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Original Article

The *Arabidopsis thaliana* Glycine-rich RNA Binding Proteins atGRP7 and atGRP2 Are Involved in Early Development

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Abstract

The glycine-rich RNA-binding proteins of *Arabidopsis thaliana* are of growing interest in the context of understanding how plants respond to biotic and abiotic stresses. In this study we focus on the phenotypic analysis of *Arabidopsis* knock-out mutants of genes that encode the glycine-rich proteins *atGRP2* and *atGRP7,* comparing them to knock-out mutants of *ABI3*, *ABI4* and *ABI5,* genes linked to cell signaling through the plant hormone abscisic acid (ABA). The results show that the abscisic acid insensitive (ABI) mutants developed faster than wild-type plants. In contrast the glycine-rich protein knock-out mutants (*grp7-1* and *grp2*) did not grow as well, suggesting an involvement of these genes in key early developmental processes. There was a significant difference in the phenotype of the knock-out mutants *grp2* and *grp7-1,* suggesting that *atGRP7* and *atGRP2* have overlapping yet distinct roles in development.

Keywords: *Arabidopsis thaliana,* glycine-rich proteins (GRP), abscisic acid (ABA), abscisic acid insensitive mutants (ABI).

1. Introduction

Arabidopsis thaliana is a small flowering spring annual plant that belongs to the *Brassicacea* family and is indigenous to Europe, Asia and northwestern Africa.

It was discovered and described by Joseph Thal in the year 1577 and since the 1940s it has been used as a model organism to study plant biology and genetics.

There are several reasons why this plant is considered an excellent model: it has a relatively small genome (only 125Mb) that was sequenced in the year 2000; there are extensive genetic and physical maps of all the five chromosomes; it has a very rapid life cycle (about eight weeks from germination to seed production); it has a prolific seed production and easy cultivation even in restricted areas; and it is easily transformed by the gram - negative bacterium *Agrobacterium tumefaciens* [9].

There are two major seed stock centers, ABRC (Arabidopsis Biological Resource Center, USA) and NASC (Nottingham Arabidopsis Stock Center, UK), where over 750 natural accessions of *Arabidopsis thaliana* are available.

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These accessions are quite different in regards to physiological features (e.g. flowering time, disease resistance) and also in regards to their morphology and development (e.g. leaf shape, hairiness) [1].

The differences between the natural accessions help researchers around the world reveal the complex genetic interactions involved in the abiotic stress response and also in the evolution of morphological features [12].

From these natural accessions several loss-of-function and gain-of-function mutant lines were created. The most important technique used for the creation of the loss-of-function mutants is the transferred-DNA (T-DNA) insertion generally achieved through the use of *Agrobacterium tumefaciens* [2].

The gain-of-function mutants are also obtained through the same technique, the only difference being that the expression of the T-DNA fragment is increased via the 35S enhancer of the cauliflower mosaic virus (CaMV) [5].

Glycine-rich proteins (GRPs) can be localized in the cell walls of many higher plants and are thought to be part of a group of structural proteins which form the cellular wall along with extensins and proline-rich proteins [10].

The glycine-rich proteins are associated with multiple independent physiological processes due to their diverse sub-cellular localization. The expression patterns of *GRP* genes are both diverse and highly tissue specific [8]. A subset of the GRP genes encode proteins that bind nucleic acids. The *Arabidopsis thaliana* genome encodes eight glycine-rich RNA-binding proteins (GR-RBP1 to GR-RBP8). These proteins are characterized by containing an RNA recognition motif (RRM) or an RNA/DNA-binding cold shock domain (CSD) at the N-terminus and a glycine-rich domain at the C-terminus [7].

These proteins appear to be involved in the adaptation to abiotic and biotic stress [11]. The analysis of their expression shows that they are strongly up-regulated when plants are exposed to cold in particular [12].

They are thought to play a prominent role in germination and seedling development of *Arabidopsis thaliana* in freezing conditions (temperature lower than 0 degrees) [4]. However, their normal developmental roles have not been studied in detail.

The aim of this study was to examine and compare the growth phenotypes of two GRP knockout lines, *grp2* and *grp7-1* to the phenotypes of well-studied *ABI* gene knockouts in which seeds fail to respond to the phytohormone abscisic acid.

2. Material and Methods

The study examined wild-type *Arabidopsis thaliana* and five mutant knock-out lines. We selected the Col0 strain as wild-type, as it is the most widely used. Three of the mutants used labeled *abi3* (N6131), *abi4* (N3836) and *abi5* (N8105) were obtained through EMS mutagenesis. These *abi* mutants are insensitive to very high concentration of the plant hormone abscisic acid [6]. The other two remaining plants used in this study are labeled *grp2* (SALK_048476C) and *grp7-1* (obtained from Prof. Dr. Dorothee Staiger, University of Bielefeld, Germany). These mutants were obtained through the T-DNA insertion technique and are verified knock-out lines for atGRP2 and atGRP7. For the phenotype analysis the plants were grown in pots in the green house under controlled climatic conditions (22 degrees °C**,** 70% moisture and 16/8h light/dark cycle). The resulting seedlings were observed over a six week period. The number of leaves was counted every second day and in the fourth week after the start of the experiment we determined the length and width of the 6th leaf, the length of the primary inflorescence, the rosette diameter, the distance between the internodes on the primary inflorescence and the number of flowers on the primary inflorescence. We also determined the bolting day of each of the plants.

3. Results and Discussions

The phenotypic analysis showed not only that the seeds of the abscisic acid insensitive (*ABI)* knockouts germinate sooner as has been previously described [11], but also that their flowering period and seed production is considerably earlier than that of the wild type (Col0). The appearance of the *ABI* mutant plants was a lot smaller in comparison to the wild-type, especially the *abi3* and *abi5* mutants (fig. 1). The analysis of the bolting day (fig. 2) shows that the *ABI* knockouts flower significantly earlier compared to wild-type. Another observation is that the *grp7-1* knockout flowered last, even though the rosette diameter (fig. 3) was similar to that of wild-type. The measurements made on the length of the primary inflorescence (fig. 4) showed that there was a significant difference between the *abi5* and *grp2* mutants in comparison to the wild type plants. Both of the mutants developed a much longer primary inflorescence. The number of flowers (fig. 5) present on the inflorescence was higher in *abi3-5*, and also in *grp2* knock-outs compared to wild-type and *grp7-1* knock-outs. This suggests that atGRP2 and atGRP7 have, to some degree, distinct developmental roles.

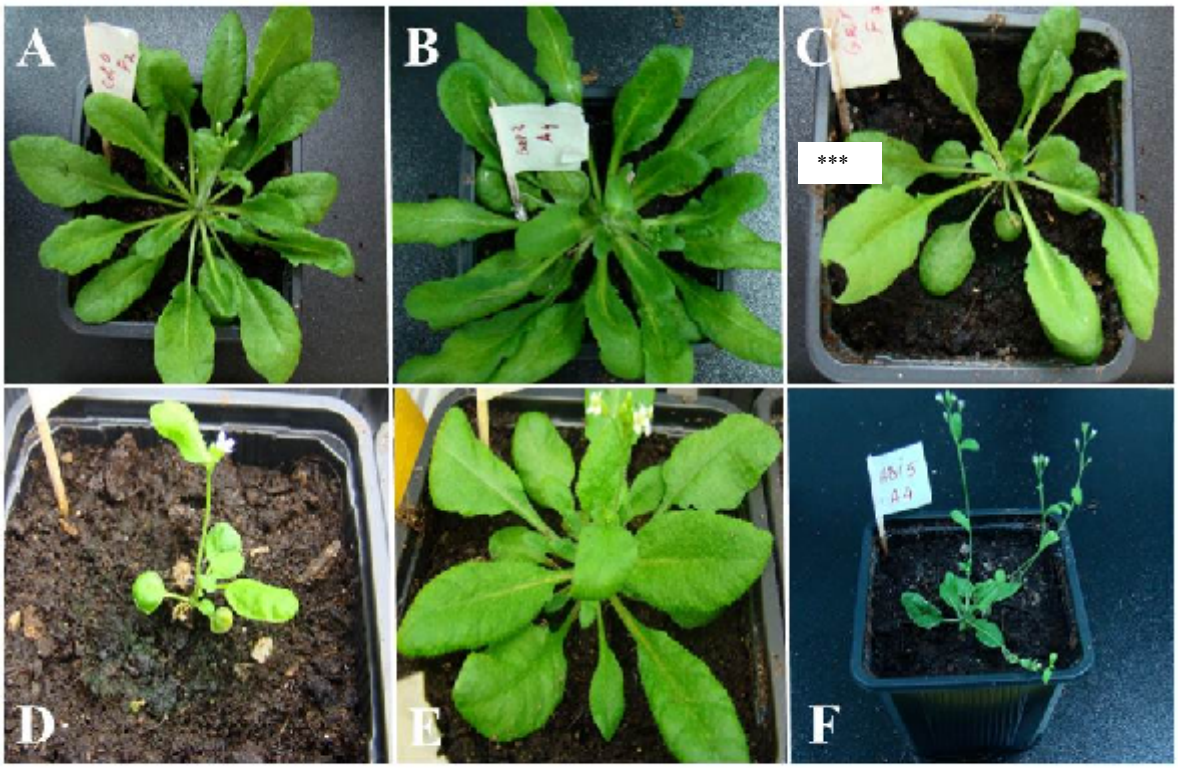

Figure 1. Representative images of wild type and knock-out strains after 4 weeks from germination. (A) Wild-type *Arabidopsis thaliana*, Col0 strain; (B) *grp2* knockout; (C) *grp7-1* knockout; (D) *abi3* knockout; (E) *abi4* knockout; (F) *abi5* knockout.

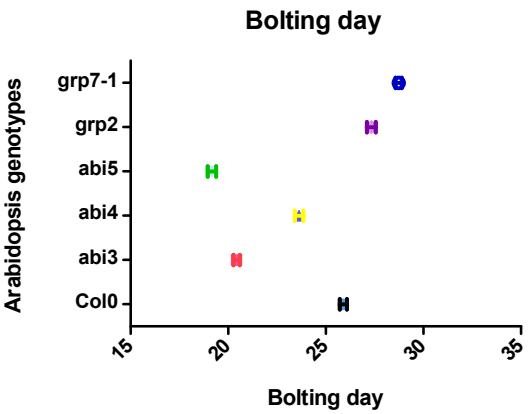

Figure 2. Bolting day of the *Arabidopsis* mutants compared to the wild type Col0

In regards to the number of leaves developed by the *Arabidopsis* mutants there were also significant differences (fig. 6).

The *abi3*, *abi5* and *grp7-1* knock-outs had a lower number of leaves as they reached the fourth week of development compared to wild-type in terms of leaf numbers.

Instead the *grp2* and *abi4* knock-outs were comparable to wild-type in terms of leaf numbers.

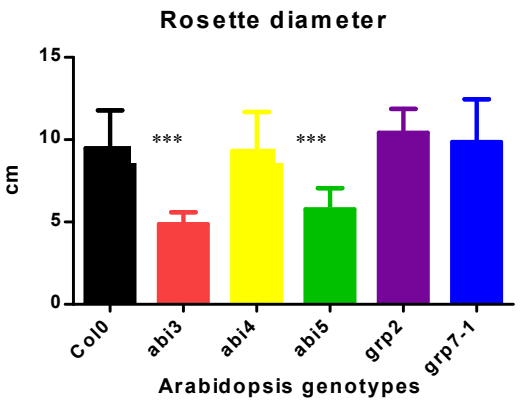

Figure 3. Comparison of the rosette diameter between the *Arabidopsis* mutants and wild type Col0. Significance for P value of 0.05

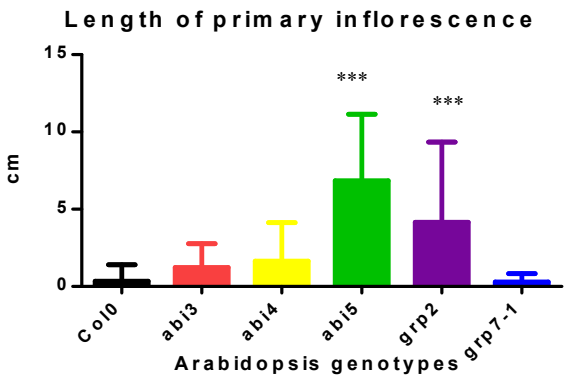

Figure 4. Length of the primary inflorescence of the *Arabidopsis* mutants compared to the wild type Col0. Significance for P value of 0.05

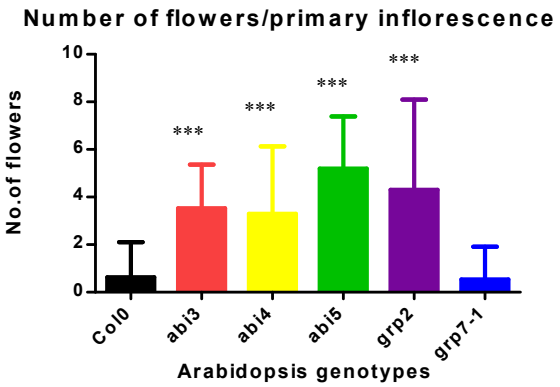

Figure 5. Number of flowers present on the primary inflorescence of the *Arabidopsis* mutants compared to the wild type Col0. Significance for P value of 0.05

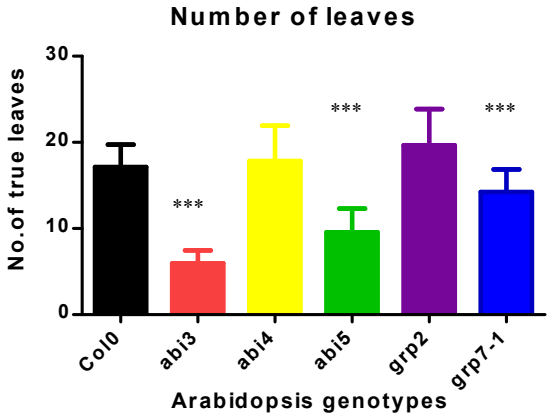

Figure 6. Number of leaves of the *Arabidopsis* mutants compared to the wild type Col0 in the fourth week of development. Significance for P value of 0.05

4. Conclusions

The analysis of phenotypes revealed significant differences in the development of the knock-out lines compared to wild-type. We observed that the *ABI* mutants have a relatively faster development and confirmed their typical early flowering period and seed production. The *GRP* knock-outs displayed slower development suggesting that these glycine-rich RNA-binding protein are involved in early developmental processes. However the phenotypes of the *grp2* and *grp7-1* knock-outs were not identical; *grp2* knock-outs had more leaves, longer inflorescence, more flowers and earlier flowering compared to *grp7-1* suggesting that the proteins have overlapping but distinct, non-redundant roles in development. Future research will consider in more detail the developmental roles of these proteins, and will determine their physiological RNA targets. It also remains to be determined what posttranscriptional processes are they are involved in. It will also be of interest to determine how their developmental roles intersect with their involvement in biotic and abiotic stress adaptation.

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