Roles of auxin and ethylene in aerenchyma formation in sugarcane roots

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ABSTRACT

Although the cross-talk between auxin and ethylene has been described during plant development, the role played by auxin upon gene expression during aerenchyma formation is poorly understood. Root aerenchyma formation results from the opening of gas spaces in the cortex. It is part of a developmental program (constitutive) or due to ethylene treatment or abiotic stress (induced) such as flooding and nutrient starvation. This process relies on programmed cell death and cell wall modifications. Here we followed development of aerenchyma formation in sugarcane along 5 cm from the root apex. As a constitutive process, the aerenchyma formation was observed in the cortex from the 3rd cm onwards. This occurred despite 1-methylcyclopropene (1-MCP) treatment, an inhibitor of ethylene perception. However, this process occurred while ethylene (and auxin) levels decreased. Within the aerenchyma formation zone, the concentration of ethylene is lower in comparison to the concentration in maize. Besides, the ratio between both hormones (ethylene and auxin) was around 1:1. These pieces of evidence suggest that ethylene sensitivity and ethylene-auxin balance may play a role in the formation of aerenchyma. Furthermore, the transcriptional analysis showed that genes related to cell expansion are up-regulated due to 1-MCP treatment. Our results help explaining the regulation of the formation constitutive aerenchyma in sugarcane.

The aerenchyma is characterized by enlarged and interconnected intercellular spaces filled with gas.\textsuperscript{1,2} They can be schizogenous and lysigenous. Whereas in the former, cells separate from one another without cell death, the second includes cell expansion, separation, cell death and cell wall modifications.\textsuperscript{2,3,4} Aerenchyma can be constitutive, i.e., its formation is included in the developmental program, or induced by abiotic stresses such as nutritional starvation and hypoxia.\textsuperscript{1} In sugarcane, the development of aerenchyma is constitutive.\textsuperscript{2,5} Its formation seems to be a result of different combinations of developmental modules,\textsuperscript{1} which are activated by plant hormones and the environment. Leite et al. (2017) reported that cell wall modifications in sugarcane roots produce a composite that apparently seals the gas spaces. They seem to function as chambers where gasses can be stored and used for respiration by the remaining living cells in the roots.

The level and tissue sensitivity to ethylene are responsible for triggering aerenchyma formation.\textsuperscript{8} In at least two species possessing constitutive aerenchyma (Juncus effusus and Oryza sativa) the role of ethylene has been demonstrated through the use of 1-methylcyclopropene (1-MCP), a substance that blocks the ethylene receptors in plants.\textsuperscript{9,10} Justin & Armstrong, (1991) reported the effect of the exogenous application of a synthetic auxin on aerenchyma formation in maize roots. However, the auxin interplay with ethylene has been neglected so far.

Here we report the levels of ethylene and auxin in root segments of sugarcane and discuss the possibility that their interplay has a role in aerenchyma formation in sugarcane. To our knowledge, this is the first report that shows both hormones measured at the same time during aerenchyma formation.

Roots were sectioned into five segments,\textsuperscript{2} and aerenchyma was present from S3 onwards in control and 1-MCP treatment (Fig. 1). No significant differences were found. Ethylene and auxin levels were measured for all five root segments (Table 1).\textsuperscript{12,13}

To assess the role of ethylene in aerenchyma formation in sugarcane root development, the inhibitor 1-methylcyclopropene (1-MCP) (EthylBloc, AgroFresh) was applied to roots at every 48 h from May/2012 to August/2012. Plant growth, root harvesting, and aerenchyma area calculation were performed as reported by Leite et al. (2017).

1-MCP affected the proportions between ethylene and auxin within the root segments. In S1 and S2, 1-MCP treated roots displayed proportionally lower ethylene concentration. Ethylene and auxin levels decreased 2.5-fold during the S1-S2 transition in 1-MCP treated roots, whereas within control plants they were kept constant (Table 1). These differences led to a higher auxin-ethylene ratio on S1 and S2 in 1-MCP plants compared to control (Fig. S1).

SHORT COMMUNICATION
Microarray and qPCR validation showed transcripational alterations. The primers used are listed in Table S2. Around 89% of the qPCR profiles confirmed the microarray results (Table S3-S7). Three expansins were among the up-regulated transcripts in 1-MCP treatment (Table S3, S4 & S5). These proteins are related to the stability of hydrogen bonds between xyloglucan and cellulose. Thus, expansin action plays a key role in cell expansion.

Although xyloglucan occurs in small proportions in sugarcane walls, it might be important in the control of cell wall architecture by holding macrofibrils together in the wall. It is, therefore, possible that expansins play a role in the cortex cell expansion in maize and sugarcane. However, the fact that 1-MCP treated roots displayed higher expression of expansins is contradictory, since this protein normally leads to expansion due to some cell wall loosening during development. The higher auxin level observed closer to the root tip (S1 and S2) suggests that this hormone might be involved in triggering the expression of cell expansion-related genes that are key elements in the cellular processes that take place before aerenchyma formation.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Table 1. Ethylene and free IAA levels along the segments in plants treated with 1-MCP.

| Segment | Control | 1-MCP | Control | 1-MCP |
|---------|---------|-------|---------|-------|
| S1      | 0.25 ± 0.02 a | 0.12 ± 0.01 a | 12.16 ± 2.04 a | 18.85 ± 2.60 a |
| S2      | 0.23 ± 0.04 a | 0.05 ± 0.02 b | 11.66 ± 0.70 b | 7.63 ± 1.30 b |
| S3      | 0.12 ± 0.02 b | 0.10 ± 0.03 b | 7.61 ± 0.51 b | 9.82 ± 1.00 b |
| S4      | 0.07 ± 0.01 b | 0.07 ± 0.02 c | 4.75 ± 0.39 c | 4.87 ± 0.33 b |
| S5      | 0.06 ± 0.01 b | 0.06 ± 0.01 c | 5.39 ± 1.02 c | 5.90 ± 0.20 b |

**a**Significant differences (p < 0.1) between root segments. Bold numbers indicate significant differences (p < 0.1) between treatments.

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