by linear growth. All that makes these leaves and roots to be suitable objects for comparative growth analysis. Otherhand, if the new *Poaceae* leaf cells are formed in the meristem only (which is quite small) and there is no cell division in the elongation zone, so it remains unclear what cytological mechanism ensures their higher linear growth rate comparing to roots. Meanwhile, these features are not properly addressed in the literature.

It should be mentioned that comparative analysis of growth of organs is usually carried out with some methodical uncertainties. First, it

Abbreviation: MB, meristem boundary

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is often performed comparing published data on different species (Ivanov, 1983; Silk, 1984; Grif et al., 2002). Also, the identification of growth differences is complicated by the fact that the comparative analysis of the growth of different organs is mainly performed on the basis of data obtained in experiments carried out in non-identical conditions (temperature, illumination, simultaneity, etc.) (Silk, 1984; Grif et al., 2002). At the same time, it should be emphasised that plant growth and its defining cytocomponents are usually highly dependent on environmental conditions, so the lack of attention to the growing conditions complicates the analysis of links between the characteristics of growth and the functions fulfilled (Grif, 1981; Durand et al., 1999). In particular, the duration of cell cycle exponentially depends on temperature and the length of elongation zone also depends on temperatute quite significantly (Grif et al., 2002; Šimkūnas, 2004). Finally, the cell division is influenced by endogeneous diurnal rhythms as well (Grif et al., 2002), so a correct comparison of the growth of different organs requires simultaneity.

The objective of our study is to estimate the differences in linear growth rates and other growth characteristics of the above-ground and under-ground organs (leaves and roots) of *Poaceae* plant *Lolium multiflorum* and to identify the growth component (meristemic or elongation growth) responsible for these differences. The comparative analysis results obtained are interpreted from the viewpoint of plant polarity, taking into account the different environments of the above-ground and under-ground organs, their different life duration and resistance mechanisms. The original climatic-hydroponics system was designed and implemented, what allows to perform accurate and simultaneous investigations of above-ground and under-ground parts of plants in controlled conditions (light, temperature, nutritional medium).

2. Materials and methods

2.1. Plant growing and primary data collection

2.1.1. Growing conditions

The plants were grown in the climatic-hydroponics system under favorable temperature (+20 °C), photoperiod − 11 h. The composition of the nutrition medium according to the demand of *Poaceae* plants was as follows (Šimkūnas et al., 2007): macroelement salts Ca $(NO_3)_2 \times 4H_2O$ 4 mM; NH_4NO_3 0.5 mM; $(NH_4)_2SO_4$ 0.185 mM; KH_2PO_4 1 mM; KNO_3 3.5 mM; $MgSO_4 \times 7H_2O$ 2 mM; microelement salts $MnSO_4 \times H_2O$ 9.1 μM; $CuSO_4 \times 5H_2O$ 0.3 μM; $ZnSO_4 \times 7H_2O$ 0.8 μM; NaCl 30 μM; $NaMoO_4 \times 2H_2O$ 0.1 μM; H_3BO_3 10 μM; iron source FeNaEDTA 26.7 μM. To maintain the stability of media, it was changed weekly.

2.1.2. Leaf growth investigation

Plants were grown for one month before taking root and leaf samples. Thus, the plants were at the tillering developmental stage when a synchronous sampling of leaves and roots was made. The analysis was conducted by choosing the third shoots (of the first order branching), which were marked with coloured rubber rings. The third leaves, approximately middle ones in the shoot (accordingly, with the average morphometric and growth characteristics), were taken for analysis (Valašinaitė et al., 2013). The following leaves were already formed in the chosen shoots: the fully expanded leaf, the growing leaf and the youngest growing leaf (emerging from the whorl of older shoots and corresponding to the phase of linear growth) (Bregard and Allard 1999). To determine the leaf linear growth rate, the length of the youngest growing leaf was measured as the starting point taking the ligule of the fully expanded leaf. The measurements were made with a ruler every day at 9.00 and 17.00 h. Till reaching approximately 30% of the final leaf length, its length increment ΔL through time Δt has been measured. It is worth to mention, that leaves grow due to blade and sheet meristem activities: till approximately 40% of the final length they grow essentially via the leaf blade meristem activity, and, when it stops, the sheat meristem located beneath starts to grow intensively (Schnyder et al., 1990; Serebryakova, 1971). So, the measurements of linear growth rate were made when leaves essentially consisted of blade tissue. Four leaves from separate plants of *L. multiflorum* were taken for analysis.

The cytological preparations were made from the youngest growing leaf after it had reached approximately 30% of the length of the fully expanded leaf (Lattanzi et al., 2004; Kavanova et al., 2008). The preparations were made by using a clearing procedure – boiling it in methanol for 10 min and then placing it into lactic acid (90%) (Fiorani, 2001). The epidermis was chosen as a convenient surface tissue, and the measurements of the epidermal cells were started from the youngest growing leaf ligule (i.e. the beginning of the blade meristem). The boundary of the meristem (MB) had been chosen as the point where the epidermal cells stop dividing and start increasing rapidly. The non-growing part of the leaf was also used for making cytological preparations via the analogical clearing procedure, and the length of mature epidermal cells (l) was measured. The abaxial leaf side was used for measurements because there were fewer stomata in it (Nelissen et al., 2013). Also, to perform these measurements, efforts were made to avoid the asymmetrical cell division, which is related with the differentiation of cells, i.e. the areas with stomata formation were omitted (Abrash and Bergmann, 2009). For that, the cell files located midway between the files containing the stomata were measured (Kavanova, 2006).

2.1.3. Root growth investigations

The adventitious roots, that prevail in the root system (their branching is weakly expressed in hydroponics), were analysed after reaching ≈ 5 cm in length, synchronously with leaf sampling described above. The adventitious roots were marked with permanent ink, 15 mm from the root end, already in the differentiation zone. When the sufficient root increment ΔL was reached (after 2 days), the samples were taken and the roots were cut at the marked-up point (Šimkūnas et al., 2007). Four adventitious roots from separate plants of *L. multiflorum* were taken for analysis.

Then, microtomical preparations were made from the root growth zone. The cell length of the root cortex in the growth zone was measured, and the meristem boundary was identified from the place where cells started to grow rapidly. This method is based on the well-know fact that the relative growth rate in the meristem remains unchanged and rises saltatory only while transiting to elongation (up to 10 times); also cell division occurs in the entire meristem, along the files of root cortex (Ivanov and Maximov, 1999: Van der Weele et al., 2002; Ivanov and Dubrovsky, 2013). As the sharp increase of cell length in root cortex occurs only while transiting to elongation, so it serves as a criterion for demarcation of the meristem boundary. The cytological preparations were also made from the part of the roots' increment, and the mature cells' length l in the root cortex was measured. Cortex cells were used for the measurements because there was no unequal cell division, which is related with cell differentiation (i.e., applying the same criterion as in leaves). Plus, the cells' measurements were performed from the cortex middle, which is characterized by typical cortex cells.

2.2. Calculations and statistical data analysis

2.2.1. Calculations and comparative analysis

Calculations of cytological growth characteristics have been performed in the case of stationary (steady-state) growth. The growth was stationary, because the plants were grown by sustaining the constant growth conditions in the climatic-hydroponics system, plus, the youngest growing leaves for analysis were chosen, as already mentioned above (corresponding to the steady-state growth phase) (Kavanova et al., 2008). Under the stationary growth condition, the number of cells and the length of the meristem and elongation zone were constant; also, the cell flow from the meristem to the elongation