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Short communication

Actin microfilaments are involved in the regulation of *HVA1* transcript accumulation in drought-treated barley leaves

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ABSTRACT

Drought is one of the stresses that limit the yield of barley. Despite extensive studies focused on the issue, the molecular mechanism of the response to drought is still not fully understood. In our previous study, we proposed drought-induced signal perception controlled by actin filaments (AFs). To test this hypothesis, we used a chemical inhibitor of AF polarization—latrunculin B. In drought-treated barley leaves, latrunculin B induced AF depolymerization and altered gene expression (mainly those controlling AF formation), notably inhibiting the expression of *HVA1*, a dehydrin encoding gene whose function in drought tolerance has been widely studied. These results suggest that AFs might be involved in water-deficit signal perception in plant cells.

1. Introduction

Drought is one of the stresses that limit the yield of barley. Despite extensive studies focused on the issue, the molecular mechanism of the response to drought is still not fully understood (Huang et al., 2012). The cytoskeleton seems to play an important role in responses to stress conditions because it represents a type of biological network in signal perception. Volkmann and Baluška (1999) proved that actin filaments (AFs) are not only a stable structural base, but function in diverse signaling pathways within the cell and between cells, e.g., in association with guard cell movements, gravitropism, pathogenesis, or wounding.

AF reorganization under stress conditions can affect gene expression (Kobayashi et al., 1997). Changes in the expression of actin depolymerizing factors (ADFs) involved in AF dynamics were observed in response to (a)biotic stress. The expression of *ADF3* was reported to be necessary for the rpg4-mediated resistance of barley to stem rust (Wang et al., 2013), while *ADF* expression was required for cold acclimation (Ouellet et al., 2001) and drought tolerance in rye (*ADF5*, Liu et al., 2003) and barley (*ADF1*, Śniegowska-Świerk et al., 2015). In our previous report (Śniegowska-Świerk et al., 2015), we showed that in a drought-tolerant cultivar of barley, a drought-induced decrease in the transcription of *ADF1* was accompanied by a decrease in an actin encoding gene (*ACT11*) expression and upregulation of the *HVA1* gene. This gene, encoding a dehydrin (group II Late Embryogenesis Abundant protein family), has been reported several times as very important for drought tolerance (Straub et al., 1994; Wójcik-Jagła et al., 2012; Chen et al., 2015). *HVA1* upregulation was observed also during drying of detached barley leaves (Wójcik-Jagła et al., 2012). Under the same conditions, Śniegowska-Świerk et al. (2015) observed extensive AF cytoskeleton reorganization and an increase in AF content. This suggested that AFs may play role in the water-deficit signaling pathway in barley leaves, which can triggered the expression of the *HVA1* gene. To determine whether, the signaling occurs through AFs-mediated *HVA1* gene expression, the latrunculin B (Lat-B) was applied. Treatment with Lat-B, a toxin synthesized by *Latrunculia magnifica* (a sponge), effects on the structural and functional AFs network properties by, especially membrane-associated, AFs degradation (Baluška et al., 2001). Interestingly, Lat-B can also suppress AF partitioning under some stress conditions, e.g., hypoosmotic stress (Liu et al., 2013). Here, we used Lat-B to perturb, the hypothesized, drought signaling cascade by the AF network disruption. The expression of key genes involved in the control of AFs polymerization/depolymerization was examined in parallel.

Abbreviations: *ACT11*, actin 11 gene; *ADF1*, *ADF3*, actin depolymerization factors encoding gene; AFs, actin filaments; Ep, epidermis; *HVA1*, dehydrin encoding gene; Lat-B, latrunculin B; Mc, mesophyll; RWC, relative water content; *SRG6*, drought-induced gene with putative transcription factor function.

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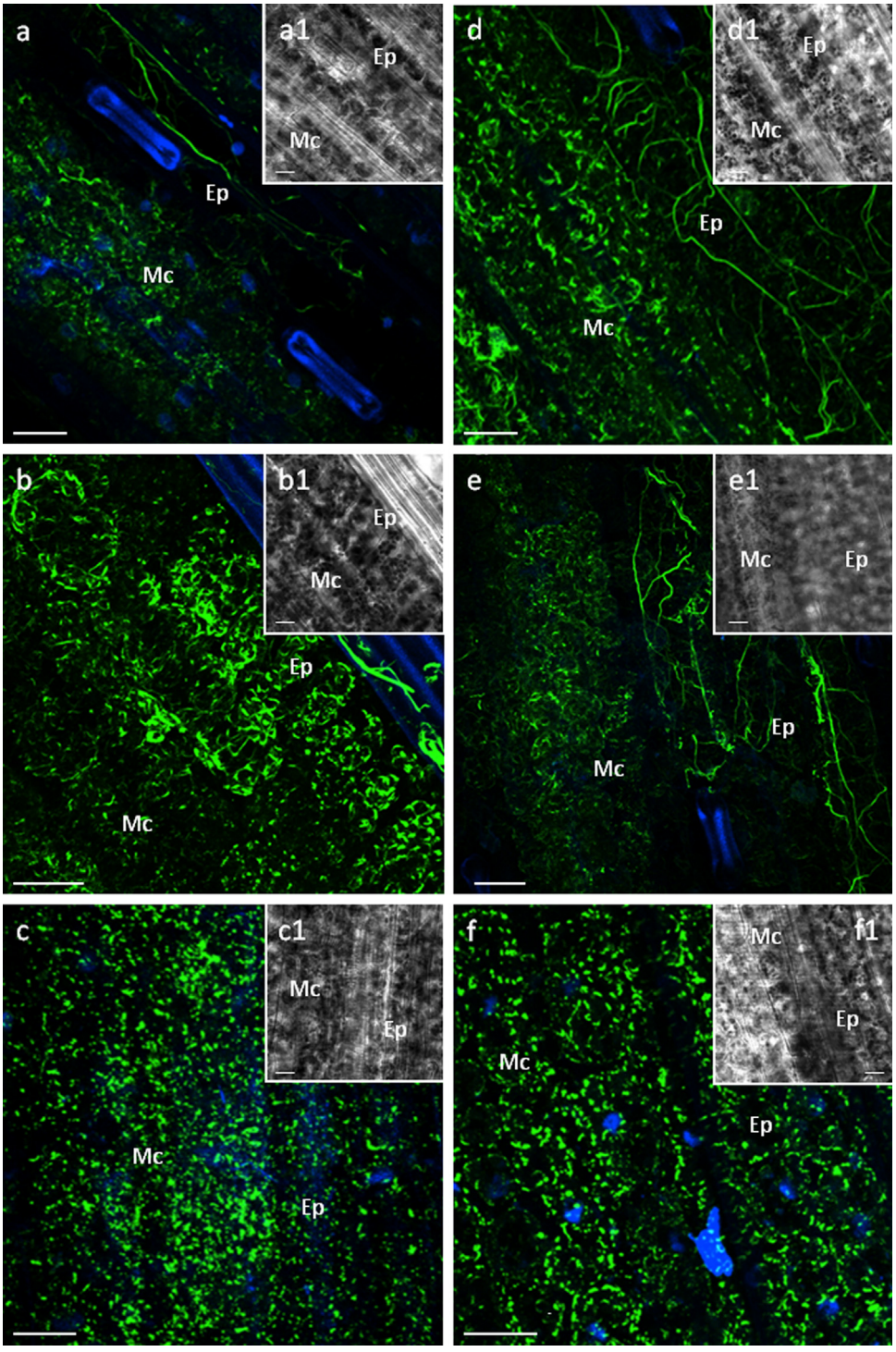

Fig. 1. Actin filaments in leaf blade sections of two barley (*Hordeum vulgare* L.) cultivars differing in their responses to drought: susceptible cv. 'Maresi' (a–c) and resistant cv. 'CAM/B1/CI' (d–f). Control (a, d), drought-treated (Lat-B^{-}) (b, e) and drought + Lat-B-treated (Lat-B^{+}) (c, f) leaves. (a) Thick and longitudinally oriented AFs in the epidermis (Ep), and randomly oriented curly aggregates of AFs in the mesophyll (Mc). (b) Numerous and longitudinal AFs arranged in thick and well-organized bundles in the Ep. A dense network of curly aggregates of AFs in the Mc. (c) AFs strongly affected by Lat-B in the Ep and Mc. AFs fully fragmented. (d) Numerous AFs arranged in well-organized bundles oriented transversally and longitudinally in the Ep. Curly aggregates of AFs in the Mc. (e) Long and thin AFs randomly oriented in the Ep and partially fragmented aggregates of AFs with weak fluorescence in the Mc. (f) AFs strongly affected by Lat-B in the Ep and Mc. Abundant AF fragments. Confocal microscopy image of Ep and Mc-merged images after Z-series projection (a–f). Inserts on the right show the corresponding transmission images (a1–f1). Actin filaments were stained with Alexa Fluor 488 phalloidin (green). Nuclei were stained with DAPI (blue). Scale bar = 20 μm.