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2017-03-28

http://hdl.handle.net/10138/181663
https://doi.org/10.3389/fpls.2017.00416

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Genomic And Phenomic Screens for Flower Related RING Type Ubiquitin E3 Ligases In Arabidopsis

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Submitted to Journal: Frontiers in Plant Science
Specialty Section: Technical Advances in Plant Science
Article type: Original Research Article
Manuscript ID: 232818
Received on: 30 Sep 2016
Revised on: 24 Jan 2017
Frontiers website link: www.frontiersin.org
Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contribution statement

MP conducted the genomic screens, performed the phenomic screens and statistical analysis of these data. KM performed the QPCR analysis and participated in phenotyping and statistical data analysis, FW designed and revised genotyping of the mutant collection. MB designed and revised the flower phenotype analysis. EC participated in the phenotyping assays and conducted the flowering time experiments. KH designed the project as a whole, approved the data and wrote the manuscript.

Keywords

Arabidopsis, RING E3 ligase, Ubiquitin, high throughput, phenomics data analysis, flower, image based phenotyping

Abstract

Flowering time control integrates endogenous as well as environmental signals to promote flower development. The pathways and molecular networks involved are complex and integrate many modes of signal transduction. In plants ubiquitin mediated protein degradation pathway has been proposed to be as important mode of signaling as phosphorylation and transcription. To systematically study the role of ubiquitin signaling in the molecular regulation of flowering we have taken a genomic approach to identify flower related Ubiquitin Proteasome System components. As a large and versatile gene family the RING type ubiquitin E3 ligases were chosen as targets of the genomic screen. To this end the complete list of Arabidopsis RING E3 ligases were retrieved and verified in the Arabidopsis genome v11. Their differential expression was used for their categorization into flower organs or developmental stages. Known regulators of flowering time or floral organ development were identified in these categories through literature search and representative mutants for each category were purchased for functional characterization by growth and morphological phenotyping. To this end, a workflow was developed for high throughput phenotypic screening of growth, morphology and flowering of nearly a thousand Arabidopsis plants in one experimental round.

Ethics statements

(Authors are required to state the ethical considerations of their study in the manuscript, including for cases where the study was exempt from ethical approval procedures)

Does the study presented in the manuscript involve human or animal subjects: No
Genomic and phenomic screens for flower related RING type ubiquitin E3 ligases in *Arabidopsis*

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Running title: Genomics and phenomics for RING E3 ligases
Key words: *Arabidopsis*, flower, RING, E3 ligase, ubiquitin, high throughput, image based phenotyping, phenomics data analysis

Abstract
Flowering time control integrates endogenous as well as environmental signals to promote flower development. The pathways and molecular networks involved are complex and integrate many modes of signal transduction. In plants ubiquitin mediated protein degradation pathway has been proposed to be as important mode of signaling as phosphorylation and transcription. To systematically study the role of ubiquitin signaling in the molecular regulation of flowering we have taken a genomic approach to identify flower related Ubiquitin Proteasome System components. As a large and versatile gene family the RING type ubiquitin E3 ligases were chosen as targets of the genomic screen. To this end the complete list of *Arabidopsis* RING E3 ligases were retrieved and verified in the *Arabidopsis* genome v11. Their differential expression was used for their categorization into flower organs or developmental stages. Known regulators of flowering time or floral organ development were identified in these categories through literature search and representative mutants for each category were purchased for functional characterization by growth and morphological phenotyping. To this end, a workflow was developed for high throughput phenotypic screening of growth, morphology and flowering of nearly a thousand *Arabidopsis* plants in one experimental round.

Introduction
Flowering time control is a complex network that integrates many modes of signal transduction promoting the transition from vegetative stage to reproduction and ultimately leading to the development of flower organs. The endogenous changes that signal the beginning of flowering are referred to as autonomous pathways (Amasino and Michaels, 2010). Multiple studies have established the major role that photoperiod has in flowering (Piñeiro and Jarillo, 2013). Flowering in *Arabidopsis* is strongly promoted in long day (LD) conditions but will also ultimately occur under short day (SD) conditions (Steffen et al., 2014). Under LDs, flower induction is dependent on the expression and protein levels of CONSTANS (CO; Suárez-López et al., 2001). Light
controls the CO transcription via the circadian clock system, inducing a CO mRNA peak during
the latter part of the day (Suárez-López et al., 2001). CO transcription is repressed by CYCLING
DOF FACTORS (CDFs; Fornara et al., 2009). Under LDs, the CO mRNA afternoon peak
coincides with a blue-light activated complex containing FLAVIN-BINDING, KELCH REPEAT,
F-BOX 1 (FKF1) and GIGANTEA (GI), which lead CO transcription repressors CYCLING DOF
FACTORS to degradation (Fornara et al., 2009; Sawa et al., 2007; Song et al., 2012). Additionally,
the FKF1-GI complex also stabilizes CO protein in the afternoon (Sawa et al., 2007; Song et al.,
2012). CO protein degradation is promoted by at least two ubiquitin E3 ligases: HIGH
EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1) and CONSTITUTIVE
PHOTOMORPHOGENIC 1 (COP1; Jang et al., 2008; Lazaro et al., 2012). In the morning, red
light promotes HOS1 interaction with CO via phytochrome B activation (Lazaro et al., 2012).
COP1 mediates CO protein degradation in a complex with SUPPRESSOR OF PHYA-105 (SPA;
Laubinger et al., 2006). In the afternoon, blue light inhibits COP1-SPA-mediated CO degradation
by activating CRYPTOCHROME 2 (CRY2) interaction with COP1 (Liu et al., 2008). Thus, both
CO transcription is up-regulated and CO protein stabilized allowing up-regulation of the mobile
flowering signal gene FLOWERING LOCUS T (FT) expression in the phloem during the afternoon
under LDs, but not under SDs (Piñeiro and Jarillo, 2013). Also regulation of flower development
is likely to involve Ubiquitin Proteasome System (UPS) components (Vierstra, 2009).

The UPS has emerged as a powerful regulatory mechanism that facilitates irreversible transitions
between developmental stages, and responses to environmental stimuli by selectively degrading
short-lived regulators, such as transcription factors and receptors (Sadhanandom et al., 2012).
Genetic analyses in plants have proposed that this pathway plays a vital role in hormone regulation,
floral homeostasis, stress responses and pathogen defense; however, very few targets have been
identified in plants apart from the hormone signaling components (Santner and Estelle, 2010). In
the UPS system, the highly conserved 76-amino acid protein, ubiquitin, acts as a covalent
molecular tag to signal target proteins for proteasome mediated degradation. Ubiquitin attachment
requires three distinct enzymatic activities: E1, ubiquitin activating enzymes; E2, ubiquitin
conjugating enzymes; and E3, ubiquitin ligase enzymes. Moreover, the UPS consists of
accompanying proteins that modulate target recognition and degradation (such as RAD23, SPA1),
deubiquitinating enzymes (DUB1) and the proteasome (26S and 20S structures). According to the
plant specific UPS database (http://plantsubq.genomics.purdue.edu) over 6 % of the Arabidopsis
proteome is potentially involved in UPS (Stone et al., 2005). However, the common strategy for
functionally addressing the role of all UPS components is still evolving. The ubiquitin E3 ligases
are the most abundant UPS components and mediate the important recognition of the target
proteins for ubiquitination (Kosarev et al., 2002; Stone et al., 2005). The E3 ligases found in plants
belong to one of four subtypes: single subunit E3-associated protein carboxyl terminus or
Homology to E6-AP C-Terminus (HECT), U-box and Really Interesting New Gene (RING) or
multisubunit cullin-RING ligases (Sadhanandom et al., 2012). The RING-type E3 proteins are the
most abundant among the single subunit E3 ligases (Kosarev et al., 2002; Stone et al., 2005).

To unravel the role of the RING type ubiquitin E3 ligase protein family, we took a reverse-genetics
approach to identify the RING E3 ligases that could be involved in regulation of Arabidopsis
flowering time and/or flower development. To this end, we first curated the RING E3 protein
family, earlier described by Stone et al. (2005), in the most recent Arabidopsis genome. The
Arabidopsis protein sequences were subjected to InterProScan for protein domain search and the
number of ubiquitin E3 ligases containing RING domains was established to be 509. Association of these RING protein encoding genes with Arabidopsis flowering and floral organs was done through the Genevestigator transcriptome database (Hruz et al., 2008). To this end, the expression profiles were divided into categories based on their specificity, high expression or enrichment in flower organs and in the developmental stages of Arabidopsis. Several already characterized regulators were identified among these genes, such as the anther dehiscence regulating DAF gene family (Peng et al., 2013), flower size regulating DA2 (Xia et al., 2013) and FRG1 involved in flowering time related DNA methylation (Groth et al., 2014). The well-established flowering time regulator COP1 fell just below the cut off criteria due to its wide expression profile. A representative mutant collection for each category was obtained from NASC stock center. Additional candidates were also selected based on literature. The genotypically verified mutant collection was subjected to systematic morphological and growth analysis using an automated imaging based plant phenotyping facility. After the thorough vegetative assessment, the flowering time parameters such as number of leaves at bolting and days to bolting were recorded together with morphological analysis of the flower structures. The phenotypic assessment indicated lines with altered growth, morphology, or flowering time. Furthermore, one of the lines showed growth defects in sepals and petals.

Materials and methods

Bioinformatic screens

In order to curate the collection of the putative ubiquitin RING E3 ligases listed on the PlantsUBQ website (http://plantsubq.genomics.purdue.edu/) the Arabidopsis thaliana genome version ARA11 was analyzed. To this end, the whole Arabidopsis proteome was downloaded from ARAPORT (https://www.araport.org/downloads/), and screened with InterProScan for protein families and domain architecture. Once the RING domains were identified, they were aligned with Jalview using ProbCons algorithm with two rounds of pre-training before the actual run. To confirm that the newly identified RING domain containing protein sequences indeed represented ubiquitin E3 ligase type RING domains, InterProScan 5 (v5.16-55.0) Gene3D, SUPERFAMILY, ProSiteProfiles, SMART, Pfam, and ProSitePatterns signatures were used. Most of InterProScan tools use Hidden Markov Models (HMMs) to detect conserved domains along protein sequences. HMMs have been developed for conserved protein domains and they define for the software, which and where critical residues should be located along the analyzed protein sequence. From the protein domain collection, the ubiquitin E3 ligase type RING domains were filtered according to the criteria provided by Kosarev et al. (2002) and Stone et al. (2005) for canonical RING domains. The metal ligand binding residues were manually inspected and corrected, and small misalignments were edited. Sequences that failed to meet the criteria of InterProScan search engines were not considered in this study.

Transcriptomic database screens

To associate the curated collection of 509 RING type ubiquitin E3 ligases with flowering the Genevestigator gene expression database software was used (Hruz et al., 2008). The experiments AT-00087, AT-00088, AT-00089 and AT-00090 containing developmental expression data of AtGenExpress initiative microarrays were selected for the analysis (Schmid et al., 2005). In the
selected experiments, hybridization probes were available for 393 RING E3 genes out of the 509.
From these experiments, the linear expression data was extracted for the developmental stages of
developed rosette, bolting, young flower, developed flower, and flower and silique. For flower
organs, the gene expression profiles were extracted for categories of shoot apical meristem (SAM),
sepal, petal, stamen, and carpels. In these categories, genes were ranked for their at least 2-fold
differential expression against the developed rosette. Their relative expression levels were
obtained by log2(FC) = log2(FL) - log2(R), where FC is fold of change, FL is flower organ or
development stage and R is rosette. The results for each category were sorted by their log2(FC)
and all genes with log2(FC) > 1 were considered as up-regulated.

**Candidate genes selected by literature**

For the candidate approach, we used interaction networks from BioGRID (http://thebiogrid.org/)
and cross-checked them with flowering pathway genes listed in the Flowering Interactive Database
FLOR-ID (Bouché et al., 2016) to identify RING E3 ligases interacting with known flowering
time regulators CONSTANS (CO), CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and
TARGET OF EARLY ACTIVATION TAGGED (EAT) 2 (TOE2).

**Plant materials and growth conditions**

For functional characterization of the identified top most differentially expressed genes and for the
selected candidates, *Arabidopsis* mutant lines were obtained from the NASC stock center
representing CATMA, SAIL, SALK and GABI-Kat collections (Alonso et al., 2003; Kleinboelting
et al., 2011; Rosso et al., 2003; Schmid et al., 2005). Altogether 49 lines were genotyped by
combination of segregation analysis and T-DNA PCR with primers listed in Supplemental Table
1. From these, 43 lines represented 30 unique gene accessions (Supplemental Table 1). As a wild
type control, Columbia (Col-0) ecotype of *Arabidopsis thaliana* was used.

For genotyping, plants were grown in vitro on MS media supplemented with the corresponding
selection. For phenotyping, seeds were sown directly on soil with 50 % peat and 50 % vermiculite.
Trays were covered with plastic wrap and stratified for three nights, after which they were
transferred to the growth chamber (FytoScope, PSI, Czech Rep.). Seven days after stratification
(DAS) the seedlings were transferred to their own pots, placed on the analysis trays and sand was
added on top of the peat to prevent growth of any green algae. From the full water saturation of
the soil, the water content was let to decrease until 70 % and was kept at this level through daily
weighing and watering. Growth conditions in the *Arabidopsis* growth chambers were 16 hours
light/ 8 hours darkness and 22 degrees Celsius. Relative air humidity of the growth chambers was
targeted at 60 %. The light intensity was set and controlled at 130 µE (MS6610, Mastech, China).

**Genotyping of the mutant lines**

Homozygous one locus mutant lines were confirmed by segregation analysis and T-DNA specific
PCRs. The PCR primers, T-DNA position and line information were summarized in Supplemental
Table 1. The transcript levels of the T-DNA targeted genes were verified by quantitative real-time
PCR (qPCR) analysis. The sample material for qPCR was harvested from the tissue indicated by
eFP browser for each gene expression pattern. Three samples were harvested for each RNA
preparation. RNA was extracted using InviTrap® Spin Plant RNA Kit (STRATEC Molecular), complementary DNA was prepared with SuperScript® IV Reverse Transcriptase (Thermo Fisher Scientific), and the qPCRs were performed using Roche Lightcycler® 480 Instrument II (Roche Diagnostics) using LightCycler® 480 SYBR Green I Master (Roche Diagnostics) with primers listed in Supplemental Table 1. Primers were primarily designed to locus downstream of the T-DNA. Significance level of the qPCR were set at 0.5-0.1 fold-up for knock-down; <0.1 fold-up for knock-out; and >2 fold-up for up-regulated (Supplemental Table 1).

High throughput Plant Phenotyping platform

The small plant phenotyping facility at the University of Helsinki Viikki campus (http://blogs.helsinki.fi/nappi-blog/) was used for the phenotypic characterization of the selected Arabidopsis mutant collection. The plants were imaged daily by overhead CCD camera for RGB images positioned in a PlantScreen™ analysis chamber with automated plant transportation between the imaging, weighing and watering stations. The RGB images were obtained for 20 plants at the time and stored in central database. The images were pre-processed online as described in Awlia et al. (2016) to allow collecting binary and RGB data for each plant. The obtained binary images were used for calculating growth parameters of area and perimeter. The obtained parameters of area, perimeter and the convex hull were then used for automatic online calculations of morphometric rosette parameters including: roundness1, roundness2, isotropy, eccentricity, compactness, Rotational Mass Symmetry (RMS) and Slenderness of Leaves (SOL) (PlantScreen™ analyzer, PSI, Czech R.). To characterize the general morphology of the mutant lines these nine morphological parameters were grouped into four categories based on their type: raw, circularity, symmetry and center distance, and compared over time (Figure 1). Raw parameters were represented by area and perimeter of the rosette and they were calculated by counting pixels of a rosette picture and the edge pixels respectively and transformed to millimeters (Figure 1A). The parameters of roundness1 and roundness2 and isotropy represented the circular parameters (Figure 1B). The parameter roundness describes rosette area in comparison to perfect circle with same perimeter and is affected by leaf slenderness, petiole length and leaf perimeter. For wild type plant, this parameter usually takes values between 0.1-0.5 while a perfect circle has value 1. Roundness value tends to decay overtime due to leaf development that at the same time increases the rosette perimeter. Roundness 2 uses rosette convex hull area and perimeter for its computation and for wild type plants this parameter appears to have values between 0.7-1.0 following an oscillating pattern with less steep peaks over time (Figure 1B). Isotropy uses the area of a drawn polygon on top of the rosette (Figure 1B). Thus, isotropy has a behavior similar to roundness 2 over time, but with less steep peaks and decreasing tendency similar to roundness. The eccentricity and RMS were symmetric parameters (Figure 1C). Eccentricity describes how elliptical the plant rosette is, where a value close to 1 correspond to a rosette with highly sharp elliptical shape, while a value close to 0 describe a circular shape. Wild type rosette shows a high eccentricity peak that decays over time with a second smaller peak by the end of growth, thus the rosette shape fluctuates between a round and an elliptical shape. On the other hand, RMS describes the symmetry of the plant rosette by making a ratio between the non-overlapping rosette convex hull area and a perfect circle of the same area centered in the plant centroid and the overlapping area of both. RMS shows a similar pattern as eccentricity, but with higher absolute values and a sharper peak. Compactness and SOL were based on the center distance (Figure 1D). Compactness is the ratio between the rosette area and the rosette convex hull area. This parameter tells about
petiole length and leaf blade width. The parameter SOL explains how sharp the leaf blades are, but it is also affected by the leaf number. SOL was derived from the ratio between squared rosette skeleton and rosette area. Thus, SOL can take values greater than 0 and below 50 in dimensionless units for wild type plants (Figure 1D).

Experimental design

Ten days old (10 DAS) Arabidopsis plants were subjected to growth and morphological characterization by top view imaging for the following 10 days. One phenotyping round was designed to accommodate a maximum of 960 Arabidopsis plants representing 36 genotypes at a time in three consecutive experimental rounds called F006 to F009 (F for flower related). The total number of lines analyzed in each round was 36 (F006), 28 (F007), 23 (F008), 20 (F009). The maximum of 36 genotypes were divided in three batches that were rotating between the growth area and the PlantScreen™ analysis chamber. Each batch consisted of three experimental units of four mutant genotypes randomized with Col-0, each represented by 20 individual plants. One experiment consisted thus of five trays of altogether 100 plants. Each experimental unit had their own Col-0 wild type in randomized block design to normalize for any local differences in the microenvironments of the PlantScreen™ or the growth area. Each line showing any phenotypic responses was analyzed in at least three independent experimental rounds. Lines that did not show differences as compared to the Col-0 wild type were excluded from the subsequent rounds thus resulting in reduced numbers of genotypes included.

Phenotypic analysis of flowering time and flower structures

After the image based growth and morphological measurements of the 20 mutant and 20 Col-0 plants in the PlantScreen™ system, the flowering time parameters were recorded. To this end, leaf numbers at bolting (LAB) and days to bolting (DTB), were manually counted for each of the plant individuals. The number of rosette leaves were counted at appearance of the flower bud (developmental stage 5.10, Boyes et al., 2001) and the DTB was recorded at the same time. The flowering time phenotypes were observed in two to three independent experimental rounds. Finally, flowers of these lines were photographed (Canon macro lens EF-S 17-85mm) and further dissected for floral organ analysis under stereomicroscope (SteREO Discovery.V20, Zeiss). Microscopic pictures of the inflorescence tips, single flowers, sepals and petals of sinal7-2 and Col-0 were taken with the attached camera AxioCam ICc3, Zeiss. The analyzed inflorescences and flowers originated from at least two independent experiments. Only the main inflorescences were considered. Flower developmental stages were determined as in Smyth et al. (1990). Flowers scored for the occurrence of aberrations of organ shape, number and identity originated from at least seven individual plants per line. Pollen grain staining according to the modified Alexander method was used to confirm pollen viability (Peterson et al., 2010). Anther images were captured using Leica DFC420 C camera attached to an optical microscope.

Statistical Analysis

The significance of the differences between mutant lines and Col-0 were computed by contrasting two fitted models to the data points using several order polynomials (Mirman, 2014). First, a model was fitted to all data points and then a second model was fitted including the factor genotype (wild...
type and mutant). These two models were then compared using a Chi square test to determine if
the second model explained more variance than the first one beyond the significance ($\alpha = 0.05$). If
the second model was statistically different from the first one, it implies that the compared
genotypes were different. These statistical analyses were conducted in R software (https://www.r-
project.org/). Number of leaves at bolting and days to bolting analysis were performed by Analysis
of Variance using GLM procedure and pairwise comparisons against Col-0 using option Dunnett
in the MEANS statement using SAS/STAT© software version 9.4 (SAS Institute Inc., Cary, NC,
USA).

Results

Curation of the Arabidopsis RING-type ubiquitin E3 ligase protein sequences

To re-confirm the published RING-type ubiquitin E3 ligase proteins encoded in Arabidopsis
genome the 27,667 Arabidopsis proteins from the latest genome annotation release (ARA11) were
scanned for RING domains. Through filtering the signatures in Gene3D, SUPERFAMILY,
ProSiteProfiles, SMART, Pfam, ProSitePatterns altogether 509 putative RING domain containing
protein sequences were obtained (Supplemental Table 2). RING gene names and descriptions were
obtained from Araport using Thalemine tool (Supplemental Table 2). Araport used curated but
also automatic gene annotation, therefore many RING domain containing proteins were annotated
as RING/Ubox protein although they did not contain Ubox domain. Similarly, some were
annotated as RING/FYVE/PHD zinc finger superfamly proteins. The 509 RING sequences were
compared to the previously described RING-type protein sequences (Kosarev et al., 2002; Stone
et al., 2005). From the 509 identified RING domain proteins 457 matched with the 490 previously
described, thus resulting in 31 non-matching sequences (Figure 2). These non-matching sequences
were thoroughly analyzed and, 6 of them were found to be merged with other gene models, 10 had
no RING domain, 3 were not found in the database, 3 corresponded to pseudogenes, 7 were split
and a new locus identifier had been assigned for them, and 2 were transposable elements
(Supplemental Table 3. A). The 50 additionally identified RING domain proteins were shown to
represent diverse RING domains such as, 1 of D type, 4 of C2 type, 20 of H2, 16 of HC, 2 of S/T,
and 7 of V (CH) type, according to the Stone et al. (2005) classification (Supplemental Table 2).

Differential gene expression data identifies 122 flower related RING ubiquitin E3 ligases

To associate the RING domain proteins with flowering or flower development two approaches
were followed: 1) identifying those with gene expression enhanced or enriched during flower
development or in flower organs, and 2) by searching RING proteins interacting with known
flower regulators. For the first approach Genevestigator (Hruz et al., 2008) tool was used to rank
the differential gene expression (DEG) of the identified RING genes over Arabidopsis
developmental stages and in flower organs relative to their expression in developed rosette (Figure
3A and B). In the selected experiments in Genevestigator database, probes were available for 393
of the 509 RING E3 ligases analyzed. The cut off for DEGs was set at two fold to be included in
the selection resulting in lists of genes of interest for each of the categories. This process was
repeated to identify gene expression enrichment at each of the development stages of bolting,
young flower, developed flower, and flower and siliques. For the developmental categories
altogether 71 DEG were identified (Figure 3C). In addition to the developmental stages,
enrichment for shoot apical meristem, sepal, petal, stamen and pistil organs were retrieved and resulted in 109 DEGs (Figure 3D). Some of the RING genes were common between these two categories and in total 122 unique RING genes were up-regulated in the flower related processes. The gene identifying AGI codes of these 122 flower related candidates are provided in the Supplemental Table 3. B.

For the second approach we identified 6 additional genes of interest through literature study and from interaction networks of CONSTANS (CO), CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and TARGET OF EARLY ACTIVATION TAGGED (EAT) 2 (TOE2) from BioGRID (http://thebiogrid.org/). Based on these interaction screens 5 RING E3 ligases were selected to the study, represented by the following mutant lines; N656705 (AT5G65683), N686069 (AT1G61620), N372291 (AT3G29270), N2037522 and N67002 (AT4G17680) and N742646 (AT2G44410). In addition, a mutant line for COP1, cop1-6, and RED AND FAR-RED INSENSITIVE 2 (RFI2) from BioGRID (http://thebiogrid.org/). Based on these interaction screens 5 RING E3 ligases were selected to the study, represented by the following mutant lines; N656705 (AT5G65683), N686069 (AT1G61620), N372291 (AT3G29270), N2037522 and N67002 (AT4G17680) and N742646 (AT2G44410). In addition, a mutant line for COP1, cop1-6, and RED AND FAR-RED INSENSITIVE 2 (RFI2) for which a role in mediating red and far-red light signaling and ubiquitination activity has been shown in vitro (Chen and Ni, 2006a; Stone et al., 2005). This E3 ligase was selected as a candidate since its expression is regulated by circadian clock and rfi2-1 mutant flowers early (Chen and Ni, 2006b). Thus, one mutant allele for RFI2 (N878610) was included in the study. Mutants representing these genes were analyzed together with the flower up-regulated RINGs and were named flower related UPS candidates in the Supplemental Table 1.

Representative mutant collection

For functional characterization of the 122 flower related UPS candidates and those selected based on literature, a mutant collection was obtained from the NASC stock center. The mutants represented lines from CATMA, SAIL, SALK and GABI-Kat collections (Alonso et al., 2003; Kleinboelting et al., 2011; Rosso et al., 2003; Schmid et al., 2005). Altogether 43 lines were shown to contain T-DNA insertion in one locus, six were doubtful and were omitted from the analysis. To confirm that the T-DNA insertion had interrupted the gene of interest, their altered expression levels were confirmed by qPCR analysis with primers listed in Supplemental Table 1. For 43 accessions representing 30 unique loci from the 122 flower related UPS candidates and the selected candidates a differential gene expression pattern was analyzed. Altogether 15 lines were knock-outs, and 12 knock-down mutants, and for 7 lines up-regulation of the gene of interested was observed (Supplemental Table 1). For one line, no differential expression was confirmed and this was excluded from the phenotyping. For 14 lines alleles were available with similar or opposite gene expression patterns.

Morphological phenotypes of the selected mutant accessions

From the genotypically and qPCR confirmed T-DNA insertion mutant lines, 43 were subjected to phenotypic characterization by top view RGB imaging using the PlantScreen™ system. Image series of each analyzed line were collected daily allowing analyzing the growth and changes in morphology over time. For scoring those lines showing phenotypes, we fitted general additive models (GAM) to each parameter of each analyzed lines (data not shown). Most of the lines showed no differences to their corresponding Col-0 controls. However, three lines were consistently significantly different across the experiments compared to Col-0 in both growth and rosette morphology, namely csu1-4 (N686069), sina17-2 (N833574) (Peralta et al., 2016) and...
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To further analyze these three lines, mixed non-linear models were fitted to their data using several order polynomials for parametric analysis of the models. This analysis confirmed the earlier observations of significant changes in growth and development for these lines over time (Table 2). Line csu1-4 showed slower growth, reduced rosette area and perimeter compared to Col-0 along the complete measured period (Figure 5A and D). For line rha1a-1 the rosette area was very similar to Col-0 being, however, slightly but significantly larger over time probably due to its higher number of leaves (Figure 5B, Table 2). Although the differences between rha1a-1 and Col-0 were small the statistical model was able to capture those. Conversely, sinal7-2 showed both area and perimeter larger than Col-0 indicating more vigorous growth (Figure 5C and F).

Morphological data for parameters of circularity that include roundness, roundness 2 and isotropy were also evaluated for these lines. Line csu1-4 showed increased roundness over the total period analyzed in comparison to Col-0 (Figure 6A-C, Table 2). However, csu1-4 roundness curve had similar pattern to Col-0 while shifted to the right (Figure 6A). Similar situation was observed for sinal7-2, where the roundness curve shape was almost identical to Col-0 but in this case was shifted to the left, showing lower roundness along the total time period (Figure 6C, Table 2). Roundness curve of rha1a-1 was neither shifted nor similar to Col-0 curve. This line showed a lower roundness than Col-0 at the beginning of the analysis, reaching a stabilization point around 16 DAS (Table 2). For Col-0 plants roundness continued decreasing until it become lower than rha1a-1 (Figure 6B).

Line csu1-4 showed a similar roundness 2 pattern as Col-0 that is shifted to the right by approximately day 2 (Figure 6D-F). Line rha1a-1, showed an oscillating pattern too, however, its roundness 2 values were constantly close to 0.9 with less steep peaks than Col-0, presenting the highest differences between days 12-16 (Figure 6E, Table 2). Similarly, to line csu1-4, line sinal7-2 presented an oscillating pattern very similar to Col-0, however, this time the curve had shifted to the left by approximately one day (Figure 6F).

Isotropy showed similar results as roundness and roundness 2, where line csu1-4 and sinal7-2 had similar oscillating pattern as Col-0, but csu1-4 curve is shifted to the right, while the curve for sinal7-2 is shifted to the left (Figure 6G-I). Line rha1a-1 showed a constant high isotropy value decreasing over time until reaching Col-0 pattern by day 23 (Figure 6H, Table 2).

The other analyzed morphological parameters were eccentricity and rotational mass symmetry (RMS). For eccentricity, line csu1-4 showed a similar pattern as Col-0 plants with a large and a small eccentricity peak, but shifted to the right (Figure 7A). Line rha1a-1 presented no shift in its curve, but it showed a rather flat peak around days 11 and 15, remaining lower than Col-0 until the end of the analysis (Figure 7B, Table 2). This result shows that rha1a-1 is less eccentric than Col-0 along the complete analysis. Line sinal7-2 showed also a similar pattern to wild type plants.
with two eccentric peaks, but slightly shifted to the left (Figure 7C). For RMS line csu1-4 showed
similar pattern as Col-0 plants, but shifted again to the right about one day for the highest peak and
remained higher than Col-0 in the last days of the analysis (Figure 7D). In the other hand, rha1a-1
presented no shift in its curve, but it showed a decrease in the peak around days 11 and 15,
decaying faster and remaining lower than Col-0 plants (Figure 7E, Table 2). Like in eccentricity,
sinal7-2 was almost indistinguishable from the Col-0 plants, except for a slight shift to the left
captured by the model (Figure 7F).

Finally, the last two morphological parameters analyzed were compactness and slenderness of the
leaves (SOL), which were based on the center distance (Figure 1). Here the line csu1-4 showed a
decay of compactness overtime in a similar way as Col-0 plants, but its curve was shifted to the
right (Figure 8A). Lines csu1-4 and sinal7-2 presented quite normal compactness curves, while
for rha1a-1 the pattern that was less compact than Col-0 plants at the beginning of the analyzed
period (Figure 8B, Table 2). The compactness later rises above Col-0, showing higher compactness
values. Like for the previously described parameters, sinal7-2 compactness curve showed slightly
lower values than Col-0, except for the last two days were Col-0 plants reached sinal7-2
compactness (Figure 8C).

Line csu1-4 showed lower SOL values than Col-0, while rha1a-1 and sinal7-2 showed higher SOL
values than Col-0 (Figure 8E and F, Table 2). The main differences in SOL could be observed
during the exponential growing phase of the rosette and reaching a plateau at the end of the
analyzed period were the differences to Col-0 plants become insignificant (Figure 8D-F).

Flowering time phenotypes

Flowering time mutants identified in the screen represented both with reduced and increased leaf
numbers at bolting (Table 1). csu1-4 line (AT1G61620) was clearly early-flowering in both
experimental replications. AT5G63970, a putative forkhead box protein, mutant line was early
flowering in one of two experimental replications. SBP (S-ribonuclease binding protein) family
protein (AT4G17680) was late flowering in both experimental replications. As already shown by
others, cop1-6 mutant was early flowering in both LAB (7) and DTB (22). In most of the mutant
lines, LAB did not differ from Col-0 in all experimental replications, but the trend was seen in
both or all. LAB or DTB of sinal7-2 did not differ from Col-0 in either of the experimental
replications.

Mutation in SINAL7 causes a flower growth phenotype

The flower morphology of the analyzed mutants was observed under stereomicroscope. In the
mutant line sinal7-2 cavities in the tip of the flower buds and wrinkled petals in mature flowers
were repeatedly observed (Figure 9A-D). The cavities were found in 14 out of 15 analyzed sinal7-2
inflorescences and in none of the nine Col-0 plants (both young and old inflorescences were
investigated). These openings were present at one or both sides of the affected buds and were
associated with tips of the lateral sepals bending inwards (Figure 9E-F). Other abnormalities
observed in sinal7-2 flower buds were vertical gaps between sepal in the middle or at base, as
well as both medial sepal tips growing inwards, as opposed to Columbia in which the abaxial
medial sepal covers the adaxial medial and both lateral sepal tips. Analysis of a number of
dissected flowers at stages late 12 to 15 revealed that in 49% of the mutant flowers (27/55) at least one lateral sepal tip was bent inwards; this phenotype was not observed in Col-0 (0/39). The analyzed flowers came from nine and seven plants, respectively, and their position at the inflorescences ranged from first to 22nd (pooled from two independent experiments; at least two plants per experiment). The dissected flowers of stage 15 were used for scoring the wrinkled petals (Figure 9C and D). During this developmental stage Col-0 petals were fully developed and their blades were relatively flat. In 16% of the sinal7-2 flowers (5/31) mild to severe wrinkling was observed, whereas this feature was not present in Col-0 (0/20; flowers between first and 19th position at the inflorescences). The bent sepals and wrinkling petals were occasionally accompanied by bent pistil (Figure 9, I-J) and stamens. Dissecting flower buds at later stage 12 revealed that the occurrence of ingrown lateral sepals was associated with the petal wrinkling, the sepals preventing elongation of the petals (Figure 9G-H). Additionally, to the above described phenotypes, we also noticed more frequent occurrence of abnormal floral organ numbers and/or identities in sinal7-2 flowers, such as five instead of six stamens or organs sharing features of petals and stamens (15% and 5%, respectively, i.e. 8/55 mutant and 2/39 Col-0 flowers).

SINAL7 has been shown to mediate ubiquitination of glyceraldehyde-3-phosphate dehydrogenase 1 (GAPC1) enzyme in vitro and to affect its enzymatic activity and subcellular localization in Arabidopsis plants (Peralta et al., 2016). In plants lacking GAPC1 male sterility has been observed (Rius et al., 2008). To investigate whether deficiency of SINAL7 impairs male fertility in sinal7-2 mutant, pollen viability was inspected (Figure 9K-L). Anthers of 12 mutants and 11 Col-0 flowers of stage 12-13 were stained, originating from seven and five plants, respectively, located at positions 3rd to 34th counting from the base of the inflorescences. However, no differences between the mutant and Col-0 pollen was observed: anthers of both lines contained almost exclusively viable pollen grains.

Discussion

Genomic knowledge in both model plants and crops is expanding at a fast pace. However, translating the knowledge from sequence to function and thereby from models to applications is hampered by bottlenecks in screening for the phenotypes associated with the genotypes. In this project, we set out to conduct a reverse genetic approach (Bolle et al., 2011), by defining a proportion of the RING type ubiquitin E3 ligases to the developmental processes of flowering time control or flower development. To this end, the RING type ubiquitin E3 ligases were curated in the most recent Arabidopsis genome annotation (ARA11) that had been improved e.g. by the next generation sequencing techniques (Krishnakumar et al., 2014). Thereby, many gene models had indeed become obsolete, split, merged or their original sequence had changed. We also found that in the annotations there are a considerable number of RING domain containing proteins annotated as RING/U-box genes. RING and U-box share similar functions and are structurally and functionally similar, both are ubiquitin E3 ligases that work as scaffolds between the ubiquitin E2 conjugase and substrate. However, at the amino acid residual level RING and U-box domains are significantly different; in the RING domain the arrangement of cysteines and histidines mediate binding of two zinc ions to stabilize the RING domain, while the U-box domains are stabilized by a set of hydrogen bonds and salt bridges (Wiborg et al., 2008).
Genomics and phenomics for RING E3 ligases

Recent studies have revealed complex molecular networks that include ubiquitin E3 ligases in regulation of flowering (Lazaro et al., 2012; Peng et al., 2013; Xia et al., 2013). To define the genomic flower related Ubiquitin Proteasome System of RING E3 ligases we verified the gene expression patterns of the curated RING genes. From the 509 RING genes, 122 were indeed associated with flowering with enrichment of gene expression prompting us to gather a representative mutant collection for phenotypic characterization. To screen for phenotypes associated with the selected mutants an automated plant phenotyping facility was utilized. To facilitate a phenotypic screening of a large Arabidopsis mutation collection a phenomics workflow established to analyze simultaneously 36 genotypes in a PlantScreen™ imaging system installed at the Viikki campus of the University of Helsinki (http://blogs.helsinki.fi/nappi-blog/). The high-throughput phenomics screen of altogether 43 genotypes singled out three mutant lines with clear growth, morphology, flowering time and/or flower structure related phenotypes.

For the Arabidopsis growth assessment, we analyzed rosette growth from day 10 to day 20 after stratification (DAS). The analysis of such longitudinal data is challenging and demands automated statistical analysis and modelling steps. The rosette growth normally follows a sigmoid pattern showing a lag phase represented by slow growth around the first 10 days, accelerating in the middle and slowing down when getting close to the transition from the vegetative to reproductive phase. The best way to model data with sigmoid behavior is by fitting a three parameter logistic regression (3PL) to explain the three stages (Neilson et al., 2015; Paine et al., 2012; Tessmer et al., 2013). However, our analysis time window captured only the lag and the exponential phases, so a 3PL model was not suitable for our data. Therefore, we used polynomials for more flexibility and a better explanation of the data for all the parameters. This was particularly useful for the initial screening of the data of the tens of lines for the complex parameters like roundness, roundness 2, isotropy, compactness and RMS.

Typically, the parameters of roundness 2, isotropy and RMS increase and decrease over time. This behavior is due to the natural cycle of leaf initiation and expansion. At the beginning when the two first true leaves are developed, the rosette has an elliptical shape that becomes more circular when the leaves 3 and 4 appear and start to expand. Because leaves 3 and 4 keep on expanding, while the leaves 1 and 2 have already stopped expanding, the rosette takes an elliptical shape around day 12 (Figure 4). This process is repeated each time two new leaves develop and expand, explaining the oscillating behavior of these parameters. The steepness of each peak decrease over time because previously generated leaves expand making the rosette more circular. Thus, recording fluctuations in these parameters allows establishing the developmental timing of leaf initiation and expansion.

Here, three lines showed consistently significant differences in growth and morphology compared to the wild type Col-0. The mutant lines csu1-4 and sinal7-2 showed similar growth curve shapes as Col-0, but shifted to the left or right, respectively, for all morphological parameters. This behavior was explained by their speed of growth over time. If two lines differ in their growth rate but were analyzed only on one particular day after germination, they could show high differences in morphological parameters. Therefore, longitudinal time course analysis of Arabidopsis rosette growth and shape became compulsory for making accurate conclusions about the effect of a mutation also on morphology. On the contrary, the rha1a-1 mutant did not show major differences in growth, but did for morphology. The increased number and serration of rosette leaves in rha1a-
rendered the rosette perimeter and the skeleton longer, thereby, reducing the roundness and increasing SOL during all time points (Figure 6B and Figure 8E). Furthermore, the increased number of leaves of rha1-1 prevented its rosette from taking overly elliptical shape, keeping it more circular than Col-0 plants over time (Figure 4). This characteristic was translated in higher roundness 2, isotropy, compactness and lower eccentricity and RMS (Figure 6E, 6H, 7B, 7E and 8B). Thus, the morphological parameters can be used not only to record developmental timing but also to explain the plant architecture in a numeric manner.

The line showing an early flowering time phenotype was COP1 SUPPRESSOR1 (CSU1). csu1-4 plants flowered three to six leaves earlier than Col-0 grown under LDs (Table 1). In addition to early flowering, csu1-4 plants showed vegetative phenotypes: plants were smaller than Col-0 (Figure 5), the eccentricity, RMS and roundness2 development started later than Col-0 (Figure 6 and 7), and SOL was smaller than in Col-0 (Figure 8). CSU1 has been shown to negatively regulate hypocotyls length in the dark, via ubiquitination of COP1 and repression of SPA1 (Xu et al., 2014). Our results indicate that CSU1 may regulate both vegetative and generative development. The line showing a late flowering phenotype, SBP (S-ribonuclease binding protein) family protein (AT4G17680), flowered one to two leaves later than Col-0 (Table 1). This gene was selected for the phenotypic analysis based on its interaction with TOE2. toe2 is late flowering, and toe1 toe2 double mutant represses FT expression (Zhai et al., 2015). Our results suggest that this SBP (S-ribonuclease binding protein) family protein could be involved in regulation of flowering time possibly through TOE2. Some SBP family members are known to regulate flowering time. Four SBP proteins, BOTRYTIS SUSCEPTIBLE1 INTERACTOR (BOI) and its three homologous repress flowering by repressing FT expression in a CO dependent manner and a CO independent manner via DELLA proteins (Nguyen et al., 2015). This evidence suggests that there might be a connection between SBP proteins and flowering time control.

In sinal7-2 mutant, defects in flower morphology were observed. SINAL7 has been shown to ubiquitinate glyceraldehyde-3-phosphate dehydrogenase 1 (GAPC1) and to regulate its enzymatic activity and movement to nucleus (Peralta et al., 2016). GAPC1 plays a role in glycolysis, thus regulating carbon metabolism and it has also been associated with cytoskeleton and mitochondria (Anderson et al., 2004; Giegé et al., 2003). Although gapc1 mutants showed male sterility phenotype (Rius et al., 2008), we did not observe increased number of aborted pollen grains in the sinal7-2 mutant (Figure 9K-L), suggesting that SINAL7-mediated GAPC1 regulation does not impact pollen maturation. Although we have not tested the effect of sinal7-2 mutation on pollen germination and pollen tube growth, the fertility of the mutant did not seem to be strongly compromised. Instead, we observed defects in sinal7-2 flower morphology, namely cavities in the flower buds and wrinkled petals. Sepal curvature is regulated by giant cells in the abaxial epidermis where the cell expansion is promoted by endoreduplication (Roeder et al., 2010, 2012). A couple of mutants have been identified in which lack of the giant cells was accompanied by their sepals bending inwards. Closer examination of sinal7-2 sepal epidermis will show whether the observed bent sepal tips and resulting flower bud cavities (Figure 9A-B, E-F) originated from endoreduplication defects, which would suggest a novel role for the SINAL7 protein. Other flower phenotypes of the mutant – wrinkling of petals, as well as bending of stamens and pistils (Figure 9C-D, G-J) – seem to be a direct consequence of abnormal shape of the sepals, being an obstacle for the developing floral organs during their growth and release from the buds. Nevertheless, at
this point it cannot be ruled out that the SINAL7 ubiquitin E3 ligase would be involved in the
development of the flower organs in other ways.

Here we showed that automated, imaging based phenotyping platform is an efficient tool to
overcome the limiting factors of manual and visual phenotypic measurements of large plant
collections. Imaging based platforms also allow deep resolution of the phenotypes and thereby
more precise association with the genotypes. Furthermore, the automated plant management and
transportation to imaging, facilitates time course experiments. Thereby, recording longitudinal
numeric values indicating changes in rosette size and morphology can be utilized in developmental
timing of plant growth and development. Here the customized solution of the PSI PlantScreen™
system by top view CCD camera in combination with online data processing was used for high
throughput phenotyping of Arabidopsis mutant collections for growth and morphological traits.
Such facilities are thus ideal tools for reverse genetics approaches that require evaluation of large
plant collections. The obtained resolution and high throughput, whereby hundreds of plants can be
analyzed in the time that normally a handful would be analyzed, is an obvious advantage.

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520.

dehydrogenase and the B subunit of the chloroplast enzyme are present in the pea leaf nucleus.
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genomics in plants. Plant Cell 13, 1499-1510.

finger protein, mediates phytochrome-controlled seedling deetiolation responses. Plant


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**Figure legends**

Figure 1. Rosette morphology parameters. A. Area and perimeter of the rosette are raw parameters and are calculated by counting pixels of rosette binary images and the edge pixels respectively and transformed to millimeters. B. The parameters of roundness and roundness 2 and isotropy represented circular parameters. C. Eccentricity and RMS were symmetric parameters. D. Compactness and SOL were based on the center distance. Pink area around RMS, compactness and roundness 2 represent rosette convex hull, and for isotropy it represents rosette polygon. The characteristics of the parameters are described in detail in the Materials and Methods section.

Figure 2. RING gene family of 509 as identified by: This study; Stone et al., 2005; and Kosarev et al., 2002. This study brought in 50 new RING genes and 31 from the earlier studies were excluded.

Figure 3. A. Differential expression profiles of developmental stage enriched RING genes relative to developed rosette (B= bolting; YF= Young flower; DF= Developed flower; FS= Flowers and siliques). B. Differential expression profiles of flower organ enriched RING genes relative to rosette; SA= Shoot apical meristem; SP= Sepals; PT= Petals; ST= stamens; PS= pistil. C. Venn diagram of RING genes expressed in the different developmental stages. D. Venn diagram of RING genes expressed in the different flower organs.

Figure 4. Rosette growth of *csu1-4, rha1a-1* and *sinal7-2* mutants. Representative rosette images are shown from day 10 to day 20 after stratification.

Figure 5. Growth measurements of area and perimeter (raw measurements) for the three mutants, *csu1-4, rha1a-1* and *sinal7-2*, from day 10 to 20. Markers = daily mean; error bars = 95%
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Figure 6. Circularity growth measurements of roundness and isotropy for the three mutants, csu1-4, rha1a-1 and sinal7-2, from day 10 to 20. Markers = daily mean; error bars = 95% confidence interval; Curves = fitted models; n = 20 plants. The experiment was repeated at least 3 times with similar results.

Figure 7. Symmetry growth measurements for the three mutants, csu1-4, rha1a-1 and sinal7-2, from day 10 to 20. Markers = daily mean; error bars = 95% confidence interval; Curves = fitted models; n = 20 plants. The experiment was repeated at least 3 times with similar results.

Figure 8. Center distance growth measurements for the three mutants, csu1-4, rha1a-1 and sinal7-2, from day 10 to 20. Markers = daily mean; error bars = 95% confidence interval; Curves = fitted models; n = 20 plants. The experiment was repeated at least 3 times with similar results.

Figure 9. Flower phenotypes of the sinal7-2 mutant. Flower developmental stages assigned according to Smyth et al., (1990). Scale bars: 1 mm (A-J) and 100 µm (K-L). A-B. Representative inflorescences of Col-0 (A) and sinal7-2 (B). All flowers and siliques older than stage 12 have been removed. Mutant flower buds contain cavities beneath the bud tip (indicated with white arrows). C-D. Petals of a Col-0 (C) and a sinal7-2 (D) flower at stage 15. White arrows pointing to wrinkled mutant petals. E-F. Adaxial surface of sepals from a Col-0 (E) and a sinal7-2 (F) flower at stage 15. White arrow pointing to an inward bending lateral sepal tip of sinal7-2. G-H. Late stage 12 flower buds of Col-0 (G) and sinal7-2 sinal7-2 (H). The medial sepals have been removed to reveal the elongating and wrinkling petals blocked by the ingrown lateral sepals. I-J. Col-0 (I) and sinal7-2 (J) flowers stage 15. K-L. Representative anthers from Col-0 (K) and sinal7-2 (L) flowers stage 12-13 stained for pollen viability.

Legends of supplemental tables

Supplemental Table 1. T-DNA mutant collection analyzed in this study, running number, AGI code, Gene name, NASC code, stock code, T-DNA position, qPCR result and interpretation, Genotyping PCR primers (forward and reverse) and qPCR primers (forward and reverse).

Supplemental Table 2. The complete list of 509 curated Arabidopsis RING E3 ligases genes (Gene ID, Gene symbol, Description, RING type, Domain sequence, Gene ontology (GO) ID, GO name, Pubmed ID and Literature). New RING domain proteins (50) curated in this study are in their own column.

Supplemental Table 3. A. (A) All RING domain proteins curated in; This study, by Stone et al., 2005 and by Kosarev et al., 2002. (B) Summary of the 31 excluded RING domain proteins with source and reason for excluding from the curated collection. B. (A) Ranking of the 122 differentially expressed RING E3 ligase genes in the developmental stages (Bolting, Young Flower, Developed Flower, Flower and Silique) and (B) flower organs (Shoot Apical Meristem, Sepal, Petal, Stamen, Pistil). (C) List of common and shared genes in the two main categories.

Supplemental Table 4. A-C Raw phenotyping data from three replicated experimental rounds for the three mutants (csu1-4, rha1a-1 and sinal7-2) showing morphological changes. A. csu1-4
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experimental rounds F0007, F0008 and F001.0 B. *rha1a-1* experimental rounds F0007, F0008 and F0011. C. *sinal7-2* experimental rounds F0007, F008 and F0011.
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## Legends of Tables

Table 1. Number of leaves and number of days to bolting in *Arabidopsis* mutant lines grown in LDs. Pairwise comparisons were performed against corresponding Col-0-line using Dunnett’s test; * indicates statistically significant difference (α=0.05). N=19-20 in each row.

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Table 2. Polynomial order and their respective Chi square probability from ANOVA test for each parameter used in this study.

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<td>&lt; 2.2e-16 ***</td>
<td>4.53e-05 ***</td>
</tr>
<tr>
<td>SOL</td>
<td>3</td>
<td>0.0006792 ***</td>
<td>3.867e-07 ***</td>
<td>0.006651 **</td>
</tr>
</tbody>
</table>

*Comparison was performed using an ANOVA test between a base model and a model including the genetic background as factor.

base model = Parameter ~ polynomial of Day + Random factor Day and Plant ID
model = Parameter ~ polynomial of Day * genetic background (Col-0 or knockout line) + Random factor Day and Plant ID
Figure 2.TIF

- This study: 50, 4, 12
- Stone et al., 2005: 107, 9
- Kosarev et al., 2002: 348, 10

In review
Figure 8.TIF

(A) Compactness vs. DAS for Col-0 and csu1-4.

(B) Compactness vs. DAS for Col-0 and rha1a-1.

(C) Compactness vs. DAS for Col-0 and sina17-2.

(D) SOL vs. DAS for Col-0.

(E) SOL vs. DAS for rha1a-1.

(F) SOL vs. DAS for sina17-2.