Meristem maintenance, auxin, jasmonic and abscisic acid pathways as a mechanism for phenotypic plasticity in *Antirrhinum majus*

Julia Weiss, Raquel Alcantud-Rodriguez, Tugba Toksöz & Marcos Egea-Cortines

Plants grow under climatic changing conditions that cause modifications in vegetative and reproductive development. The degree of changes in organ development i.e. its phenotypic plasticity seems to be determined by the organ identity and the type of environmental cue. We used intraspecific competition and found that *Antirrhinum majus* behaves as a decoupled species for lateral organ size and number. Crowding causes decreases in leaf size and increased leaf number whereas floral size is robust and floral number is reduced. Genes involved in shoot apical meristem maintenance like *ROA* and *HIRZ*, cell cycle (*CYCD3a; CYCD3b, HISTONE H4*) or organ polarity (*GRAM*) were not significantly downregulated under crowding conditions. A transcriptomic analysis of inflorescence meristems showed Gene Ontology enriched pathways upregulated including Jasmonic and Abscisic acid synthesis and or signalling. Genes involved in auxin synthesis such as *AmTAR2* and signalling *AmANT* were not affected by crowding. In contrast, *AmJAZ1*, *AmMYB21*, *AmOPCL1* and *AmABA2* were significantly upregulated. Our work provides a mechanistic working hypothesis where a robust SAM and stable auxin signalling enables a homogeneous floral size while changes in JA and ABA signalling maybe responsible for the decreased leaf size and floral number.

In contrast to animals, plants produce organs throughout development and environmental conditions play a key role in the size and type of organs produced. The degree of developmental plasticity of lateral organs seems to be determined by a combination of organ identity, the species under study and the type of environmental conditions that may affect its ontogeny. Some organs are highly robust and show little variation. One proposed mechanism to establish robust traits is the existence of genetic redundancy and highly interactive genetic networks. The other side of the coin is plasticity. Many developmental processes are highly plastic such as root formation, leaf development or flowering time as they respond to environmental cues.

Aerial organs such as leaves and flowers are generated from the shoot apical meristem (SAM). Plants may show adaptation to changing environments in the SAM via modifying the output of organ number and/or size. Cells produced in the SAM, are displaced to side positions and become recruited to form lateral organ primordia. In *Antirrhinum majus*, stem cells are maintained undifferentiated by the homeobox gene *ROSULATA* (*ROA*), expressed in the quiescent zone, just underneath the shoot apical meristem. Orthologs of *ROA* like *WUSCHEL* from Arabidopsis or *TERMINATOR* from Petunia show a conserved function. Cells in the SAM retain a meristematic identity due to the expression of *HIRZINA* (*HIRZ*) and *INVAGINATA* (*INA*), two homeobox genes belonging to the *KNOTTED*, and *SHOOT MERISTEMLESS* family. The identity of lateral primordia will depend on the developmental stage of the SAM. If the floral program is initiated, lateral primordia will adopt a floral identity.

The development of lateral organs seems to be initiated by an increase in the local levels of auxins. Local changes in auxin synthesis maybe important in adaptation to the environment as the Arabidopsis auxin synthesis gene *TRYPTOPHAN AMINOTRANSFERASE 1 TAA1* plays a role in shade avoidance. The AP2 transcription...
factor AINTEGUMENTA has a dual function in activation of the polarity genes and the establishment of floral organ identity. It also plays a role in lateral organ size via control of cell division and expansion in Arabidopsis, Petunia and Antirrhinum, and is downstream of the auxin signalling pathway. As lateral organs initiate, organ polarity genes take over and a combination of transcription factors and siRNAs establish the growth planes of lateral organs. The YABBY genes fulfil a key role in establishing the proximo-distal polarity of lateral organ formation and in Antirrhinum the gene GRAMINIFOLIA plays a function in lateral organ formation.

Floral organs are formed as a result of the coordinated expression of several genes that give rise to sepal, petals, stamens and carpels whose identity is established by combinations of MADS-box genes. The proper activation of MADS-box genes are also responsible for the final size and shape of the floral organs, and play a key role in maintenance of basic cell division and expansion in the flower.

Although the basic functioning of meristem maintenance is understood, the influence of environmental cues on meristem output, understanding it as a combination of lateral organ formation and organs that attain a certain size, is not. Both biotic and abiotic stresses tend to cause a decrease in the number and/or size of lateral organs produced by plants. Integration of stress is thought to occur via changes in the levels of plant growth regulators like brassinosteroids, jasmonic acid (JA), abscisic acid (ABA) or auxins, all of which can show interactions with each other. Thus changes in signalling may account for modified SAM activity.

We found by serendipity that Antirrhinum majus flower size was robust and highly resilient to changes under different growth conditions, whereas vegetative development, specifically leaf size was strongly affected by the environmental conditions. We established a system based on plant crowding, used in ecology and in agriculture. We had previously found that intense leaf removal has little or no effect on floral size in Antirrhinum majus. In a large set of F2 populations used to create an Antirrhinum map, we found that plants left on small pots after picking to larger trays where segregations took place ended up with a very small vegetative size, but floral size appeared to be normal. Experiments were carried out several times in autumn, winter and spring in order to estimate effects of crowding on development and to verify the preliminary observations. We used pots with 1, 5 or 10 plants but we performed all analysis on control (1 plant per pot) and the most extreme treatment (10 plants per pot).

Growth of plants subject to crowding was analysed during development (Table 1). We measured the length of the first three internodes and found that they were significantly shorter (Table 1), indicating that the effect of crowding on growth occurred from early stages of development. A detailed kinetic analysis of shoot length showed that at early stages of growth (8 days), crowded plants were significantly shorter than controls (P = 0.0321). However, this trend was abrogated during later growth as from day 15 onward crowded plants caught up with the controls and differences were non significant (Fig. 1a).

Changes from double decussate to spiral phyllotaxis have been described in Antirrhinum as a point of floral transition. We did not observe changes in the number of double decussate leaves indicating that transition to flower was not affected by the crowding conditions (Table 1). However we found a significant increase in the number of spiral leaves before flower primordia appeared (p < 0.05), pointing to a delayed acquisition of the floral program in the SAM. As a result the total number of leaves produced under crowding was significantly larger (P < 0.01). Leaf area was significantly decreased indicating that despite the increased number of leaves, there was a strong reduction in size.

We measured ten parameters describing floral size (Fig. 1b). Two floral parameters, sepal length and dorsal petal expansion were significantly larger in crowded plant (Table 2). Both stamen and gynoecium length were significantly shorter in crowded plants as compared to control flowers, albeit the actual differences were of 5.2% (P = 0.017) in stamens and 4.5% in gynoecia (P = 0.023). The number of flowers per plant was strongly reduced to 52% in crowding conditions (P = 0.001) (Table 1). To assess a cause-effect relation between foliar area variations and flower production we made a correlation analysis between both characters. Plants grown under normal conditions did not show any type of correlation between floral number and leaf area (Spearman Coefficient 0.0629, p = 0.739) but under crowding conditions we found a statistically significant positive correlation (p = 0.013).

| Internode 1 | Internode 2 | Internode 3 | Total height | Number of flowers | Double decussate leaves | Spiral leaves | Total leaves | Leaf area (cm²) |
|-------------|-------------|-------------|--------------|-------------------|-------------------------|--------------|-------------|----------------|
| 1           | 2           | 3           | 479 ± 5      | 16.97 ± 3.3       | 7.20 ± 1.27             | 5.63 ± 3.66  | 12.83 ± 2.56 | 145.70 ± 25  |
| 10          | 2           | 10          | 295 ± 5      | 8.13 ± 4.71       | 6.96 ± 1.49             | 8.30 ± 3.44  | 15.26 ± 2.42 | 105.18 ± 28  |
| 1 vs 10     | –12.18***   | –14.62***   | –8.89***     | –38.33***         | –52.08***               | –3.38        | 47.41*      | 18.92**       | 27.81***      |

Table 1. Effect of crowding on vegetative growth in Antirrhinum majus. Numbers on the first column correspond to 1 plant per pot 10 plants per pot and the difference. Values represent average ± standard deviation. Differences expressed as treatment versus control 100%. We performed student T-tests. P values correspond to *P < 0.05. **P < 0.01. ***P < 0.001. Measurements correspond to 30 individuals per treatment.
The low value of the Spearman coefficient (0.5853) suggests that there are other factors affecting floral number production under crowding beyond leaf area reduction.

**Effect of environmental stress on basic meristem maintenance.** We analysed the effect of crowding on gene expression in inflorescence meristems harvested when floral primordia started to appear. We measured gene expression levels that define basic meristem functions, meristem maintenance by ROA and meristem growth by HIRZ. We found that both HIRZ and ROA showed a trend towards down regulation in crowding but this trend was not significant (HIRZ P = 0.513; ROA P = 0.465) (Fig. 2). We measured three cell division markers, CYCLIN D3b (CycD3b), CYCLIN D3a24 and HISTONE H4 (H4), marking mitotic index25. Both CycD3a and CycD3b showed non significant down regulation, indicating that local levels of cell division could be maintained in the centre of the SAM and lateral primordia. The expression of H4 was similar in control and crowded plants (relative expression level 1.549 P = 0.518) indicating that general mitotic index was not affected (Fig. 2).

![Figure 1](image.png)

**Figure 1.** (a) Growth kinetics of C, control and CR, crowded plants, numbers refer to days after sowing. The Y axis refers to height in cm. (b) Typical inflorescences of crowded plants at 1, 5 and 10 plants per pot. The picture shown in figure B was taken by MEC.

| Organ                  | Control | Crowding | p-value |
|------------------------|---------|----------|---------|
| Tube length            | 14.8    | 15.2     | 0.706   |
| Ventral petal length   | 25.9    | 27.3     | 0.274   |
| Petal height           | 22.6    | 20.5     | 0.115   |
| Sepal length           | 7.8     | 7.5      | 0.044   |
| Tube width             | 12.1    | 11.7     | 0.175   |
| Dorsal petal length    | 37.3    | 36.5     | 0.117   |
| Ventral petal expansion| 23.3    | 23.4     | 0.963   |
| Dorsal petal expansion | 26.9    | 29.6     | 0.012   |
| Stamen length          | 23.5    | 22.3     | 0.017   |
| Gynoecium length       | 20.2    | 19.3     | 0.023   |

**Table 2.** Comparison of floral parameters between control and crowded grown plants. Values represent millimeters.
Formation of lateral primordia is marked at very early stages by the YABBY gene GRAMINIFOLIA, involved in lateral organ polarity. The expression levels of GRAM were unchanged (relative expression level 1.004 P = 0.459) indicating that the relative effect of crowding on lateral primordia initiation could not be identified by changes in levels of organ polarity acquisition.

Altogether we can conclude that crowding had non-significant effect on meristem maintenance genes, cell division or organ polarity establishment.

Transcriptomic analysis of SAM under crowding conditions. We performed a large scale transcriptomic analysis to uncover possible mechanisms of stress integration in the SAM. A total of 1134 genes were significantly upregulated and 361 were down regulated (Supplementary Table S1). A gene ontology enrichment analysis identified biological processes in the up and down regulated genes (Supplementary Table S2). Despite showing a lower number of down regulated genes, the number of significantly different down regulated GO terms was substantially larger with 11 biological processes, 5 cellular components and one molecular process affected. There were only 4 biological processes, 2 cellular components and one molecular function with significant up regulation.

We used the corresponding Arabidopsis orthologs to identify enriched biological pathways. The result of the analysis showed two sets of genes significantly up regulated, but discrete downregulated pathways were not found. One upregulated pathway corresponds to genes involved in JA signalling including the MYB transcription factors JAZ1 and MYB21 as central genes. The second upregulated pathway was related to ABA synthesis and was centred on ABA2 (Fig. 3). These results indicated that signalling into the SAM could occur via JA and ABA. Surprisingly auxin signalling or synthesis was apparently unaffected.

As we used multiple displacement amplification for cDNA amplification and biases have been found to occur as a result of this technique in GC rich templates, we tested several genes that were not significantly modified and those that appeared as upregulated networks in the microarray.

Auxin signalling is not modified by stress. There are several hormone-signalling pathways that play a role in meristem function. Amongst them auxin plays a key role in lateral organ initiation. Despite the importance of auxins in lateral organ formation and general plant development there was no obvious change in expression of genes involved in auxin signalling, transport or synthesis. We identified 16 genes with annotation related to auxins (Table 3). Only two genes showed significant changes in gene expression. INDOLEACETIC ACID-INDUCED PROTEIN 16 was significantly down regulated (~2.04 fold) and SMALL AUXIN UPREGULATED RNA 51 was significantly up-regulated (2.03 fold). Genes with well established roles in auxin transport like PIN-FORMED 4 or signal transduction like AUXIN RESISTANT 2 did not show significant differences in gene expression.
Furthermore, two Arabidopsis genes, **AUXIN INDUCIBLE 2-11**, and **DORMANCY-ASSOCIATED PROTEIN 1** were apparently duplicated in *Antirrhinum*. Whilst one paralog was non-significantly up-regulated (Snap112107_cn5436 and Snap112107_cn2840), a second one was non-significantly down-regulated (Snap112107_cn5435 and Snap112107_cn2580) (Table 3) suggesting that altogether the auxin signalling pathway was not affected by crowding. In order to further verify this result, we identified an *Antirrhinum* clone (AJ794078) with high homology to the **TRYPTOPHAN AMINOTRANSFERASE** (*TAA*) and **TRYPTOPHAN AMINOTRANSFERASE RELATED** genes involved in auxin synthesis and shade avoidance 8,28. The expression of AJ794078 was not significantly affected in the microarray (−1.19 fold downregulation). We performed a phylogenetic analysis of AJ794078 to identify its degree of homology with the *TAA1-TAR* family from Arabidopsis. The *Antirrhinum* clone clearly clustered together with *TAR2* from Arabidopsis and in a different clade from *TAA1* (Supplementary Fig. 1). We confirmed the expression level of *AmTAR2* under crowding, which was virtually identical to control plants (expression 0.901; P = 0.498) indicating that auxin biosynthesis was not affected by crowding (Fig. 4). As auxin signalling maybe localized in primordia, we tested the expression of *AmAINTEGUMENTA*, an AP2 transcription factor involved in auxin signaling and lateral organ formation13,29. As found for *AmTAR2* expression, *AmANT* was non-significantly down-regulated (expression 0.901; P = 0.546) indicating that auxin synthesis and signalling was not affected by the imposed stresses.

**Crowding activates JA and ABA signalling.** The *Antirrhinum* genes corresponding to the Arabidopsis MYB genes *JAZ1* (AJ787051), *MYB21* (AJ797639) and the short chain alcohol dehydogenase *ABA2/GIN1* (AJ802690) were used to verify the degree of sequence homology with the Arabidopsis genes by phylogenetic analysis. The EST corresponding to *AmJAZ1* was found to be on a single clade together with *AtJAZ1* and *AtJAZ2* (Fig. 5). The putative *AmMYB21* was in a clade together with *AtMYB21* and *AtMYB24* from Arabidopsis and was clearly separated from other *MYB* genes like *VENOSA* or *ROSEA* involved in anthocyanin patterning and colour intensity in petals30.
A quantitative PCR analysis showed significant up-regulation of both genes that closely corresponded to those found in the microarray analysis (Fig. 4). Indeed AmMYB21 showed significant overexpression in the microarray of 3.43 fold and of 1.84 by qPCR with a p value of 0.000, AmJAZ1 had a 2.81 up regulation in the microarray and a 3.63 upregulation in the QPCR analysis (p = 0.001).

There are several 4-coumarate C0 ligases in the Arabidopsis and the OPC-8:0 COA LIGASE1 (OPCL1) is involved in jasmonic acid biosynthesis31,32. We identified an Antirrhinum gene that showed high homology with the OPCL1 gene from Arabidopsis. We confirmed this finding by a phylogenetic analysis that showed a high homology to OPCL1 and a clear separation of other clades containing other acyl CoA ligases that are not involved in JA synthesis (Supplementary Fig. 2). An expression analysis showed that it was significantly up-regulated in crowded plants as compared to control plants (4.13 fold P = 0.001).

The ABA2/GIN1 gene encodes a short-chain alcohol dehydrogenase catalyzing the conversion of xanthoxin to abscisic aldehyde33,34. We found two paralogs in Antirrhinum (Fig. 4). One of them (A.majusABA2; AJ802690) was significantly upregulated in the microarray analysis (3.13 fold). The corresponding gene was found to be 3.00 fold up regulated (p = 0.001) in QPCR assays.

Our results show first that the multiple displacement amplification may be suitable for amplification of cDNA libraries. And second, both JA synthesis, signalling and ABA synthesis are upregulated in crowded plants indicating that the identified decrease in leaf area and floral number may be the result of adjustments governed by these two pathways. A robust auxin signalling and meristem maintenance may be required to acquire normal floral size.

Discussion
There are several paradigms in plant biology regarding growth and development. One is the role of the SAM as the generator of cells forming lateral organ primordia. Second is the fact that many species display correlations between vegetative and reproductive organ phenotypes35,36. And third is that the so-called floral organ identity genes may impose a morphogenetic program that is thought to be a departure from a default vegetative
pathway. Flowers and lateral organs can be considered as different phenotypic modules from a developmental and evolutionary perspective provided they have uncoupled mechanisms of control. In the present work we have identified the potential of Antirrhinum majus as a model to study decoupling of vegetative and reproductive development. The current hypothesis is that floral size may play a key role in pollination, and changes may modify fitness. Although the number of flowers is not related to leaf area under normal growth conditions our results clearly show that crowding imposes growth constrains where floral number becomes somehow dependent on leaf area. This also indicates that testing coupling or decoupling requires several growth conditions whereby covariations may appear as a result of limiting resources.

Although our initial hypothesis was that changes in organ number production would be reflected by changes in basic meristem functions and cell division, our data proved us wrong. Our current interpretation is that the complete SAM has a coordinated reduction in functions in such a way that it maintains a coherent relationship between stem cell formation, lateral recruitment, cell division and primordia initiation. Furthermore cell division is carefully controlled in all different areas and does not show changes overall. If meristem maintenance and cell division are maintained it may explain the formation of perfect flowers irrespective of growth conditions.

The signalling and synthesis of several plant hormones is involved in meristem function, including auxins, brassinosteroids or JA. Light governs stem cell division via local changes in auxin levels involved in leaf positioning and phylotaxis. Thus we would have expected changes in auxin synthesis or signalling. However neither the transcription level of AmTAR1, an aminotransferase-related gene involved in auxin synthesis, nor AmANT1, a gene responsive to auxin signalling and organ primordia initiation, were altered under the two treatment conditions.

Taken together our results indicate that a strong homeostasis of SAM maintenance and auxin signalling may be a requirement for floral size maintenance. The fact that leaf number is not decreasing whereas floral number does, indicates that there are two distinct mechanisms that may be dependent on the floral identity program. The vegetative phase may be dependent on a certain number of lateral organs that should be achieved in order to start flowering. Flowering time mutations and environmental conditions affect the number of leaves produced in Arabidopsis, Antirrhinum and other plants. The floral identity program may have an intrinsic mechanism controlling the number of cells per primordium thus ensuring achievement of the correct floral size.

JA signalling plays a dual role as they inhibit growth by suppressing mitosis in apical meristems, but is also required for stamen development. We found a significant up regulation of AmJAZ1, AmMYB21 and AmMYB24 involved in Jasmonic acid signalling at various levels and of AmOPCL1 involved in early steps of JA synthesis. JAZ proteins are repressors of JA signalling. But JAZ proteins interact with AtMYB21 and AtMYB24 controlling anther development. The phylogenetic relationship of A. majus MYB21 with PhybrideaEOBI, A. majus MYB305 or Phybrida_ODORANT1 is close enough to suggest additional functions of A. majus MYB21. In fact AtMYB21 is required for terpenoid synthesis in Arabidopsis.

Altogether crowding may be considered a combined stress and the emergence of the ABA synthesis pathway as upregulated in crowded plants is probably expected. ABA is a major integrator of abiotic stress in plants. The ABA2 gene is involved in ABA synthesis and plays an additional role in sugar sensing. Activation of the ABA synthesis pathway should be responsible for improved resilience to abiotic stress. This translates in Antirrhinum plants with smaller but not fewer leaves. It may also define the pace of lateral organ formation once flowering starts, causing a general decrease in the number of flowers produced that are otherwise perfect. Pending further experimental evidence based on gain and loss of function of genes involved in the pathways analysed, we can speculate that a combination of robust behaviour and plastic expression of auxins, JA, ABA and meristematic functions may be involved in robustness and phenotypic plasticity of plant aerial organs.

Materials and Methods

Plant material, growth conditions and treatments. Seeds of Antirrhinum majus inbred line 165E were germinated on fine vermiculite and transplanted after two weeks to the final growth conditions on pots. Plants were watered as required with an automatic drip irrigation system in a greenhouse.

We transplanted 40 pots (650 ml volume) with one plant, and 20 with five or ten plants when the first pair of true leaves had emerged. The different vegetative parameters were measured in 30 individuals when plants flowered and at least one flower on the primary stem had opened. Twenty three percent of the plants in the 10 plants/pot treatment developed full leaf number but did not flower and were included in the measurements of plant size.

Plant measurements. Total height was measured from the base to the top of the plants. Growth kinetics was recorded by measuring plant height every 7–14 days. Once the inflorescence meristems formed we measured additional vegetative parameters. Leaves were counted including leaves in the double decussated vegetative part and spirally organized single leaves in the inflorescence. Bracts subtending flower were not counted as leaves.

The leaves and bracts of the plants were cut as close to the stem as possible and all together were ordered on a paper sheet using a ruler as an internal standard. Leaves were scanned and leaf area was analyzed using the ImageJ program (http://rsb.info.nih.gov/ij/).

Once floral organs were fully developed, the size of the different organs and parts of the flower were measured with a millimetre calliper as described. The parameters measured were: 1, tube length; 2, ventral petal length; 3, petal height; 4, sepal length; 5, tube width; 6, dorsal petal length; 7, ventral petal expansion; 8, dorsal petal expansion; 9, stamen length and 10, gynoecium length.

Statistical analysis. We performed statistical analysis with the R program (www.r-project.org), comparing 10 plants/pot against 1 plant/pot. One-way ANOVA was used for all the parameters that showed a normal distribution, and the Kruskall and Wallis analysis in case that one of the parameters under comparison deviated from normality.
a normal distribution. The parameters were considered significantly different among treatments when the P value was smaller than 0.05.

Microarray analysis. Inflorescence shoot apical meristems of Antirrhinum plants were sampled when the first flower opened, total RNA was extracted and first strand cDNA was synthesized as described previously. We gathered enough apical meristems to obtain 60–100 mg of tissue. The number of meristems required was about 50. As the quantity of mRNA obtained and cDNA was minimal (0.1–1 ng), we used Multiple Displacement Amplification to obtain enough cDNA for microarray analysis as described previously49. Microarray analysis was performed on two control samples and three crowded samples by MoGene using a microarray based on 12,497 Antirrhinum genes publicly available, and comprising 11959 single genes from A. majus (Omnibus accession number GSE36356)50,51. Microarray data were deposited in the NCBI Gene Expression Omnibus database (Accession number GSE72818).

Bioinformatic analysis. Genes identified in Antirrhinum were compared by TBLASTN against Arabidopsis using translated sequences. In order to ascertain the degree of homology and the putative orthology of the sequences used for Q-PCR, we downloaded sequences obtained by TBLASTN from NCBI. Protein sequences were aligned using CLUSTALX, and phylogenetic trees were built using the built in Neighbour Joining algorithm52. Trees were rendered with NJPlot53.

We used the REST program to perform a Gene Ontology enrichment analysis and identify pathways defined in Gene Ontology with significant changes in gene expression using g:Profiler26. Genes with significant differences in gene expression were blasted using BLAST2GO44, and the corresponding annotated genes were used to create a set of conserved Arabidopsis orthologs. The Arabidopsis orthologs were used to perform a Gene Ontology enrichment analysis and identify pathways defined in Gene Ontology with significant changes in gene expression using g:Profiler26.

Quantitative PCR. Genes were amplified in a Stratagene Mx3000 qPCR machine (www.agilent.com), with sequence-specific primers (Table S1) synthesized by Invitrogen (www.invitrogen.com) using Takara SYBR-Green (www.thermofisher.com). We used the gene ubiquitin as a control for normalization52. PCR efficiency was calculated as described before55. Statistical analysis of gene expression was performed using group-wise comparison with the REST program56. We performed two independent experiments with three biological and two technical replicates.

References
1. Kafri, R., Levy, M. & Pilpel, Y. The regulatory utilization of genetic redundancy through responsive backup circuits. Proc. Natl. Acad. Sci. USA 103, 11653–8 (2006).
2. Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K. & Relyea, R. A. Ecological consequences of phenotypic plasticity. Trends Ecol. Evol. 20, 685–692 (2005).
3. Kieffer, M. et al. Analysis of the transcription factor WUSCHEL and its functional homologue in Antirrhinum reveals a potential mechanism for their roles in meristem maintenance. Plant Cell 18, 560–573 (2006).
4. Schoof, H. et al. The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. Cell 100, 635–644 (2000).
5. Stuurman, J., Jaggi, F., Kuhlemeier, C. & Jaggi, F. Shoot meristem maintenance is controlled by a GRAS-gene mediated signal from differentiating cells. Genes Dev. 16, 2213–2218 (2002).
6. Gola, I. J., Feek, E. J. & Hudson, A. Spontaneous mutations in KNOX genes give rise to a novel floral structure in Antirrhinum. Curr. Biol. 12, 515–522 (2002).
7. Reinhardt, D., Mandel, T. & Kuhlemeier, C. Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell 12, 507–518 (2000).
8. Tao, Y. et al. Rapid synthesis of auxin via a new tropoblast-dependent pathway is required for shade avoidance in plants. Cell 133, 164–176 (2008).
9. Nole-Wilson, S. & Krizek, B. A. AINTEGUMENTA contributes to organ polarity and regulates growth of lateral organs in combination with YABBY genes. Plant Physiol. 141, 977–987 (2006).
10. Manchado-Rojo, M., Weiss, J. & Egea-Cortines, M. Validation of Aintegumenta as a gene to modify floral size in ornamental plants. Plant Biol. 12, 1053–1065 (2014).
11. Mizukami, Y. & Fischer, R. L. Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. Proc. Natl. Acad. Sci. USA 97, 942–947 (2000).
12. Krizek, B. A. Ectopic expression AINTEGUMENTA in Arabidopsis plants results in increased growth of floral organs. Dev. Genet. 25, 224–236 (1999).
13. Hu, Y. C., Xie, O., Chua, N. H. & Xie, Q. The Arabidopsis auxin-inducible gene ARGOS controls lateral organ size. Plant Cell 15, 1951–1961 (2003).
14. Husbands, A. Y., Chitwood, D. H., Plavskin, Y. & Timmermans, M. C. P. Signals and prepatterns: new insights into organ polarity in plants. Genes Dev. 23, 1986–1997 (2009).
15. Gola, J. F., Roccaro, M., Kuzof, R. & Hudson, A. GRAMINIFOLIA promotes growth and polarity of Antirrhinum leaves. Development 131, 3661–3670 (2004).
16. Causier, B., Schwartz-Sommer, Z. & Davies, B. Floral organ identity: 20 years of ABCs. Semin Cell Dev Biol 21, 73–79 (2010).
17. Manchado-Rojo, M., Delgado-Benarroch, L., Rao, M. J., Weiss, J. & Egea-Cortines, M. Quantitative levels of DEFICIENS and GLOBOSA during late petal development show a complex transcriptional network topology of B function. Plant J. 72, 294–307 (2012).
18. Dornelas, M. C., Patreze, C. M., Angenent, G. C. & Immink, R. G. H. MADS: the missing link between identity and growth? Trends Plant Sci. 16, 89–97 (2011).
19. Krishna, P. Brassinosteroid-Mediated Stress Responses. J. Plant Growth Regul. 22, 289–297 (2003).
20. Divi, U. K., Rahman, T. & Krishna, P. Brassinosteroid-mediated stress tolerance in Arabidopsis shows interactions with abscisic acid, ethylene and salicylic acid pathways. BMC Plant Biol. 10, 151 (2010).
21. Bayo-Canha, A., Delgado-Benarroch, L., Weiss, J. & Egea-Cortines, M. Artificial decrease of leaf area affects inflorescence quality but not floral size in Antirrhinum majus. Sci. Hort. (Amsterdam.) 113, 383–386 (2007).
22. Schwartz-Sommer, Z. et al. A molecular recombination map of Antirrhinum majus. BMC Plant Biol. 10, 275 (2010).
23. Bradley, D., Vincent, C., Carpenter, R. & Coen, E. Pathways for inflorescence and floral induction in Antirrhinum. Development 122, 1535–1544 (1996).
The authors declare no competing financial interests.

Competing financial interests:

Acknowledgements
This work was funded by BFU-2010-15843 and BFU-45148-R. Part of the material used belongs to the Proyecto Vitalis Campus Mare Nostrum “Espacio Mediterráneo de Investigación en Red en Alimentos y Salud” - CEI10-2-0002.

Author Contributions
J.W. and M.E.C. conceived and designed the experiments, R.A., T.T., J.W. and M.E.C. performed experiments, J.W. and M.E.C. analysed data and produced figures and tables. M.E.C. wrote the manuscript and J.W. corrected the manuscript. All authors reviewed the manuscript.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

24. Gaudin, V. et al. The expression of D-cyclin genes defines distinct developmental zones in snapdragon apical meristems and is locally regulated by the cycloidea gene. Plant Physiol. 122, 1137–1148 (2000).
25. Groot, E. P., Sinha, N. & Gleissberg, S. Expression patterns of STM-like KNOX and Histone H4 genes in shoot development of the dissected-leaved basal eudicot plants Chelidonium majus and Eschscholzia californica (Papaveraceae). Plant Mol. Biol. 58, 317–331 (2005).
26. Reimand, J., Arak, T. & Vilo, J. G:Profiler - A web server for functional interpretation of gene lists (2011 update). Nucleic Acids Res. 39, 1–9 (2011).
27. Pinard, R. et al. Assessment of whole genome non-coding RNA and protein-coding genes in silico and high-throughput, massively parallel whole genome sequencing. BMC Genomics 7, 216 (2006).
28. Stepanova, A. N. et al. TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. Cell 133, 177–191 (2008).
29. Delgado-Benarroch, L., Causier, B., Weiss, J. & Egea-Cortines, M. FORMOSA controls cell division and expansion during floral development in Antirrhinum majus. Planta 229, 1219–1229 (2009).
30. Yoshida, S., Mandel, T. & Kuhlemeier, C. Stem cell activation by light guides plant organogenesis. J. Exp. Bot. 59, 403–419 (2008).
31. Cheng, W. et al. A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. Plant Cell 14, 2723–2743 (2002).
32. Gonzalez-Guzman, M. et al. The short-chain alcohol dehydrogenase ABA2 catalyzes the conversion of xanthoxin to abscisic aldehyde. Plant Cell 14, 1833–1846 (2002).
33. Chini, A. et al. Plant Hormone Signalling: Farnesylated JAZ proteins as repressors of the SCF(COI1) complex. Plant Cell 19, 2657–2677 (2007).
34. Song, S. et al. Jasmonate-regulated stamen development in Arabidopsis. J. Exp. Bot. 59, 296–302 (2008).
35. Ishiguro, S. The DEFECTIVE IN ANther DEHISCENCE1 Gene Encodes a Novel Phospholipase A1 Catalyzing the Initial Step of JASMONATE Biosynthesis, Which Synchronizes Pollen Maturation, Anther Dehiscence, and Flower Opening in Arabidopsis. Plant Cell 13, 2191–2209 (2001).
36. Sanders, P. M. et al. Arabidopsis DELAYED DEHISCENCE1 Gene Encodes an Enzyme in the Jasmonic Acid Synthesis Pathway. PLANT CELL ONLINE 12, 1041–1062 (2000).
37. Thines, B. et al. JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. J. Exp. Bot. 59, 403–419 (2008).
38. Zelaya, R., Schneider, T. & Kuhlemeier, C. Stem cell activation by light guides plant organogenesis. Genes Dev. 25, 1439–50 (2011).
39. Lancaster, A. et al. A regulatory network for coordinated flower maturation. PLoS Genet. 8, e1002506 (2012).
40. Seki, M., Umezawa, T., Urano, K. & Shinozaki, K. Regulatory metabolic networks in drought stress responses. Curr. Opin. Plant Biol. 10, 296–302 (2007).
41. Arenas-Huerta, F., Arroyo, A., Zhou, L., Sheen, J. & León, P. Analysis of Arabidopsis glucose insensitive mutants, gins5 and gins6, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. Genes Dev. 14, 2085–96 (2000).
42. Gibson, S. I. Control of plant development and gene expression by sugar signaling. Curr. Opin. Plant Biol. 8, 93–102 (2005).
43. Vincent, C. A. & Coen, E. S. A temporal and morphological framework for flower development in Antirrhinum majus. Can. J. Bot. 82, 681–690 (2004).
44. Nordlund, J. et al. Application of whole genome amplification and quantitative PCR for detection and quantification of spoilage yeasts in orange juice. Int. J. Food Microbiol. 126, 195–201 (2008).
45. Bey, M. et al. Characterization of Antirrhinum petal development and identification of target genes of the class B MADS box gene DEFCIENS. Plant Cell 16, 3197–3215 (2004).
46. Muhlemann, J. K. et al. Developmental Changes in the Metabolic Network of Snapdragon Flowers. PLoS One 7 (2012). doi: 10.1371/journal.pone.0040381
47. Larkin, M. A. et al. Clustal W and clustal X version 2.0. Bioinformatics 23, 2947–8 (2007).
48. Perrière, G. & Gouy, M. WWW-Query: An on-line retrieval system for biological sequence banks. Bioinformatics 11, 364–369 (1996).
49. Gota, S. et al. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. 36, 3420–3435 (2008).
50. Mallona, L., Weiss, J. & Egea-Cortines, M. pcrEfficiency: A web tool for PCR amplification efficiency prediction. BMC Bioinformatics 12, 404 (2011).
51. Pfaffl, M. W., Horgan, G. W. & Dempfle, L. Relative expression software tool (REST(C)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucl. Acids Res. 30, e36– (2002).

Acknowledgements
This work was funded by BFU-2010-15843 and BFU-45148-R. Part of the material used belongs to the Proyecto Vitalis Campus Mare Nostrum “Espacio Mediterráneo de Investigación en Red en Alimentos y Salud” - CEI10-2-0002.

Author Contributions
J.W. and M.E.C. conceived and designed the experiments, R.A., T.T., J.W. and M.E.C. performed experiments, J.W. and M.E.C. analysed data and produced figures and tables. M.E.C. wrote the manuscript and J.W. corrected the manuscript. All authors reviewed the manuscript.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.
How to cite this article: Weiss, J. et al. Meristem maintenance, auxin, jasmonic and abscisic acid pathways as a mechanism for phenotypic plasticity in Antirrhinum majus. Sci. Rep. 6, 19807; doi: 10.1038/srep19807 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/