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# *Arabidopsis thaliana* Dynamic Phenotypic Plasticity in Response to Environmental Conditions

**Azim M. Merchant, Karolina M. Pajerowska-Mukhtar***

Department of Biology, University of Alabama at Birmingham, Birmingham, USA

**Abstract** Living organisms exhibit a nearly ubiquitous property of phenotypic plasticity that enables them to display a range of phenotypes under diverse environmental conditions, including both biotic and abiotic stresses. Phenotypic plasticity in plants can affect single cells, tissues, organs as well as the whole plant phenotypes including morphology, physiology and ecological relationships with other organisms. Leaf traits including shape and size are closely tied to photosynthetic capacity and thus are considered as important indicators for investigating plasticity. Here we describe a comprehensive study aimed to understand the roles of environmental conditions in shaping up the leaf morphology. We showed that wild type *Arabidopsis thaliana* leaves reduce their length and width when grown under environmentally suboptimal conditions. We also performed a comparative study of a loss-of-function mutant plants corresponding to eukaryotic GCN2 (general control nonderepressible 2) kinase. We demonstrated novel contributions of this universal regulatory factor in leaf architecture under stress-free and environmentally imposed stress conditions. Our data shed light on comprehending the underlying mechanisms of leaf shape plasticity in *Arabidopsis thaliana*.

**Keywords** *Arabidopsis thaliana*, Phenotypic plasticity, Leaf morphology, GCN2

## 1. Introduction

Plants, as sessile organisms, are constantly challenged with various biotic and abiotic stresses and phenotypic plasticity constitutes an effective way to help cope up with the environmental stresses [1-3]. This phenomenon, defined as the pattern of response to changes in environmental conditions, can affect many morphological traits in plants but is best manifested in leaf shape. Leaf size and shape is one of the most complex phenotypes of angiosperms that is tightly controlled by environmental and genetic factors, spatially and temporally coordinating cell expansion and cell cycle activity [4-6]. Environmentally controlled variations in leaf size and parameters related to it, such as length and width, have been well documented in a number of plant species [4, 6-10].

*Arabidopsis thaliana* (Arabidopsis), a dicot commonly used as a plant model system, has been nearly exclusively studied under controlled laboratory conditions, where light intensity, photoperiod, humidity and temperature are maintained constant throughout the life cycle of the plant [11, 12]. However, a limited number of studies were conducted over the last years that used field-grown Arabidopsis plants. Typically, these reports were focused on understanding the environmental influence on well-studied phenotypic responses, such as fruit number, germination, seed length and width, flowering time and flooding response [13-17]. A handful of studies published to date investigated the overall fitness of a specific Arabidopsis mutant grown under field conditions and addressed morphological changes likely resulting from varied environmental influences [18-20]. In addition, the reports on the use of transgenic Arabidopsis or mutant plants that specifically focus on the phenotypic plasticity of leaf shape as the function of environment are also limited.

Eukaryotic GCN2 (general control nonderepressible 2) is a serine/threonine protein kinase that is involved in sensing starvation-induced stress affecting multiple cellular processes. GCN2 encodes a multidomain containing protein harboring histidyl-tRNA synthetase (HisRS) and kinase domain [21, 22]. In yeast and mammals, the uncharged tRNAs accumulate under amino acid starvation and bind with the HisRS domain. This, in turn, activates the kinase activity [23, 24] to trigger the downstream signaling pathway. Upon activation, the GCN2 phosphorylates α-subunit of eukaryotic initiation factor 2 (eIF2α) to derepress the translation of downstream target genes encoding transcription factors, *i.e.* activating transcription factor 4 (ATF4) and general control nonderepressible 4 (GCN4) in mammals and yeast, respectively [23, 25, 26]. A wide range of GCN2 functions in diverse eukaryotes has been already documented. This includes starvation sensing, growth and development, differentiation, immune responses and tumor

* Corresponding author:
kmukhtar@uab.edu (Karolina M. Pajerowska-Mukhtar)
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cell survival [26]. Arabidopsis also possesses a conserved GCN2-eIF2α signaling pathway and it has been demonstrated that this cascade can be activated by a wide array of stimuli and stresses including wounding, phytohormones and herbicide treatment [27-30]. Recently, GCN2 has also been reported to function in the normal growth and development, including seed germination, chlorophyll accumulation and leaf shape [31]. While the roles of GCN2 in response to diverse stresses are emerging, the involvement of GCN2 in phenotypic plasticity remains unclear. Here, we report a comprehensive study describing the morphology of five most prominent rosette leaves of Arabidopsis wild type and GCN2-deficient mutant plants. The objectives of this study were to shed light on the extent of environmental influence on lengths and widths of individual leaves in a cruciferous rosette, and to understand the contribution of *GCN2* gene to the plant's ability to accurately execute these plastic responses. We discuss varied influence of the growth conditions on the leaf shape depending on the developmental stages of the plant. Our data provide novel insights into the involvement of a universal eukaryotic regulatory factor in shaping leaf length and width.

# 2. Materials and Methods

Wild-type and *gcn2* Arabidopsis plants used in this study are from the Landsberg erecta (Ler) ecotype. The Ler seeds were obtained from the Arabidopsis Biological Resource Center (ABRC; Ohio State University, OH, USA). The *gcn2* Genetrap insertion line GT8359 was obtained from Cold Spring Harbor Laboratory, New York, USA (http://genetrap.cshl.org). The Genetrap lines carry a transposable element insertion (Ds) in Ler background [32]. Seeds were incubated for 72 h at 4°C to break dormancy and subsequently grown on MetroMix 360 (Sun Gro Horticulture) soil for 4 weeks. All plants were grown in 72-well flats and positions of the two genotypes were randomized. Two different types of growth conditions were used. The first set was grown in a tightly controlled growth room under 12 h light/12 h dark cycle at 21°C, 65% humidity and light intensity of 250 μmol $m^{-2}$ $sec^{-1}$. The second study was conducted during late spring/summer months (May and June) in the University of Alabama at Birmingham partially controlled greenhouse facility. Plants were grown under natural photoperiod (~14h light/10h dark) with temperatures varying between 15-25°C, humidity of 45-75% and light intensity ranging from 80-500 μmol $m^{-2}$ $sec^{-1}$ on overcast and sunny days, respectively. Once the plants reached to developmental stage # 3.50-3.70 (when rosette size reaches to 50%-70% of the final plant size) [33], leaves were numbered in chronological order, and leaves #5, 6, 7, 8 and 9 were harvested. Fifteen leaves per genotype per replicate were collected. Leaves were placed on moist paper towels to avoid dehydration and manual measurements of lengths and widths of the leaf blades were taken in triplicates. Data were subjected to statistical analyses (Student's *t*-test) using SAS 9.3 software package (SAS Institute, Cary, NC).

# 3. Results and Discussion

### 3.1. Environmental Factors Affected the Dynamics of Rosette Area Development

Plants alter their morphology, physiology and cellular functions in response to varied environmental conditions. Leaf size and shape respond to changes in light levels and temperature and are important components of growth and development. We compared the leaf length and width as indicators of morphological plasticity under controlled growth room and greenhouse conditions. Four-week-old plants grown under either tightly controlled conditions in a growth room or partially controlled greenhouse conditions were at developmental stages # 3.50-3.70 (when rosette size is 50%-70% final size) [33].

Using the partly environmentally controlled greenhouse facility had a unique advantage in mimicking the field conditions for the following reasons. First, while the conditions were fluctuating depending on the weather and thus not optimal, they never fell out of the accepted range for Arabidopsis, thus ensuring a 100% plant survival and eliminating the possible adverse influence of sublethal temperature stress or presence of uncontrolled infectious agents. In addition, given that the local regulations don't permit growing mutant or transgenic Arabidopsis in the field, using the greenhouse facility was the only feasible experimental system to be employed for our studies on the contribution of GCN2 to phenotypic plasticity.

To quantify leaf size parameters, 16 plants per genotype per growth condition were subjected to systematic measurements of leaf length and width (Table 1). The length and width of Ler leaves #5–9 grown under stress-free conditions range from an average of 1.34 to 2.20 cm and 0.82 to 1.31 cm, respectively (Figure 1A, 1B).

Comparative analyses between the two growth conditions revealed that both the length and width of Ler leaves were significantly reduced in the greenhouse compared to the leaves of the plants grown in growth room (Figure 2A and 2B). A reduction of up to 37.5% in leaf length was observed for the leaves # 5, 6, 8 and 9. The decrease of leaf blade length for the leaf # 7 was not as remarkable and accounted for an average of approximately 15% (Table 1; Figure 2A). On the other hand, leaves number 5 through 8 exhibited a reduction of 15-22% in leaf width, when grown in greenhouse (Figure 2B). The leaf width for leaf 9 was only reduced by up to 10%; however, the effect was still statistically significant. Overall, we observed a general reduction in both leaf width and length for all the Ler leaves under investigation, when grown under greenhouse conditions. This indicates that the fluctuating environmental conditions can adversely affect the growth and development of the wild type Arabidopsis plant (Table 1; Figure 1B and Figure 2A, 2B).

**Table 1.** Ler and *gcn2* leaf lengths and widths measured in plants grown under growth room and greenhouse. Student's *t*-test was conducted to determine statistical significance in differences between genotypes

| Leaf size parameter | Leaf number | Ler Average [cm] | *gcn2* average [cm] | two-tailed p-value |
|---|---|---|---|---|
| Growth room leaf length | 5 | 1.7625 | 1.8685 | 0.1253 |
| | 6 | 1.9688 | 2.1142 | 0.1041 |
| | 7 | 2.2094 | 2.3143 | 0.3244 |
| | 8 | 1.5719 | 1.7229 | 0.0274 |
| | 9 | 1.3438 | 1.5429 | 0.0004 |
| Greenhouse leaf length | 5 | 1.1185 | 1.4828 | 0.0001 |
| | 6 | 1.2296 | 1.5828 | 0.0001 |
| | 7 | 2.2093 | 2.3143 | 0.3244 |
| | 8 | 1.0444 | 1.3276 | 0.0001 |
| | 9 | 0.9593 | 1.1448 | 0.0009 |
| Growth room leaf width | 5 | 1.0875 | 0.9486 | 0.0002 |
| | 6 | 1.2063 | 1.0314 | 0.0001 |
| | 7 | 1.3094 | 1.18 | 0.0038 |
| | 8 | 0.975 | 0.8314 | 0.0001 |
| | 9 | 0.8281 | 0.7143 | 0.0012 |
| Greenhouse leaf width | 5 | 0.8741 | 0.8897 | 0.6497 |
| | 6 | 0.9519 | 0.9793 | 0.4286 |
| | 7 | 1.0222 | 1.0724 | 0.2029 |
| | 8 | 0.8222 | 0.8034 | 0.5139 |
| | 9 | 0.7407 | 0.7483 | 0.7964 |

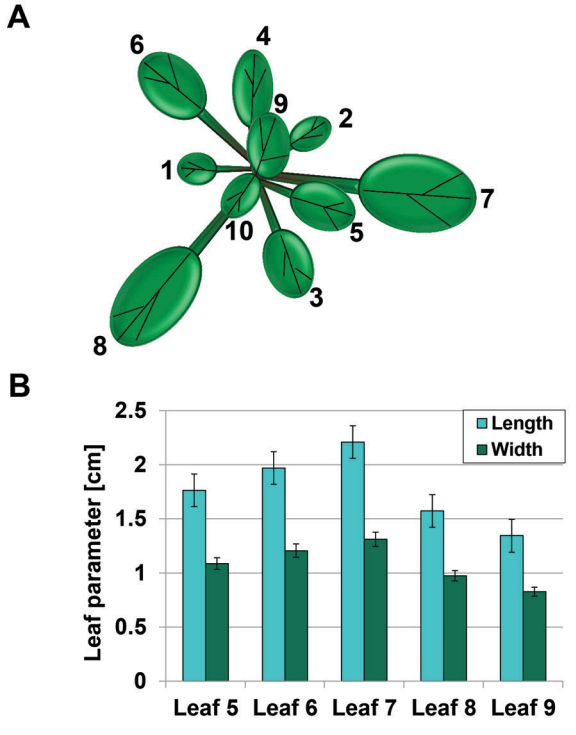


**Figure 1.** **A.** Schematic representation of an adult Arabidopsis rosette at a developmental stage 3.50-3.70. Leaves are numbered in the order of their emergence. **B.** Length and width of wild type growth room-grown Ler rosette leaves #5-9. Error bars represent standard error. Experiment was performed in triplicate with similar results

### 3.2. Influence of AtGCN2 on Leaf Phenotypic Plasticity

GCN2 is a global regulatory factor that is involved in controlling evolutionarily conserved signal transduction pathways responsible for sensing starvation [24]. We grew a loss-of-function *gcn2* mutant under growth room and greenhouse conditions and compared leaf growth of leaves # 5 through 9 to wild type Ler plants. In the overall comparison between the *gcn2* mutant plants grown under two contrasting conditions, we observed that plants grown in the greenhouse display a smaller leaf size compared to the growth room conditions. These findings are in agreement with the overall reduction of leaf length and leaf width determined for Ler plants. However, the measurements of the individual *gcn2* leaves generated certain intriguing results as summarized in Figure 2A and 2B and Table 2. The percent reduction of length for the leaves of *gcn2* mutant grown under greenhouse conditions ranges between 74 and 79%. While statistically highly significant for all the leaves under investigation, this length reduction for *gcn2* leaves is of a lesser extent compared to the percent length reduction in the corresponding Ler leaves (Figure 2A).

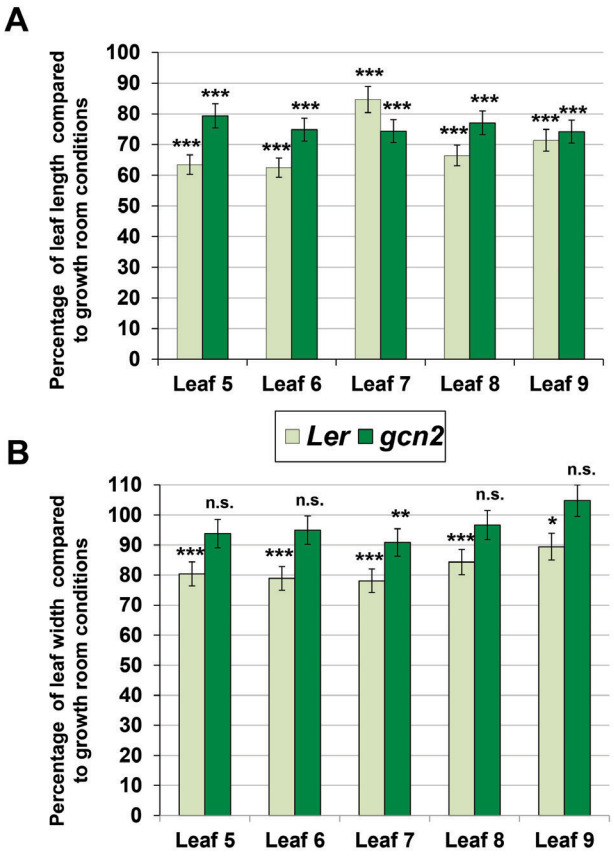


**Figure 2.** **A.** Percentage of leaf length reduction in greenhouse-grown Ler and *gcn2* plants compared to growth room-grown counterparts. Error bars represent standard error. Experiment was performed in triplicate with similar results. *** - p<0.0001, Student's *t*-test. **B.** Percentage of leaf width reduction in greenhouse-grown Ler and *gcn2* plants compared to growth room-grown counterparts. Error bars represent standard error. Experiment was performed in triplicate with similar results. n.s. – p>0.05, * - p<0.05, ** - p<0.001, *** - p<0.0001, Student's *t*-test

In sharp contrast to our findings with the leaf lengths, however, the percent leaf width was only mildly (~3-6%) and not significantly reduced for the majority of greenhouse-grown *gcn2* leaves tested (leaves # 5, 6, and 8) (Figure 2B). Intriguingly, leaf #9 (the youngest of the five leaves tested) displayed an opposite trend with a slight increase in width (104%) that was not, however, statistically significant. The largest leaf of the rosette, leaf #7, was the only one to exhibit a reduction as high as 9% that was statistically significant (Figure 2B).

Taken together, we concluded that the differences in the leaf length and leaf width between the *gcn2* and Ler plants were overall clearly pronounced, but did not seem to fit into a common general pattern. To comprehensively evaluate the specific contributions of factors such as the growth conditions, and variables such as the leaf number, we compiled a systematic comparison focused on the outcome for each of the five tested leaves under both contrasting growth conditions, along with a proposed explanation of a potential developmental mechanism that might be causing the phenotype observed, or is activated to counteract the adverse environmental effects (Table 2). Our results might reflect one way through which phenotypic plasticity manifests its effects through recruitment of general regulatory molecules in plants. GCN2 and other such molecules can control the action of an intricate network of compensatory mechanisms aimed to produce an optimal green canopy required for fulfilling photosynthetic needs of the plant. Our results might indicate that the possible onset of a compensatory assimilation mechanism was more efficiently coordinated in the *gcn2* mutant plants compared to the Ler wild type. Existence of such mechanisms has been proposed previously [2, 34, 35] but wasn't specifically linked with a single plant gene.

**Table 2.** Summary of the *gcn2* phenotypes associated with leaves # 5-9 while grown under contrasting conditions. Phenotype descriptions are based on data and statistical significance of differences between Ler and *gcn2* calculated in Table 1. Possible biological mechanisms underlying the observed phenotypes are proposed

| Growth conditions | Leaf number | *gcn2* mutant phenotype | Possible underlying mechanism |
|---|---|---|---|
| Growth room | 5 | Leaves narrower, but not longer | Decrease in leaf area due to reduced cell expansion |
| | 6 | | |
| | 7 | | |
| | 8 | Leaves both longer and narrower | Cell elongation is triggered to compensate for reduced expansion |
| | 9 | | |
| Greenhouse | 5 | Leaves longer, but not wider | Increase in leaf area due to cell elongation |
| | 6 | | |
| | 7 | | |
| | 8 | | |
| | 9 | | |

### 3.3. Contribution of GCN2 in Overall Plant Fitness

To gain a deeper understanding of the GCN2 contributions to complex leaf shape phenotypes under varied environmental conditions, we next calculated the ratios between leaf lengths and widths between greenhouse and growth room conditions, which is a widely accepted way to study the leaf size [31]. To obtain a comparative measure of the differences between the two genotypes tested while including the varied input of growth conditions, we calculated ratios of leaf length and leaf width between the individual leaves of *gcn2* and Ler grown under growth room and greenhouse conditions (Figure 3; example formula below).

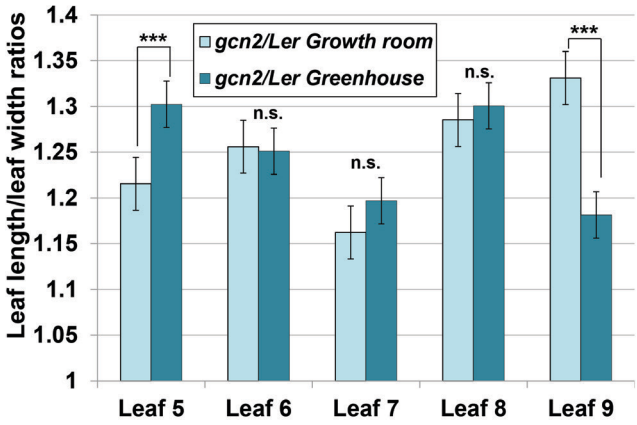


**Figure 3.** Ratios of leaf length and leaf width between the individual leaves of *gcn2* and Ler grown under growth room and greenhouse conditions. n.s. – $p>0.05$, *** - $p<0.0001$, Student's *t*-test

We did not discern a significant difference between the ratios of *gcn2*/Ler obtained for growth room and greenhouse condition for the leaves #6, 7 and 8. Remarkably, however, we observed significant and opposite effects for the leaves #5 and 9 (the oldest and youngest among the leaves tested). The ratio between leaf lengths/leaf widths when comparing *gcn2*/Ler for leaf #5 is significantly higher under greenhouse conditions (1.3) compared to growth room conditions (1.21). In contrast, we found the corresponding value for leaf #9 to be significantly lower for the greenhouse conditions (1.18) than under growth room conditions (1.33). This implies that GCN2 imposes both positive and negative regulatory roles on the fitness of leaves #5 and 9, respectively, when grown under conditions that are outside the optimal range for Arabidopsis. This result likely reflects leaf age-dependent adaptive assimilation challenges imposed by fluctuating environmental conditions and distantly resembles a trend observed previously in an unrelated experiment [14].

$$gcn2 \text{ /Ler greenhouse} = \frac{gcn2 \text{ leaf length / } gcn2 \text{ leaf width in greenhouse}}{\text{Ler leaf length / Ler leaf width in greenhouse}}$$

Overall, we concluded that the variable environment of greenhouse had adverse effects on leaf sizes in both genotypes tested, but was manifested differentially for each individual leaf and more profound in the Ler plants

compared to the *gcn2* mutants. Moreover, we determined that leaves # 5 and 9 can specifically serve as markers to capture the influence of GCN2 on leaf phenotypic plasticity. This discovery will allow for more targeted analyses in the future.

The regulation of leaf size in Arabidopsis is still only partially understood, and proposed to rely on a number of processes including a complex spatial and temporal coordination of cell expansion and cell cycle activity [36, 37]. Any perturbation within these processes, such as loss of function of a specific gene, might translate into altered size and shape of a leaf. In general, smaller leaves are produced as a result of decreased cell size, resulting from reduced expansion and elongation, or a combination of both factors [36, 37].

Recently, we described the roles of AtGCN2 in plant hormone gibberellic acid (GA)-mediated regulation of germination and chlorophyll content [31]. The phenotypes of the *gcn2* plants are reminiscent of plants deficient in GA biosynthesis or signaling. It has been previously shown that GA can control leaf size and shape through regulation of cell division and cell expansion [38]. For example, ectopic overexpression of GA 20-oxidase 1 (GA20OX1), which catalyzes important steps in GA synthesis, causes an enlargement of younger leaves when ectopically expressed in Arabidopsis [39, 40]. GAs have been also implicated in the control of cell proliferation. In the quadruple DELLA mutant, in which these growth repressors in GA signaling are down-regulated, cell proliferation and cell expansion rates were shown to increase [41]. In addition, a handful of reports indicate a role of GAs in cell elongation in various plant systems [42-44]. Consistent with this evidence, our data illustrate that in the growth room-grown *gcn2* plants, the youngest leaves (# 8 and 9) are both narrower and longer (Table 2), indicating that strongest impact of decreased GA signaling. In contrast, and further corroborating the age-specific role of GA in foliar morphology, the older *gcn2* leaves (# 5, 6 and 7) showed only effects on the leaf blade width (Table 2). The role of GA in phenotypic plasticity has been proposed previously [45, 46] and our study lends additional insights into the possible links to this important phytohormone in shaping plant environmental responses.

## 4. Conclusions

Overall, our results demonstrate an important function of GCN2 kinase in controlling phenotypic plasticity of global leaf shape under varied environmental conditions, which may be accomplished by influencing GA biosynthesis or signaling in Arabidopsis.

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