Potassium Deficiency and JA-dependent Responses to Biotic Stress in Barley

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Background

Potassium (K) is an essential element in plants and is required in large quantities to maintain normal growth, development and metabolism (1). In a microarray study investigating transcriptome changes of *Arabidopsis thaliana* during K starvation and after resupply Armengaud *et al* (2) identified genes related to jasmonic acid (JA) signalling as one of the largest functional categories within K-responsive genes. A rise in JA as well as other oxylipins was subsequently confirmed in K-deficient plants.

Jasmonic acid is a phytohormone involved in a number of processes in growth and development as well as responses to abiotic and biotic stress (3), in particular wounding. JA is part of a complex signal network involved in the response to pathogen attack acting in conjunction with ethylene (ET) and salicylic acid (SA) (Fig.1). While ET is involved in K-sensing in the roots (1), SA is not affected by K-deficiency.

This project aims to answer the following questions:

Does the JA-response to K-deficiency occur in plants other than *A. thaliana*?
Do increased JA levels in K-deficient plants interfere with defence, and how?

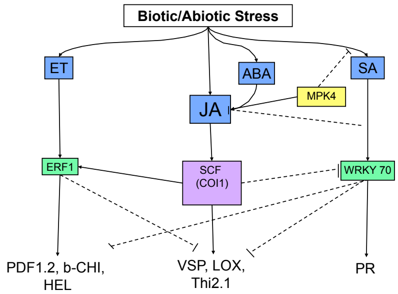

Fig. 1. Plant stress signalling network.
Different types of biotic or abiotic stress, such as pathogen infection or wounding, induce the synthesis and subsequent activation of several hormonal pathways (i.e. JA, ET, SA and ABA). Modified from (4).

Are JA levels increased during K deficiency in Barley?

Experimental approach: Barely (Optic) plants were germinated on paper then transferred to either full nutrient or K free hydroponic systems after 5 days (Fig. 2a) . 5 plants were sampled and pooled every 3 days.

Fig. 2a. Growth system.

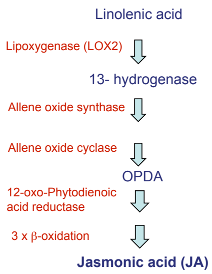

Fig. 2b. JA biosynthesis.

Lipoxygenase is a crucial enzyme in JA biosynthesis (Fig. 2b) and LOX2 is known to be induced by JA and K-deficiency in Arabidopsis (2). The level of LOX2 transcription in barley was determined by qPCR with RNA isolated from plants grown in full nutrient (▪) and -K (▫) media. 5 plants were pooled every 3 days, the mean expression level (± SE) of 3 replicate experiments is shown in Fig. 2c. As early as Day 3 (after transfer to hydroponics) an increase in LOX2 can be seen in the K-starved plants. The largest difference is seen on Day 12.

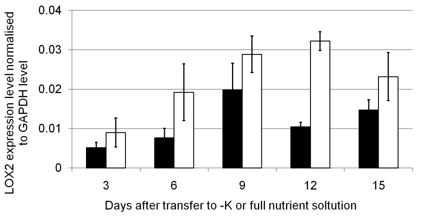

Fig. 2c. LOX2 expression over time in K starved plants

To confirm the increase in JA related gene expression in K starved plants, the expression level of proteins reported to be upregulated in response to JA in barley were analysed. As shown in Fig. 2d Jasmonate Induced proteins JIP23, JIP37 (unknown function) and JIP60 (a ribosome inactivating protein) were all expressed at higher levels in K starved (▫) samples (Day 12) compared to full nutrient (▪) tissue

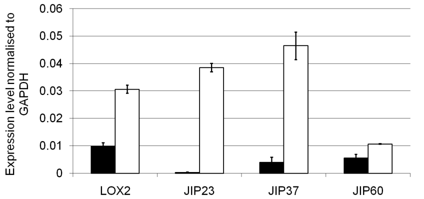

Fig. 2d. JIP expression at 12 days after K withdrawal

Does K-deficiency affect the wounding response?

Barley plants grown for 10 days in full nutrient (▪) or K free (▫) hydroponic solution were wounded using a wire brush. 5 plants were pooled every 2 h for each treatment. LOX2 transcript level was determined using qPCR, the mean (± SE) of 3 replicate experiments is shown. The LOX2 expression in full nutrient plants reached a peak after 4 h while in the K deficient plant LOX2 expression continued to increase until up to 8 h

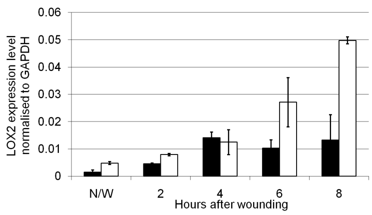

Fig. 3: LOX2 expression after wounding.

Does K-deficiency affect defence against Rhynchosporium?

Rhynchosporium secalis, commonly known as scald or leaf blotch, is a hemi-biotrophic fungus that grows without symptoms under the leaf cuticle in early infection, before producing conidia and causing visual symptoms (Fig. 4a).

Plants were grown in full nutrient (▪) or K free (▫) nutrient solution for 14 days, and leaf segments prepared. After 24h each segment was inoculated with spore solution. The length of the lesion was measured.

Fig. 4a. Leaf lesions caused by *R. secalis*.

18 days after inoculation the mean lesion size is significantly larger on base segments of leaves from K deficient plants than on the corresponding segments from K-sufficient plants (Fig. 4b).
No significant difference is apparent on tip and middle segments.

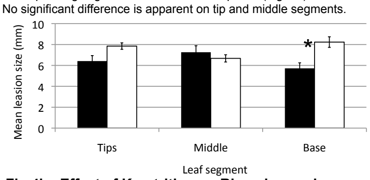

Fig 4b. Effect of K nutrition on Rhynchosporium infection

Does K-deficiency affect powdery mildew infection?

Powdery mildew (*Blumeria graminis*)
Is an obligate biotroph apparent as white fluffy fungal growth on the leaf surface (Fig. 5a).It reduces yield by draining nutrients from the plant.

Plants were grown in full nutrient or K free nutrient solution for 14 days. The 2nd leaf was cut into 4cm pieces and separated into tips, middle and base segments. After 24h recovery time the segments where inoculated with powdery mildew.

Fig. 5a. Leaf segments infected with powdery mildew.

The number of colonies on each segment were recorded (Fig. 5b). 10 days after inoculation no significant difference was seen in the number of colonies per mm² between treatments, for any of the segment types.

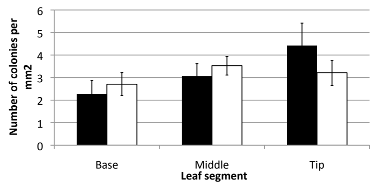

Fig. 5b. Effect of K deficiency on powdery mildew infection

The transcript level of LOX2 in leaf segments 2 days after inoculation was analysed , 4 leaf segments were pooled per sample. In the full nutrient plants the LOX2 expression was lower in infected (+) plants than in uninfected (-), possibly due to suppression by the salicylic acid pathway. While in the K deficient plants the LOX2 expression is increased in the tips and base, no change is seen in the middle segment.

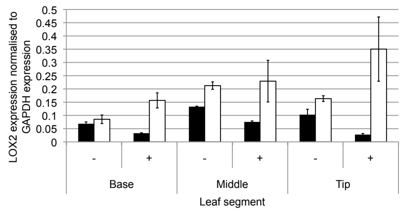

Fig. 5c. Effect of K deficiency on LOX2 expression after powdery mildew infection

References
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2. Armengaud *et al.* (2004) Plant Physiol. 136, 2556-2576.
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Conclusions

- Up-regulation of a JA biosynthesis gene LOX2 is seen in K starved barley plants. An increase in transcript levels is also observed for Jasmonate-Induced Proteins JIP23, JIP37 and JIP60. These results provide strong indication for increased JA levels in K starved barley plants.
- The increase in LOX2 expression after wounding seems to be prolonged in K starved plants.
- Lesions induced by Rynchosporium infection are larger in K-deficient plants. This could indicate increased JA levels lead to increased susceptibility to this heterotrophic fungus.
- After infection with powdery mildew (a biotrophic fungus) LOX2 transcript levels are decreased in nutrient sufficient plants but increased in K starved plants. This could indicate attenuation of SA mediated defence in K-starved plants. However, no significant effect on infection was apparent.