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Research article

Endopolyploidy levels in barley vary in different root types and significantly decrease under phosphorus deficiency

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ABSTRACT

Increased endopolyploidy is important for plant growth and development as well as for adaptation to environmental stresses. However, little is known about the role of reduced endopolyploidy, especially in root systems. In this report, endopolyploidy variations were examined in different types of barley (*Hordeum vulgare* L.) roots, and the effects of phosphorus (P) deficiency and salinity (NaCl) stress on root endopolyploidy were also studied. The results showed that the endopolyploidy levels were lower in lateral roots than in either primary or nodal roots. The lower endopolyploidy in lateral roots was attributed to cortical cells. P deficiency reduced the endopolyploidy levels in lateral roots and mature zone of primary roots. By contrast, salinity had no effects on the endopolyploidy levels in either lateral or primary roots, but had a minor effect on nodal roots. Transcript analysis of cell cycle-related genes showed that multiple cell cycle-related genes were more highly expressed in lateral roots than in primary roots, suggesting their roles in lowering endopolyploidy. P deficiency reduced *HvCCS52A1* transcripts in the mature zone of primary roots, but had little effect on the transcripts of 12 cell cycle-related genes in lateral roots, suggesting that endopolyploidy regulation differs between lateral roots and primary roots. Our results revealed that endopolyploidy reduction in root systems could be an integrated part of endopolyploidy plasticity in barley growth and development as well as in adaptation to a low P environment.

1. Introduction

Endoreduplication exists in more than 90% of herbaceous angiosperms, and only occurs in some somatic cells through a unique cell cycle mode (i.e., the endocycle). In the endocycle, cells undergo DNA replication without cell division, leading to higher endopolyploidy (Breuer et al., 2014). The chimeric cell ploidy derived from endoreduplication differs from genome polyploidy, which leads to a uniform increase in cell ploidy for all cells in the organism.

Endoreduplication plays an important role in plant growth and development and in adaptation to environmental stresses (Barow, 2006; Maluszynska et al., 2013; Scholes and Paige, 2015). Increased endopolyploidy can meet the need of specialized cells in growth, development and metabolic activity, such as fruit development (Pirrello et al., 2013), endosperm development (Kladnik et al., 2006), formation of multicellular trichome (Breuer et al., 2014) and rhizobium nodulation and mycorrhizal colonization (Kondorosi and Kondorosi, 2004; Wildermuth, 2010). Environmental factors can also modulate endopolyploidy levels. Darkness leads to higher endopolyploidy levels in Arabidopsis hypocotyl (Gendreau et al., 1997). UV-B irradiation enhances endoreduplication in epidermal cells of cucumber cotyledons (Yamasaki et al., 2010), which could contribute to plant growth under high incident UV radiation (Gegas et al., 2014). However, water deficit

Abbreviations: CCS52, cell cycle switch 52; CDK, cyclin-dependent kinase; Co/epi, cortical/epidermal; C value, the amount of DNA per haploid cell; CYC, cyclin genes; DW, dry weight; FW, fresh weight; Lrm, the mature zone of lateral roots; Lrt, lateral root tips; Lrt/m, lateral roots including the meristem/elongation zone and mature zone; Nrm, the mature zone of nodal roots; Nrt, nodal root tips; P, phosphorus; Prb, the basal segment of primary roots; Prm, the mature zone of primary roots; Prt, primary root tips.

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and shading result in the reduction of endopolyploidy levels in Arabidopsis leaves (Cookson et al., 2006). Low phosphate availability in soil decreases endopolyploidy levels in leaves (Smarda et al., 2013). Therefore, the reduction of endopolyploidy also appears to be important for plant adaptation to some environmental stresses. Much endopolyploidy plasticity has been learned from the above-ground tissues of plants (Scholes and Paige, 2015), but limited information is known for root systems.

Root systems are vital for land plants to acquire water and nutrients from soils and are the target for crop improvement (Gewin, 2010). Barley is an economically important temperate cereal and has a dual root system that consists of primary roots of embryonic origin, nodal roots that develop from basal nodes of main stems and tillers, and lateral roots that develop from both primary and nodal roots (Smith and De Smet, 2012). These three root types are morphologically distinct and functionally different. Many studies of root endopolyploidy have focused on young seedlings, which generally contain primary roots, while less is known about lateral roots and nodal roots (Ceccarelli et al., 2006; Silva et al., 2012). Lateral roots are predominant in terms of the total root length of mature plants (Pierret et al., 2005) and play a pivotal role in the acquisition of water and nutrients. Plants exposed to many abiotic and biotic stresses from soil demonstrate changes in root system development which is significant in adaptation to environmental stresses (Chapin and Bieleski, 1982). A significant portion of the photosynthate produced by plants is used for the construction and maintenance of root systems (Hammond and White, 2008). Stressed plants increase carbon allocation to the root system compared to non-stressed plants (Hermans et al., 2006). Understanding the fundamental aspects of root system biology is important for the optimization of crop growth and yield.

Cell cycle-related genes are known to be involved in the endoreduplication of the model plant Arabidopsis (Edgar et al., 2014; Scofield et al., 2014). Cyclin-dependent kinases (CDKA and CDKB) are key regulators of the cell cycle in plants. The cell cycle phase-specific expression of cyclins (CYCs) activates CDKs through direct interaction. Different CDK-cyclin pairs form complexes, regulating different phases of the cell cycle. Generally, A-type cyclins (CYCAs) control the S-to-M phase progression, and CYCBs control the G2/M transition and M phase progression (Inzé and De Veylder, 2006). CYCDs control the G1/S transition, but some are expressed in the G2/M phase (Inagaki and Umeda, 2011). To exit the mitotic cycle to the endocycle, the activity of certain CDK-CYC complexes is down-regulated. Degradation of CYCs is a key trigger of endocycle entry. The cell cycle switch 52 (CCS52), an activator of E3 ubiquitin ligase, promotes transition from the mitotic cycle to the endocycle by proteolysis of CYCs (Kondorosi and Kondorosi, 2004; Mathieu-Rivet et al., 2010). The down-regulation of CDK activities through phosphorylation of the CDK subunit by WEE1 kinases can also promote the transition from the mitotic cycle to the endocycle (Shimotohno et al., 2006). However, little is known about the involvement of these cell cycle-related genes in the endoreduplication of temperate cereals such as barley.

In this work, we examined endopolyploidy variations in different segments of three root types of barley in response to P deficiency and salinity stress. We also determined the transcript levels of 12 cell cycle-related genes in primary and lateral roots to determine the molecular mechanisms underlying endopolyploidy adjustment as well as the effects of P deficiency. Our results revealed that the endopolyploidy levels in barley varied in different root types and significantly decreased under P deficiency, suggesting that the endopolyploidy reduction could be an integrated part of endopolyploidy plasticity in barley growth, development, and adaptation to environmental stresses.

2. Materials and methods

2.1. Plant materials and growth conditions

Barley seeds (*Hordeum vulgare* L. cv Clipper) were surface sterilized with 70% ethanol for one min, rinsed with distilled water three times, placed in petri dishes with moist filter paper, and kept at room temperature in the dark overnight. Germinated seeds were transferred to square pots (8.5 × 8.5 × 17.5 cm) containing University of California potting mix with complete fertilizers (Tiong et al., 2014), and watered every two days to maintain a field capacity of 8% water content (w/w). Plants were grown in a controlled-environment growth room with 12 h light/12 h night cycle at 300 μmol m^{-2} s^{-1} photon flux intensity at the plant level, and temperatures of 15 °C day/10 °C night were used to simulate the winter temperature in southern Australia for early barley growth. In each experimental group, there were four independent replicates and two plants per replicate.

Root segments from 26-day-old barley plants were cut from nodal roots of 4–10 cm in length, primary roots of 8–17 cm, and lateral roots of 1–3 cm. These root segments were: Primary root tips (Prt, 0–5 mm from the apex); the mature zone of primary roots (Prm, 5–10 mm from the apex); the basal zone of primary roots (Prb, 5 mm from the root-shoot junction); lateral roots (Lrt/m, 0–10 mm from the apex); nodal root tips (Nrt, 0–5 mm from the apex) and the mature zone of nodal roots (Nrm, 5–10 mm from the apex).

For ploidy measurements of cortex/epidermis and stele derived from lateral and nodal roots, barley plants (cv. Sahara) were grown in potting mix as described above. Plants 37 days old were used for separation of the cortex/epidermis and stele from lateral roots, and plants 52 days old were used for separation of the cortex/epidermis and stele from nodal roots. The lateral roots taken from the middle part of the primary roots (approximately 10–15 cm in length), and nodal roots approximately 5–10 cm in length, were used for tissue separation. The lateral root apex (0–5 mm) was cut and discarded, and root segments of 5–20 mm were used to prepare cortical/epidermal and stele cells by peeling off the cortex/epidermis with a sharp razor blade under a dissection microscope.

2.2. Flow cytometric analysis of root segments and tissues

Cell ploidy levels were determined using flow cytometry. Fresh root segments and tissues were chopped with a sharp razor blade for 30 s–60 s in 500 μl of nuclei extraction buffer (CyStain UV precise P Nuclei Extraction Buffer; Partec) and filtered through a nylon sieve with 20 μm pore size. Then, 1.5 ml of staining solution (CyStain UV precise P Staining Buffer, Partec) with DAPI was added into the filtrate, and the mixture was used to measure ploidy levels with a Ploidy Analyser (Partec, Germany). For each sample, at least 1000 nuclei were analysed. Mean C values (the amount of DNA per haploid cell) were calculated using the formula, mean C value = (2 × n2C + 4 × n4C + 8 × n8C)/(n2C + n4C + n8C) described by Barow and Jovtchev (2007), where n2C, n4C and n8C are the numbers of nuclei with the corresponding C value (2C, 4C and 8C).

2.3. Root sectioning

Root segments from 26 day-old barley plants were collected from the mature zone (5–10 mm from the root apex) of three types of roots and fixed in ethanol-glacial acetic acid (3:1, v/v) at 4 °C for 24 h. Dehydration and embedding in paraffin or epoxy resin was performed similar to the method described by Sharma et al. (2010).