Effect of GA, ABA and water stress on leaf elongation and XET activity in barley *Hordeum vulgare* L.*

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The relationship between xyloglucan endo transglycosylase (XET) and leaf elongation was investigated in barley (*H. vulgare* L.). Imposing of treatments of gibberellic acid (GA), abscisic acid (ABA) and water deficit were used to attain stimulation and inhibition of growth respectively. In GA-responsive dwarf mutant M489, leaf elongation rate and XET activity increased following treatment with GA. Temporal aspects of GA stimulation indicated that XET is probably involved in GA-stimulated leaf elongation. Feeding 4μM ABA decreased leaf length to 50% by 3 and 4 days after treatment. XET activity assayed on day 3 was found to be significantly reduced in ABA fed leaves as compared to distilled water controls. Water stress reduced the length of both 3rd and 4th leaves compared to well-watered controls from 4days onwards after withholding water. XET activity decreased considerably under water stress in both leaves and followed similar trend as that of leaf elongation. Overall, the results point to a link between extractable XET activity and rates of leaf elongation, which indicates the potential role of XET in leaf elongation.

Xyloglucan endo transglycosylase (XET) which catalyses cleavage of the hemicellulose xyloglucan and transfers the newly formed potentially reducing terminus to a new XG9 oligomer is believed to be a key enzyme in cell wall reconstruction [1-3]. XET has been suggested to allow cell expansion by cleaving xyloglucan molecules that tether adjacent cellulose microfibrils and rejoining the cut ends of cleaved tethers [1]. XET is thus believed to be important in control of cell expansion and organ growth. A wide variety of plant species including bryophytes, dicots and monocots have been shown to exhibit XET activity [1]. Moreover, the highest levels of activity have been demonstrated to be associated with regions of active elongation in both dicots and monocots [4-5]. A gene family encodes XET enzymes and five CDNA clones related to the barley XET were characterized and spatial distribution of the corresponding mRNAs were determined throughout the elongating leaf using clone specific hybridization probes [6]. High levels of XET activity and its association with regions of active elongation in monocots is particularly interesting, as unlike dicots wherein xyloglucan is the major cell wall hemicellulose, monocots contain glucurono arabinoyxylans as their predominant hemicellulose with mixed linkage β-D glucans and xyloglucan each contributing approximately 20% on a mole % basis [7].

In view of the above the present study was undertaken to investigate the relationship between leaf elongation and XET activity in barley. Treatments like use of gibberellic acid (GA), abscisic acid (ABA) and water deficit were imposed to attain stimulation and inhibition of growth respectively.

**Materials and Methods**

**Plant culture**—Barley (*Hordeum vulgare* L.) was grown in 20x12x10 cm plastic containers with 1:1 mixture (v/v) of perlite and vermiculite in artificially lit growth cabinets maintained at 18/13°C day/night and 12 h photoperiod in Phytocon at Canberra. Plants were irrigated with deionized water and Hoagland ½ N nutrient solution on each morning and afternoon respectively. The following experiments were conducted using the plant culture protocol.

**Experiment 1**: Time course study of induction of XET activity and leaf elongation in response to gibberellic acid (GA) application:

GA responsive dwarf mutant M489, which had been isolated from var. Himalaya following sodium azide mutagenesis and subjected to back crossing with the parent Himalaya by Dr. P.M.Chandler, Division of Plant Industry, CSIRO, Canberra was used in this experiment. As the leaf 2 emerged,
lengths of all the plants in the containers maintained in the growth cabinet were measured (0 hr). Five microfilters of GA3 (1 μg/μl) was then applied at the base of the leaf whorl in 1/2 of the population, while the other 1/2 received an equal amount of control solution which contained 50% acetone. Data on leaf length as well as XET activity were recorded at 6, 24, 48, 72 hr after GA application. There were 4 and 3 replicates for leaf length measurements and XET assay respectively.

Experiment II: Effect of ABA feeding on the leaf growth and XET activity: Grains of var. Himalaya were surface sterilized, washed in HCl solution, rinsed in sterile water and germinated in sterile filter paper envelopes. The envelopes were transferred to ABA solutions 24hr after planting and grown under continuous low intensity fluorescent lighting at 20°C for 4d before harvesting. Seedlings grown in H₂O were used as controls. Data on leaf lengths and XET activity were recorded on leaf 1 in 10 and 2 replicates respectively.

Experiment III: Effect of water deficit stress on leaf elongation and XET activity: Barley (var. Himalaya) plants were grown at 15/13°C d/n and 50% RH. Water deficit treatment was imposed by withholding water as soon as leaf 3 was visible. Leaf elongation, leaf water potential and XET activities were recorded in the leaf 3 during development of stress. There were 33 replicates for leaf length measurements and 2 for XET extractions.

Leaf length from base of the pot was monitored during the experiments. On each day, leaf length measurements were done between 10.30 and 12.00 hrs in water stress experiments. For the assay of in vitro XET activity leaf samples were homogenized on ice in 20 mM MES(pH 6.0) containing 10 mM CaCl₂ and 10 mM Na iso-ascorbate using a pestle and mortar. Homogenates were cleared by centrifugation at 1000 rpm for 10 min and the supernatant decanted and stored at -80°C until XET activity was assayed using nasturtium xylglucan as substrate. The soluble protein content of extracts was determined by the dye binding method.

Leaf water potential (Ψ₁): Plant water status was quantified at the time of sampling in water stress experiment by measuring Ψ₁ using a pressure chamber (Soil Moisture Corp., USA). The water potentials were monitored in the fully expanded leaf, which was leaf 2.

Results

Experiment I: Leaf elongation and XET activity in response to GA application: Fig 1 shows the changes in leaf length as well as in XET activity in response to GA application in a GA responsive dwarf mutant M489. Leaf 2 lengths of GA treated plants increased remarkably by 24hr and later. XET activity also showed similar trend to leaf elongation. The slight decline in XET activity at 72hr after a peak at 48hr corresponded with a leveling off of the leaf elongation rate by 72hr. The results demonstrated that treatment with GA3 stimulated leaf elongation and extractable XET activity.

Experiment II: Leaf elongation and XET activity in response to ABA: Results presented in Fig 2 show the effect of 4μM ABA feeding on leaf lengths and XET activity in leaf 1 of barley var. Himalaya. Leaf elongation was decreased to 50% by 3 and 4 days after treatment. XET activity assayed on day 3 was
found to be significantly reduced in ABA fed leaves as compared to controls growing on distilled water (Fig.2 inset).

Experiment III: Leaf elongation and XET activity in response to water deficit: Water stress reduced the length of both 3rd and 4th leaves compared to well watered controls from 4 days onwards after withholding water (Fig.3a and b). Difference in leaf water potential (Δψw) between water stressed and well-watered plants was 0.5 and 0.65 MPa by 6 and 8 days respectively. XET activity decreased considerably under water stress in both leaves and followed similar trend as that of leaf elongation (Fig.3, b and d).

Discussion

Various treatments to alter leaf elongation were used to explore the relationship between XET and leaf growth. In GA-responsive dwarf mutant M489, leaf elongation rate and XET activity increased following treatment with GA3. Temporal aspects of GA stimulation showed that XET is involved in GA-stimulated leaf elongation. In maize roots and barley leaves highest levels of XET activity are known to be associated with regions of rapid elongation 5,7,8.

ABA is known to inhibit leaf elongation 11-14. In our experiments, ABA at the concentration of 4μM inhibited XET, the key enzyme involved in cell wall loosening corresponding to decrease in elongation rate of leaf1. ABA is known to mediate several stress responses and to regulate many stress genes15. Several workers have found that ABA plays a role in inhibition of shoot and leaf growth under water deficit stress11,13. Our results indicate that inhibition of XET activity could be a crucial event in the manifestation of ABA-mediated inhibition of leaf elongation.

Water deficit resulted in reduction in lengths as well as XET activity of 3rd and 4th leaves of barley var. Himalaya. Although it is known that water deficit reduces leaf growth 16,17 but the mechanism of inhibition of leaf growth and biophysical and biochemical processes involved are not clear. The fall in growth rate is apparently in response to non-hydraulic signals from roots in drying soil, which is or is related to ABA11,18-20. The stress modulation of leaf growth generally operates through cell expansion although in very young leaves there is a modulation of cell number as well21. Cell expansion requires wall loosening which in turn has to be manifested via a reconstruction or rearrangement of cell wall framework. It is in this context that our results on activity of XET and leaf elongation in response to water deficit are important as they highlight a close parallel relationship between the two processes.

An important aspect of these experiments is that the stimulation and inhibition of leaf elongation were studied in the similar genetic background as the GA-responsive dwarf mutant M489 had been isolated from var. Himalaya and went through several back crossing generations on to the parent Himalaya. However, data on in vitro XET activity levels need to be interpreted with caution20. The in vitro XET activities based on an oligosaccharide acceptor may be more related to in vivo activities between polymeric XG molecules within a growing cell wall. There is also a possibility that the activity observed is a composite of several isozymes with the potential for different pattern of expression under stimulation and cessation of growth. Spatial resolution of the regulation of XET activity in response to the changing leaf elongation rates could be useful in probing further the role of XET in leaf growth.

Overall, the results indicate that higher levels of extractable XET activity are associated with elevated rates of leaf elongation and experiments to study the
stimulation cessation of leaf elongation showed parallel changes in leaf elongation and XET activity.

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References