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Introduction

Cell expansion is controlled by cell extensibility, which is regulated by expansin and xyloglucan endotransglycosylase (XET) proteins (Cosgrove,1999). The expression of expansin and XET has been associated with cell elongation controlling leaf, stem, and root elongation in various plant species. It is well known that plant growth is regulated by hormones. Gibberellic acid (GA) primarily stimulates leaf elongation and GA inhibitors such as trinexapac-ethyl (TE) reduce the leaf elongation rate (Isabel S,2001). However, whether growth effects of GA or its inhibitor on the elongation of leaves are associated with changes in expansin and XET expression is not clear.

Drought stress inhibits plant growth through influencing leaf elongation and initiation. Mild drought can reduce leaf expansion. Severe drought stresses can even cease leaf growth. However, the effects of drought stress on leaf elongation in tall fescue and the genes controlling the elongating processes under drought stress are not well known.

Objective

The objective of this study was to investigate how GA and a GA inhibitor (trinexapac-ethyl, TE) regulate leaf elongation at cellular and molecular levels under well-watered and drought conditions.

Materials and Methods

Plant Materials

Tall fescue (*Festuca arundinacea*) – ‘K-31’

Growing Conditions

- Seeds were sown in 50-cell plastic trays.
- Plants were watered every other day and fertilized with half-strength Hoagland’s nutrient solution twice a week when the first leaf appeared.
- After 2 weeks, uniform-sized seedlings were transferred into plastic containers filled with half-strength Hoagland’s nutrient solution. The solution in each container were aerated using an aquarium pump.
- Growth chamber conditions: 22/18° C, 650 PAR, 60% RH
- Drought Stress : -0.5MPa, -1MPa, -1.5MPa
- Treatment: adding PEG8000 into the nutrient solution after the 3rd leaf emergence and elevating the PEG concentration every other day
- GA treatment: plants were treated in nutrient solution with 50 μM GA₃ for 24h, then transferred to normal solution
- TE treatment: plants were foliar sprayed by TE (2%)
- All treatments were performed in 4 replicates

Physiological Measurements

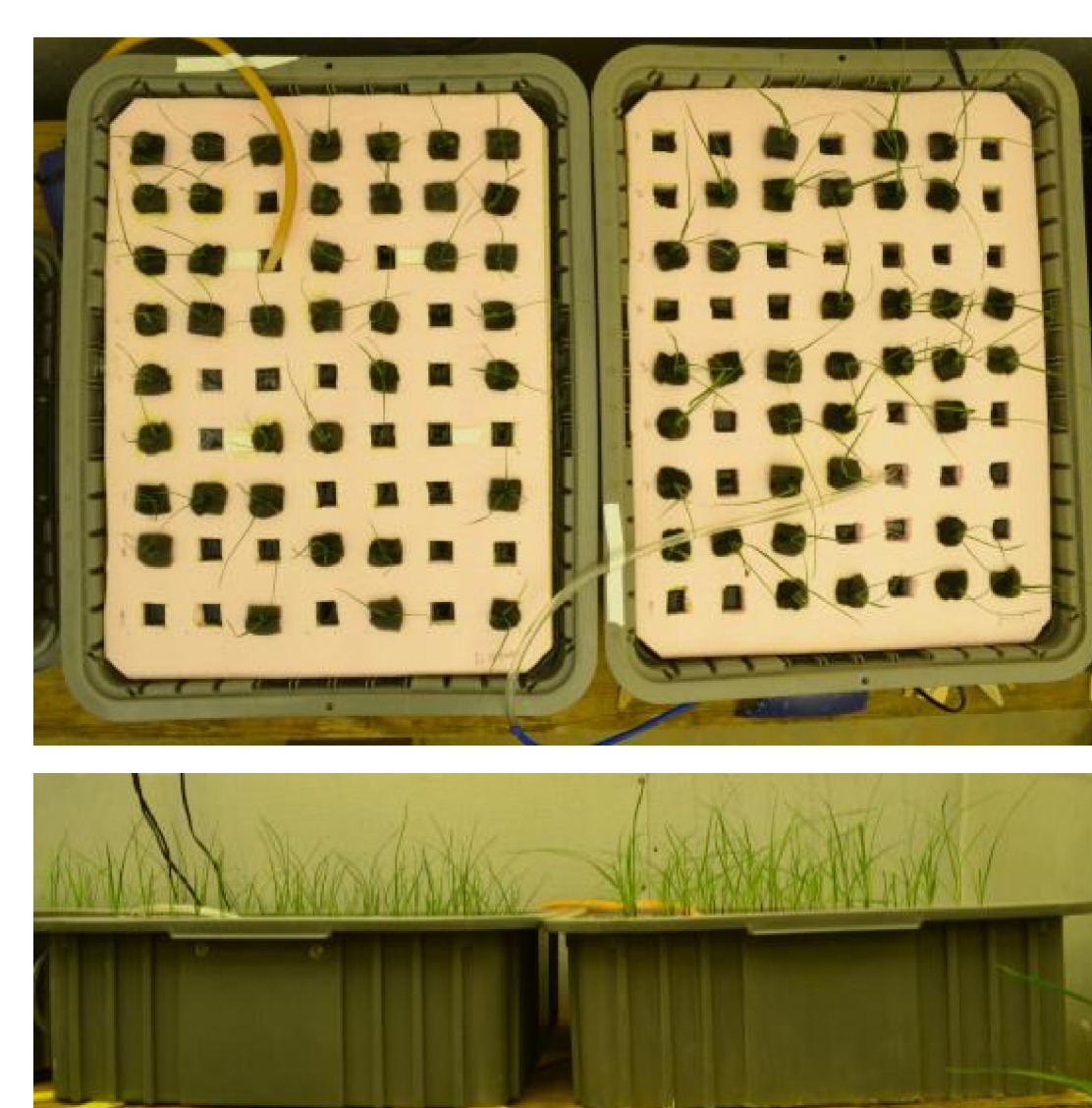
Leaf length (mm)
Leaf elongation rate (mm/d)
Epidermal cell length
Leaf relative water content (RWC)

Gene Expression Analysis

5 expansin genes (A1,A5,A9,B2,B11) and 3 XET (*XET1,XET2,XET3*) genes were tested by quantitative RT-PCR

Statistic Analysis

Treatment effects by ANOVA using SAS 9.2
LSD means tested at P<0.05 level



Hydroponic System

Conclusions

The leaf growth rate of K-31’ was elevated by GA under both well-watered and drought conditions.

Drought stress inhibited tall fescue leaf growth partly through reducing the epidermal cell length.

Three expansin genes were up-regulated by GA treatment : A1, A5, B2.

Four expansin genes (A1,A9, B2 and B11) and one XET gene (*XET2*) were down-regulated by drought stresses.

Two expansin genes (A9, B2) were regulated by both GA and drought stress.

Results and Discussion

Fig. 1 Leaf elongation rate of ‘K-31’ with GA, GA inhibitor (TE) and water under well-watered conditions

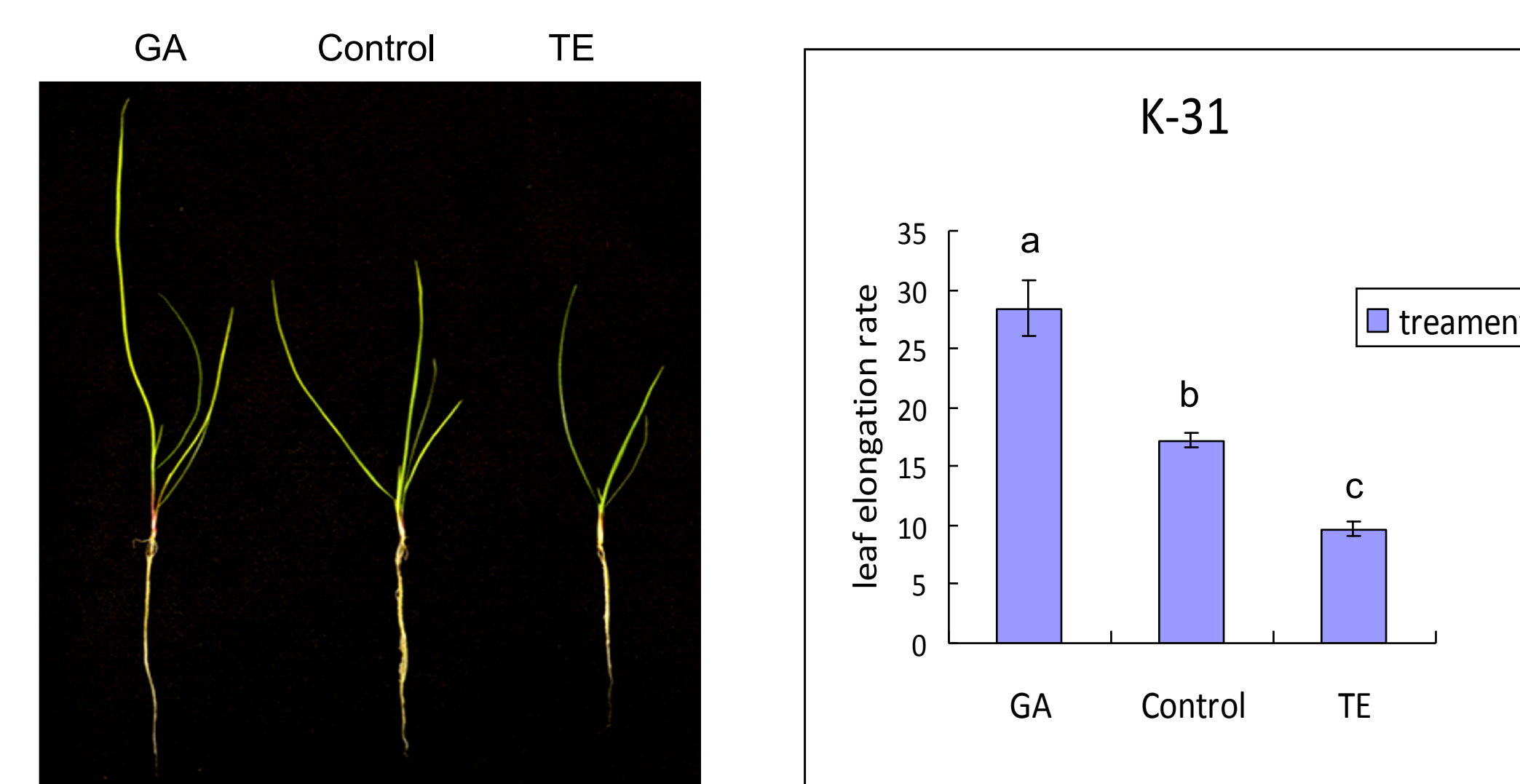
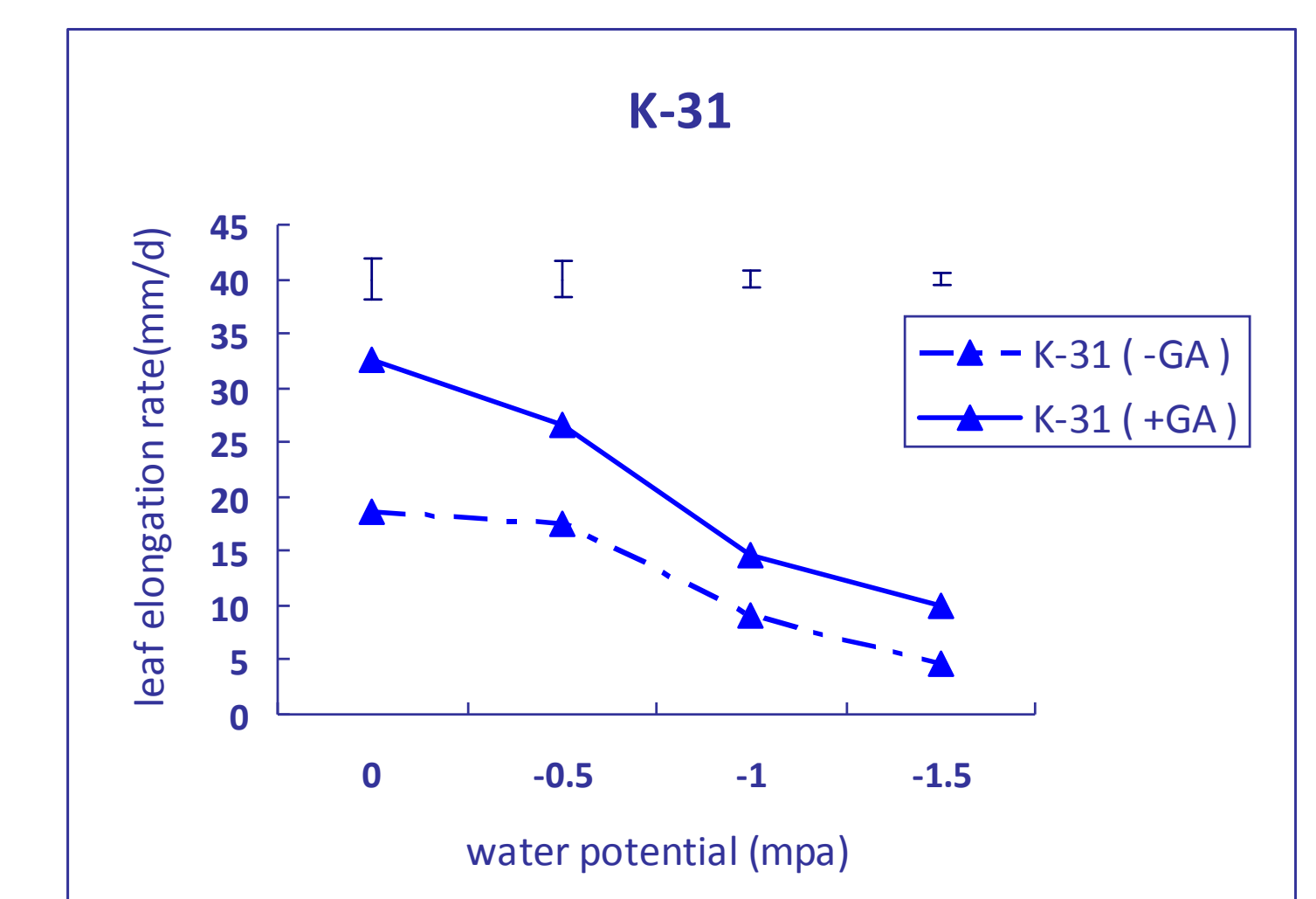


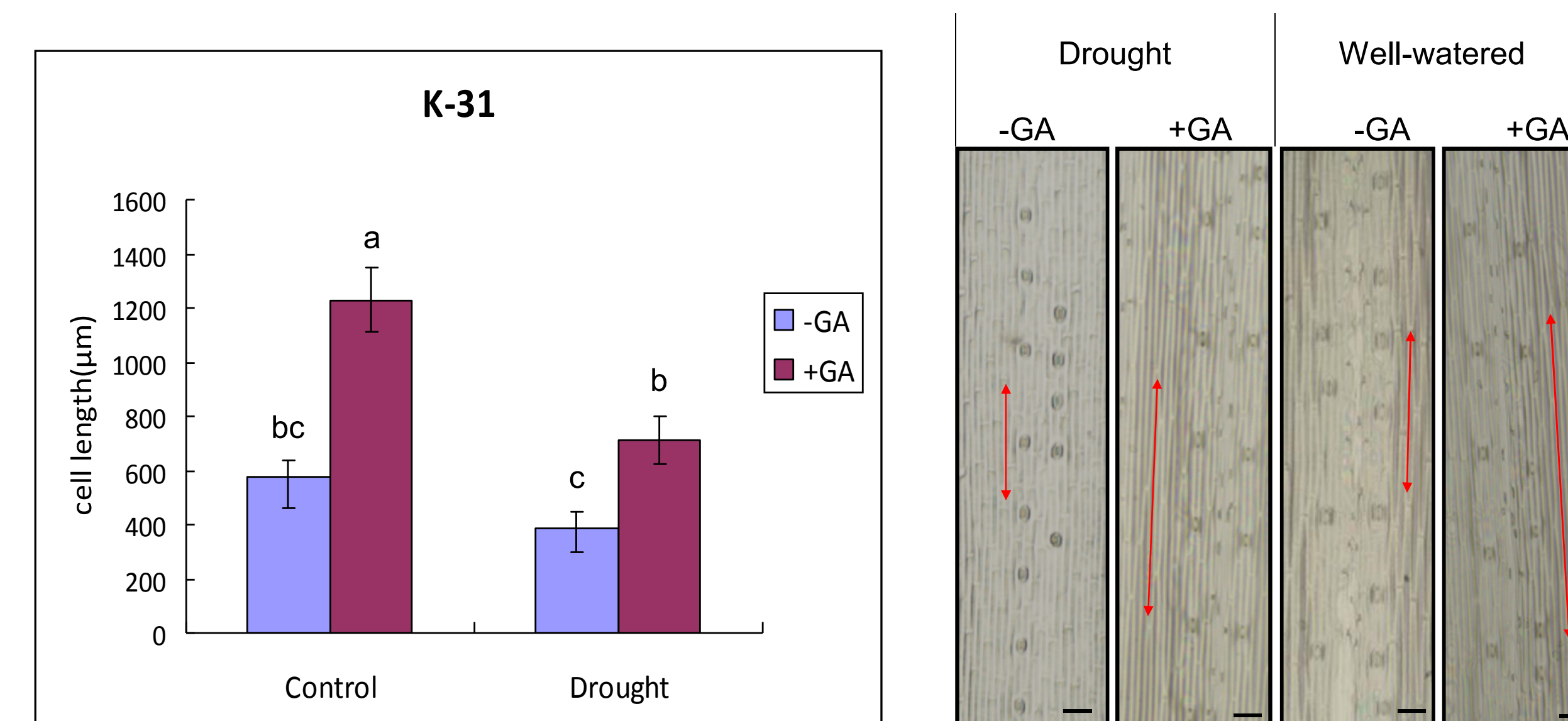
Fig.2 Leaf elongation rate of ‘K-31’ with and without GA treatment under well watered and drought conditions



For K-31, the 3rd leaves treated with GA were as 1.65-fold long as controls (water instead of GA), and the leaves treated with TE were as 0.56-fold long as controls.

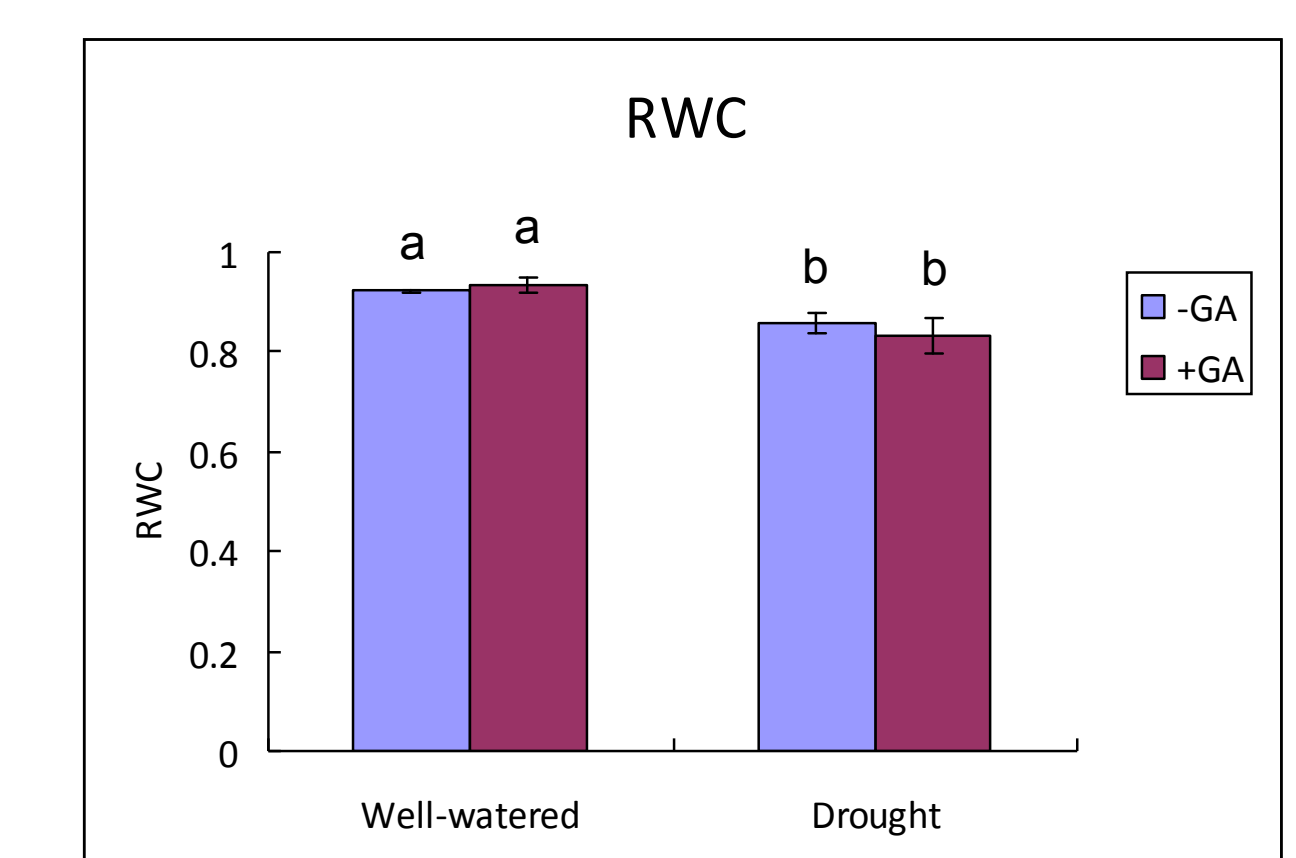
Drought stress inhibited the ‘K-31’ leaf elongation rate at -1MPa and -1.5MPa osmotic potential level. Plants treated with GA had much higher elongation rate than that without GA treatment under both well-watered and drought conditions.

Fig.3 leaf epidermal cell length of ‘K-31’ with GA and without GA treatment under well-watered and drought conditions



Drought stress significantly inhibited the leaf epidermal cell elongation rate and reduced the final cell length. GA promoted the cell elongation under both well-watered and drought conditions.

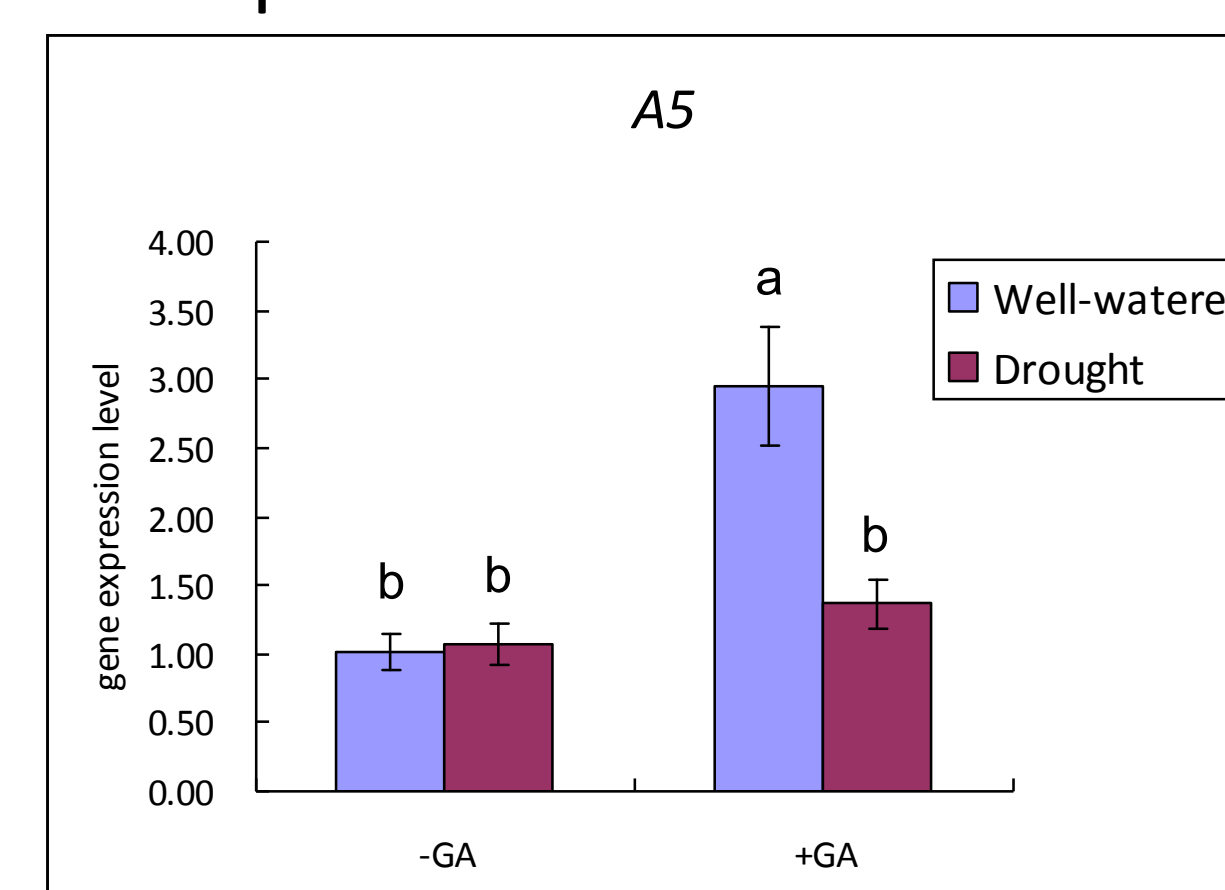
Fig.4 leaf relative water content of ‘K-31’ with GA and without GA treatment under well-watered and drought conditions



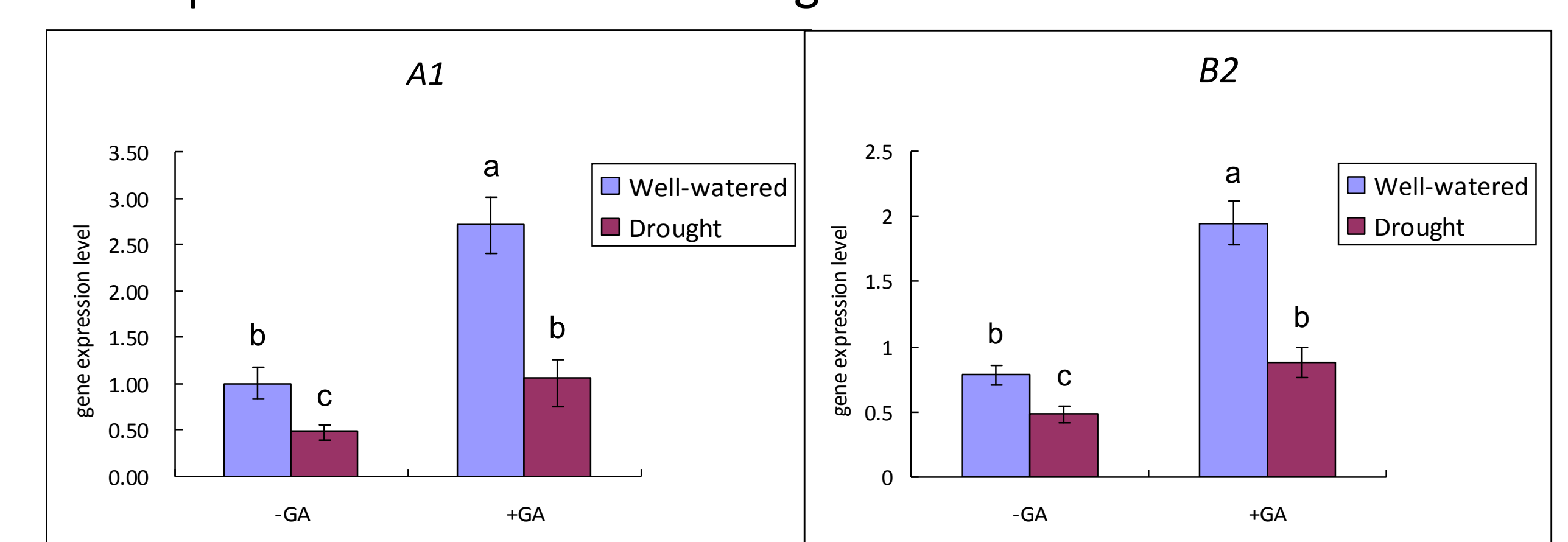
Drought stress reduced the leaf RWC of grasses with GA treatment and without GA treatment.

Fig. 5 Gene expression of expansins and XETs response to GA treatment and drought stress(-1MPa)

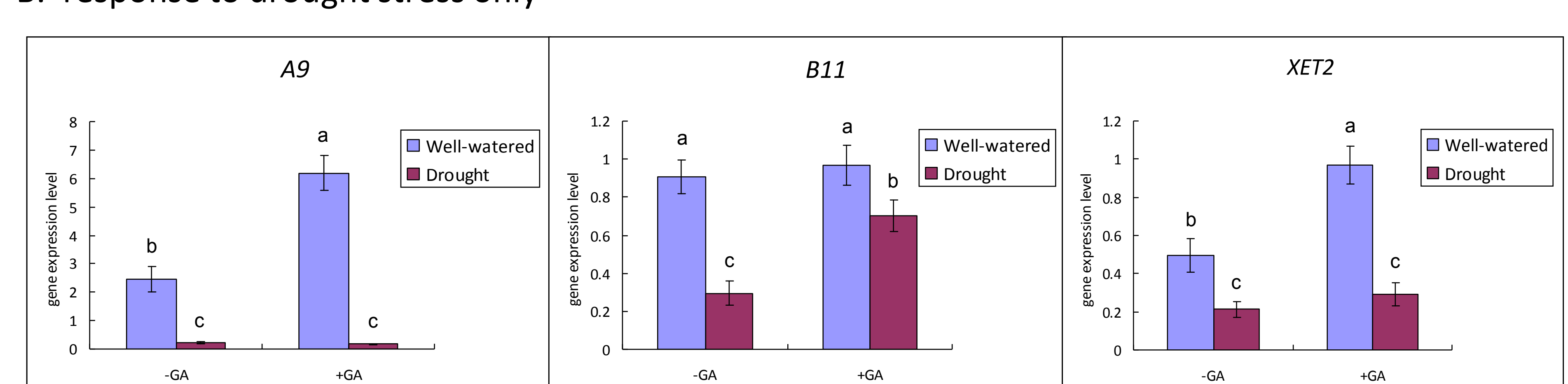
A. response to GA treatment only



C. response to both GA and drought



B. response to drought stress only



Drought stress reduced the expression level of the four expansin genes and one XET gene, two of which also response to GA treatment. One expansin gene (A5) only response to GA treatment.