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# *MGOUN1* and *MGOUN2:* two genes required for primordium initiation at the shoot apical and floral meristems in *Arabidopsis thaliana*

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## SUMMARY

**We report two new recessive mutations in *Arabidopsis*, *mgoun1* and *mgoun2* which cause a reduction in the number of leaves and floral organs, larger meristems and fasciation of the inflorescence stem. Although meristem structure is affected in the mutants, we provide evidence that its overall organisation is normal, as shown by the expression patterns of two meristem markers. Microscopical analyses suggest that both mutations affect organ primordia production. *mgo1* strongly inhibits leaf production in a weak allele of *shoot meristemless, stm-2*. In addition, *mgo1* and *2* severely reduce the ability of the *fasciata1* and *2* mutants to initiate organs, although meristem formation per se was not inhibited. The strong allele, *stm-5*, is epistatic to *mgo1*, showing that the presence of meristematic cells is essential for *MGO1* function. These results suggest a role for the *MGO* genes in primordia initiation although a more general role in meristem function can not be excluded.**

**We describe a form of fasciation which is radically different from that described for *clavata*, which is thought to have an increased size of the meristem centre. Instead of one enlarged central meristem *mgo1* and *2* show a continuous fragmentation of the shoot apex into multiple meristems, which leads to the formation of many extra branches. The phenotype of *mgo1 clv3* and *mgo2 clv3* double mutants suggest that the *MGO* and *CLV* genes are involved in different events**

**In conclusion, our results reveal two new components of the regulatory network controlling meristem function and primordia formation. A model for *MGO* genes is discussed.**

Key words: Primordia, Meristem, *Arabidopsis*, *MGOUN1*, *MGOUN2*, Fasciation

## INTRODUCTION

The number and arrangement of the aerial organs of plants are entirely determined by the activity of small populations of mitotic cells, called shoot apical meristems (SAMs). These structures therefore determine an important part of plant architecture.

Based on analyses of ultrastructure and mitotic frequencies, it has been proposed that SAMs are organised into three distinct zones (reviewed by Steeves and Sussex, 1989 and more recently by Clark, 1997; Kerstetter and Hake, 1997): a central zone (CZ), a peripheral zone (PZ) and an underlying rib zone (RZ). The CZ contains slowly dividing cells, and is thought to ensure a constant renewal of stem cells. The progeny of these stem cells is subsequently recruited into the PZ, where organ primordia initiation takes place, or into the RZ which gives rise to the pith and central tissues of the shoot axis. According to this model, the number and arrangement of organs will depend on two parameters: (i) the number of cells produced by the CZ and the PZ that are available for organ initiation, and (ii) the way these cells are subsequently partitioned into the primordia.

Recent genetic and molecular studies have confirmed this general model and allowed a first insight in the regulation of organ initiation. In particular, in *Arabidopsis*, mutants with abnormal organ arrangements and numbers have been isolated. One class of mutants is mainly perturbed in meristem maintenance and cell proliferation within the meristem. This is thought to lead indirectly to the production of aberrant numbers of organs since the number of cells produced by the CZ and which become available for primordium formation is modified in these mutants. Examples of this class are *shoot meristemless* (*stm*) (Barton and Poethig, 1993; Endrizzi et al., 1996) and *wuschel* (*wus*) (Laux et al., 1996), which are unable to maintain a functional meristem, and *clavata* (*clv 1,2,3*) and *fasciata* (*fas1,2*), which have enlarged meristems (Leyser and Furner, 1992; Clark et al., 1993, 1995). So far only the *STM* and *CLV1* genes have been isolated. *STM* belongs to the *KNOTTED* class of homeobox genes, and it is expressed in the central parts of the shoot apical and floral meristems but not in the primordia (Long et al., 1996). *CLV1* encodes a putative receptor kinase, that is expressed in the inner parts of the meristem (Clark et al., 1997). It has been proposed that the CLV1 protein could function in cell-to-cell signalling. Another class of mutants appears to affect more directly the partitioning of cells into leaf, flower or floral organ primordia. The *pin-formed* and *pinoid* mutants of *Arabidopsis*, are unable to initiate flower primordia (Bennett et al., 1995; Okada et al., 1991), and in the *perianthia* mutant, the typical *Arabidopsis* crucifer-type flower

is changed into a flower bearing 5 organs in the 3 outermost whorls (Running and Meyerowitz, 1996).

Although these results have provided a first insight in the molecular basis of organ initiation, it is obvious that we need to identify additional factors involved in this process. For this purpose, we have screened for mutants showing abnormal numbers of leaves and floral organs. Here we describe two mutants (*mgoun1* and *2*) which show such defects throughout development: abnormal leaf numbers and floral organ numbers are observed in combination with increased stem fasciation. The observed phenotypes and the genetic analyses suggest that the *MGO* genes are necessary for the proper initiation of primordia.

## MATERIALS AND METHODS

### Plant growth conditions and plant strains

Plants were grown in vitro as described by Santoni et al. (1994). Two- to three-week-old plants were transferred to the greenhouse for further analyses.

The following strains were used: *fas1-1*, *fas2-1*, *clv2-1* obtained from the ARBC, the Ohio State University, US. *stm-5* was a generous gift from Dr T. Laux, Universität Tübingen, Germany. *clv3-1* and *stm-2* were obtained from Dr S. Clark, University of Michigan, Ann Arbor, US. All strains were in the Landsberg *erecta* (L*er*) background except *fas1-1* which was in the Enkeim background.

### Mutant isolation

Mutants were identified either in vitro at the seedling stage, or later in the greenhouse, by determining the number of leaves or floral organs. *mgo1-3* was identified after EMS mutagenesis (Columbia ecotype (Col); Santoni et al., 1994). *mgo1-1*, *mgo1-2*, and *mgo2* were identified in a T-DNA insertion mutagenesis screen (Wassilewskaya ecotype (WS); Bechtold et al., 1993; Bouchez et al., 1993) The *mgo2* phenotype is linked to the T-DNA kanamycin resistance marker (all mutants tested are kanamycin resistant $n$=1 054).

### Mapping

For mapping, *mgo1-1* and *mgo2* (WS background) were crossed with the wild-types L*er* and Col. Forty-four seedlings homozygous for *mgo1-1* and *mgo2* were selected in the $F_2$ population. We determined the chromosome location of *mgo1-1* and *mgo2* mutations by searching for linked WS-type alleles of cleaved amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel, 1993).

### Double mutant analysis

In all cases, double mutants were identified, in the $F_2$ generation, as plants with a new phenotype. Frequencies of these plants are compatible with a segregation ratio of 1:16 confirming that they correspond to the double mutants. In addition, the different genetic backgrounds did not have any major effect on the phenotypes of the single mutants which could be clearly recognised in the segregating $F_2$ population.

*mgo1-1 mgo2*, *mgo1-1 fas1*, *mgo1-1 fas2*, *mgo2 fas2* and *mgo1-1 stm-2* double mutants could be clearly distinguished 2 weeks after germination. When quantifying the floral organs and primordia of *stm* and double mutants (Table 2 and 3), we used *stm* mutants (*stm-2* and *stm-5*) which segregated in $F_2$ populations, deriving from crosses with WS wild-type, as controls. The double mutants *clv3-1 mgo1-1* and *clv3-1 mgo2* were identified in the greenhouse as plants with an extremely fasciated stem. Since they were fertile, further analysis was performed on the progeny of these plants. No *mgo2 fas1* and *mgo2 stm* double mutants were found due to the tight linkage of these loci: *stm*, *mgo2* and *fas1* map respectively at position 75, 82 and 88 of the classical genetic map (see Results and http://mutant.lse.okstate.edu/genepage/classical_map.html).

### Histological analysis and GUS staining

Histological sections were prepared as described by Traas et al. (1995) except that the sections were stained in toluidine blue (0.01% in water). No in situ data are available for the expression pattern of the *KNAT2* gene but experiments using a *KNAT2* promoter GUS fusion suggest that this gene is likely to be a L3 marker (Dockx, 1995). GUS was detected using standard procedures. Sections (unstained) were made as described above and viewed in a Nikon FXA microscope using dark-field illumination.

### In situ hybridisation

Plants were fixed in 4% formaldehyde (fresh from paraformaldehyde) in PBS under vacuum for 2× 20 minutes, and left in fixative overnight. After fixation, plants were washed, dehydrated, and embedded in paraffin, essentially as described by Jackson (1991). Paraffin sections (8-10 μm thick) were cut with a disposable metal knife and attached to precoated glass slides (Fischer Scientific, US). Sense and antisense probes of a full length cDNA of *STM* (a generous gift from Dr K. Barton, University of Wisconsin-Madison, US). were synthesised using digoxigenin (DIG-UTP; Boehringer Mannheim) according to the manufacturer's instructions. In situ hybridisation was carried out as described by Jackson (1991). Immunodetection of the DIG-labelled probes was performed using an anti-DIG antibody with phosphatase as described by the manufacturer (Boehringer Mannheim). Sections were stained with calcofluor white (0.01% in water).

### Confocal microscopy and scanning electron microscopy

Plants were fixed, and then stained with propidium iodide to visualise DNA, as described by Clark et al. (1995). Optical sections were made using a Leica TCSNT confocal microscope. Meristem structure was studied using low-temperature scanning electron microscopy as described by Traas et al. (1995).

## RESULTS

### Mutant isolation and genetic characterisation

In our screen we identified four mutants with a similar phenotype. While these four mutants are all the same size as the wild type, at the adult stage they all produce fewer leaves and have enlarged and flattened (fasciated) stems (Fig. 1A-I). The mutants have flowers with abnormal numbers of organs that are of variable sizes (Fig. 1J-L).

Genetic tests showed that we have identified four recessive mutations falling into two complementation groups : *mgoun1* (3 alleles) and *mgoun2* (1 allele). Mgoun is a ridge-shaped mountain in the Atlas range which reminded us of the fasciated meristem of these mutants (see later). The three *mgo1* alleles are very similar and most of the results presented here were obtained with *mgo1-1*.

*mgo1* was mapped to the bottom of chromosome 5: 11.8 cM distal to LFY3, and 6.8 cM and 19.2 cM proximal to g2368 and m555, respectively. *mgo2* was mapped to chromosome 1 between GAPB (16.4 cM away) and ADH (22.3 cM away), which corresponds to position 82 on the genetic map. *mgo2* shows complementation with *clv2*, *stm* and *fas1* mutants.

### Organ production in *mgo1* and *mgo2* mutants

#### Production of leaves and floral organs is reduced in *mgo1-1* and *mgo2*

In wild type, the first two opposite leaves emerge 5 days after germination under our in vitro conditions and 5 to 6 leaves will develop before bolting (Table 1). In *mgo1-1* and *mgo2*, the first leaves emerge 2 days later than in the wild type and the number of rosette leaves is variable, with a mean value of about 2

(Table 1). Mutant leaves are lanceolate or asymmetrical. In *mgo1-1*, one of the two first leaves is replaced by a finger-like structure in 23% of the plantlets (Fig. 1G-I). Root growth is unaffected in *mgo1-1*, whereas *mgo2* has a shorter root than the wild type. The position of the cotyledons is abnormal in 45% of *mgo1-1* plantlets.

The number of floral organs is constant in the wild-type: all flowers have 4 sepals, 4 petals, 2 carpels. The average stamen number is 5.2±0.1 due to the absence in some flowers of 1 or 2 of the expected 6 stamens (Table 2 and Fig. 1J).

In *mgo1-1* and *mgo2*, the number of floral organs in each whorl is highly variable (Table 2 and Fig. 1K, L). For instance, 3-6 sepals are found in *mgo1-1*, and 1-7 stamens are found in *mgo2* flowers. Although the average number of sepals is close to WT (4), the average number of petals and stamens is reduced in both mutants (Table 2). There are slightly more carpels in *mgo1-1* and *mgo2*. The average total number of floral organs is reduced in both mutants compared to the wild type (Table 2). Both mutants are fertile, but *mgo1-1* shows reduced fertility.

In addition, organ width is variable, mainly in the first whorl, although overall organ shape is normal (Fig. 1K-L). Filamentous structures in the second or third whorl are visible in some flowers.

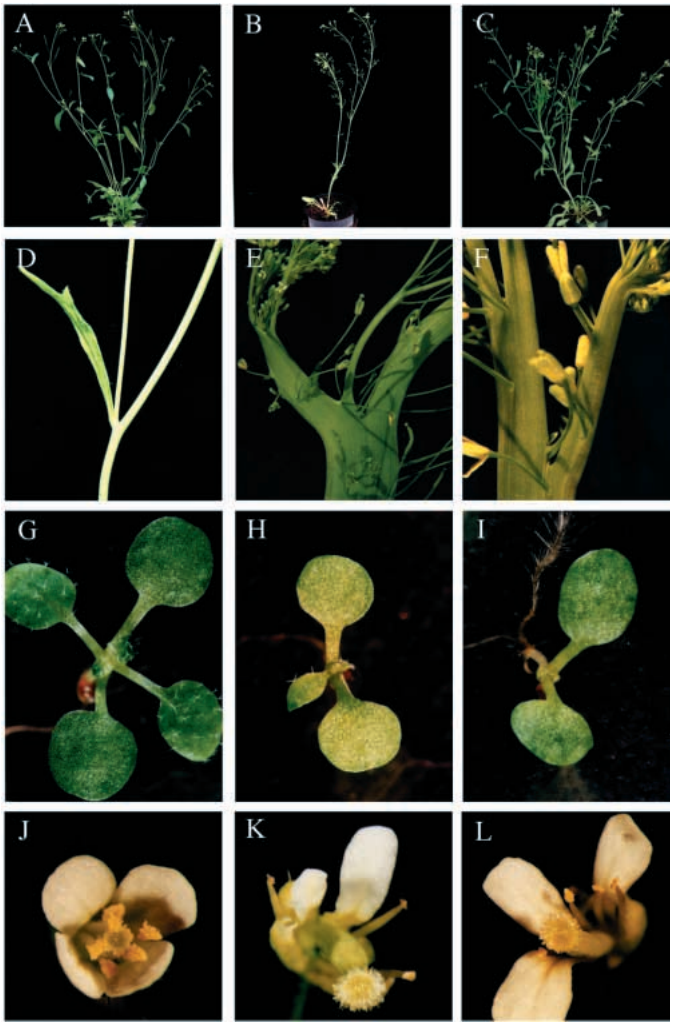


**Fig. 1.** Morphology of wild- type, *mgo1-1* and *mgo2* plants. (A) Wild-type adult plant. Final size of *mgo1-1* (B) and *mgo2* (C) plants is unaffected. (D) Branching of wild-type stem: a caulin leaf subtends an axillary branch. In *mgo* mutants stems are flat and wider than wild-type stems (fasciated). (E) Extreme fasciation of *mgo1-1*. (F) a *mgo2* stem. Note that stem branching in mutants is different from wild type occurring without the presence of a cauline leaf. 12-day-old wild-type (G), *mgo1-1* (H) and *mgo2* (I) seedlings. Leaf production is reduced in both *mgo* mutants. Wild-type flowers (J) have a regular and constant structure: four petals and six stamens are visible. In contrast *mgo* flowers have reduced and variable floral organ numbers: the *mgo1-1* flower shown in K bears 2 petals (note the variable size of the two petals); the *mgo2* flower has 3 petals and 4 stamens (L).

## Organ initiation in flower meristems

We subsequently investigated the stage at which organ development is perturbed in both mutants. We chose to study sepal initiation as an indicator of organ development because these organs are easily accessible for microscopy. In wild-type plants, the abaxial sepal primordium arises first, followed by the adaxial, and finally, the two lateral primordia (Fig. 2A,B; see also Smyth et al., 1990).

Both *mgo1-1* and *mgo2* show similar defects in sepal formation (Fig. 2C-E). Floral meristem partitioning into whorls is not perturbed by these mutations (Fig. 2C,D). Cells are allocated to the outer whorl where sepal initiation will take place, but the further partitioning into sepals is altered. Primordium size and position are irregular and abnormal in both mutants. Some sepal primordia appear to be partially fused (see arrows Fig. 2D). The number of sepal primordia (arrow Fig. 2E) is variable. In 12 developing floral buds of *mgo2*, we observed from 3 to 7 sepal primordia. We frequently observed that some sectors of the first whorl failed to initiate sepal primordia even at a stage where the petal and stamen primordia were already visible. This suggests that in some sectors the partitioning into sepals does not take place at all. Together these observations suggest that the *MGO* genes are required for the proper initiation of floral organs.

## Organ initiation in double mutants.

In order to obtain more insight into the role of the *MGO* genes, we analysed the genetic interactions between *mgo1-1*, *mgo2* and other mutants which have reduced organ numbers, namely *fas1*, *fas2* and *stm*.

The double mutant *mgo1-1 mgo2* has the same characteristics as the mutants. However both the effects on organ numbers (Table 1 and 2) and stem fasciation (see below) are greatly enhanced in the double mutant.

The *STM* gene is necessary for shoot meristem formation during embryogenesis and meristem maintenance during postembryonic development (Barton and Poethig, 1993; Clark et al., 1996; Endrizzi et al., 1996). In *stm-2*, a weak allele, meristematic-like cells are formed during embryogenesis, and are entirely incorporated into the first primordia (Clark et al., 1996; Endrizzi et al., 1996 and Table 3). The mutant is therefore able to initiate primordia, but incapable of maintaining a SAM. *stm-5* has a stronger phenotype as most seedlings do not form any meristematic cells at all, and only a few seedlings form primordia (Endrizzi et al., 1996 and Table 3).

When *mgo1-1* is introduced into a *stm-2* background, most of the double mutant embryos do not form any leaf primordia or clusters of meristematic-like cells (Table 3). About half of the double mutants do not develop a visible shoot after 19 days (Table 3 and Fig. 3G,K). Very few double mutants develop

**Table 1. Number of leaves produced during the rosette stage**

| Genotype | *n* | Range | Mean ± s.e. |
|---|---|---|---|
| Wild type (WS) | 59 | 4-6 | 5.2±0.1 |
| *mgo1-1* | 30 | 1-4 | 2.2±0.2 |
| *mgo2* | 66 | 0-5 | 2.0±0.1 |
| *mgo1-1 mgo2* | 32 | 0-5 | 0.7±0.2 |
| *clv3-1* | 23 | 6-8 | 7.3±0.2 |
| *mgo1-1 clv3-1* | 22 | 2-6 | 4.1±0.3 |
| *mgo2 clv3-1* | 25 | 1-4 | 2.2±0.2 |

Range and average numbers of leaves produced in vitro during vegetative stage.
*n*, numbers of plants analyzed.

flowers, but when present they show reduced floral organ numbers when compared to *mgo1-1* or *stm-2* mutants (Table 2). Together, these data indicate that *mgo1* reduces the capacity of the plant to initiate organs of *stm-2* throughout development.

In a 19-day-old $F_2$ population of a cross between the heterozygotes of the *mgo1-1* and *stm-5* mutations no new phenotype was visible and we found 219 wild type, 69 *mgo1-1* seedlings and 93 *stm-5*-like seedlings (segregation 9/3/4, $\chi^2$=0.24). This indicates that *stm-5* is epistatic to *mgo1*. No *mgo2 stm* double mutants were found (see Materials and Methods).

*fasciata 1* and *2* mutants described by Leyser and Furner, (1992) have an enlarged shoot apical meristem, disturbed leaf production (Fig. 3E,F) and fewer floral organs. After 19 days, SAMs of *fas* (*1* or *2*) *mgo* (*1* or *2*) double mutants have formed a round dome (Fig. 3H-J,L-M). No leaves are produced by *mgo2 fas2* double mutants (Fig. 3J,M), whereas finger-like structures develop on the meristem flank of *mgo1-1 fas2* and *mgo1-1 fas1* double mutants (Fig. 3H,I,L). In addition, *mgo1-1 fas1* double mutants have a short root and narrow cotyledons (Fig. 3H). No *mgo2 fas1* double mutants were found (see Material and Methods).

In conclusion, leaf production is extremely inhibited when *fas1* or *fas2* are combined with *mgo*. This suggest that *MGO* genes are absolutely required for leaf production in a *fas* background.

## The SAM is perturbed in *mgo1* and *mgo2*

### Histological analyses of wild-type and mutant meristems

The morphology of the apical meristem was analysed on longitudinal sections of wild-type and mutant apices. In the wild-type mature embryo, the SAM is made up of an average of 25-35 small dense cells organised into 3-4 layers (Fig. 4A); the two outermost cell layers (L1 and L2) are well defined. At 8 days, the wild-type SAM is flat and 4-5 cells deep, and by 12 days, it has formed a convex structure (Fig. 4D,G). Cells in the two outer layers divide preferentially in the anticlinal plane; periclinal divisions occur at the periphery of the second layer. Cells in the underlying L3 layer do not show any preferential division plane.

The *mgo1-1* mutation affects meristem organisation in the embryo: cell shape is irregular and the layered structure is less evident (Fig. 4B). SAMs of *mgo2* embryos are normal (Fig. 4C) and the first difference from the wild type is visible after 6 days (data not shown). In both *mgo* mutants, the organisations of 8- and 12-day-old SAMs are perturbed in the same way (Fig. 4E,F,H,I). Cell layers are not clearly visible and cell division planes are not well oriented. The meristems enlarge progressively between 8 and 12 days until they reach a diameter which is 2 to 3 times wider than in the wild type. On the flank of *mgo2* meristems, cells become bigger and vacuoles form (Fig. 4I).

In conclusion, *mgo1-1* and *mgo2* affect meristem structure and lead to disorganisation of the cell division plane alignment and to progressive overgrowth. *mgo1-1* affects the SAM during embryogenesis, whereas *mgo2* only has a role in postembryonic SAM development.

### *STM* and p*KNAT2::uidA* expression patterns in the SAM

Since the organisation of *mgo1-1* and *mgo2* meristems is perturbed, we looked at the identity of cells within the meristem by analysing the expression patterns of meristem markers. In this way, we tested two members of the *KNOTTED* gene family, *STM* and *KNAT2*.

The activity of the *KNAT2* promoter controlling the *uidA* gene was studied in 10-day-old plants. In the wild type, GUS expression is restricted to the SAM, the base of developing young leaves and the vascular strands near the apex (Fig. 5A). Within the SAM, the GUS signal is only visible in the lower part corresponding to the L3 layer and is more concentrated to the periphery. The expression pattern of p*KNAT2::uidA* in mutant seedlings is similar to the wild-type pattern (Fig. 5B,C).

The *STM* expression pattern was studied by in situ hybridisation on sections of 10-day-old seedlings. As described by Long et al. (1996), *STM* is expressed in the wild type in all shoot apical and floral meristems but it is absent from the incipient primordia (Fig. 6A,D). No major changes in the overall pattern of *STM* expression were found in mutant seedlings; *STM* expression is visible in the central part of the SAM and absent from domains at the periphery of the meristem which could be the developing primordia (Fig. 6B,C,E,F).

**Table 2. Floral organ numbers**

| | | Sepals | | Petals | | Stamens | | Carpels | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | *n* | Range | Mean±s.e. | Range | Mean±s.e. | Range | Mean±s.e. | Range | Mean±s.e. | Range | Mean±s.e. |
| Wild type (WS) | 192 | 4 | 4.00 | 4 | 4.00 | 4-6 | 5.2±0.1 | 2 | 2.00 | 14-16 | 15.2±0.1 |
| *mgo1-1* | 60 | 3-6 | 4.3±0.1 | 0-4 | 2.4±0.2 | 1-6 | 3.5±0.2 | 2-3 | 2.3±0.1 | 8-16 | 12.5±0.3 |
| *mgo2* | 109 | 2-7 | 4.0±0.1 | 1-7 | 3.1±0.1 | 2-8 | 4.7±0.1 | 1-4 | 2.1±0.1 | 9-21 | 13.9±0.1 |
| *mgo1-1 mgo2* | 20 | 4-6 | 4.7±0.2 | 0-3 | 1.0±0.2 | 1-4 | 2.1±0.3 | 1-3 | 2.3±0.2 | 8-12 | 10.1±0.3 |
| *stm2* | 23 | 2-5 | 3.8±0.2 | 0-3 | 2.0±0.2 | 2-5 | 3.8±0.2 | 0 | 0 | 6-12 | 9.6±0.4 |
| *mgo1-1 stm2* | 32 | 2-5 | 3.4±0.2 | 0-2 | 0.1±0.1 | 0-3 | 0.6±0.2 | 0 | 0 | 2-8 | 4.1±0.3 |
| *clv3-1* | 29 | 4-6 | 4.5±0.2 | 4-6 | 4.4±0.2 | 6-8 | 6.8±0.2 | 3-6 | 4.5±0.2 | 17-24 | 20.2±0.4 |
| *mgo1-1 clv3-1* | 19 | 4-6 | 4.7±0.2 | 1-6 | 2.9±0.4 | 2-6 | 4.3±0.3 | 3-5 | 3.9±0.2 | 12-20 | 15.8±0.5 |
| *mgo2 clv3-1* | 37 | 3-7 | 4.5±0.2 | 1-5 | 3.4±0.2 | 3-8 | 6.0±0.2 | 2-8 | 4.3±0.3 | 13-23 | 18.2±0.4 |

*n*, numbers of flowers analyzed.

**Table 3. Primordia formation in mutants and double mutants**

| | 6 days after germination | | | | | 12 days after germination | | | | | 19 days after germination | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | Shoots | Pr | MLC | – | *n* | Shoots | Pr | MLC | – | *n* | Shoots | Pr | MLC | – |
| *stm-2* | 15 | 0% | 100% | 0% | 0% | 22 | 100% | 0% | 0% | 0% | 38 | 100% | 0% | 0% | 0% |
| *mgo1-1* | 13 | 0% | 100% | 0% | 0% | 21 | 100% | 0% | 0% | 0% | 28 | 100% | 0% | 0% | 0% |
| *mgo1-1 stm2* | 22 | 0% | 9% | 9% | 82% | 32 | 22% | 50% | 0% | 28% | 33 | 55% | 6% | 9% | 30% |
| *stm-5* | 19 | 0% | 0% | 11% | 89% | 15 | 0% | 0% | 7% | 93% | 34 | 3% | 6% | 12% | 79% |

Plants were scored after 6, 12 or 19 days growth for shoot development. Plants without shoots were observed under the confocal microscope and scored for leaf primordia (Pr) and clusters of meristematic-like cells (MLC). The remaining plants had no visible primordia or meristematic cells (–).
*n*, numbers of plants analyzed.

However, the domain expressing *STM* is more extensive than in the wild-type, confirming that the SAM in *mgo* is larger. When the mutant apex is composed of several domes, *STM* is expressed in several distinct domains (Fig. 6G). In some apices (Fig. 6H) *STM* is expressed in two distinct domains although the apex still forms a single morphological unit.

These results indicate that, although the SAM in the mutants is perturbed, the general organisation of the SAM into functional domains is maintained.

### *mgo1* and *mgo2* stems are fasciated and show increased branching

After bolting, wild-type plants form an inflorescence stem which bears cauline leaves and lateral inflorescence shoots. Flowers are produced along the stem in a regular spiral phylotaxy (Fig. 1A). The inflorescence stems of *mgo* mutants are fasciated: they are wider and flatter than in the wild-type (Fig. 1B,C,E,F). Some of the young emerging stems have a wild-type morphology, while in others, fasciation occurs immediately after bolting. The stem enlarges progressively and then bifurcates, restoring a wild-type structure which will enlarge again (Fig. 1F). This process, which is repeated several times, leads to a bushy phenotype for older plants. Note that this process is different from wild-type branching where an axillary meristem subtended by a cauline leaf develops and forms an accessory branch (Fig. 1D). In both *mgo* mutants, flower position along the stem is irregular, in that more than one flower stalk may start from the same stem point.

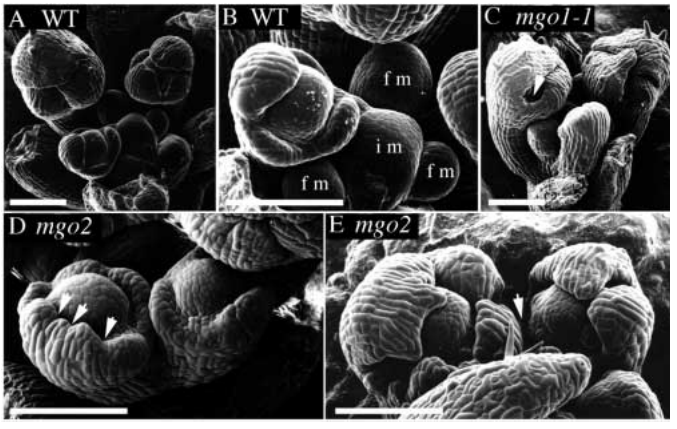


**Fig. 2.** Flower development in wild-type plants and *mgo* mutants. In wild-type (A,B) flowers are initiated by the inflorescence meristem (im) following a regular pattern. The floral meristem (f m) initiates four sepal primordia. (C) *mgo1-1* apex showing floral meristems at different stages. Note the abnormal spacing between the primordia (arrow) and a flower with 3 sepals (left). (D) meristem partitioning into whorls is not perturbed but further partitioning into primordia is perturbed. Note the irregular primordia number, position and width. Partial primordia fusion may occur (arrow). (E) More advanced flowers with stamen and petal primordia visible, but lacking sepals in a flower sector (arrow). Bars, 100 μm.

Using scanning electron microscopy, we observed that *mgo* apices do not appear as one continuous structure but as juxtapositions of several meristematic domes (Fig. 7A). Some of these domes are clearly separated, which could represent early stages of bifurcation. In *mgo1-1 mgo2* double mutants, the fasciation of the meristem is enhanced (Fig. 7B). The domes do not separate when they become morphologically distinct, as this would not lead to the observed stem fasciation but to an extreme increase in the number of stems, which is not the phenotype of the mutants.

### *clv* and *mgo* define two types of fasciation

It has been proposed that in *clavata1* and *3* meristem overgrowth and fasciation is due to the increased size of the CZ (Clark et al., 1995, 1996). The fasciated meristem of *clavata*, however, is different from that observed in *mgo*, as it forms a continuous structure with no individual subunits visible (Fig. 7C). In addition, organ production is increased in *clv*, whereas it is reduced in *mgo*.

In order to study the interaction between *mgo* and *clv*, *mgo1-1 clv3-1* and *mgo2 clv3-1* double mutants were constructed. Up to 2 weeks after germination, double mutants are almost identical to single *mgo* mutants. Later on, double mutants show additive defects. Organ numbers are intermediary between those of single mutants (Table 1 and 2). In double mutant flowers, organ width and number are variable just as in the *mgo* mutants and, a fifth inner carpel whorl develops as in *clv3-1* flowers (data not shown). Stems are extremely fasciated and can be up to 1 cm wide. SAMs are fasciated and, as in *mgo*, composed of juxtaposed domes (Fig. 7D,E). These results indicate that there is no epistatic relationship between *MGO1*, *MGO2* and *CLV3* and that the *MGO* and *CLV3* genes are still active in a *clv3* and *mgo* mutant background respectively.

## DISCUSSION

### MGO1 and MGO2 are required for specifying the correct number of organs throughout development by promoting primordia formation

In vitro, *mgo1* and *mgo2* meristems produce just two leaves before bolting, in contrast to the five leaves which are initiated by wild-type plants. *mgo1* and *mgo2* flowers show an overall reduction in the number of organs, with variable numbers in all

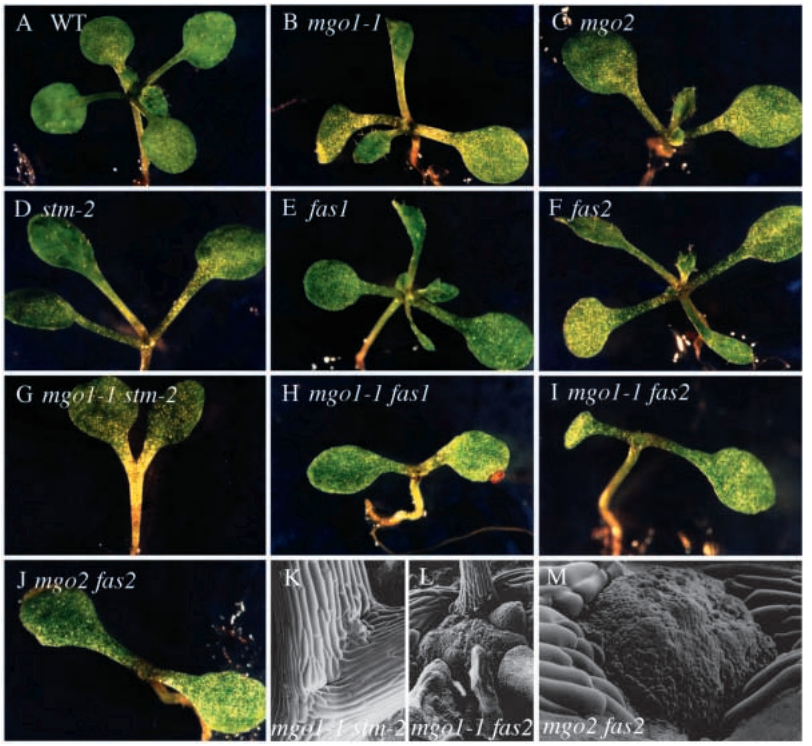


**Fig. 3.** Phenotype of 19-day-old mutants and double mutants. (A) Wild type. (B) *mgo1-1* and (C) *mgo2* have reduced leaf numbers. In *stm-2* (D) a single leaf is visible. *fas1* (E) and *fas2* (F) have abnormal leaves. No leaf is visible in *mgo1-1 stm-2* double mutants (G,K). *mgo1-1 fas1* (H), *mgo1-1 fas2* (I) and *mgo2 fas2* (J) show no leaf development and have enlarged meristems. *mgo1-1 fas2* have finger-like structures (L) whereas *mgo2 fas2* (M) do not show such structures.

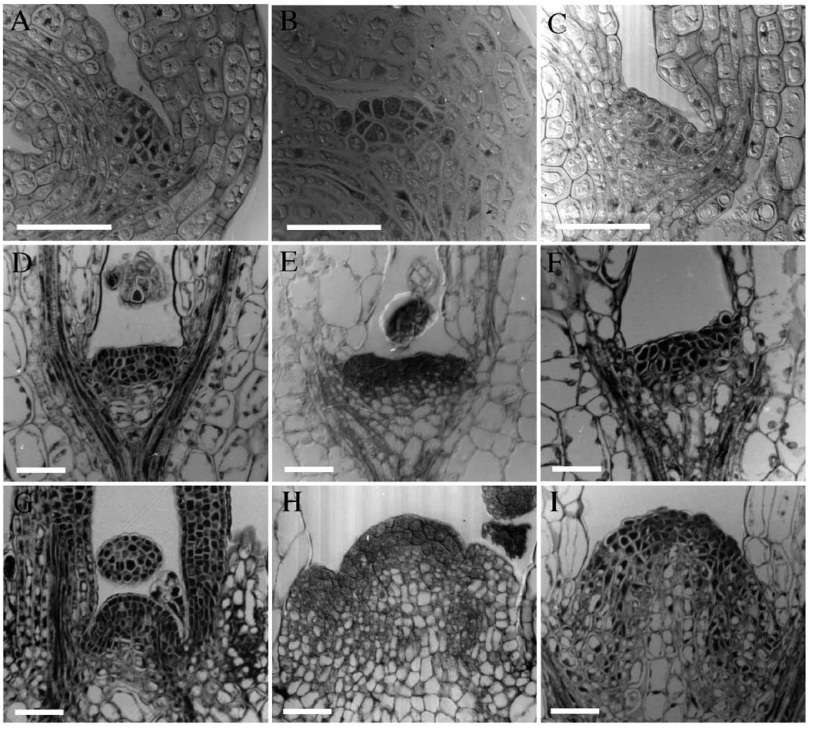


**Fig. 4.** Meristem structure in wild-type and *mgo* mutants. SAMs of mature embryos (A-C), 8-day-old (D-F) and 12-day-old (G-I) seedlings. (A,D,G) Wild-type meristems show a regular structure. In constrast, *mgo1-1* meristems (B,E,H) are disorganised from the embryo stage onwards and *mgo2* meristems (C,F,I) show abnormal organisation from 8 days after germination onwards. Meristem enlargement occurs in both mutants between 8 and 12 days. Bars, 50 μm.

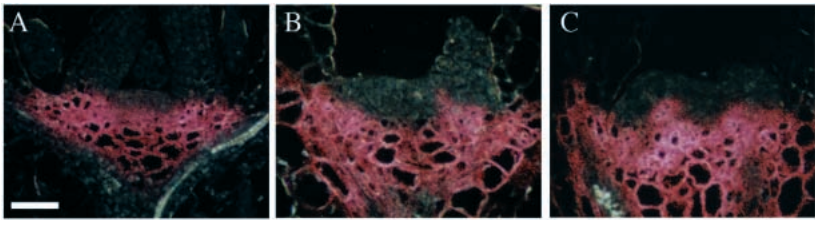


**Fig. 5.** pKNAT2::GUS expression in 10-day-old wild type and *mgo* mutants. In wild-type plants (A), only the basal part of the apex is stained. In *mgo1* (B) and *mgo2* (C) there is a similar staining pattern. Bar, for all panels, 50 μm.

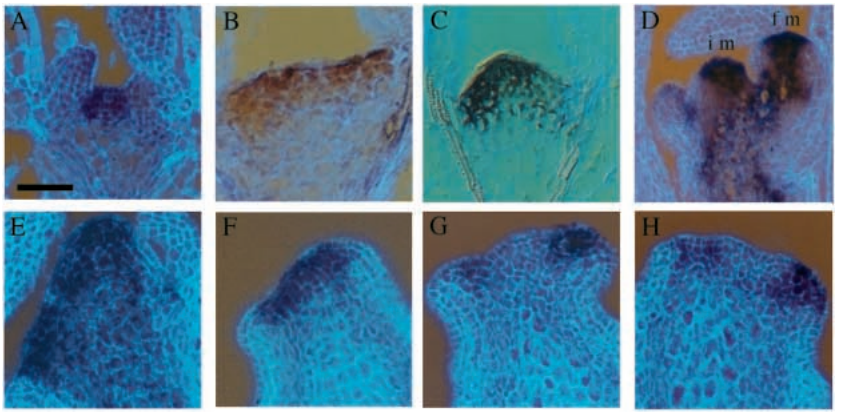


**Fig. 6.** *STM* expression in wild-type and *mgo* mutants. In situ hybridisation was done on 10-day-old (A-C) or 3-week-old (D-H) plants. In wild type (A) *STM* expression is restricted to the meristem and absent from developing primordia. In *mgo1-1* (B) and *mgo2* (C) the domain expressing *STM* is clearly enlarged, although the general expression pattern is unchanged. In 3-week-old wild type, *STM* is expressed in the shoot and floral meristems and absent from primordia (D). The domain expressing *STM* is wider in *mgo1-1* (E) and *mgo2* (F). (G) *STM* is expressed in the two domes of a *mgo2* apex. (H) a *mgo2* apex forming a unique dome but with two separated stained domains. i m, inflorescence, f m floral meristem. Bar, for all panels, 50 μm.

four whorls. These results indicate that *MGO1* and *MGO2* are necessary for determining the number of organs produced during vegetative and floral development. This phenotype may be due to defects in the SAM at two levels: the CZ and the PZ. Abnormal functioning of the central parts of the meristem could result in decreased or increased cell numbers flowing to the PZ, which in turn would modify the number of cells available for primordium initiation and thus influence organ arrangements. However, as mentioned earlier, the mutants may also be perturbed in the process of primordium initiation at the meristem periphery.

Several observations indicate that the *MGO* genes are involved in the process of organ primordia initiation itself. First, the vegetative meristem produces fewer leaves, despite the fact that there are enough cells. In fact, the *mgo* meristem is larger than the wild-type meristem. Secondly, SEM observations of developing flowers clearly show that the initiation of sepals at the periphery of the meristem is perturbed. Cells are apparently allocated to the outer whorl, but then fail to be partitioned correctly. Floral organ number is abnormal in all four whorls showing that the *MGO* genes are necessary throughout flower development whenever organ initiation takes place. *mgo* mutants are therefore radically different from mutants such as *clv*, *stm* or *wus*, that are supposedly perturbed in the CZ, and where the number of organs is positively correlated with the number of cells produced by the meristem. It was proposed that in *clavata*, the enlarged meristem is due to an increase in size of the CZ (Clark et al., 1993, 1995, 1996). In *mgo* mutants, meristem overgrowth could be a secondary effect of the perturbed primordia formation; cells, instead of being 'used' by the primordia, remain in the meristem.

Genetic analysis also supports the hypothesis that *MGO1* and *MGO2* are involved in primordium production. Like *mgo*, the *fas1* and *fas2* mutants show variable organ numbers. Interestingly, double mutants between *fasciata* and *mgoun* develop vegetative meristems that are almost incapable of forming leaves. Since we do not know at this stage whether all the alleles are null alleles, it is impossible to say whether *fas* and *mgo* act in the same pathway, or in parallel pathways, which affect the same process. The results do show, however, that *mgo* genes become an absolute requirement for primordium initiation in the absence of *fas*, and vice versa. Analysis of *stm mgo1* double mutants could be interpreted in a similar way. *STM* is thought to maintain undifferentiated source cells in the meristem and not to be required for primordium initiation per se (Long et al., 1996). It is this ability to initiate primordia which is severely reduced when *mgo1* is introduced into a *stm* background.

Although the overall organisation of the meristem into distinct zones, as shown by p*KNAT2::uidA* and *STM* expression patterns, is apparently not perturbed, both mutations affect the fine structure of the vegetative SAM. The L1, L2 and L3 layers are less well-defined and division planes are abnormal. It could be that this is an indirect effect of the aberrant partitioning in the periphery, but we cannot rule out that *MGO1* and *MGO2* are also directly involved in setting up the architecture of the entire meristem. Indeed, some of the genetic evidence could also be interpreted in this way. For instance, if both *fas* and *mgo* affect the structure of the meristem itself, it is conceivable, that the accumulated effect of the mutations could lead to such a perturbation of meristem function, that primordium initiation would be completely abolished.

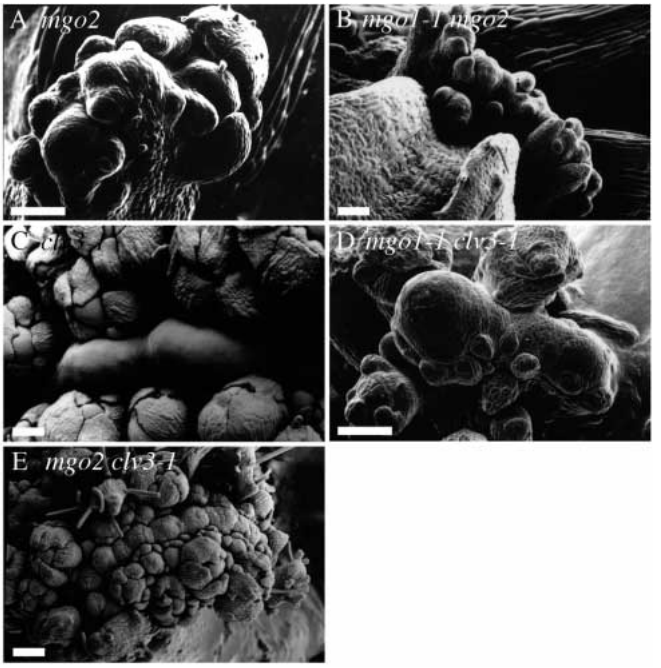


**Fig. 7.** Fasciated shoot meristems in mutants and double mutants. Apices of (A) *mgo2* and (B) *mgo1-1 mgo2* double mutants are composed of several juxtapositioned domes. No subunits are visible in a *clv3-1* fasciated meristem (C). In *mgo1-1 clv3-1* (D) and *mgo2 clv3-1* (E) double mutants several domes form the fasciated shoot meristem. Each dome is larger than in *mgo* single mutants. Bar, 100 μm.

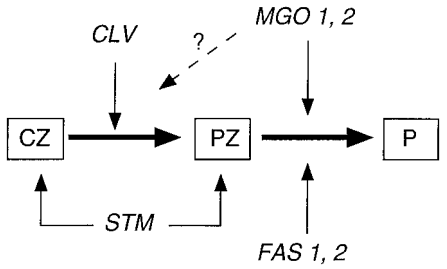


**Fig. 8.** Schematic representation of the respective roles of MGO, CLV, FAS and STM based on genetic and phenotypical evidence. In the meristem, successive generations of cells transit from the CZ to the PZ before being partitioned (P) into primordia.

### *mgo* and *clv* define two different types of fasciation

One of the most obvious characteristics of the *mgo* mutants is their fasciation. Meristem fasciation is not specific to the inflorescence meristem as it can also occur during vegetative development, when mutants are grown in SD (data not shown). As in *clavata*, this phenotype is affected by growth conditions, but usually the stems are 3-4 times as wide as in the wild type. Clear differences from *clavata* become apparent when the meristems are viewed in the electron microscope. As described earlier (Clark et al., 1993, 1995), *clavata* mutants show one central meristem which can be up to 1 cm wide. Fasciated apices in *mgo*, however, have a large dome, which consists of a variable number of juxtapositioned meristems. This is particularly clear in the double mutant *mgo1-1 mgo2* shown in Fig. 8B. The observed fasciation of *mgo* can be explained in several ways: it could be the result of the continuous fragmentation of the existing SAM, or, the ectopic formation of new meristems. We have repeatedly observed mutant apices forming a unique dome expressing *STM* in two separate cell clusters. This observation suggests that fragmentation of the *STM*-expressing domain is an early event of meristem bifurcation. We never observed the small domains of *STM* expression that would be expected in the case of early steps of ectopic meristem formation. Although we cannot rule out either of these hypotheses, these observations suggest that fragmentation occurs within the meristem**.** Mutants homozygous for both *mgo* and *clv3* still have an apex consisting of several juxtapositioned domes, each dome being significantly larger than in the *mgo* single mutants. This indicates that the full activity of *MGO1* and *MGO2* is not required to maintain the integrity of the large central meristem of the *clv* mutant.

## CONCLUSION

The two complementation groups described here have comparable effects on reducing organ formation and altering meristem structure, while the *mgo1-1 mgo2* double mutant has an enhanced phenotype. Both mutations have the same phenotypic effect in *clv* and *fas* backgrounds. These results suggest that these two genes have comparable roles during postembryonic plant development. *MGO1* has an additional role during embryonic development. We propose that these genes are necessary for correct primordia development in all meristems. They may stimulate cells to leave the meristem and may also be necessary for the partitioning into primordia (Fig. 8).

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