Combinatorial control of meristem identity in maize inflorescences

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SUMMARY

The architecture of maize inflorescences, the male tassel and the female ear, is defined by a series of iterative branching events. The inflorescence meristem initiates spikelet pair meristems. These in turn initiate spikelet meristems which finally produce the floret meristems. After initiating one meristem, the spikelet pair and spikelet meristem convert into spikelet and floret meristems, respectively. The phenotype of reversed germ orientation1 (rgo1) mutants is the production of an increased number of floret meristems by each spikelet meristem. The visible phenotypes include increased numbers of flowers in tassel and ear spikelets, disrupted rowing in the ear, fused kernels, and kernels with embryos facing the base of the ear, the opposite orientation observed in wild-type ears. rgo1 behaves as single recessive mutant. indeterminate spikelet1 (ids1) is an unlinked recessive mutant that has a similar phenotype to rgo1. Plants heterozygous for both rgo1 and ids1 exhibit nonallelic noncomplementation; these mutants fail to complement each other. Plants homozygous for both mutations have more severe phenotypes than either of the single mutants; the progression of meristem identities is retarded and sometimes even reversed. In addition, in rgo1; ids1 double mutants extra branching is observed in spikelet pair meristems, a meristem that is not affected by mutants of either gene individually. These data suggest a model for control of meristem identity and determinacy in which the progress through meristem identities is mediated by a dosage-sensitive pathway. This pathway is combinatorially controlled by at least two genes that have overlapping functions.

Key words: rgo, Maize inflorescence development, Nonallelic noncomplementation, Spikelet, Meristem identity, Gene dosage, ids1

INTRODUCTION

Plant growth consists of iterative developmental events that determine the morphology of the mature organism. Meristems, self-organizing pools of undifferentiated cells, give rise to most plant organs including other meristems. The above ground portion of the plant is elaborated from the shoot apical meristem (SAM), which produces leaves, stems and axillary meristems (together called a phytomer) in a repetitive manner (Galinat, 1959; Steeves and Sussex, 1989). If meristem maintenance is disrupted, production of phytomers ceases and plant development arrests (Long et al., 1996; Vollbrecht et al., 2000). Therefore, proper plant development depends on normal meristematic activity. The reproductive structure of plants, the inflorescence, is produced by an inflorescence meristem (IM), which is derived from the SAM and/or axillary meristems. Inflorescences exhibit a wide range of meristematic behaviors in different plants, leading to the varying reproductive morphologies seen in the plant kingdom. Secondary and higher order meristems can be initiated from the primary IM. Each order of meristems represents an order of branching, thus meristem function and inflorescence shape are directly related (Kellogg, 2000). In the grasses, inflorescences range from simple and unbranched spikes to highly branched panicles. This variability can be observed within single species (Chapman and Peat, 1992). During the domestication of corn, Zea mays, from teosinte, there has been an increase in inflorescence complexity that reflects human selection for agronomically important traits (Doebley and Stec, 1991; Galinat, 1996; Iltis, 1988).

Maize is a monoecious species that has two types of inflorescences, the male tassel at the apex of the plant, and female ears in the axils of leaves. Tassel and ear development are similar until after flowers are initiated, at which point organ abortion in the tassel and the ear produce separate unisexual inflorescences (Dellaporta and Calderon-Urrea, 1994; Irish, 1996). At the top of the plant the SAM elongates and converts into an IM, which gives rise to the tassel. At approximately the same time, the axillary meristems initiate a prophyll (the first leaf on a shoot) and eight to 14 husk leaves, and then convert into a lateral IM, which produces the ear. The top (youngest) ear primordium grows faster than lower primordia and often develops into a single dominant ear. The IM gives rise to 2°, 3°, and 4° meristems (Fig. 1A). Each IM produces an indeterminate number of spikelet pair meristems (SPM, 2°) in an acropetal (from the base to the apex), polystichous (multiple rows) manner. In the tassel, the first few SPMs become branch meristems and produce long tassel branches that initiate SPMs in a distichous pattern. All other SPMs initiate one spikelet meristem (SM, 3°) and then convert into SMs. Each SM then initiates a pair of bract-like glumes. Subsequently, each SM initiates the lower floret meristem (FM, 4°) and converts into...
Fig. 1. A model of inflorescence development. (A) The inflorescence meristem (IM, 1°) initiates (black arrows) spikelet pair meristems (SPM, 2°). The SPM then initiates a spikelet meristem (SM, 3°) on its flank, and then converts (blue arrows) into a SM. In a reiterative manner, each SM initiates one floret meristem (FM, 4°) and converts into a FM. Adapted from Irish (Irish, 1997b). (B) In the ear, wild-type spikelets (top) initiate the lower floret (1) which aborts (red) and then the spikelet converts into the upper floret (2) which develops into a functional flower (green). In rgo1 plants (bottom), the spikelet produces a third flower (3). In many cases the second flower also develops (green/red gradient). Owing to the distichous pattern of floret initiation by the spikelet, any odd numbered flower (1, 3, 5, …) will face the base of the ear, while even numbered flowers (2, 4, 6, …) face the tip of the ear. Any mutation that either increases or decreases floret number may result in a rgo phenotype.

the upper FM. The FMs make the floral organs, the palea/lemma, lodicules, anthers and pistils. The pistils abort in the tassel, while the anthers and lower pistil abort in the ear (Cheng et al., 1983). This results in a single kernel developing from each ear spikelet. A large number of mutants that affect these meristem initiation and identity conversion events have been identified and make the maize inflorescence a powerful system for studying meristem function (McSteen et al., 2000; Veit et al., 1993).

Maize inflorescence mutants affect tassel and ear development in many ways. Interestingly, mutant phenotypes often differentially affect the two inflorescence types (Postlethwait and Nelson, 1964). These mutants interact with each other, often synergistically. The class II tasselseed mutants (ts4, Ts6) affect both floral organ abortion and branching. Pistil abortion in the tassel is inhibited resulting in a feminized tassel with seeds. In addition, SPMs fail to convert into SMs in ts4, and SM to FM transitions are delayed in Ts6. Both of these mutations result in extra branching in the ear (Irish, 1997a; Irish, 1997b). The ramosa mutants (ra1, ra2, ra3) have indeterminate SPMs; these make long branches that produce many SMs (Postlethwait and Nelson, 1964; Veit et al., 1993). in determinate spikelet1 (ids1) mutants delay the SM to FM conversion resulting in the production of extra florets, which is a more branched and less determinate condition (Chuck et al., 1998). indeterminate floral apex1 (ifa1) affects determinacy of all inflorescence meristems, resulting in extra SPMs, SMs and an indeterminate FM. ids1 and ifa1 interact synergistically, with SMs converting into branch meristems in the ear and into SPMs in the tassel (Laudencia-Chingcuancoc and Hake, 2002).

Perturbations of regular rowing and kernel orientation are sensitive indicators of defects in inflorescence development. Increased or decreased numbers of florets produce an easily recognizable reversed germ orientation (rgo) phenotype (Fig. 2D). Several reversed germ and fused kernel mutants have been previously described in maize. Kiesselbach describes the occurrence of reversed and fused kernels in various inbreds and hybrids, as well as documenting reports of these phenotypes dating back to 1810 (Kiesselbach, 1926). In this and subsequent reports (Jackson, 1996b; Joachim, 1956), the reversed orientation of the kernels was attributed to the development of the lower instead of the upper floret. Fused kernels were attributed to the development of both florets in a spikelet. The development of both florets occurs in Country Gentleman sweet corn and results in reversed kernels (Micu et al., 1983). Diverse patterns of floret development are found in the grasses; basal fertile florets with non-fertile apical florets are found in the pooids, while the opposite pattern occurs in the panicoids (Chapman and Peat, 1992).

The reversed germ orientation1 (rgo1) mutant was isolated by Sachan and Sarkar. They suggested that the phenotype was due to the differential growth of the integuments and the nucellus causing rotation of the kernel (Sachan and Sarkar, 1980). In this paper, we show that the rgo1 reversed kernel phenotype is actually due to the production of an extra floret by the spikelet meristem. We demonstrate that rgo1 and ids1 have overlapping functions in regulating meristem identity. The rgo1; ids1 double mutant is synergistic and affects more meristem types than either mutant alone. A dosage based model is proposed for the regulation of meristem identity conversion in the maize inflorescence.

MATERIALS AND METHODS

Plant strains and growth

A rgo1-Sarkar stock (904G) was obtained from the Maize Genetics Cooperation Stock Center (MGSC, Urbana, IL) (Jackson, 1996a). This was introgressed seven times into B73 (Pioneer Hi-Bred, Des Moines, IA). The B73 introgression was used for complementation and allelism crosses and double mutant analyses. Plants were grown in fields in Berkeley, CA and San Jose, CA during the summer. Plants were grown in greenhouses in Berkeley, CA and on Molokai, HI during winter seasons. The phenotype of rgo1 did not vary in these different growth conditions. ids1-mum1 (Chuck et al., 1998) was a gift from G. Chuck (USDA, ARS, Albany, CA). ids1-VI and ids1-Burr were gifts from J. Jackson (MGSC, Urbana, IL). Originally described as rgo2-VI and rgo2-Burr, complementation and allelism tests with both ids1-mum1 and rgo1, as well as mapping data, showed that they are alleles of ids1 (Jackson and Kaplinsky, 2000). To observe double mutants, plants used for the ids1; rgo1 complementation test were selfed. The genotype of representative double mutant individuals was confirmed molecularly using an ids1 cDNA and umc114 as RFLP probes, and umc1267 primers to detect a linked SSR. Some double mutants were constructed by crossing wx1 rgo1 lines generated during mapping (see below) to wx1 plants. Selfed progeny from this cross segregated wx1 kernels, which were enriched for rgo1. Some double mutants were constructed by crossing wx1 rgo1 lines generated during mapping (see below) to wx1 plants. Selfed progeny from this cross segregated wx1 kernels, which were enriched for rgo1. Genetic nomenclature follows the standards for maize genetics (Burr et al., 1995); unlinked genes are separated by a semicolon, linked genes by a space.

rgo1 mapping

Wx1 rgo1 plants were crossed to a wx1 stock and 33 wx1 translocation lines representing 18 chromosome arms. The progeny of these crosses were self-pollinated and the resulting waxy (wx1) and opaque (Wx1)
seed were planted separately. Linkage to an arm is indicated by repulsion from a given translocation marked by \( w_{x1} \) (Laughnan and Gabay-Laughnan, 1996). After mapping to a chromosome arm, finer molecular mapping was performed using RFLP and SSR markers.

**Scanning electron microscopy**

1-3 cm developing ears were dissected from 6- to 8-week old plants and fresh tissue was used for imaging. 1-3 cm tassel primordia were fixed in FAA (3.7% formaldehyde, 5% glacial acetic acid, 50% ethanol) overnight at 4°C, dehydrated through an ethanol series, and critical-point dried. Glumes were dissected away to expose the floret primordia, and the samples were then sputter coated with 15 nm of gold/palladium (Polaron SEM coating system). An Electroscan E3 Environmental Scanning Electron Microscope (ESEM) was used to image samples at 20 kV. Images were digitally captured using IAAS software (Electroscan Corp.). Electron microscopy was performed in the Electron Microscope Laboratory at the University of California at Berkeley.

**Histology**

Tissue was fixed overnight at 4°C in FAA, dehydrated through an ethanol series, and stained with 0.1% Safranin. The samples were then embedded in paraffin (Paraplast, Oxford Labware, St Louis, MO). The samples were mounted on stubs, and 10 \( \mu m \) sections were cut and then mounted on slides. The slides were deparaffinized in Histoclear (National Diagnostics, Atlanta, GA), hydrated through an ethanol series, and stained for 30 seconds to 1 minute with 0.05% Toluidine Blue O. Slides were then rinsed, dehydrated, and mounted with Merckoglas (Mikroskopic, Germany) (Ruzin, 1999). Images were digitally captured using a Zeiss Axiophot light microscope.

**Quantitative analysis**

Tassel phenotypes were quantified by counting the number of tassel branches, the number of spikelets on the central spike of the tassel, and the number of florets per spikelet. Between 20 and 50 spikelets from the central spike were counted to determine the number of florets per spikelet, and the numbers from siblings were pooled.

**RESULTS**

The **reversed germ orientation1** phenotype affects both ear and tassel development

To analyze the \( rgo1 \) phenotype, the \( rgo1 \) mutation was introgressed into a B73 background. During vegetative growth, \( rgo1 \) plants are indistinguishable from wild-type siblings. However, \( rgo1 \) ears have irregular rows of kernels compared to wild-type siblings (Fig. 2A, B). Closer examination of \( rgo1 \) ears reveals kernels with their embryos facing both the tip and the base of the ear (Fig. 2B, D). Often, reversed and normal orientation kernels are found in the same spikelet surrounded by a shared pair of glumes. \( rgo1 \) ears also contain fused kernels, each containing an embryo (Fig. 2B). The frequency of fused kernels varies from ear to ear. Open pollination of \( rgo1 \) plants produced fused kernels where one kernel was fertilized by a pollen grain containing a color gene, the other without. Fig. 2E and F show the top and bottom of a fused kernel, each with a visible embryo.

In wild-type female spikelets the upper floret develops into the kernel with the embryo facing the tip of the ear, while the lower floret arrests and aborts. In wild-type male spikelets, two florets develop and produce three anthers each (Fig. 2G). In \( rgo1 \) tassels spikelets have three florets, each with three anthers, for a total of nine anthers per spikelet (Fig. 2H). The penetrance of the tassel phenotype is background dependent, and ranges from 39% in B73 to 91% in Gaspe Bay Flint (GBF). The total number of spikelets and number of tassel branches are not affected in \( rgo1 \) mutants (Table 1).

**rgo1 is a single recessive mutation and maps to chromosome 9**

Self pollinations of heterozygous \( rgo1/+ \) plants produced 25% \( (n=48) \) mutant progeny, and crosses of \( rgo1/+ \) heterozygotes to
rgo1 homozygotes produced 50.6% (n=81, \(\chi^2=0.012, \text{d.f.}=1, P=0.91\)) mutant progeny. This indicates that rgo1 behaves as a single recessive mutation when introgressed into B73. waxy1 (\(wx1\)) marked reciprocal translocation stocks were used to map rgo1 to a chromosome arm. rgo1 was in repulsion to all of the \(wx1\) marked translocations as well as to a \(wx1\) stock, indicating that rgo1 is located on chromosome 9 and linked to \(wx1\). Test crosses place rgo1 and \(wx1\) approximately 11 cM apart.

Further mapping with RFLP and SSR molecular markers locate rgo1 within 2 cM of the SSR marker umc1267 in bin 3, close to the centromere.

The spikelet meristem initiates an extra flower in rgo1 mutants

To define the developmental defects in rgo1 mutants, early ear development was observed using electron microscopy and histology. Inflorescence meristems (IMs) of rgo1 ears initiate multiple rows of SPMs in the normal polystichous pattern and appear identical to wild-type IMs (Fig. 3A,B). SPMs in rgo1 plants initiate a SM correctly from the flank of each SPM (Fig. 3C,D), and are thus not responsible for the disrupted rowing observed in rgo1 ears (Fig. 2B).

Differences between normal and mutant development become evident upon examination of the SM. Normally each SM initiates a set of outer and inner glumes, and then the lower floret meristem (Fig. 3E). The SM itself converts into the upper floret meristem, and initiates floral organs, including three anthers (a) and the pistil primordial (p). In wild-type spikelets the anthers are arranged with the middle anther facing the tip of the ear (G), whereas the anthers face the base of the ear in rgo1 (H). Finally, each floral meristem produces the gynoecial ridge (gr), which is in the reversed orientation in rgo1 (J) relative to wild-type (I). The second floret (arrowheads, J) often develops in rgo1 spikelets. Bars, 200 \(\mu m\) (A,B), 100 \(\mu m\) (C-J).

### Table 1. The effect of genetic background on penetrance of tassel phenotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Florets per spikelet</th>
<th>Spikelets on the central spike</th>
<th>Tassel branch number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±s.d.</td>
<td>n</td>
<td>Mean±s.d.</td>
</tr>
<tr>
<td>rgo1^B73x6</td>
<td>2.6±0.5*</td>
<td>154</td>
<td>206.1±25.0</td>
</tr>
<tr>
<td>wt sibling</td>
<td>2.0±0.0</td>
<td>450</td>
<td>209.4±21.1</td>
</tr>
<tr>
<td>rgo1^GBFx1</td>
<td>2.9±0.3*</td>
<td>138</td>
<td>nd</td>
</tr>
<tr>
<td>wt sibling</td>
<td>2.0±0.0</td>
<td>355</td>
<td>nd</td>
</tr>
<tr>
<td>(rgo1^B73x4 · ids1-Vf) rgo1, ids1 double mutants</td>
<td>3.6±1.0**</td>
<td>120</td>
<td>260.0±19.6</td>
</tr>
<tr>
<td>rgo1 and ids1 single mutants</td>
<td>2.4±0.5*</td>
<td>120</td>
<td>264.5±18.4</td>
</tr>
<tr>
<td>wt sibling</td>
<td>2.0±0.0</td>
<td>120</td>
<td>273.4±23.1</td>
</tr>
</tbody>
</table>

The number of florets per spikelet, spikelets on the central spike, and number of tassel branches were counted in mutants and their wild-type siblings from segregating families. *Significantly (\(P<0.001\)) different from wild type; **significantly (\(P<0.001\)) different from both wild type and single mutants using the Student’s two-tailed t-test. s.d., standard deviation; n, number of spikelets counted (florets per spikelet) or number of plants counted (spikelets per spike, tassel branch number); nd, not determined.
floral meristem. \(rgo1\) SMs also initiate two glumes and a FM, but then continue to make a second FM at 180° from the first FM (Fig. 3F). Subsequently both wild-type and \(rgo1\) SMs become FMs and initiate floral primordia (Fig. 3G,H). However, the orientation of the floral organs on \(rgo1\) spikelets is at 180° from normal, with the middle anther primordium on the same side as the outer glume (Fig. 3H), as opposed to the inner glume as in wild-type spikelets (Fig. 3G). Later in development (further down the developing ear), the FM makes the gynoecial ridge, two fused carpels that elongate to become the silk (Nickerson, 1954). Like the anther primordia, the orientation of the developing silk on the top flower is reversed in \(rgo1\) spikelets (Fig. 3J). In \(rgo1\) spikelets the lowest FM arrests and aborts similar to wild-type development, but in many spikelets the second flower continues to develop (arrowheads in Fig. 3J). The developmental defects in tassel spikelets mirror those observed in ear spikelets, with no defects observed until FMs are initiated. Wild-type SMs initiate only one FM (Fig. 4A), while \(rgo1\) SMs initiate two (Fig. 4B). The orientation of the third flower in \(rgo1\) tassel spikelets is reversed compared to wild-type apical florets (Fig. 4C,D).

\(rgo1\) affects floral abortion of subapical florets producing fused kernels and disorganized rowing

During normal development the lower flower aborts while the upper flower makes a silk (Fig. 5A). The flower is then fertilized, and develops into a kernel. The lower flower undergoes programmed cell death which is detectable using the TUNEL assay for DNA fragmentation (Jones et al., 2001) and the accumulation of autofluorescent compounds in the cell wall (data not shown). After cell death, collapsed cell walls are all that remains of the aborted flower (af in Fig. 5E). In \(rgo1\) plants, developing flowers have several fates, reflecting the variable expressivity of the mutation. Mirroring wild-type development, the apical flower can develop while the two lower flowers abort (Fig. 5B,F). However, in many \(rgo1\) spikelets the second flower does not abort and the two upper flowers develop while the lowest flower aborts (Fig. 5C,G). Two kernels are produced in spikelets where the two apical flowers develop. The top (third) flower produces a reversed kernel, while the middle (second) flower’s kernel has a normal orientation (Fig. 1). These two flowers can fuse during development, producing fused kernels (Fig. 5D,H). In spikelets with two developing flowers, the flowers are crowded and displace each other resulting in the disrupted rows of kernels observed in mature \(rgo1\) ears (Fig. 2B).

\(rgo1\) and \(ids1\) exhibit nonallelic noncomplementation

\(indeterminate\) spikelet1 (\(ids1\)) is a maize gene with homology to the AP2 class of transcription factors. Mutations of the \(ids1\)

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**Fig. 4.** \(rgo1\) tassel defects are similar to ear defects. (A) Wild-type tassel spikelet meristems (sm) initiate one floret meristem (fm) while (B) \(rgo1\) spikelet meristems initiate two. Earlier events (not shown) and later events including floral organ initiation (C, wild type; D, \(rgo1\)) are unaffected. a, anther primordia; p, pistil primordia; l, lodicule. Bars, 100 μm (A), 200 μm (B), 50 μm (C,D).

**Fig. 5.** \(rgo1\) spikelet development has several possible outcomes. (A,E) Wild-type spikelets have one silk (si), a single ovule primordium (op) and an aborting lower floret (af) surrounded by the outer glume (og) and inner glume (ig). (B-D,F-H) \(rgo1\) mutant spikelets have three florets. The two lower florets abort in some spikelets (B,F). In other spikelets, only the first floret aborts, resulting in two functional flowers (C,G) and a pair of opposed kernels. Rarely, the two developing florets are fused (D,H), and produce a fused kernel. Bars, 100 μm (A-D), 250 μm (E-H).
Homozygous *rgo1* and *ids1* mutants were crossed to each other. The resulting *ids1/+; rgo1/+* plants were grown and self-pollinated to produce the double mutant. Since *ids1* maps to chromosome 1 and *rgo1* maps to chromosome 9, it was a surprise that the ears of the doubly heterozygous plants had disturbed rows and reversed kernels, reminiscent of the *ids1* and *rgo1* single mutants (Fig. 6). When two recessive mutations at different loci fail to complement each other, the genetic behavior is known as nonallelic noncomplementation (Yook et al., 2001). Penetrance of this phenotype was 26% in *ids1-Burr*, 84% in *ids1-mum1* and 100% with *ids1-VI*. Penetrant *ids1-VI* and *ids1-mum1* ears were used for further studies. Nonallelic noncomplementation suggests that genetically, *rgo1* and *ids1* have overlapping functions. The occurrence of a mutant phenotype with the loss of two functional copies (either a *rgo1* or *ids1* homozygote or the *ids1/+; rgo1/+* double heterozygote) implies that at least three wild-type copies of these two genes are needed for correct regulation of spikelet meristem activity.

**rgo1; ids1 double mutants affect multiple meristems in the ear**

In order to observe double mutants, families of the selfed progeny of double heterozygote plants were grown. Three classes of F2 ears were observed in these families: normal ears, ears with reversed kernels and disrupted rowing like *rgo1* or *ids1* single mutants, and ears with proliferated spikelets and long branches (Fig. 6). The expectation based on the observation of nonallelic noncomplementation is that 1/16 of the plants (all plants missing two or more copies of *ids1* or *rgo1*) would exhibit a mutant phenotype, and 1/16 would be double mutants. Table 2 lists the observed numbers of ears in each of these phenotypic classes. All families segregated the three phenotypic classes as expected, although the severity of the double mutant phenotype varied from family to family.

While single mutants only affect the SM, the *ids1; rgo1* phenotype affects the SM as well as the SPM. To analyze the double mutants, we define meristems that produce glumes or florets as SMs, and meristems that produce spikelets as SPMs. A range of effects on the SM is observed, from retardation of identity transitions to identity reversions. As expected from the single mutant phenotypes, extra florets are produced by each SM, resulting in reversed and fused kernels (Fig. 7A,E). In addition, the double mutant SMs make supernumerary glumes (Fig. 7B,G). The SM can initiate one or more florets, elongate into a branch, and then initiate one or more spikelets (Fig. 7C,E). Both the primary and reiterated spikelets are identified by the presence of subtending glumes (Fig. 7C,D).

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**Table 2. Numbers of ears in each phenotypic class**

<table>
<thead>
<tr>
<th>Family</th>
<th>Pedigree</th>
<th>Wild-type ears</th>
<th>Ears with reversed kernels</th>
<th>Ears with long branches</th>
<th>$\chi^2$ P value, n.n.</th>
<th>$\chi^2$ P value, n.n.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK612</td>
<td><em>(rgo1</em>W23/M14s2 × <em>ids1-VI)</em></td>
<td>37</td>
<td>92</td>
<td>5</td>
<td>0.25</td>
<td>8.5×10⁻¹³</td>
</tr>
<tr>
<td>NK1153</td>
<td><em>(rgo1</em>B73x4 × <em>ids1-VI)</em></td>
<td>63</td>
<td>115</td>
<td>13</td>
<td>0.80</td>
<td>1.9×10⁻¹⁰</td>
</tr>
<tr>
<td>NK1580/1</td>
<td><em>(rgo1</em>B73x4 × <em>ids1-VI)</em></td>
<td>100</td>
<td>207</td>
<td>18</td>
<td>0.83</td>
<td>7.7×10⁻²²</td>
</tr>
<tr>
<td>NK1154/7</td>
<td><em>(ids1-mum1 × wxl rgo1)</em>, wxl kernels</td>
<td>4</td>
<td>35</td>
<td>10</td>
<td>0.69</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Families of *rgo1/+; ids1/+* heterozygotes were selfed (*). F2 ears were scored as either wild type, *rgo1*- or *ids1*-like (reversed kernels), or double mutants with long branches (long branches). $\chi^2$ P values are indicated for each family, and do not rule out the possibility of nonallelic noncomplementation (n.n.) with a double mutant phenotype. The $\chi^2$ P values for the null hypothesis, no nonallelic noncomplementation, were all <1×10⁻⁴. For families NK612, 1153, 1580 and 1581 the expected ratio of wt:rgo:long was 5:10:1 (n.n.) and 9:6:1 (no n.n.). Families 1154 and 1157 consisted of only *wxl* kernels from an ear segregating *wxl* linked to *rgo1*. The expected ratios (0.9:12:3.1) were based on a 11% recombination rate between *rgo1* and *wxl*.

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![Diagram of *rgo1* and *ids1* phenotypes](image)

Fig. 6. *rgo1* and *ids1* exhibit nonallelic noncomplementation and have a synergistic double mutant phenotype. *rgo1* and *ids1* homozygous plants were crossed (top). The double heterozygote progeny had a reversed kernel and disrupted row phenotypes (middle). When these plants were self-pollinated, wild type (wt), *rgo1*-like (rgo), and double mutants with long branches (branched) were produced (bottom).

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*Table and text continued*
In cases where the SM is producing spikelets, it reverts to SPM identity. SPMs produce too many spikelets in the double mutant. This phenotype can be restricted to the base of the ear (Fig. 6, Fig. 7A) where the SPM produces multiple SMs. In the most severe cases, all of the SPMs on the ear become indeterminate (Fig. 7F), and produce multiple spikelets (Fig. 7G). These spikelets make many glumes (arrowheads, 7G) and only rarely produce florets as evidenced by the absence of silks. Since glumes are produced by the SM, in these spikelets the transition from producing glume primordia to floret primordia is delayed.

Unlike the synergistic interaction observed in the ear, \textit{rgo1}; \textit{ids1} tassels have an additive phenotype. More florets are produced by each spikelet, but there is no evidence that the SMs in the tassel have changed identities or that SPMs are affected (Table 1).

**DISCUSSION**

\textit{rgo1} is required for conversion from spikelet to floret meristem identity

The progression from vegetative to floral meristem identities during plant growth is critical for normal plant reproduction. We have shown that progression of meristem identity from the spikelet to the floret stage is retarded (i.e. the spikelet meristem does not convert into a floret meristem, and thus continues to initiate florets) in the \textit{rgo1} mutant (Fig. 8A,B). As a result, extra flowers are produced, and because of the distichous pattern of floret primordia initiation on the spikelet axis, kernels with a reversed germ orientation are observed (Fig. 1B). Many \textit{rgo1} ear spikelets form two fertile flowers. Some of these bi-floral spikelets have fused flowers (Fig. 5D). This may indicate that conversion of the SM to FM identity occurred before two distinct flowers formed. The abnormal survival of sub-apical florets may be a result of a lack of suppression by the apical flower (Novoplansky, 1996).

If \textit{ids1} is placed into the framework of meristem identity changes it would perform a similar role as \textit{rgo1} (Fig. 7C), promoting the SM to FM change. The overlapping functions of \textit{rgo1} and \textit{ids1} revealed by their mutants and genetic interactions support the idea that these genes perform similar
Like the SM, SPM conversion is defective in and ids1 also produces long branches with multiple SMs (Fig. 7A,F). Both double mutants have more severe effects in the ear than in the tassel. In both cases, meristem identity has changed and results in a decrease in determinacy. rgo1 and ifa1 do not exhibit a synergistic interaction like ids1 and ifa1 do (data not shown), although ids1 interacts synergistically with both rgo1 and ifa1. This illustrates that although rgo1 and ids1 functions overlap, these genes play unique roles in inflorescence development.

The conversion model of maize inflorescence development proposes that SPMs initiate a SM and then convert into SMs, and SMs initiate a FM and then convert into FMs (Fig. 8A). This model is based on the class II tasselseed phenotypes, in which meristem identity conversions are delayed (Irish, 1997a). Determinacy is simply a function of correct identity transitions in the conversion model. A SM that converts into a FM is determinate, while a SM that never changes its identity is indeterminate. An alternative model of inflorescence development, the lateral branching model, does not involve identity conversion (Chuck et al., 1998). Instead, Chuck et al., suggest that all florets, including the terminal floret, are initiated laterally by the SM. A band of ids1 expression between the developing upper and lower floret was interpreted to be the remnant SM, which is not suppressed in ids1 mutants. This theory was partially reconciled with the conversion model by Irish who suggested that the remnant of the SM is morphologically indistinguishable but still present on the flank of the FM (Irish, 1998). In the lateral branching model, ids1 functions to suppress indeterminate growth of the SM. One of the predictions of this model is that ids1 mutant SMs should make more FMs, but as SM identity does not change, only FM primordia should be produced by the SM.

Observations from our research favor the conversion model, which can explain mutant behavior without invoking remnant meristems. In the double mutant, SMs produce supernumerary FMs, but also initiate additional glumes and SMs, indicating that SM identity has changed (Fig. 8D). The lateral branching model does not implicate ids1 in meristem identity changes because in this model the SM never becomes a FM. The conversion model explicitly implicates identity change as a mechanism, and thus can explain the double mutant phenotype. The lateral branching model was partially based on the observation that ids1-mum1 spikelets do not terminate in florets, because in this model the florets are lateral branches of the spikelet. Upon introgression into B73, ids1-mum1 plants can make functional terminal florets, as do the ids1-Burr and ids1-VI alleles. ids1-VI ears make kernels and do not exhibit indeterminate spikelets on the ear (Fig. 6). Thus, in certain genetic backgrounds, ids1 mutant spikelets can terminate in fertile florets, suggesting that ids1 is involved in the conversion of SMs into FMs.

**Meristem identity is regulated in a dosage-sensitive manner by multiple genes**

Nonallelic noncomplementation can be explained by a genetic pathway that is sensitive to the total dosage of two genes (Stearns and Botstein, 1988; Yook et al., 2001). Based on the nonallelic noncomplementation and synergistic double mutant phenotypes of rgo1 and ids1, we propose a dosage based model for meristem conversion. In this model, SPM→SM and SM→FM conversions are regulated by both ids1 and rgo1, although the threshold for normal behavior is lower in SPMs than in SMs (Fig. 9). Signals from the vegetative portion of the maize plant are known to affect meristem identity transitions. In the maize indeterminate 1 (ids1) mutant the transition from vegetative to reproductive development is extremely delayed or never happens. ids1 is expressed in immature leaves, but acts in a non cell-autonomous manner and affects the apical meristem (Colasanti et al., 1998). Meristem culture experiments also support the idea that the SAM integrates signals from the whole plant and does not keep track of its identity internally (Irish and Karlen, 1998; Irish and Nelson, 1991). If rgo1 and ids1 are in the genetic pathway that responds to these signals coming from the rest of the plant, these mutants could make the meristem less sensitive to the signal needed for meristem conversion (this is equivalent to raising the signaling threshold).

Control of meristem identity transitions in the maize inflorescence is under the combinatorial control of multiple genes. The penetrance of the ids1+/+; rgo1+/ nonallelic noncomplementation phenotype and the severity of the ids1; rgo1 double mutant phenotype are variable (Fig. 7), suggesting that there are genetic modifiers in the maize genome (and probably environmental effects as well) that affect this phenotype. Three additional lines of experimental evidence provide support for the presence of modifiers. To test the dosage model of nonallelic noncomplementation, we produced plants missing one copy of the long arm of chromosome 1 (which carries ids1) using B centromere translocation lines (Birchler, 1996). Chromosome 1L hypoploids exhibited a reversed kernel phenotype. This shows that there are other genes on 1L (possibly tbl, knl, and/or ts6, which all have effects on meristematic behavior) that in combination with a missing dose of ids1 have an effect on spikelet meristem identity. Similarly, a directed Mutator transposon mutagenesis experiment aimed at cloning rgo1 produced hundreds of plants with rgo1-like phenotypes. Only one or two disruptions of the rgo1 gene would be expected, suggesting that rgo1 heterozygotes uncover many branching modifiers. Finally, a large screen of over 50,000 F1 Mutator active ears for dominant or semi-dominant reversed germ

![Fig. 9. Gene dosage model for SM and SPM identity. SPMs are wild type (green) with even one dose of ids1 or rgo1, and mutant (red) in the absence of any ids1 or rgo1. SMs are more sensitive to ids1 and rgo1 dosage, with a threshold somewhere between two and three doses.](http://www.someurl.com)
phenotypes identified several ears with strong reversed germ phenotypes. These ears transmit fascicled (ears with more than one main spike due to multiple IMs), ramosa (ears with long branches at their base due to indeterminate SPMs), and reversed germ (SM) phenotypes in the F2 families in a recessive manner. In these families, heterozygotes uncover a reversed germ (i.e. SM) phenotype. However, homozygotes affect both the SM and SPM and IMs. These data suggest there are many genes in the maize genome that, when disrupted, can contribute to the dosage effect that creates reversed kernels.

Several other examples of dosage-sensitive developmental pathways have been described in plants. **LEAFY (LFY)** is an *Arabidopsis* gene that regulates the transition to flowering by promoting and maintaining floral meristem identity (Weigel et al., 1992). Heterozygous *lfy/+* plants are normal under long day conditions. However, in short day conditions floral reversion results in inflorescences being elaborated from the floral meristem (Okamura et al., 1996). A second example can be found in the regulation of meristem maintenance. The **CLAVATA1** (**CLV1**) and **CLV3** genes in *Arabidopsis* are involved in maintaining meristem size by balancing the rates of cell division and primordial initiation. **CLV1** encodes a receptor kinase, and **CLV3** is its ligand (Trotchoud et al., 2000). **CLV1** and **CLV3** exhibit a form of dosage sensitivity. The weak **clv1/+** phenotype is strongly enhanced in a **clv3/+** background (Clark et al., 1995). In addition, **stm/+** heterozygotes suppress **clv1** semidominance (Clark et al., 1996). Finally, hemizygosity of either **liguleless1** or **liguleless2**, genes necessary for ligule development in maize, affect the null phenotype of the other mutant (Harper and Freeling, 1996). These examples may reflect a general sensitivity to dosage in many aspects of meristem function.

**Conclusion**

**Kellogg** (Kellogg, 2000) has proposed a generalized model for inflorescence development. The model states that meristems can produce either more meristems or a set of determinate floral organs on their flanks. In a reiterative manner, any subsequent meristems must make the same developmental decision. This simple model can explain the wide variety of inflorescence forms found in plants. If our results can be generalized, then progress through the various orders of meristem identity will depend on the dosage of **ids1** and **rgo1**-like genes and the concentration of their products. Meristem identity is established by varying a threshold, with different thresholds for different identities. Duplication or loss of these genes would explain variations in the number of iterations of this basic process, and thus could be responsible for the great diversity in inflorescence forms.

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