Molecular and Functional Analysis of *AGL2*-like MADS-box Genes in Maize (*Zea mays* ssp. *mays*)

Indications for their involvement in grass inflorescence architecture

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1 INTRODUCTION

1.1 Development

Organisms can be seen as complex chemical system of self-maintenance, in which the acting factors primarily consist of proteins, encoded by genes (Margulis & Sagan, 1995). These genes are embedded in the smallest unit of the living world, the cell. Functionally, genes are orderly inter-linked and cross-wired into a network (Theißen & Saedler, 1995). The genetic network ultimately governs basic characteristical programs, such as metabolism and transfer of energy, transmission of heritable information and reproduction, development and aging, that make the living world alive (Westhoff *et al*., 1998). Development is the total set of changes in form and function that an individual of a certain species is going through. The predefined course of these changes is controlled by the spatial and temporal regulation of developmental genes in the network. This wildtype development can be altered when the normal expression is changed in single genes guiding a particular step in development. This can lead to an abberant shape of the individual, called a mutant phenotype. By isolating mutant alleles of the same gene one can analyze how the gene acts in the developmental process on a molecular level.

The blueprints (`*Baupläne´*) for both plant and animal overall development are realized by developmental control genes. These direct the expression of multiple developmental genes bringing about the formation of a whole organ or body part. Mutations in these genes result in dramatic disruptions of morphogenesis, leading to the formation of organs at the wrong positions (homeotic transformations). These homeotic genes tend to code for transcription factors. The most important animal homeotic selector genes belong to the family of homeobox genes (Gehring, 1994). These factors have characteristic helix-loop-helix elements allowing them to bind to promoter regions of downstream target genes. The homeo-box genes are some of the most stable in evolution and are highly conserved in all eukaryotic organisms. In animals, these homeo-box containing genes are organized in clusters, that have also been conserved during evolution. The arrangement of homeo-box genes is correlated with the anterior-posterior position of the body segments they specify during the embryonic stage of the animal. For example, a consequence of *antennapedia* mutants is the formation of legs in place of antenna in adult flies (*Drosophila melanogaster*) (Lawrence, 1992). In addition to this, the ectopic expression in flies of a mouse homeobox gene (*hoxB6*) leads to a similar homeotic tranformation of antennae into

legs (McGinnis & Kuziora, 1994). This means that even the function -the formation of a certain part of the overall pattern- has been conserved in evolution during millions of years. This is called ´synteny of function´(Theißen & Saedler, 1995).

1.2 The ABC-model of flower development

The concept of hierarchical control of development in animals has strongly influenced plant developmental biology. The pattern of body segments laid down by developmental control genes during the embryonic stage of *Drosophila* has been compared with the pattern of a series of leaf-like structures laid down during the development of a flower (Theißen & Saedler, 1995). Flower formation is a function of floral meristems. Upon flowering, the shoot apical meristem changes fate, giving rise to an inflorescence meristem out of a vegetative one. The inflorescence meristem undergoes further transitions to produce several lateral floral meristems. The meristems grow in size, and from around the periphery of the meristem dome some highly mounded structures bulge out, the floral organ primordia. These primordia emerge in four concentric rings, called whorls. In the outer whorl the sepals are formed, enclosing the petals in the second whorl. Together they make up the perianth. Inside of these, in whorl three, the stamina are formed. In the innermost whorl the carpels develop.

Work in plants was mainly based on two dicot plant model systems, Thale cress (*Arabidopsis thaliana*) and Snapdragon (*Antirrhinum majus*). Two groups of mutant plants were investigated that show homeotic transformations of floral meristems and floral organs. In the former group, the identity of the floral meristem is not specified. As shown in *squamosa* in *Antirrhinum*, the lateral floral meristems are transformed into an inflorescence-like growth at the site of the flower (Huijser *et al.*, 1992). Within the latter group, the phenotypes of the homeotic transformations could be classified into three functional classes, labeled A-, B- and C- function mutations. Per class different but overlapping sets of organs were affected (for an overview, see: Schwarz-Sommer *et al*., 1990; Coen & Meyerowitz, 1991; Weigel & Meyerowitz, 1994; Meyerowitz, 1994). In the first class the perianth was transformed, as can be seen in *apetala1* and *apetala2* in *Arabidopsis* (Mandel *et al*., 1992; Jofuku *et al*., 1994). Sepals are changed into carpels, and petals into stamens. In the second class, examplified by *pistillata* and *apetala3* in *Arabidopsis*, or *deficiens* and *globosa* in *Antirrhinum*, the petals are converted into sepals, and the stamens into carpels (Goto & Meyerowitz, 1994; Jack *et al*., 1992; Sommmer *et al*., 1990; Tröbner *et al*.,

1992). As can be seen in *agamous* in *Arabidopsis* or *plena* in *Antirrhinum*, the last class of floral homeotic mutants has the stamens converted into petals and the carpels are transformed into sepals. The meristem becomes indeterminate, creating a reiterated pattern of sepals and petals (Yanofsky *et al*., 1990; Bradley *et al*., 1993). It was observed that A-function genes work antagonistically to C-function genes, and vice versa. In doing so, they mutually exclude each other of functioning in the same whorls. In addition to that, B-function genes can be expressed in the same whorl with either A-, or C- function genes. Since the function of these genes in a whorl specifies which organ is formed, these homeotic genes where called floral organ identity genes. Depending on the combination of functions present in a particular whorl, the respective floral organ is developed. A genes specify sepals; A+B genes specify petals; B+C genes specify stamens; C genes specify carpels. Genetic crosses of B- and C-function mutations showed an indeterminate flower consisting of a reiteration of only sepals. A-, B, and C-function triple mutants revealed that all floral organs are reverted to their ground state and appear as leaves (Weigel & Meyerowitz, 1994). The combinatorial action of the floral organ identity genes has been called the ´ABC-model´. Lately, the model has been extended to include a D-function, specifying ovule identity (Angenent *et al*., 1995). In petunia (*Petunia hybrida*) ectopic expression of the genes *FBP7* and *FBP11* lead to the formation of ovules on the perianth.

As the genes have been isolated, the molecular mode of action could be elucidated. The site of expression of the floral meristem and organ identity genes closely correlated with the site of the mutant phenotype, except for *AP2* that is more widely expressed. This indicates that the wildtype expression can give a strong suggestion to the function the gene exerts. It showed that all floral meristem and organ identity genes, except *AP2*, are MADS-box genes. This name is an acronym, derived from *MCM1* (yeast), *AGAMOUS* (*Arabidopsis*), *DEFICIENS* (*Antirrhinum*) and *SRF* (Human). MADS-box genes code for a class of transcription factors, that is present in all eukaryotic species (Schwarz-Sommer *et al*., 1990). The MADS domain is a highly conserved motif of approximately 57 amino acids long, that can bind to DNA (Riechmann & Meyerowitz, 1997). In plants, the MADS-domain also contributes to the dimerizing capacity of the protein with other proteins, as does a second domain, the K-domain. The K-domain has sequence conservation with other proteins, that can form keratin-like coiled coil structures, involved in protein–protein interaction (Shore & Sharrocks, 1995). In *Antirrhinum*, *in vitro* transcribed and translated DEF and GLO protein have been shown to bind to DNA as heterodimers. Together they recognize a DNA sequence with CArG motifs containing a palindromic core (Schwarz-Sommer *et al*., 1992). Furthermore, MADS-domain proteins can even form ternary complexes in

yeast (Egea-Cortines *et al*., 1999), as examplified by the heterodimer DEF-GLO interacting with a homodimer of SQUAMOSA. The complex has increased DNA binding affinity compared to either of the two dimers. This type of interaction may augment the capacity with which MADS-domain transcription factors exert their function within the regulatory network. The intervening region between MADS-box and K-box was termed I-region. The C-terminal part of the protein, or C-region, might act as a transactivation domain. In plants, most of the MADS-box genes have this structure composed out of four modules and are therefore called MIKC-type genes (Münster *et al*., 1997).

1.3 *AGL2*-like MADS-box genes

The high level of sequence similarity between functionally syntenic MADS-box genes (orthologs such as *AGAMOUS* and *PLENA*; see above), lead to the understanding that genes from different species can sometimes function more alike than related genes from within the same species (paralogs). Phylogenetic reconstructions, based on sequence comparison, revealed that the MADS-box genes coding for a floral organ identity can be classified into well-defined monophyletic clades (Theißen et al.,1996). The majority of the member genes exert a highly similar function within these clades, based on mutant or transgenic analysis. Other MADS-box genes from a variety of species have now been isolated that do not cluster in clades that have been functionally defined. In one clade, the MADS-box genes always cluster together with the *Arabidopsis* gene *AGL2*. The first genes were isolated based on the homology with *AGAMOUS*, hence the name *AGAMOUS-LIKE2*. These genes have been around for at least 300 million of years, as these have been isolated from angiosperms (dicot and monocots) and from gymnosperms (Cacharrón *et al*., 1999; Mouradov *et al*, 1998). This suggests that the last common ancestor of seed plants already had at least one *AGL2*-like gene. The strong selection pressure during more than 300 million years to retain *AGL2*-like genes in higher plants points to the importance of their function (Münster *et al*., 1997).

Untill recently, no mutants in any *AGL2*-like gene were known that could shed light on the function of the genes. A transgenic approach was therefore taken by two groups working on *Solanaceous* dicot model systems. By down-regulating the level of endogenous *AGL2*-like gene transcript due to the integration of a transgene into the genome, a phenotype was obtained in petunia for the gene *FBP2*, and in tomato (*Lycopersicon esculentum*) for *TM5* (Angenent *et al*.,

1994; Pnueli *et al*., 1994). *FBP2* and *TM5* are expressed in petals, stamens, and carpels in the wildtype. In the transgenic plants, both dicots have flowers in which the three inner whorls were converted to sepals, or that have sepaloid petals (i.e. the conversion is partial). In flowers with a strong phenotype, the central floral meristem became indeterminate. In addition to this, in transgenic petunia plants, a new inflorescence-like primordium was formed in the axils of the innermost floral organs. Hence, the phenotype suggests that these *AGL2*-like genes function by mediating between floral meristem identity genes and floral organ identity genes.

Transgenic approaches using co-suppression or antisense technology may not always lead to an unambiguous result as to the specific disruption of only the target gene. The endogenous gene is left intact. Its expression is reduced by the transgene due to interfering with the transcript based on sequence homology. Therefore, the obtained phenotype may result from a reduction of expression of a highly similar gene, or might not be a null-phenotype (100% reduction of the endogenous transcript), thereby obscuring the proper phenotype that gives clues to the real function. In *Arabidopsis* a ´reverse genetics´ approach (from gene to mutant phenotype, as opposed to the classical way of isolating the gene causing a mutant phenotype, termed forward genetics) was used. T-DNA and transposon insertion populations were screened to find mutants in three *AGL2*-like genes. Mutant loci of *AGL2* and *AGL4* showed no phenotype, and *AGL9* showed only slightly sepaloid petals (Pelaz *et al*., 2000). In the wildtype, *AGL2* and *AGL4* are expressed in all 4 whorls (Flanagan *et al*.,1994; Savidge *et al*.,1995), whereas *AGL9* expression is found in whorl 2, 3 and 4 (Mandel & Yanofsky, 1998). In the *AGL2*/*AGL4*/*AGL9* triple mutant however, all floral organs were converted to sepals, resembling the B/C-function double mutant. The *AGL2*-like genes were hence renamed *SEPALLATA* (*SEP1-3*). The genes act redundantly, and are required in combination with the B- and C-function genes to specify the floral organ identity in the distinct whorls. Therefore, they were included into the ABC-model as the E-function genes (Theißen, 2001). Additionally, ectopic expression of the E-function genes together with A, B and C-function genes is sufficient to convert leaves into flower organs (Honma & Goto, 2001; Pelaz *et al*., 2001), supporting the idea that floral organs represent modified leaves (von Goethe, 1790; Theißen & Saedler, 2001).

An extensive functional analysis of all *AGL2*-like genes within a plant species has not been published to date. For *Arabidopsis* an extra *AGL2*-like gene is known, *AGL3*, that is also expressed in leaves (Huang *et al*., 1995). From different plant species it is clear that other *AGL2*-like genes may have a different expression pattern than the one from *SEP1-3*, *FBP2* or *TM5*

(Theißen *et al*., 1996, Cacharrón *et al*., 1999). Furthermore, it has been observed that other *AGL2*-like genes have obtained a different function during evolution (Theißen *et al*., 1996). *GRCD1*, an *AGL2*-like gene from gerbera (*Gerbera hybrida*), only specifies whorl three floral organ identity in female marginal florets (Kotilainen *et al*., 2000), even though the gene is expressed in all four whorls. The *AGL2*-like gene *GRCD2*, however, confers determinacy to the two outer regions of the capitulum (i.e. the inflorescence), where of the ray and transflorets are found (Kotilainen *et al*., 1999; Teeri, pers. comm.). Since the *AGL2*-like clade is a rather diverse group of genes, there is a need to investigate the function of these genes in its endogenous plant model systems. Until now, MADS-box genes have primarily been investigated in dicot model plants such as *Arabidopsis* and *Antirrhinum*. Monocots have been much less characterized functionally, although they include the agronomically important cereal grass species (family *Poaceae*), like maize (*Zea mays* ssp. *mays*) and rice (*Oryza sativa*). Information about MADS-box gene function in monocots would enable one to make predictions about the degree of conservation of function within the ABC model beyond the dicot-monocot split around 200 milion years ago (Savard *et al*., 1994), and the way MADS-box genes helped to shape the inflorescences and flower-like structures on these, as compared to the dicot ones. First, an introduction to inflorescence development must be discussed to address the question as to whether the ABC model can be applied to grasses. Maize is described as an example.

1.4 Maize inflorescence development

Maize is a monoecious plant, having two distinct unisexual inflorescences per plant, in which the male and female floral organs are organized (Cheng *et al*., 1983). The staminate inflorescence, the tassel, is located at the apex of the plant, whereas the pistillate inflorescence, the ear, develops in the leaf axils of the seventh until the tenth leaf, surrounded by husk leaves. In the first developmental stages, the two inflorescences are morphologically almost identical. Via specific abortion of the organs of the opposite sex, the unisexual flowers are created out of initially bisexual floral primordia. Like in all grasses, the flower-like structures are called florets (fig.1.1.A).

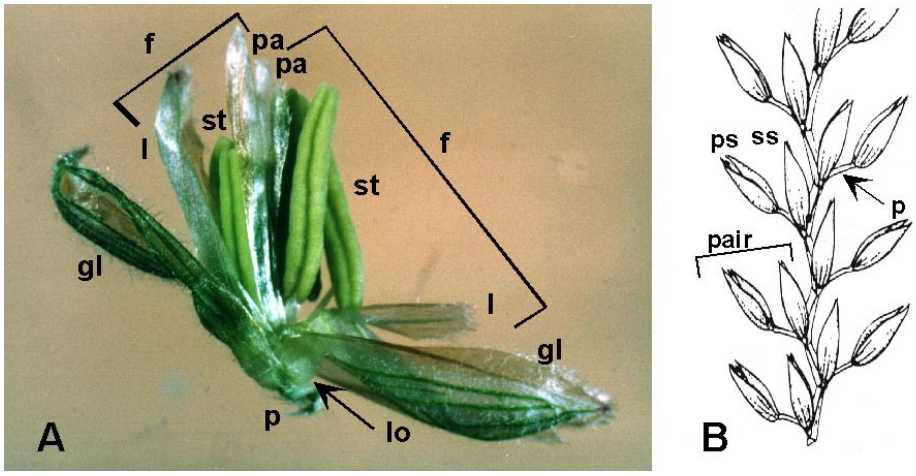

Fig 1.1.A-B. Spikelet and spikelet pair architecture. **A.** Fully developed wild-type male spikelet of maize having two florets. Each male floret consists of a lemma and palea, encircling three stamens and two lodicules. **B.** Side branch of the tassel showing spikelets pairwise aligned. Each pair consists of a sessile spikelet having a short stalk (pedicel), and a pedicellate one having a longer pedicel. f=floret, l=lemma, pa=palea, gl=glume, lo=lodicule, st=stamen, p=pedicel, ps=pedicellate, ss=sessile spikelet, pair=pair of spikelets.

In maize these consist of two papery scales, called lemma and palea, that enclose three stamens and a gynoecium, made up of three carpels. In the male floret also two highly reduced petal-like structures form, the lodicules. The florets are pairwise organized, with an upper and lower floret. These are placed together within two bracts, called glumes. The whole entity, hold together by the glumes, is called the spikelet, which is the basic unit of grass inflorescences (fig.1.1.B.). In maize also the spikelets are pairwise organized. In the tassel one spikelet has a longer stalk (the pedicellate spikelet) than the other (the sessile one). In the ear, however, all spikelets are placed close to the main inflorescence stem. Furthermore, in the ear the lower floret within each spikelet is aborted.

The complex architecture of the maize inflorescence of paired florets within paired spikelets, is the result of the sequential differentiation of the inflorescence meristem (IM) (Irish, 1997). This model of maize inflorescence architecture was based on the SEM analysis of two *tassel seed* mutants (*ts4* and *Ts6*), that show, apart from a lack of abortion of the gynoecia in the tassel, an abberant branching pattern in the inflorescence. After the transition to flowering has taken place in wild-type plants, the IM gives rise to spikelet pair meristems (SPM) in an acropetal fashion (fig.1.2).

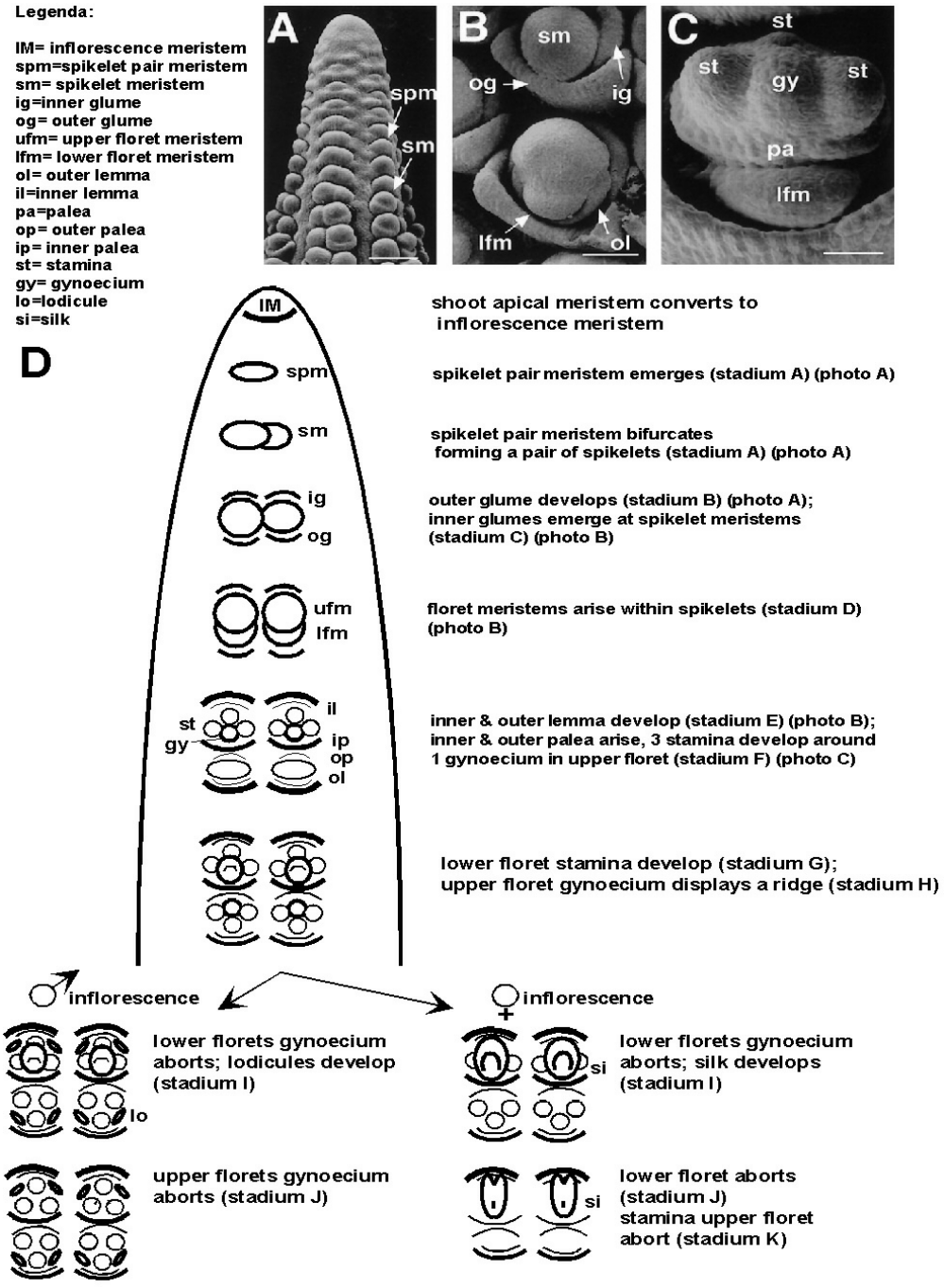

Fig.1.2. A-D Developmental stages in the maize inflorescence based on scanning electron microscopic analysis. **A.** Initiating wild-type spikelet pair primordia and spikelet primordia on a developing ear. Bar =300µm **B.** Unbranched spikelet meristem (top). Slightly older spikelet meristem laterally branched to form the lower floret meristem (bottom). Bar =100µm **C.** Development of floral organ initials at the upper floret. Bar =80µm. SEM pictures (fig. 1A-C) from Chuck *et al*. (1998). **D.** Schematic representation of the developmental stages. The inflorescence meristem is converted into spikelet pair meristem (SPM). The SPM creates two spikelet meristems (SM). The SM initiates glume initials first, then produces two floret meristems (FM). The FM forms all the floral organs, before sex specific abortion leads to unisexual florets. Modified after Cheng *et al.* (1983).

The SPM gives rise to two spikelet meristems (SM), by first producing an extra SM, before converting into an SM itself. Each SM in turn forms two floret meristems (FM), via a similar pattern in which the initial meristem undergoes a change of fate into the next level of meristem identity. The floret meristems finally produce the different floral organs. The sequential emergence of the different meristems is unidirectional. The meristems are only able to initiate a meristem on the next level, not the other way around. This indicates that the level of determinacy is increased with each step from SPM via SM to FM. The two tassel seed mutations show the wild type gene to act on the conversion of SPM to SM (*ts4*), or on the transition of SM to FM (*Ts6*) (fig.1.3.A).

The Irish-model of grass inflorescence architecture was challenged by Chuck and co-workers (1998) after analyzing another branching mutant, *indeterminate spikelet1* (*ids1*). The phenotype shows the spikelet meristem to be more indeterminate by producing three to ten florets in stead of the wildtype two. All florets appear to have been initiated laterally by the SM on a stalk-like structure in the center of the spikelet, the rachilla (fig.1.3.B). In wild-type maize spikelets, the rachilla is not visible, unlike in other grass species that have a higher number of florets per spikelet.

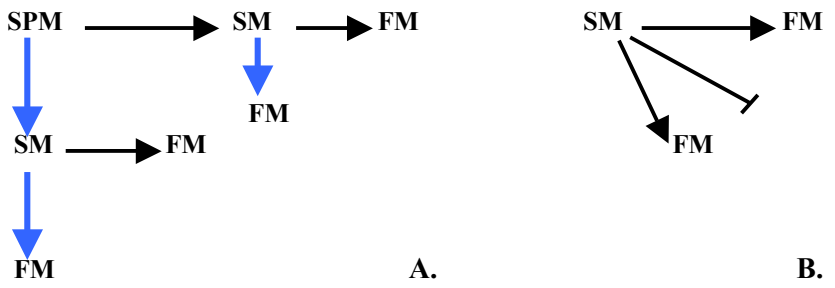

Fig.1.3. Competing models of maize spikelet architecture. Derived from Irish, 1998. **A.** Model of spikelet formation in maize after Irish, 1997. Black arrows indicate the initiation of meristems; blue arrows indicate the conversion of fate of the meristems. The spikelet pair meristem initiates a single spikelet meristem, then converts also to a spikelet meristem. Each spikelet meristem initiates a single floral meristem, then also converts to a floral meristem. **B.** Model of spikelet formation in maize after Chuck *et al*, 1998. The spikelet meristem laterally initiates two floral meristems, then terminates activity.
Abbrev.:SPM= spikelet pair meristem, SM= spikelet meristem, FM= floral meristem.

Due to the fact that grass florets are distinct in structure from dicot flowers and differently organized -in spikelets- on the inflorescence, it remained to be proven, whether the ABC model is applicable to them. Especially the identity of the floral organs and bracts enclosing the two inner

whorls, i.e. the glume, lemma, palea, and lodicules was widely debated. A maize homeotic mutant, called *silky*, was investigated and found to encode a B-function homolog of *DEFICIENS* (Ambrose *et al*., 2000) The phenotype shows that stamens were converted into carpels and lodicules into paleas. Hence, the lodicules in grasses are homologous structures to petals in dicots. Furthermore it showed that the paleas, previously classified as bract-like scales, are homologous to sepals in higher eudicots.

Also in rice, a homolog of *AGL2*-like genes, *OsMADS1*, was found to be mutated in *leafy hull sterile1* plants (Jeon *et al*., 2000). The wildtype spikelets in rice have, in contrast to maize, only one floret. The mutant phenotype shows a partial conversion of lodicules to paleas, which is consistent with paleas being the grass representatives of dicot sepals. If the spikelet had a strong phenotype, the glumes were surrounding a reiterated set of paleas, resembling the B/C double mutant in *Arabidopsis*, or likewise, the *SEP(1-3)* triple mutant. Additionally to the E-function phenotype, rice *lhs1* spikelets had sometimes an extra floret, indicating that *OsMADS1* controls the level of spikelet meristem determinacy as well. This resembles the lack of function of *fbp2* in petunia. This is further evidence that the ABC model of eudicot flower development can be applied to grass species.

1.5 Goal of the thesis

The goal of this thesis work was a molecular and functional analysis of *AGL2*-like genes in maize. *AGL2*-like genes have been shown to be important in governing inflorescence and flower development in higher eudicots, by mediating between floral meristem and organ identity genes. *AGL2*-like genes can have highly distinctive patterns of expression, suggesting a diversification in gene functions. The differences in inflorescence ´Bauplan´ between grasses and non-grasses (see above), raises the question as to whether the various *AGL2*-like genes can have a role in guiding this specific development of the architecture of the complex inflorescences. This is all the more suggested for grass *AGL2*-like genes that can have expression patterns conferring novel positional information not known from dicots. Though *AGL2*-like genes in some dicot species have rendered a clear phenotype, and hence function, a comparison of these species shows differences in the correlation between expression pattern and phenotype, and in the level of redundancy among *AGL2*-like genes. A more thorough characterization of the *AGL2*-like subfamily of MADS-box genes in maize may eventually lead to a better understanding in their

putative role as control genes of grass inflorescence development. In maize, 8 AGL2-like genes have been isolated that have different expression patterns (Theißen & Saedler, 2001). Among these, two showed extraordinary expression patterns, fitting to only a subset of the total number of primordia, each at only one of the different grass inflorescence meristem levels.

The gene *ZMM6* is strongly expressed in both the tassel and the ear in very early stages of development (fig.1.4.). Expression is turned on after the transition of the inflorescence meristem to spikelet pair meristem (SPM) (stage A, after Cheng *et al*., 1983) (Cacharrón, 1994; Cacharrón *et al*., 1995). In the SPM the expression is low. After the spikelet pair meristem bifurcates, *ZMM6* is expressed in only one of a pair of spikelet primordia (late stage A), and discriminates therefore between the sessile and pedicelate spikelet.

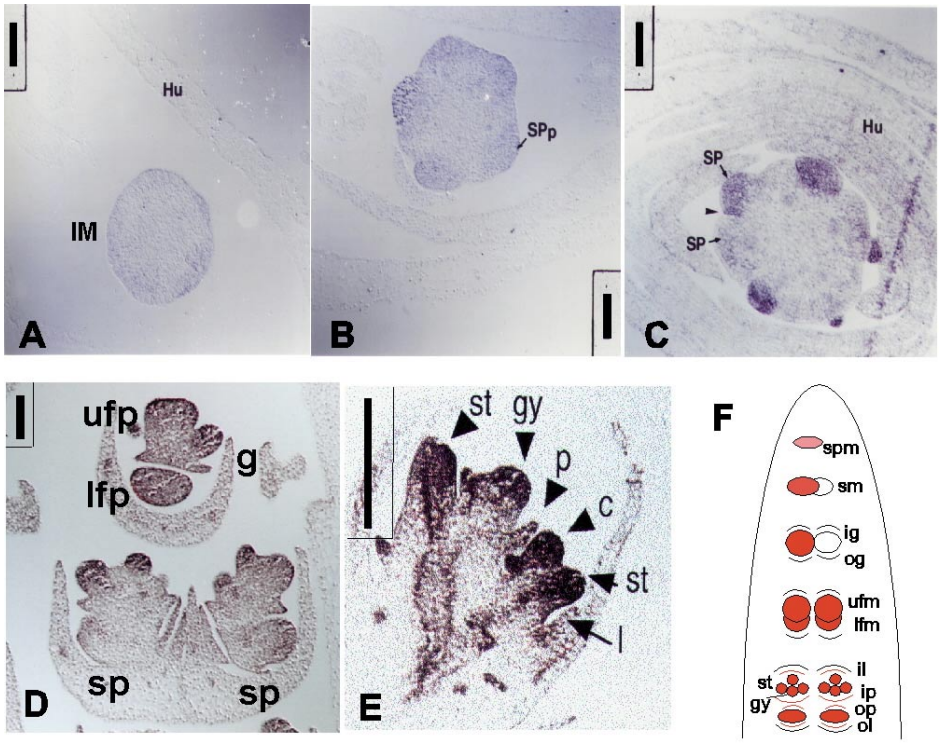

Fig.1.4. Expression of *ZMM6* transcript in inflorescences, as revealed by *in situ* hybridization with digoxigenin-labeled antisense riboprobes. Bar=100µm. **A-C.** Transverse cross-sections of female inflorescence, probed with antisense *ZMM6*. **A.** IM shows no expression. **B.** At the early stage A, a weak expression is shown in the spikelet pair primordia (SPp). **C.** At late stage A, expression is only in one spikelet primordium (SP) out of a pair. **D.** Tangential section through female spikelets at stage F, showing expression in both upper and lower floret, but not in the glumes (g). Both spikelets of a pair show *ZMM6* expression. **E.** Median longitudinal section through a male spikelet at stage H. Expression is visible in all floral organs, of upper and lower floret primordia. **F.** Schematic representation of *ZMM6* expression (in red) during inflorescence development at stage A-F. SPp=spikelet pair primordium, SP=spikelet primordium, ufp=upper floret primordium, lfp= lower floret primordium, g=glume, Hu=husk leaf, remaining abbreviations as fig1 & 2. (After Cacharrón, 1994).

After the differentiation of the spikelet meristems to floret meristem, *ZMM6* is continuously expressed in only one spikelet, at the same level in the upper as well as the lower floret inititials, but not in the glumes (stage A to C). In later stages the gene is also turned on in the second spikelet primordium, except in the glumes (stage D and further). *ZMM6* is then expressed in all floral organs.

The gene *ZMM8* is strongly expressed in both male and female inflorescences (Cacharrón, 1998; Cacharrón *et al*., 1999). Expression is turned on starting at stage D, after the spikelet meristem has produced the two floret primordia. *ZMM8* transcript is only detected in the upper floret initial. Expression is shown equally in all floret organs, but not in the two glumes surrounding them. The gene continues to be transcribed in only the upper floret primordia, throughout the development of the floret initials (stage H and further). The sustained absence in the lower floret primordia, therefore does not seem to be linked to the delayed development of this primordium. *ZMM8* is a developmental marker, discriminating between the upper and the lower floret primordia.

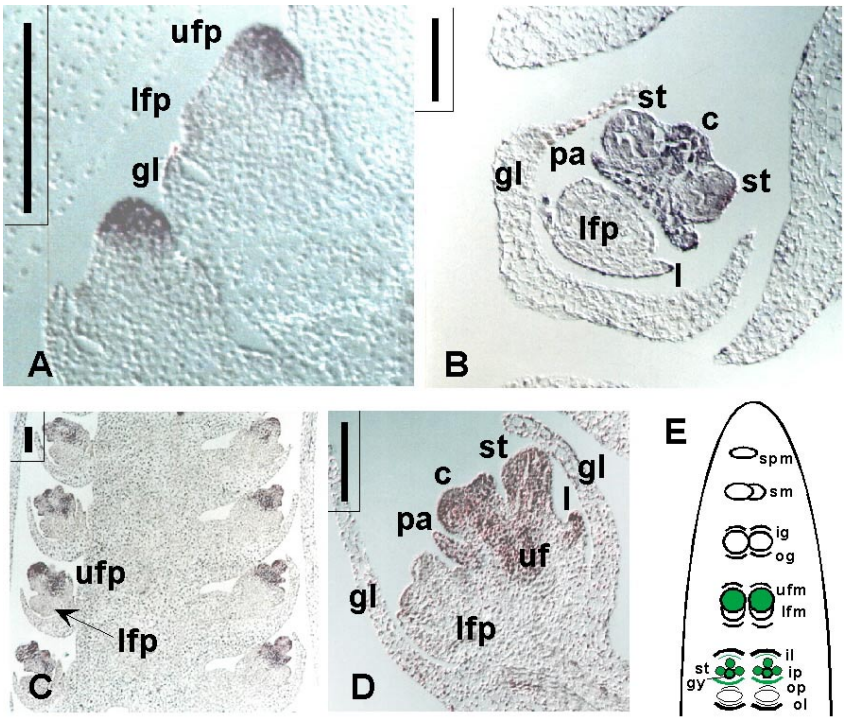

Fig.1.5. Expression of *ZMM8* transcript in inflorescences, as revealed by *in situ* hybridization with digoxigenin-labeled antisense riboprobes. **A-D.** Probed with antisense *ZMM8.* Bar= 100µm. **A.** Median longitudinal section through female spikelets at stage D, when expression in upper floret primordium starts. **B.** Transverse cross-section through a male spikelet at stage F. **C.** Median longitudinal section through female inflorescence at stage F. **D.** Close-up of a median logitudinal section through a male spikelet primordium at stage H, showing a continuation of expression in only the upper floret primordium. **E.** Schematic representation of *ZMM8* expression (in green) during inflorescence development at stage A-F. Abbreviations as in fig 4. (After Cacharrón *et al*., 1999).

This thesis describes a functional characterization of the *AGL2*-like genes *ZMM6* and *ZMM8*. Based on the present day models of grass inflorescence architecture, these genes may exert their function at the respective meristem levels in which they are expressed. This may indicate that *ZMM6* might act during the transition of spikelet pair meristem to spikelet meristem, since it is differently expressed during development in the two spikelet primordia. *ZMM6* may confer sessile versus pedicellate spikelet identity, or regulate the level of determinacy of the spikelet pair primordium, ordering the SPM to stop initiating more spikelet primordia. A similar way of reasoning holds for *ZMM8*, that might exert its function during the transition of spikelet meristem to floral meristem. *ZMM8* might confer upper floret identity to the meristem, thereby preventing it from being aborted as the lower floret is. Another hypothesis is that it might determine the degree of determinacy of the spikelet meristem, signaling to the meristem to stop initiating more floret primordia. Alternatively or additionally, *ZMM6* and *ZMM8* may function as E-function genes that allow the B- and C- function genes to confer the identities to the respective whorls. As no candidate mutants mapped at the genomic loci of these genes (Neuffer *et al*, 1997), a transgenic approach was taken to reveal the function.

The *AGL2*-like gene family in maize is more analyzed in detail by a screen to obtain genomic clones of this family and by a structural characterization of the total genomic sequence of a few members. Furthermore, a new member of the subfamily is presented and analyzed phylogenetically to reveal how it relates to the other members.

2 MATERIAL AND METHODS

2.1 Materials

2.1.1 Media

All media were autoclaved before use.

LB	1 % (w/v) NaCl, 1 % (w/v) bactotryptone, 0.5 % (w/v) yeast extract.
NZY	0.5 % (w/v) NaCl, 0.2 % (w/v) $MgSO_4$, 0.5 % (w/v) yeast extract, 10 % (w/v) NZ Amine (casein hydrolysate), adjusted to pH=7.5 with NaOH.
NZY plates	NZY broth with 1.5% (w/v) agar.
NZY top agar	NZY broth with 0.7 % (w/v) agarose.

2.1.2 Buffers

Denhardt´s (5x)	0.1 % (w/v) BSA, 0.1 % (w/v) Ficoll, 0.1 % (w/v) PVP.
SSC (20x)	3 M NaCl, 0.3 M sodium citrate.
SM	0.58 % (w/v) NaCl, 0.2 % (w/v) $MgSO_4$, 50 mM Tris-HCl (pH 7.5), 0.01 % gelatin.
TBE (1x)	0.9 M Tris-HCl, 0.9 M boric acid, 25 mM EDTA.

2.1.3 Cloning vectors

pGEM-T	(Promega)
pBluescript II KS(+)	(Stratagene)
pRT104	(Töpfer *et al*., 1993)
pAHC25	(Taylor *et al*., 1993)
pK225	(Dr. Thompson lab)
p35SAcS/GCM5::GUS	(Dr. Thompson lab)

2.1.4 Plant material

Zea mays ssp. *mays* L. cv. A69Y+ (backcross partner)
Zea mays ssp. *mays* L. cv. B73+ (backcross partner)
Zea mays ssp. *mays* L. cv. HE89+ (for transformation purposes)
Zea mays ssp. *mays* L. cv. T232+ (for cloning purposes)
Zea mays ssp. *mays* L. , Ts6/ts6 x ts6/ts6 segregating population (MGSC stock 116 I)

2.1.5 *E. coli* strains

DH10B:	*F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80dlacZΔM15 ΔlacX74 deoR recA1 endA1 araD139 Δ(ara leu)7607 galU galKλ⁻rpsl nupG* (GIBCO BRL)
XL1-Blue MRA:	*Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 gyrA96 relA1 lac* (Stratagene)

2.2 Methods

2.2.1 Plasmid DNA isolation

Plasmid DNA was isolated via the ´alkali lysis minipreparation´ protocol (Sambrook *et al*., 1989). Sequencing grade DNA was obtained with the Qiagen mini-plasmid kit. DNA for plant transformation was obtained in bulk amounts with the Qiagen midi- and maxi plasmid kit, followed by an additional purification with phenol/chloroform and subsequent isopropanol precipitation, ethanol wash and resuspension in de-ionized water (MQ).

2.2.2 Genomic library screening

A genomic library containing DNA of maize inbred line T232, cloned into phage ´Lambda DASH II vector´ (Stratagene) within the *Bam*H I restriction site, was kindy provided by prof.dr. G. Theissen. The titer was $2.5*10^6$ plaque-forming units (pfu) per ml. Isolation of genomic clones was performed according to the protocol of the Lambda DASH II/*Bam*HI vector kit (Stratagene).

Host cells of *E. coli* strain XL1-Blue MRA were grown for 4 to 6 hrs at 37°C in LB with 0.2% (v/v) maltose and 10 mM $MgSO_4$ up to an OD_{600}=1.0. The bacteria were pelleted at 2000 rpm for 10 minutes, and subsequently resuspended in twice the volume of sterile 10 mM $MgSO_4$ to OD_{600}=0.5. Aliquots of 6 µl of the library suspension containing 15,000 pfu were mixed with 200 µl host cells and incubated for 15 minutes at 37 °C under gentle shaking. Then 3 ml of NZY top agar of 48 °C was added and the mixture was plated on NZY plates of 90 mm. The plates were incubated at 37 °C for 8 hrs, and chilled for 2 hrs at 4°C. The plaques were transferred to a nitrocellulose membrane for 2 minutes. The orientation of the membrane to the plate was marked by sticking with a needle a few wholes it. After lifting it, the membrane was denatured by submerging it in 1.5 M NaCl + 0.5 M NaOH for 2 minutes. Subsequently the membrane was neutralized for 5 minutes in 1.5 M NaCl + 0.5 M Tris-HCl (pH 8.0). Then the membrane was rinsed in 0.2 M Tris-HCl + 2 x SSC (pH 7.5) for 20 seconds, and briefy blotted onto Whatmann 3MM paper. The DNA was crosslinked to the filter with a UV crosslinker (Stratagene) by applying 120,000 $\mu J*cm^{-2}$ of energy. The filter was baked in an oven at 80 °C for 2 hrs.
The filters were prehybridized, hybridized and washed under non-stringent conditions with labelled probe as described below prior to exposure to an X-ray film. Positive clones were picked after proper orientation of the film to the plate by using a cut-off blue tip. The clones were transferred to 1 ml SM + 20 µl chloroform and vortexed. The suspension was 100 fold diluted in SM buffer. Of this 2 µl was used to re-infect 200 µl of host cells as described above for the second screening. The whole procedure was usually repeated for a third time as most clones were too close to the background plaques.

2.2.3 Phage lambda DNA isolation

The prelysate was made by infecting 25 µl host cells with 250 µl plaque isolate. The mix was incubated for 15 minutes at 37 °C before 1 ml NZY was added. Then it was grown overnight at 37 °C and 150 rpm. 100 µl prelysate was mixed with 100 µl host cells and 300 µl SM. The mix was incubated for 15 minutes at 37 °C and 25 ml NZY was added. This was incubated overnight at 37 °C and 150 rpm. From this lysate 10 ml was mixed with 5 µl RNaseA (20 mg/ml) + 4 µl DNase (50 mg/ml) in corex tubes and incubated for 3 to 4 hrs at 37 °C. Then 5 ml PEG_{6000} + 1.5 M NaCl was added and after mixing it, it was incubated for 1hr on ice. Subsequently it was

centrifuged for 10 minutes at 4 °C at 10,000 rpm. The phage pellet was resuspended in 1 ml TE (pH 8.0) and transferred to 2ml Eppendorf tubes. To this 40 µl 0.5 M EDTA + 20 µl 5 M NaCl + 50 µl 10% Triton X-100 was added. After mixing it, it was incubated for 15 minutes at 70 °C. Then the DNA was extracted with 1 volume of phenol/chloroform, and subsequently with 1 volume of 24:1 chloroform/isoamylalcohol. The DNA was precipitated with 1 volume of isopropanol, centrifugated for 15 minutes at 13,000 rpm and washed in 1 volume of 70 % ethanol. The pellet was dried in the air and dissolved in 50 µl TE (pH 8.0).

2.2.4 Plant genomic DNA isolation

Genomic DNA was extracted from maize plants by a protocol derived from the method of Junghans and Metzlaff (1990). 3 g of leaf material was ground by mortar and pestle in liquid nitrogen to a fine powder. 15 ml DNA extraction buffer consisting of 100 mM Tris (pH 8.0), 500 mM NaCl, 50 mM EDTA (pH 8.0) with freshly added β–mercaptoethanol was poured onto the powder. Then 2 ml of 20 % SDS was added and the mixture was gently stirred and incubated for 10 minutes at 65 °C before 5 ml of 5 M potassium acetate was added. This was gently mixed and incubated for 20 minutes on ice. After centrifugation for 15 minutes at 5,000 rpm the supernatant was collected through a miracloth and transferred to a new tube. The sample was treated with 10 µl RNaseA (100 mg/ml) for 30 minutes at 37 °C. Then 1 volume of phenol/chloroform was added and the extraction was shaken. Subsequently it was centrifuged at 5,000 rpm for 10 minutes and transferred to a new tube. The 1 volume of chloroform was added. After gentle shaking the mixture was centrifuged at 5,000 rpm for 10 minutes. The DNA in the supernatant was precipitated with 1 volume of isopropanol and centrifuged at 10,000 rpm for 30 minutes. The pellet was rinsed in 70 % ethanol and subsequently dried at room temperature for 30 minutes. The DNA was dissolved overnight in 1 ml TE (pH 8.0). Extracted DNA was quantified by comparison of band-intensity on ethidium bromide stained agarose gels with a DNA molecular weight marker.

2.2.5 DNA digestion, separation and blotting onto membranes

10 µl purified DNA was digested to completion and subsequently loaded and separated overnight via electrophoresis in 0.7 % agarose gels with 0.5 x TBE buffer. The DNA was depurinated with 0.125 M HCl for 10 minutes. The gel was soaked for 45 minutes in denaturing solution (1.5 M NaCl + 0.5 M NaOH) and then for 30 minutes in neutralizing solution (1.5 M NaCl + 1 M Tris-Cl, pH 7.5). After washing in 20 x SSC, the DNA was transferred to a Nylon N membrane (Amersham) according to the standard capillary transfer procedure (Sambrooke *et al*., 1989). The DNA was crosslinked to the filter with a UV crosslinker (Stratagene) by applying 120,000 µJ*cm^{-2} of energy. The filter was baked in an oven at 80 °C for 2 hrs.

2.2.6 Random prime labelling

Probes were prepared from purified DNA fragments that have been isolated from agarose gel using the gel purification kit (QIAGEN). The labelling was caried out in 30 µl of the random prime labelling mix described below. The reaction was incubated for 4 hrs at RT. The probe was purified with Qiagen PCR purification Kit and denatured by boiling for 5 minutes and subsequently chilled on ice directly before use.

random prime labelling mix

20 µl denatured DNA (50 ng)
3 µl oligo labelling mix (10 x)
1 µl 100 x BSA (DNase free)
5 µl [α^{32}P]dCTP (10µCi/µl)
1 µl Klenow polymerase (2 U/µl)

10x oligo labelling mix

2.5 µg/ml pd $(N)_6$
0.5 M Tris-Cl (pH7.5)
0.1 M $MgCl_2$
0.5 M dATP, dTTP, dGTP
70 mM β-mercaptoethanol

2.2.7 Hybridization of DNA

Pre-hybridization and hybridization were carried out in hybridization solution in glass tubes (30 cm x 4 cm) at 65 °C (if under non-stringent conditions, at 58 °C) under continuous rotation in a hybridization oven (Bachofer, Reutlingen, Germany). The pre-hybridization was performed for at least 4 hrs. Upon adding the denatured radio-active probe, the hybridization was performed for at least 16 hrs.

After hybridization the filter was washed accordingly:

1. twice 50 ml 2 x SSC + 0.1 % SDS at RT for 10 minutes
2. twice 50 ml 1 x SSC + 0.1 % SDS at 65 °C for 10 minutes
3. once 50 ml 0.1 x SSC + 0.1 % SDS at 65 °C for 15 minutes

The filter was wrapped in thin plastic foil (Saran film) and exposed overnight to a Kodak X-ray film (X-Omat AR) in a cassette with Trimax intensifying screen at –80 °C.

hybridization solution

5 x Denhardt`s solution
5 x SSC
0.1 % (w/v) SDS
100µg/ml Herring sperm DNA

The probe was removed from the blot for subsequent re-hybridization by triple incubation at 65 °C for 30 minutes with stripping solution (5 mM Tris-HCl (pH7.5), 2 mM EDTA, 0.1 % SDS, 0.01 % Ficoll, 0.02 % PVP).

2.2.8 Polymerase Chain Reaction (PCR)

PCR reactions were performed in 50 µl reaction volume in a heating-lid thermo-cycler (Biometra, Göttingen). PCR reactions with the *Taq*-system were performed in order to screen for positive clones or transgenic plants. For cloning and subsequent sequencing purposes the Expand high-fidelity (HF) PCR system (Roche) was used in combination with a manual hot-start. 5 ng plasmid and lambda DNA was used as template, whereas for genomic DNA 200 ng was used.

Taq-reaction:

10 x *Taq* buffer	5 µl
dNTPs (10 mM each)	5 µl
primer forward (10 pM)	0.5 µl
primer reverse (10 pM)	0.5 µl
MQ water	37.5 µl
DNA	1.0 µl
Taq-pol. (5 U/µl)	0.5 µl

Taq-conditions:

1. 96 °C 2 min.
2. 96 °C 15 sec.
3. 58 °C 15 sec.
4. 72 °C 1 min/kb

(30 cycles)

5. 72 °C 5 min.
6. 15 °C ∞

HF-reaction:

10 x HF buffer	5.0 µl
dNTPs (1 mM each)	2.5 µl
primer forward (1pM)	0.75 µl
primer reverse (1pM)	0.75 µl
MQ water	39.25 µl
DNA	1.0 µl
Polymerase-mix (3.5 U/µl)	0.75 µl

HF-conditions:

1. 96 °C 2 min.
2. 94 °C 15 sec.
3. 58 °C 15 sec.
4. 68 °C 45 sec/kb

(10 cycles)

5. 94 °C 15 sec.
6. 58 °C 15 sec.
7. 68 °C 45 sec/kb+10 sec/cycle

(20 cycles)

8. 68 °C 7 min.
9. 15 °C ∞

2.2.9 Isolation of RNA

Total RNA extraction was performed using the RNeasy plant minikit (Qiagen) according to the manufacturer´s protocol. Tassel and ear material was harvested in the latest stages of development (J-K) as described by Cheng *et al.,* 1983. Extracted RNA was quantified by comparison of the intensity of the rRNA fraction on ethidium bromide stained agarose gels and by absorbance at λ = 260 nm.

2.2.10 Northern analysis

Equipment was rinsed thoroughly with DEPC treated water to avoid contamination with RNases. DEPC was added to 0.1 % (v/v), and left to stirr overnight prior to autoclaving. 20 µg total RNA was mixed with one fourth volume of 5 x RNA loading buffer, heated to 60 °C in a waterbad for 10 minutes and cooled on ice before loading. The samples were separated on a 1.2 % (w/v) denaturing agarose gel (1.2 g agarose, 700 µl formaldehyde, 10 ml 10 x FA buffer, 0.5 µl (1 mg/ml) ethidium bromide in 100 ml MQ) in 1 x FA buffer (see below).

The gel was rinsed twice in water for 2 minutes and then soaked in 20 x SSC for 20 minutes. The RNA was transferred to a Biodyne B membrane (PALL) according to the standard capillary transfer procedure (Sambrooke *et al*., 1989). The RNA was crosslinked to the filter with a UV crosslinker (Stratagene) by applying 120,000 µJ*cm^{-2} of energy. The filter was baked in an oven at 80 °C for 30 minutes.

10 x FA buffer

200 mM MOPS
50 mM NaAc
10 mM EDTA
set to pH=8.0
with 10 N NaOH

5 x RNA loading buffer

2.7 % (v/v) formaldehyde
20 % (v/v) glycerol
30 % (v/v) de-ionized formamide
40 % (v/v) 10x FA buffer
0.05 % (w/v) bromophenol blue
4 mM EDTA

2.2.11 Hybridization of RNA

Pre-hybridization and hybridization were performed in hybridization solution in glass tubes (30 cm x 4 cm) at 42 °C under continuous rotation in a hybridization oven (Bachofer, Reutlingen, Germany). The pre-hybridization was performed for at least 2 hrs. After adding the denatured radio-active probe, the hybridization was performed for at least 16 hrs. The same procedure was used for labelling as for hybridization of DNA.

After hybridization the filter was washed accordingly:

1. twice 50 ml 2 x SSC, 0.1 % SDS at RT for 10 minutes
2. twice 50 ml 0.2 x SSC, 0.1 % SDS at 42 °C for 10 min.

After washing the filter was kept from drying. Detection of radio-active signals and stripping were performed as described in §2.2.7.

hybridization solution

100 µg/ml Herring sperm DNA
50 % formamide
5 x SSC
5 x Denhardt`s solution
0.1 % (w/v) SDS

2.2.12 Rapid amplification of genomic ends (RAGE)

Flanking DNA to partial genomic clones and/or regulatory elements of unknown sequences were unidirectionally amplified from genomic DNA via a method derived from the Genome Walker kit (Stratagene). Genomic DNA was digested to completion with a specific restriction enzyme creating either a blunt end or a 5‘ overhang. The overhang of a 100 µl restriction digest of 2 µg genomic DNA was then filled-in by adding 1 µl 3,5 U/µl Klenow polymerase and 1 µl 10 mM dNTPs and by incubating at 37 °C for 30 minutes. The blunt end genomic fragments were purified via PCR spin columns (QIAGEN) and then ligated to an adaptor made up of the following 2 primers: W620 and W621 (App.7.1). The adaptor was created by annealing the 2 primers in equimolar concentrations in 1 x PCR *Taq* buffer and slowly cooling down the mixture from 80 °C to RT. The ligation mix was spin column purified. Nested PCR with the high-fidelity PCR system (see above) was performed with 2 gene specific primers and the adaptor primers W622 and W623 (nested one; App.7.1). The first PCR sample was spin column purified to remove the first set of primers and then diluted 50 fold in 10 mM Tris-HCl (pH 8.0). Of this, 1 µl was used for the ultimate amplification with the nested set of primers.

2.2.13 Electro-competent cells

E. coli DH10B cells were grown overnight at 37 °C from a glycerin stock in LB medium without antibiotics. This was used to grow a 300 ml LB overnight culture. Of this culture approximately 25 ml was used to start a 500 ml culture of OD_{600} = 0.2 that was grown at 16 °C. The culture was stopped after about 7 hrs when the OD_{600}=0.4. The cells were pelleted for 10 minutes at 5,000 rpm at 4 °C, and resuspended in pre-cooled 350 ml MQ water. The suspension was centrifuged for 10 minutes at 5,000 rpm at 4 °C, and resuspended in pre-cooled 250 ml MQ water. The centrifugation and subsequent resuspension were repeated in increasingly smaller volumes of 50 ml, 25 ml and 5 ml. Finally the cells were resuspended in 800 µl 7 % DMSO and alliquotted in 50 µl batches that were snap-freezed in liquid nitrogen.

2.2.14 Cloning and subcloning

The DNA fragment to be cloned was purified after restriction digestion from agarose gel using the gel purification kit (QIAGEN) The plasmid pBluescript (S/K+) into which it was to be cloned was linearized with the same restriction enzyme. 2 µg vector was dephosphorylated with calf intestine alkaline phosphatase (CIAP) (Pharmacia Biotech) to prevent self-ligation with 0.2 Weiss units by a 30 minute incubation at 37 °C in a buffer consisting of 10 mM MgAc + 10mM Tris-Ac + 50 mM KAc. The phosphatase was heat inactivated at 85 °C for 15 minutes. The vector was then gel purified in the same way as the insert DNA. Alternatively, if the DNA fragment was a PCR fragment, it was cloned into pGEM-T (Promega), with neighter restriction digestion, nor dephosphorylation. 100 ng insert was ligated into 50ng vector in 10 µl T4 DNA ligase buffer (30 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP) using 3 Weiss units T4 DNA ligase at 16 °C overnight. The ligation mix was precipitated with 3 volumes ethanol after adding a 10^{th} volume 3 M NaAc (pH 5.2). The pellet was washed with 70% ethanol, dried in the air for 15 minutes and dissolved in 2 µl MQ water. A `gene pulser` cuvette (0.1 cm gap) (Biorad) was pre-cooled on ice. Then 1 µl ligation mix was added to a batch of 50 µl electro-competent cells that was slowly thawn on ice. This was transferred to the voltage cubicule of a Biorad electroporator and a discharge of 1.8 kV was given for 4.5 mseconds. Immediately 500 µl LB without antibiotic was added and the cells were incubated for 2 hrs at 37 °C. Then they were plated out on LB medium containing 100 µg/ml ampicillin for plasmid selection and 0.5 mM IPTG + 80 µg/ml X-

gal for blue-white screening for insert selection. Single colonies were grown overnight for subsequent PCR selection prior to verification via sequencing. Fragments were agarose gel purified and cloned into pGEM-T.

2.2.15 Cloning and transformation of the *ZMM6* and *ZMM8* constructs for anti-sense and over-expression in maize

A full length cDNA fragment of *ZMM6* was amplified from clone pBLUE/ZMCDK5 with the high-fidelity PCR system using primers M601 and M602 to facilitate cloning of the construct for over-expression (for primer sequences, see App.7.1). Primer M601 contained a restriction site for *Nco*I in order to conserve the ATG start codon of the gene. M602 had a restriction site for *Eco*RI at the 5´end. The primers were placed between bp-15-7 and bp756-769 respectively, relative to the start codon. The full length *ZMM6* fragment was cloned after digestion with the respective enzymes into the plasmid pRT104 between the CaMV 35S promoter and the NOS-terminator of transcription (*NOS*-ter) for constitutive expression (Töpfer *et al*., 1993). The use of *Nco*I ensured a translational fusion between promoter and coding sequence in order to obtain a functionally active protein *in planta* upon translation.

The construct for anti-sense expression of *ZMM6* was made by cloning in reverse orientation the 3´part of the cDNA clone pBLUE/ZMCDK5 encoding the IKC-domains (see §1.2). The MADS-box was excluded in order to prevent the possible down regulation of other highly similar genes. Cloning followed an analogous PCR amplification step as described above, using primers A601 and A602. The 5´end of primer A601 contained a restriction site for *Eco*RI, primer A602 introduced with a *Nco*I restriction site a novel ATG start codon. The primers were placed between bp173-199 and bp740-766 respectively, relative to the start codon of pBLUE/ZMCDK5. The fragment was cloned into the multiple cloning site of pRT104 between the CaMV 35S promoter and the *NOS*-terminator.

The casettes harbouring sense and anti-sense fragments of *ZMM8* were cloned into pRT104 using the same PCR-based approach. The fragments were amplified from the cDNA clone pGEM/SW24. For the sense construct driving the over-expression of *ZMM8*, the primers M801 and M802 were used, having a restriction site of *Bal*I (=*Mlu*NI) and *Bam*HI, respectively. The *ZMM8* antisense fragment, lacking the MADS-box, was amplified using A801 and A802, having

a *Xba*I and a *Nco*I restriction site respectively. The positions of the four primers M801, M802, A801 and A802 are, relative to the start codon of clone pGEM/SW24, bp-19-8, bp760-786, bp192-220 and bp610-635.

As a control an antisense construct of the *ZMM15* gene was used. *ZMM15* is a member of the maize *SQUA*-like subfamily of MADS-box genes (Cacharrón, 1998). An antisense fragment was amplified from clone pGEM/JC3 using primers aJ31 and aJ32, positioned between bp182-213 and bp725-751 from the ATG codon. The fragment was cloned into pRT104 into *Eco*RI and *Nco*I.

The cassettes, consisting out of promoter-ORF-terminator, were in turn cloned into the plant transformation vector pK225 using the unique restriction site *Hin*dIII (fig.2.1). The plasmid pK225 is a derivative of pAHC25 (Taylor *et al*., 1993) containing a small DNA fragment insertion that enlarges the multiple cloning site (Dr. R. Thompson, pers. comm.). Additionally, the plasmid pK225 contained the ubiquitin (UBI) promoter (Christensen *et al*, 1992) for constitutive expression of the *BAR* gene as a selectable marker for transgenic plantlets.

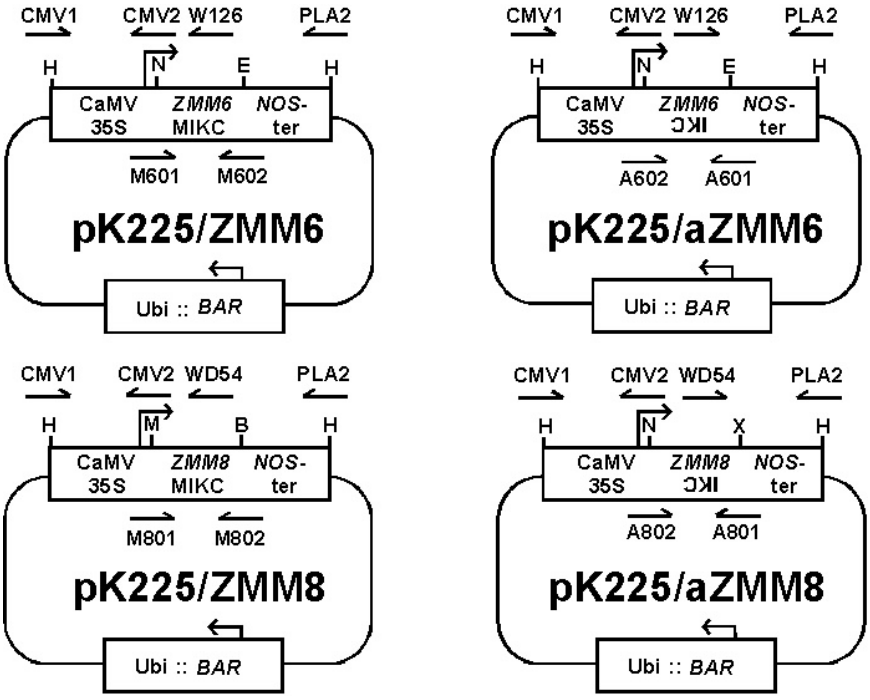

Fig.2.1. Structure of the sense and anti-sense *ZMM6* and *ZMM8* contructs for maize plant transformation. H=*Hin*dIII, N=*Nco*I, E=*Eco*RI, M=*Mlu*NI, X=*Xba*I. Arrows indicate primers. Bend arrows indicate transcription start point.

The *BAR* gene encodes phosphinotricin acetyl transferase (PAT), a protein that inactivates phosphinotricin, the active component of the herbicides Bialaphos and Basta (Thompson *et al*., 1987). After the cloning procedure, the cassettes were sequenced to ascertain that the PCR step involved did not introduce any base pair exchanges. Furthermore, for each construct a clone was picked in which the MADS-box gene cassette was inserted *in trans* with the *BAR* gene. In this way a possible interference of transcription was avoided stemming from a back-to-back positioning of the CaMV 35S and UBI promoter.

In addition to the antisense *ZMM15* construct, a empty pK225 plant transformation vector was used as a control. Furthermore, to increase the number of controls for comparison, inflorescences of plants with the maize p35SAcS/GCM5::*GUS* construct were kindly provided by Dr. R. Thompson & R. Bhat. This is a pAHC25 derivative in which the *BAR* gene is fused with the CaMV 35S promoter, and *GUS* expression is driven by the promoter of the un-related, non-MADS-box gene *GCM5* involved in chromatin deacetylation (Dr. R. Thompson, pers.comm.).

PEG-mediated transformation of maize embryogenic protoplasts of line HE89+ was performed in Dr. Steinbiss´ group at the Max Planck Institute Institute for Breeding research (MPI), Cologne with the sense and anti-sense constructs, and with sense constructs only at Hoechst AgrEvo, Frankfurt (Mórocz *et al*., 1990). Transformed calli were obtained after selection with the herbicide Basta, after which a regeneration step lead to Basta resistant plantlets. All plants obtained from Hoechst AgrEvo were already in the first daughter generation T_1, back-crossed to line B73+. The MPI T_0-plants were back-crosses to line A69Y+ for further functional analysis of the phenotype with a larger sampling, and to investigate the inheritance of the transgene.

2.2.16 Crosses and back-crosses

Controlled pollinations of maize female inflorescences were performed based on protocols described at http://www.agron.missouri.edu/IMP/WEB/pollen.htm. The transgenes were rescued into the next generation in order to observe the inheritance of the trait. The ear shoots were bagged prior to the emergence of silks from the husk leaves to avoid contamination. Then, one day old silks were cut back with a knife for pollination the next day. The old pollen was released from the tassel before bagging the previous night and discarded. In the next morning viable pollen was applied to the silks within 10 minutes after collection.

2.2.17 Mapping with Recombinant Inbred (RI´s)

Mapping was performed based on a RFLP (restriction fragment length polymorphism) found between parental alleles in recombinant inbreds (RI´s) (Burr & Burr, 1991). An RFLP was obtained after Southern analysis of a restriction digestion of genomic DNA of the RI plants. RI´s are derivatives of a segregating F_2 population of inbred parents, in which linked blocks of parental alleles are essentially fixed. Two families were used for mapping, that are called after the parents used in the cross, TxCM (for T232 x CM37) and $COxT_x$ (for CO159 x T_x303). The parental allele distribution pattern, as shown by the polymorphic bands of the RFLP, is compared to the database at the Brookhaven National Laboratory (http://burr.bio.bnl.gov/acemaz.html) in collaboration with Prof. B. Burr to reveal the map position, relative to previously mapped markers. The mapping filters were kindly provided by Prof. Dr. G. Theißen.

2.2.18 Computer & sequence analysis

DNA sequencing was performed at the MPI-sequencing unit (ADIS) with an automatic DNA sequencer (model 377, Applied Biosystems). Sequences were analyzed on a `DEC Alpha Workstation` with help of sequence analysis programs of the Wisconsin Packet Version 8.0 from GCG (Genetics Computer Group, Wisconsin). Multiple protein sequences were compared with Clustal W (Thompson *et al*., 1994) from the MacVector™ Packet Version 6.0. Blast searches were performed in the WWW at the NCBI server (http://www.ncbi.nlm.nih.gov) (Altschul *et al*., 1990).

2.2.19 Morphological analysis and photographical dataprocessing

Pictures were taken with assistance of Mrs. Kalda, MPI-photo Laboratory. Photos were scanned, processed and assembled using Adobe Photoshop 5.5 (Adobe Systems Inc.) and Canvas 6.0 (Deneba Systems Inc).

2.2.20 Quantitative and statistical analysis

Phenotypic values for all characters, that were morphologically different from the wild-type as defined in §1.4, of transgenic and control plants were arranged in Excel (Microsoft). Absolute phenotypic values were converted into phenotypic ratios to take into account the different numbers of spikelets per inflorescence due to a difference in inflorescence size. Phenotypic ratios were calculated by dividing the absolute number of morphologically different structures with the absolute numbers of spikelets per inflorescence. The standard deviations of the means of the phenotypic ratios per trait were calculated in Excel as described by Kesel and co-workers, 1999. The Excel files containing the phenotypic ratios were loaded into the statistical program SPSS for statistical evaluation (Voß, 2000). As the analysis of the means and their standard deviations suggested that the occurrence of the phenotypes displayed a non-normal distribution, the comparisons of the groups of plants were statistically evaluated using the non-parametrical Mann-Whitney (U)-test (Kesel *et al*., 1999; Voß, 2000). The starting hypothesis was H_0: $\mu 1=\mu 2$. $\mu 1$ consists of the trait of the batch of control plants, whereas $\mu 2$ consists of that trait in either the sense or the anti-sense plants of either *ZMM6* or *ZMM8* transformed plants in the T_0. The starting hypothesis was overthrown when the probability of the asymptotic significance p was $0 \leq p \leq 0.05$ (Kesel *et al*., 1999). In that case, the compared character was not equal between the groups in a statistically significant way.

Furthermore, the quantitative analysis was performed by displaying the phenotypic ratios per group of transgenic plants in a graphic, compared to those of the control group. Only independent lines were included when the number of analyzed plants in that line was more than three. As the phenotypes in the plants showed a non-normal distribution, the strength of the phenotypes is only indicative. The distinctly counted phenotypic ratios were combined with respect to the type of inflorescence meristem that was affected. In the tassel, non-basal monopedicellate spikelet pairs were clustered with non-basal triplets, and 3-floretted spikelets were clustered with 4-floretted spikelets. The height of these mean values indicate how many spikelet pairs or spikelet meristems, respectively were affected. In the ear, the ratios of the triplets were grouped with those of the quadruplets, since these phenotypes both result from a loss of spikelet pair meristem determinacy.

3 RESULTS

3.1 Molecular analysis of *AGL2*-like genes in maize

A molecular analysis of maize *AGL2*-like genes was performed to obtain data that could render information about this subfamily of MADS-box genes. This subfamily has been shown to play an important role in inflorescence development of higher plants by mediating between floral meristem and floral organ identity genes. Most of the previously characterized members stem from dicotyledonous plants that produce simple inflorescences, contrary to the more compound inflorescence structures in grass flowers, such as maize. *AGL2*-like genes in maize have very extraordinary expression patterns suggesting that these genes may have been recruited to establish novel positional information not found within eudicot inflorescences. As the exact expression of these genes is directed by regulatory elements within genomic sequences, such as promoter and first intron, the characterization was started by screening a genomic library. A phylogenetic evaluation, the chromosomal localization and a structural characterization of members of the *AGL2*-like subfamily was performed using sequence information of genomic and cDNA clones. Furthermore, a functional analysis of members of the *AGL2*-like gene subfamily has been performed via a transgenic approach and a candidate gene approach.

3.1.1 Genomic library screen

A wild-type maize (*Zea mays* ssp. *mays* var. T232) genomic library, cloned into phage lambda DASHII/*Bam*HI, was kindly provided by Prof. dr. G. Theißen. The genomic library was screened by plaque hybridization under non-stringent conditions with a radio-actively labeled probe. The probe consisted of a mixture of the 3´part of cDNA fragments from four *AGL2*-like genes. Three maize genes, being *ZMM3*, *ZMM8* and *ZMM24*, were kindly provided by Prof. dr. G. Theißen. Their cDNA fragments were partially amplified with primers $CER_{(RQVT)}2$ and P018 from the respective clones pGEM/WFE030, pGEM/WFE031 and pGEM/WFE068 (App.7.1). The fourth gene was from lily (*Lilium regale*) (*AK21*), and was kindly provided by dr. A. Kanno. Its fragment was partially amplified using the primers P018 and P038 from cDNA clone pGEM/LRM70. The gene fragments were subsequently labeled using the random prime labeling method. Approximately $1{,}8*10^6$ pfu´s were screened in three rounds, and positives plaques having a strong signal were re-plated for removing contaminating clones. The DNA of 70 single

recombinant λ phage clones was isolated. The DNA was used for a restriction digestion analysis by a double digestion with *Eco*RI and *Bam*HI in order to remove the duplicate clones. The restriction digestion pattern was revealed by separation of the restriction fragments via gel electrophoresis (Fig.3.1).

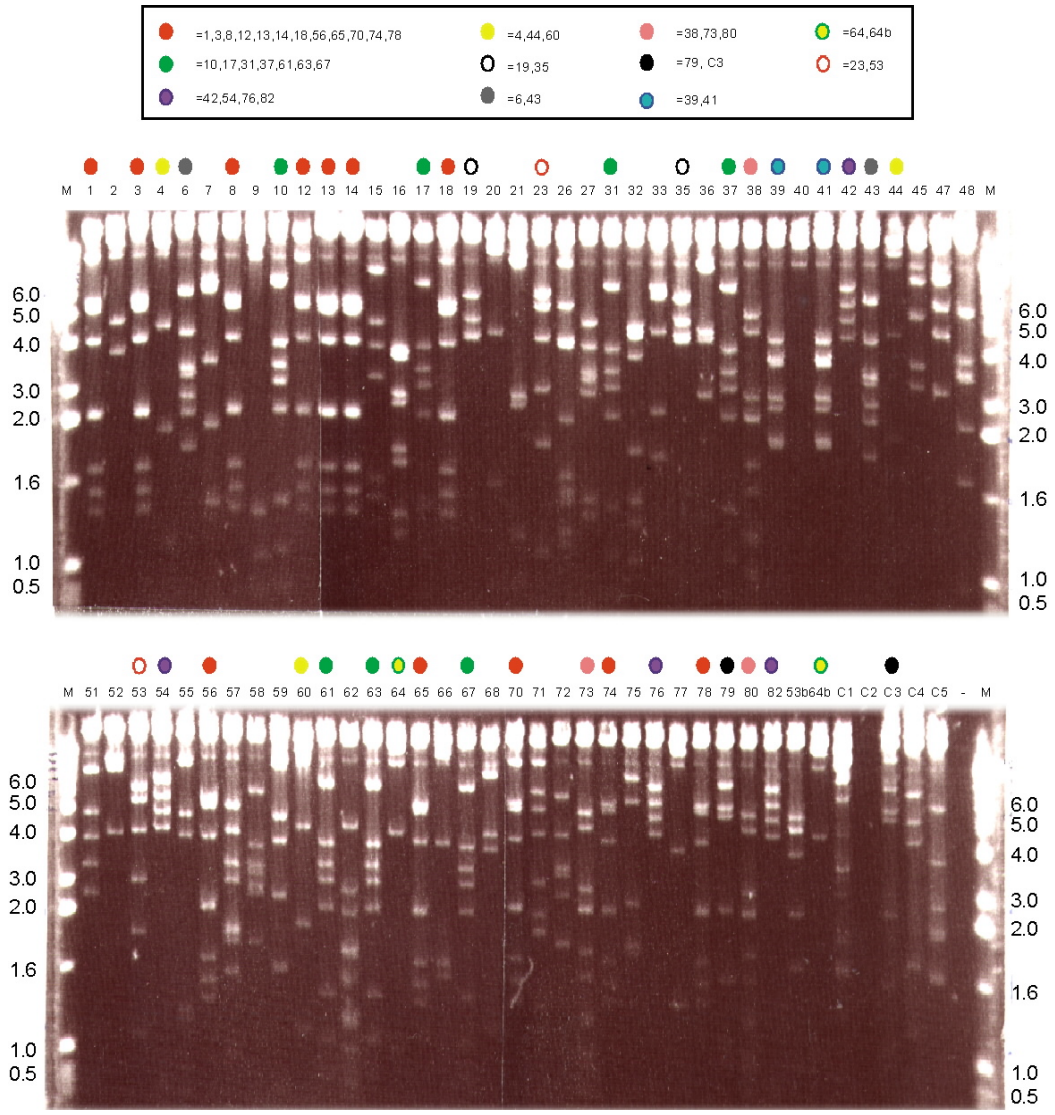

Fig.3.1. Restriction digestion analysis of putative positives from an AGL2-like MADS-box gene genomic screen. Restriction digestion was performed using *Eco*RI and *Bam*HI. Numbers indicate the isolated genomic clones. Colored dots indicate duplicate clones. Marker fragment sizes of the 1.6kb-ladder (lane M) are indicated in kb. Control MADS-box gene genomic clones were kindly provided by prof. dr. G. Theißen (lane C1-C5). (C1-C3, unpubl. results; C4 & C5, Theißen *et al*., 1995). C1=λEMBL4-B1,2N (*ZMM6*, teosinte (*Z. m*. ssp. *parviglumis*)); C2=λDASHII-3-1-1.1 (*ZMM6*, maize); C3=λDASHII-14-2-1 (*B9_20*); C4=λEMBL4-I16b (*ZMM1*); C5=λEMBL4-I17b (*ZAG2*).

Upon comparison of the restriction fragment patterns, 10 clones showed to have been isolated more than once. Additionally, clone λDASHII-wd79 had been isolated before as shown by

comparison with control lane C3 that contains a genomic clone of the gene *B9_20*. In total, 42 genomic clones displayed a unique pattern, indicating these contained independent genomic inserts. In order to look for putative *AGL2*-like MADS-box gene containing genomic clones among these, the gel was blotted onto a nylon membrane for Southern analysis and hybridized under stringent conditions with labeled probes of the eight maize *AGL2*-like MADS-box genes *ZMM3*, *ZMM6*, *ZMM7*, *ZMM8, ZMM14*, *ZMM24, B9_20* and *WFH24*, and of a MADS-box fragment from *ZMM6* (table 3.1).

Table.3.1. Southern blot analysis of restriction digested genomic clone DNA, hybridized with the 3´part of cDNA´s of the AGL2-like genes *ZMM3*, *ZMM6*, *ZMM7*, *ZMM8, ZMM14*, *ZMM24, B9_20* and *WFH24*, and with the MADS-box of *ZMM6* (indicated as ´MADS´). The genomic clones are placed in rows, the different cDNA probes are aligned per column. A positive signal is indicated as a ´+´, a weak signal as ´+/-´, no signal is left blank.

clone	MADS	*ZMM3*	*ZMM6*	*ZMM7*	*ZMM8*	*ZMM14*	*ZMM24*	*B9_20*	*wfh243*
1							+		+
2					+				
4	+	+	+	+	+		+	+	
6	+	+	+	+			+	+	
7									
9	+	+	+	+	+		+	+	
10	+	+	+	+	+		+	+	
15							+		
16	+		+						
19	+		+				+	+	
20	+/-	+	+				+	+	
21	+		+						
23	+	+	+	+	+		+	+	
26									
27	+/-								
32									
33	+	+	+				+	+	
36	+						+	+	
38	+/-						+		
39									
40									
42	+		+	+	+			+	
45									
47									
48									
51	+	+	+		+	+		+	
52									
55							+		
57	+	+	+	+	+		+	+	
58	+		+	+			+	+	
59	+	+	+		+	+	+	+	
62									
64	+	+	+	+	+		+	+	
66									
68									
71	+		+		+		+	+	
72	+		+	+			+	+	
73	+/-						+		
75									
77	+/-	+	+	+			+		
79	+		+	+	+		+	+	
53b									

As to the genes *ZMM3*, *ZMM8* and *ZMM24*, the fragments for labeling were produced as described above. The MADS-box fragment and the 3´part cDNA fragments of *ZMM6*, *ZMM7*, *ZMM14*, *B9_20* and *WFH243* were amplified from the respective cDNA clones pBLUE/ZMCDK5, pBLUE/ZMCDK5, pGEM/WFE023, pGEM/WFI005, pGEM/WFH231 and pGEM/WFH243 by the primer pairs WD02/M602, WD01/WD09, WD01/WD06, W258/W270, WD01/W268 and W257/W263 respectively (App.7.1).

Of the 42 clones, 18 clones showed no signal to the MADS-box probe. Therefore, these could be partial MADS-box gene genomic clones, or no MADS-box genes at all. Some clones were showing no positive signals. These clones, λDASHII-wd7, -26, -32, -39, -40, -45, 47, -48, -52, 62, -66, -68, -75, -53b, most probably do not contain *AGL2*-like MADS-box genes, or even MADS-box genes at all, and were therefore excluded from further analysis. Of the remaining clones, fragments were amplified for sequencing via PCR with the high fidelity EXPAND system. In this way, laborious subcloning procedures could be avoided. In the case a clone showed a positive signal to the MADS-box probe, a MADS-box fragment was amplified using one primer annealing to a highly conserved region in the MADS-box (WD01 or $CER_{(RQVT)}2$) and one primer fitting to either site of the multiple cloning site of the vector phage lambda (LDL1 or LDR1; App.7.1). The conserved region codes for a peptide domain -consisting of the amino acid sequence ´RQVT´- that is present in the majority of the MADS-box genes including members of the AGL2-like subfamily (dr.T. Münster, pers. comm.). In case a genomic clone did not seem to have a (part of the) MADS-box, the whole insert was amplified using LDL1 in combination with LDR1. The same procedure was performed when a clone containing a MADS-box could not be amplified using the MADS-box specific primer. The fragments were purified over a QIAGEN PCR purification column and sequenced in order to find out the exact sequence identity of the gene contained by it (table 3.2). Sequencing was initiated from one of the primers used for amplification. Sequence identity was obtained by a sequence alignment based on comparative analysis in the PILEUP program of the GCG package with other maize MADS-box gene sequences from the MADS-database (http://www.mpiz-koeln.mpg.de/mads/).

An amplification product for further sequence analysis could not be obtained from roughly half of the clones, and for some partial genomic clones the initial sequencing reactions did not lead to a sequence that showed homology to any known MADS-box gene sequence upon alignment.

Table.3.2. Sequence analysis of the inserts of the genomic clones from the *AGL2*-like screen. The sequences of the inserts were compared to MADS-box gene sequences of known genes taken from the MADS-database (http://www.mpiz-koeln.mpg.de/mads/) and aligned by PILEUP of the GCG package, using a gap weight of 3.0 and a gap weight of 0.1. Different subfamilies (=subf.) are defined by monophyletic clades, described in Theißen *et al*., 2000. *TMZ1* indicates the clone contains an *AG*-like MADS-box sequence in a transposable element, as described in Fischer *et al*., 1995.

clone	gene	clade	clone	gene	clade
1	*ZMM24*	*AGL2*	9	*ZAG4*	*AG (TMZ1)*
2	*ZMM8*	*AGL2*	20	*ZAG4*	*AG (TMZ1)*
6	*ZMM7*	*AGL2*	57	*ZMM2*	*AG*
58	*ZMM7*	*AGL2*	38	*SW159*	*SQUA*
72	*ZMM7*	*AGL2*	64	*ZMM4*	*SQUA*
10	*ZMM3*	*AGL2*	19	*ZMM5*	*TM3*
33	New !	*AGL2*	36	*ZMM5*	*TM3*
51	*ZMM14*	*AGL2*	23	*ZAG5*	*AGL6*
79	*B9_20*	*AGL2*	71	*ZAG5*	*AGL6*
16	*ZMM16*	*GLO*			

Of the nineteen other clones, eighteen contained genomic sequences belonging to previously known MADS-box genes belonging to six different subfamilies The subfamilies are *AGL2*-like, *GLO*-like, *AG*-like, *TM3*-like (for *TomatoMADS3*), *SQUA*-like and *AGL6*-like (for *AGAMOUS*-like6) subfamily (for review, see Theißen *et al*., 2000). All of these genes share the MIKC-type domain structure of MADS-box genes from higher plants. Only 47% of the clones were members of the *AGL2*-like subfamily that was screened for with specific probes, due to the non-stringent hybridization conditions. However, these conditions allowed for the isolation of new genes. One clone, λDASHII-wd33, contained an unknown and untill now unpublished member of the *AGL2*-like subfamily. This makes the total number of *AGL2*-like MADS-box genes in maize to be nine. Clone λDASHII-wd33 is a partial genomic clone, and harbours the promoter, MADS-box and 3kb of the first intron. Its sequence is displayed in appendix 7.2. The MADS-box of λDASHII-wd33 was used to reveal its phylogenetic relationship to the previously isolated *AGL2*-like genes. Some of the genomic clones containing *AGL2*-like gene sequences were analyzed in detail, in order to reveal and compare the exon-intron structures.

3.1.2 Structural characterization of *AGL2*-like genes

The genomic sequence of *ZMM3*, *ZMM6*, *ZMM14* and B9_20 were characterized structurally (fig.3.2). The *ZMM3* sequence was obtained from λDASHII-wd10. A partial genomic clone of

ZMM6, λDASHII-3-1-1.1, spanning part of the Ist intron and the 3´part of the gene, kindly provided by Prof. dr. G. Theißen, was sequenced. The MADS-box and remaining part of the first intron were amplified from genomic T232 DNA using the primer pair WD32/W126 (App.7.1) from the 5´UTR of the cDNA pBLUE/ZMCDK5 and the 5´end of the genomic clone λDASHII-3-1-1.1 respectively (App.7.3). A 1.1kb promoter fragment was amplified based on sequence information of the *ZMM6* genomic locus from teosinte (*Z. m*. sp. *parviglumis*) (M. Tur, pers. comm.) using primer pair PT059/WD34 (App.7.1). An extra 1.5kb upstream promoter fragment was isolated via RAGE with gene specific nested primers W618 and W619 (App.7.1). The partial genomic clone of *ZMM14*, λDASHII-wd51 was totally sequenced and the 3´part was directly amplified from genomic T232 DNA with primer pair W756/W762 (App.7.1). The genomic B9_20 sequence of clone λDASHII-14-2-1 was kindly provided by Dr. Zh. Meng, as well as the genomic *ZMM1* sequence of λEMBL4-I16b for comparison (Theißen *et al*, 1995). The *AGL9*/*SEP3* genomic and cDNA sequence of the dicot *Arabidopsis thaliana* was extracted via Blast (acc. no. AC002396, chromosome I, BAC F2I6; AF015552) from the database at NCBI (http://www.ncbi.nlm.nih.gov) for comparison (Mandel & Yanofsky, 1998). The *AGL2*-like sequences used for the structural analysis are displayed in App.7.3.

All *AGL2*-like genes, including the dicot one, were shown to have 8 exons, instead of the 7 exons of the *AG*-like gene *ZMM1*. The extra intron is positioned in the C-region, coding for a more variable domain. The length of the exons IV, V and VII were conserved. As the former two exons are part of the region coding for the highly conserved K-box, believed to be involved in protein dimerization, the importance of this may be reflected by the structural conservation.

Some of the introns in the maize *AGL2*-like sequences were clearly much larger than those of the dicot *AGL9* gene. Among the maize *AGL2*-like genes, however, there was no conservation in size of the expanded introns, nor conservation as to which introns where larger. Additionally, some of these introns were also much larger than in the maize *ZMM1* gene. As introns have been shown to contain regulatory sequences, apart from the promoter, the variation in the lengths of the different *AGL2*-like introns may point to the large diversity in expression patterns of these genes, not seen in dicots, nor in *AG*-like genes.

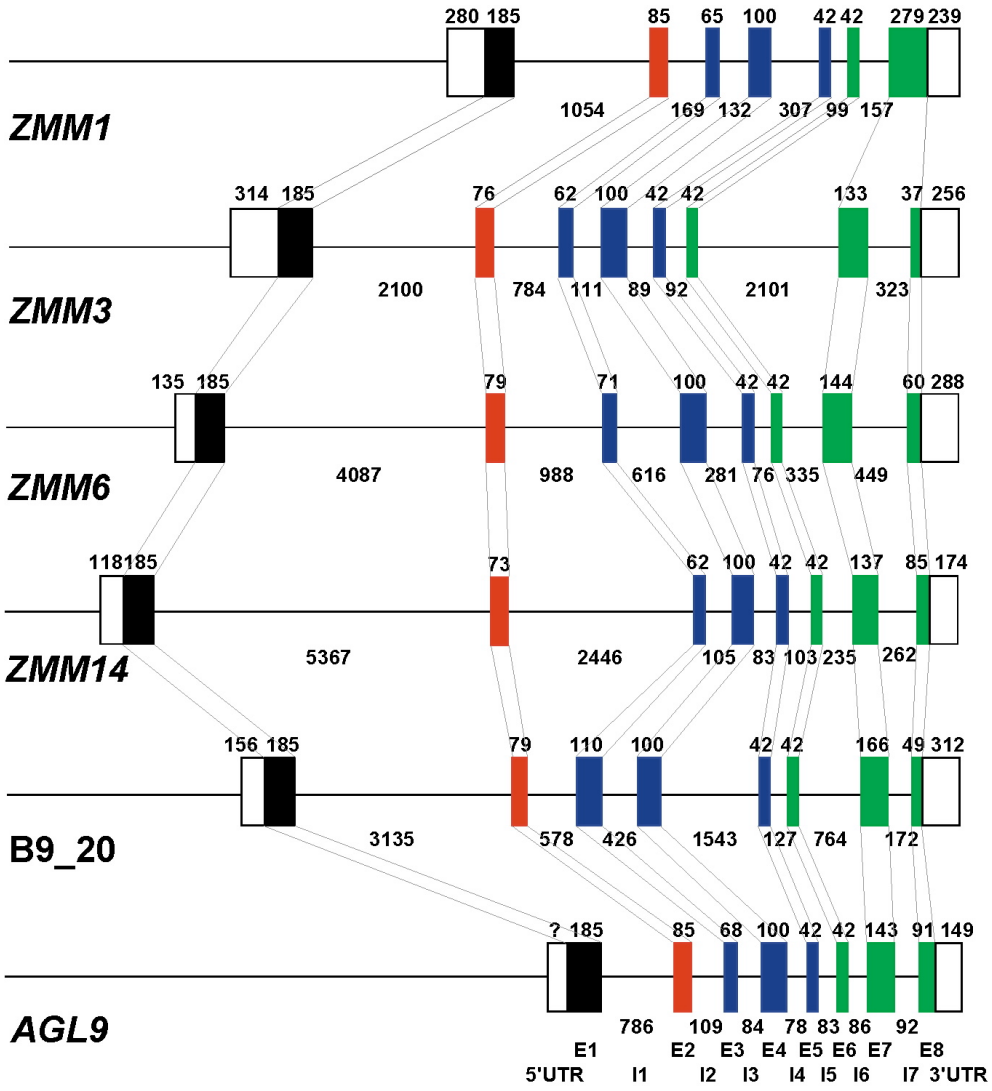

Fig.3.2. Genomic structures of *AGL2*-like genes. The exon-intron structures of *ZMM3*, *ZMM6*, *ZMM14* and *B9_20* are compared to the *A. thaliana AGL2*-like gene *AGL9*, and to *ZMM1*, a maize *AG*-like gene. Exons of the different genes encoding highly related protein domains are connected with thin lines. Exons are indicated as E1-E8, introns as I1-I7. The length of the exons and introns (in bp) are indicated above or below the respective regions. Question marks refer to regions of undetermined structural organization. The MADS-box is indicated in black, the I-region in red, the K-box in blue, and theC-region in green. UTR´s are shown in white boxes.

Nearly all of the introns had the typical plant gene dinucleotides GU and AG at the 5´-donor and 3´-acceptor site, respectively, as well as a significantly higher amount of adenosine and uridine residues, as part of the only loosely conserved plant intron recognition and splice site sequences (Lou, *et al*, 1993; Luehrsen *et al*, 1994).

3.1.3 Phylogenetic analyses

The phylogenetic relationships between members of the *AGL2*-like subfamily were investigated. The sequences were obtained from databases on the world wide web and from the MADS-homepage (http://www.mpiz-koeln.mpg.de/mads/). Based on sequence comparison of the MIK-domain region, an alignment was made using the program PILEUP and the relations were subsequently visualized using the program PLOT, both from the GCG package. The algorithm used to construct the tree was Neighbour Joining method. The relationship among the genes could be inferred from the way the genes were positioned at the branches of the tree (fig.3.3).

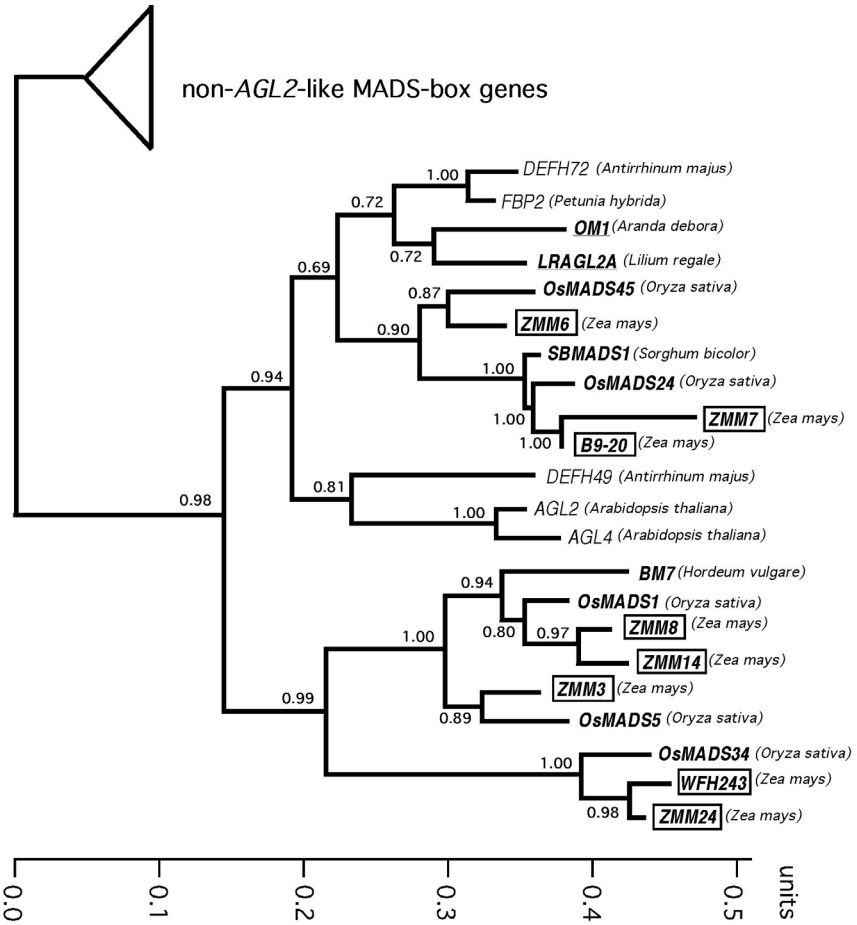

Fig.3.3. Phylogenetic analysis of *AGL2*-like MADS-box genes. The resulting phylogenetic tree was produced with the ´Neighbour-Joining ´ method using default parameters and standard conditions. The amino acid sequence used for comparison was the 3´ part of the genes, consisting of the MIK domains. The species name is given in between brackets behind the gene name. Monocot genes are written in bold, unlike dicot ones. The monocot genes that do not belong to the grass family (*Poaceae*) are underlined. The depicted tree, that contains only *AGL2*-like genes, is part of a larger tree containing also other genes. These latter ones are grouped together on a branch indicated ´non-*AGL2*-like MADS-box genes´. The bootstrap values are indicated next to the branching point. The units indicate the relative degree of difference of a gene to its most recent common ancestor with other genes.

A similar phylogenetic tree could be obtained by using other algorithms such as ´maximum parsimony´, indicating that this method is capable of reliably depict the degree of relationship among the genes. The genes within the tree predominantly cluster in groups that are correlated with the described taxonomical, and hence evolutionary, relationship. The dicot genes built two groups separately from the two monocot gene containing clades. Among the monocots, the grass genes cluster together as do non-grass genes. Some of the genes show to be more closely related to genes from different species than they are to genes from the same species. More clearly, within the order of the *Liliiflorae*, the genes *OM1* and *LRAGL2A* from *Aranda* and *Lilium* revealed their shared origin, by clustering closely together. These genes are called ´orthologous´genes since they have arisen by a speciation event.

Most of the maize *AGL2*-like genes cluster pairwise on the tree, reflecting the allotetraploid nature of the maize due to a recent whole genome duplication (Gaut & Doebley, 1997). The genes *ZMM8* and *ZMM14*, and *ZMM7* and *B9_20*, and *ZMM24* and *WFH243* are subclustered in pairs. These gene couples are young paralogous genes. They were created during a hybridization event in the phylogenetic line leading up to a single species. The paralogous maize genes often cluster closely together with only a single rice gene. Rice and maize are members of the grass family and therefore related among the monocots. The allotetraploidization event that generated the maize genome occurred after the lineages that led to maize and rice had already been separated. These rice genes are therefore the orthologous genes to both of the paralogous maize genes in the subclade. However, it could be that more related genes are present in the rice genome, so that the paralogy of their orthologous maize genes is not supported anymore. Alternatively, in case of more thorough sampling in maize, one could find a paralogous gene partner to a gene that has none at present. This latter situation could be proven or disproven by a chromosomal localization of the genes involved, since the map positions of the genes should normally be found in duplicated regions of the genome.

In order to establish the phylogenetic relationship of the newly isolated gene *WD33* to the other maize genes, a phylogenetic tree was made. Due to fact that only the MADS-box was available on the partial genomic clone, this part of the genes was used for comparison (fig.3.4).

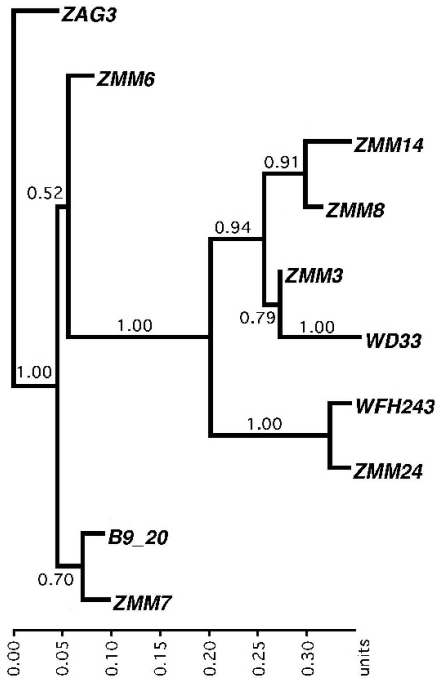

Fig.3.4. Phylogenetic analysis of maize *AGL2*-like MADS-box genes. The resulting phylogenetic tree was produced with the ´Neighbour-Joining ´ method using default parameters and standard conditions. The nucleotide sequence used for comparison was the MADS-box. *ZAG3*, a member of the closely related *AGL6*-like family, was included to form the outgroup. The bootstrap values are indicated next to the branching point. The units indicate the relative degree of difference of a gene to its most recent common ancestor with other genes.

The pairs of paralogous maize genes found in the previous tree were still observed, suggesting the tree reflect most likely the true phylogenetic relationship among these genes. Furthermore, the latter tree also confirms the close relationship of *ZMM3* to the *ZMM8*/*ZMM14* gene pair. Interestingly, the gene *ZMM3* clusters even more closely together with *WD33*. Whether the suggestion of a new gene pair is true has to be investigated further by mapping the new gene, isolation of new subfamily members, as well as expression studies and functional studies, although young paralogs could have changed expression or function after the allotetraploidization event.

In order to further establish the degree of relatedness between the different members of the *AGL2*-like gene subfamily their amino acid sequences were compared. The sequence comparison among the conceptual amino acid sequences of eight full length cDNAs of these *AGL2*-like genes showed sequence identities between 51.3% and 94.4%, and sequence similarities between 61.3% and 96.8% (table 3.3).

Table 3.3. Sequence comparison of the conceptual amino acid sequences encoded by cDNAs of *AGL2*-like genes. Comparisons are made using the program GAP of the GCG package. Parameters for gap creation penalty is 8.0, for gap extension penalty is 2.0. Above the diagonal lining are indicated the sequence identities, below are indicated the sequence similarities, both in percentages. The upper most and lower most values are indicated in grey.

	ZMM3	*ZMM6*	*ZMM7*	*ZMM8*	*ZMM14*	*ZMM24*	*B9_20*	*WFH243*
ZMM3	-	58.9	61.4	77.2	75.2	59.3	61.5	61.8
ZMM6	66.7	-	76.3	54.6	53.8	50.0	78.2	53.7
ZMM7	70.4	84.5	-	55.7	55.3	51.7	94.4	56.8
ZMM8	83.6	63.8	65.8	-	89.1	53.6	55.4	63.2
ZMM14	81.2	63.3	66.2	92.3	-	53.4	56.6	59.1
ZMM24	67.7	61.3	62.1	63.2	62.6	-	51.3	89.5
B9_20	69.3	84.8	96.8	64.5	66.8	61.3	-	56.9
WFH243	71.1	66.8	68.0	73.6	68.5	91.5	67.6	-

The comparison of the MADS domain, including the new *AGL2*-like gene λDASHII-wd33, showed sequence identities between 75.4% and 100.0%, and sequence similarities between 82.0% and 100.0% (table 3.4). Although *WD33* is most similar to *ZMM3*, *ZMM3* is more similar to *ZMM8* and *ZMM14*. This indicates that *WD33* and *ZMM3* probably do not form a gene pair as is suggested from their position on the phylogenetic tree (fig.3.4)

Table 3.4. Sequence comparison of the conceptual amino acid sequences in the MADS domain of *AGL2*-like genes. Comparisons are made using the program GAP of the GCG package. Parameters for gap creation penalty is 8.0, for gap extension penalty is 2.0. Above the diagonal lining are indicated the sequence identities, below are indicated the sequence similarities, both in percentages. The upper most and lower most values are indicated in grey.

	ZMM3	*ZMM6*	*ZMM7*	*ZMM8*	*ZMM14*	*ZMM24*	*B9_20*	*WFH243*	*WD33*
ZMM3	-	86.9	83.6	95.1	95.1	85.2	85.2	83.6	90.2
ZMM6	91.8	-	93.4	86.9	86.9	80.3	96.7	78.7	77.0
ZMM7	90.2	95.1	-	83.6	83.6	78.7	96.7	77.0	73.8
ZMM8	95.1	91.8	90.2	-	100.0	85.2	85.2	85.2	85.2
ZMM14	95.1	91.8	90.2	100.0	-	85.2	85.2	85.2	85.2
ZMM24	91.8	88.5	85.3	91.8	91.8	-	78.7	98.4	77.0
B9_20	90.2	96.7	98.4	90.2	90.2	86.9	-	77.0	75.4
WFH243	91.8	88.5	85.2	93.4	93.4	100.0	86.9	-	75.4
WD33	91.8	83.6	82.0	86.9	86.9	85.2	82.0	85.2	-

3.1.4 Chromosomal localization of *AGL2*-like genes

The map position of genes can be important to obtain information about their phylogenetic relationships. In maize, large duplicated regions can be found that have arisen after a recent genome duplication event (Gaut & Doebley, 1997). When paralogous genes map in such a region, most likely their paralogous partner gene maps in the duplicate partner region. Furthermore, when a map position of a gene correlates closely with the map position of a mutant, this becomes a likely candidate gene conferring that phenotype upon mutation. Hence, the chromosomal position can be informative as to the genealogical relationship as well as to the function of a gene.

For six of the nine *AGL2*-like genes restriction fragment length polymorphisms (RFLP) have been found with which these genes could be mapped in recombinant inbreds (RI) (Prof. dr. G. Theißen, W. Faigl, and Prof. dr. B. Burr, pers. comm.; Cacharrón *et al*., 1999; Fischer *et al*., 1995). The positions of the following genes are indicated in the map of the Brookhaven National Laboratory (BNL) (http://burr.bio.bnl.gov/acemaz.html) (table 3.5):

Table 3.5. Map positions of *AGL2*-like genes in maize. Indicated is on which chromosomal arm the genes lay, the position in centimorgans (cM) and the marker name of the gene on the map.

gene	chromosome	cM	marker
ZMM3	short arm chromosome 9	(9S034)	mpik25
ZMM6	long arm chromosome 1	(1L131)	mpik23A
ZMM7	long arm chromosome 7	(7L095)	mpik27
ZMM8	long arm chromosome 9	(9L115)	mpik28
ZMM14	short arm chromosome 1	(1S058)	mpik38
ZMM24	long arm chromosome 1	(1L253)	kws7

B9_20, *WFH243* and λDASHII-wd33 could not be mapped as no RFLP could be found between the parental alleles of the mapping population of the RI´s. The position of the genes on the maize chromosomes is depicted schematically in figure 3.5.

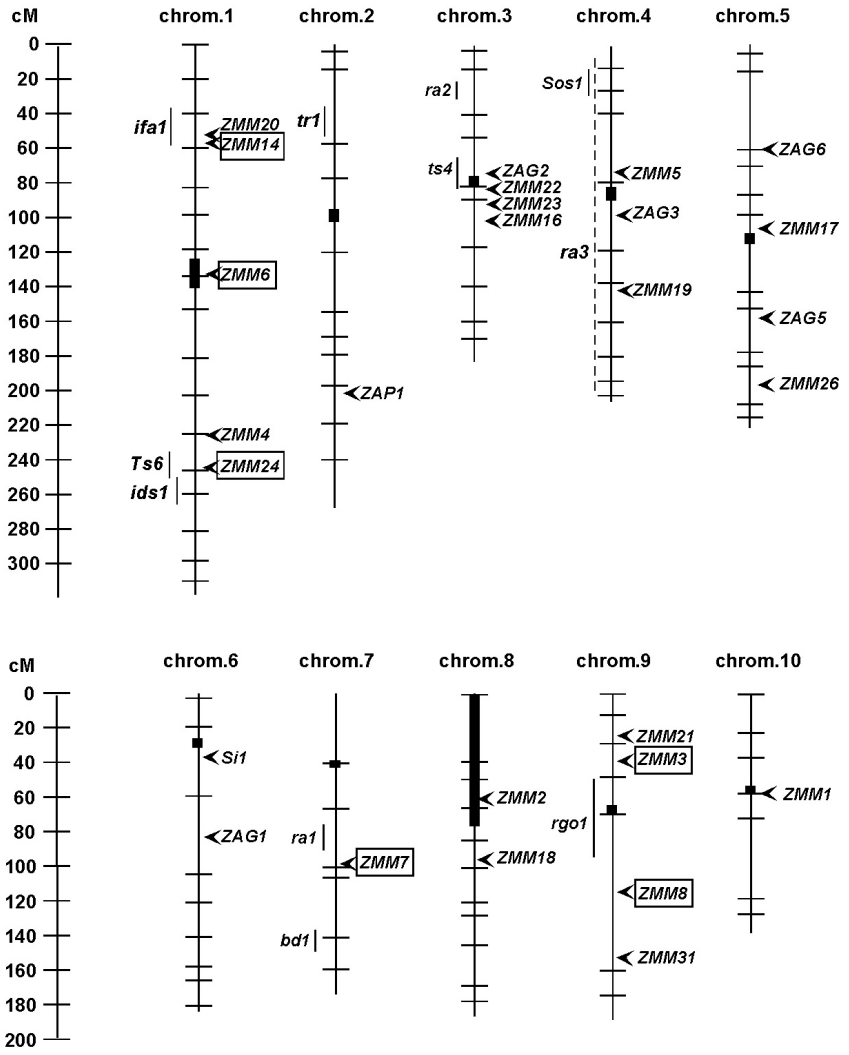

Fig.3.5. Chromosomal localization of MADS-box genes and inflorescence branching mutants in maize. The map positions are based on the map of the Brookhaven National Laboratory (BNL) (http://burr.bio.bnl.gov/acemaz.html). The genes are indicated right of the chromosomes and their approximate map positions are indicated by arrow heads. *AGL2*-like genes are shown in boxes. The information about the genetic loci of the mutants originate from Neuffer (1997) and the maize database (MAIZE DB; http://www.agron.missouri.edu) (App.7.9). Integration of the mutant loci on the map is indicated by a vertical line left of the chromosome. Thick black boxes indicate the approximate positions of the centromeres. Abbreviations: cM= centimorgan, *bd1=branched silkless1*, *ids1= indeterminate spikelet1*, *ifa1= indeterminate floral apex1*, *ra1= ramosa1*, *ra2= ramosa2*, *ra3= ramosa3, rgo1=reverse germ orientation1, si1=silky1*, *Sos1= suppressor of sessile spikelet1*; *ts4= tasselseed4*, *tr1= two ranked ear1*; *Ts6= Tasselseed6*.

The *AGL2*-like genes are dispersed over the genome, as is the case for other MADS-box genes as well. The map position of two of the *AGL2*-like genes correlated with the approximate loci of two developmental mutants. The *ZMM14*/*ifa1* candidate pair has been described previously (Cacharrón *et al*., 1999). The newly mapped *Ts6* dominant mutation was localized in the same region as the recently isolated *AGL2*-like gene *ZMM24*. *Ts6* maps at 1L254, between *npi238* and

bnl8.29a. This candidate couple, described in §3.1.5, has been preliminarily investigated since it was discovered just recently.

3.1.5 RFLP and expression analysis of the *ZMM24*/*Ts6* candidate loci

Out of a population segregating for the mutant *Ts6*-phenotype (Irish, 1997), one wild-type and one mutant sibling were investigated. A Southern analysis was performed with genomic DNA isolated from the two siblings to identify an RFLP (fig3.6). The DNA was digested with several restriction enzymes, and the blotted DNA was hybridized to a labeled 5´UTR fragment of *ZMM24* clone pGEM/WF1557. The fragment was amplified via PCR using primers T7/W785 (App.7.1). The Southern showed an RFLP with 4 out of 5 restriction enzymes.

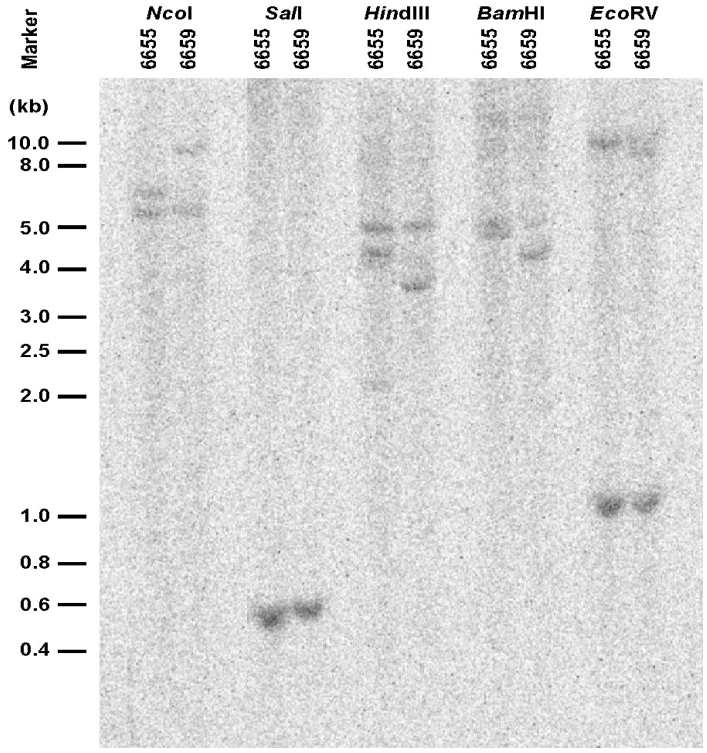

Fig.3.6. Southern analysis of the *ZMM24*/*Ts6* candidate locus. A mutant sibling plant 6659 and a wild-type sibling, plant 6655, were investigated. Genomic DNA of the siblings was restricted with *Nco*I, *Sal*I, *Hin*dIII, *Bam*HI and *Eco*RV. All enzymes but *Sal*I show a polymorphism.

The correlation between the 'segregation' of the dominant mutant phenotype and the polymorphism in the promoter region, did not falsify the hypothesis that the loci of the gene and the mutant might be the same. Therefore, the expression of *ZMM24* was analyzed in male inflorescences in siblings of the segregating population. The transcript level of two wild-type siblings, plant 6655 and 6657, were compared to the one from phenotypic plant 6659 (fig.3.7)

after hybridization of the blot to a 3´specific probe. The probe was amplified by primers W812/W813 amplified from pGEM/WFE068 (App.7.1). In the dominant mutant the *ZMM24* transcript was more abundant than in the wild-type siblings, strengthening the hypothesis of the candidate locus *Ts6* to the *AGL2*-like gene *ZMM24*.

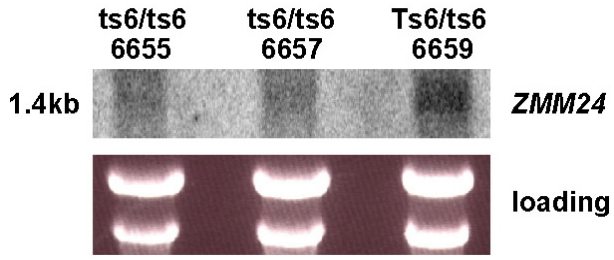

Fig.3.7. Northern blot analysis with total RNA from the male inflorescence of wild-type siblings 6655 and 6657 (*ts6*/*ts6*) and the mutant sibling 6659 (*Ts6*/*ts6*). The blot was hybridized with a 3´-specific probe of the *AGL2*-like gene *ZMM24*. As a control for RNA quality and loading amounts the loaded rRNA was quantified by comparison of band-intensity by ethidium bromide staining of the blotted agarose gel. Apparent transcript size was 1.4kb.

3.2 Functional analysis of *ZMM6* and *ZMM8* in transgenic maize plants

A functional analysis of *ZMM6* and *ZMM8* was performed. Mapping data for these genes were compared with all presently mapped morphological mutants. Mapping of classical mutants as well as molecular markers in maize is coordinated by the MAIZE genetic stock center, where continuously up-dated maps and mapping data are placed onto the homepage of the center, directly available for the maize community (http://www.agron.missouri.edu). Although the mutant *ifa1* mapped close to *ZMM14*, the duplicate gene of *ZMM8*, (Cacharrón *et al*., 1999), no mutants´ map position correlated with the map positions of *ZMM6* and *ZMM8*. Therefore, a transgenic approach was undertaken to reveal the function of these genes. Cloning and transformation of the *ZMM6* and *ZMM8* constructs for antisense and over-expression in maize are described in §2.2.15.

3.2.1 Regeneration and analysis of transgenic *ZMM6* plants

A PCR was performed on genomic DNA isolated from young leaf material to screen for the presence of the transgene in the genome. The primers used in the assay depend on the construct to be screened (for primer positions, see §2.2.15; for primer sequences, App.7.1). For the sense

construct the primers CMV1 and W126 were used, spanning the CaMV 35S promoter and part of the ORF of *ZMM6*, rendering a 0.7kb fragment. For the anti-sense construct PLA2 and W126 were used, spanning part of the ORF of *ZMM6* and the *NOS*-terminator, resulting in a 0.4kb fragment. As an example a genomic PCR of pK225/ZMM6 transgenic plantlets of the T_0 generation is presented in fig.3.8.

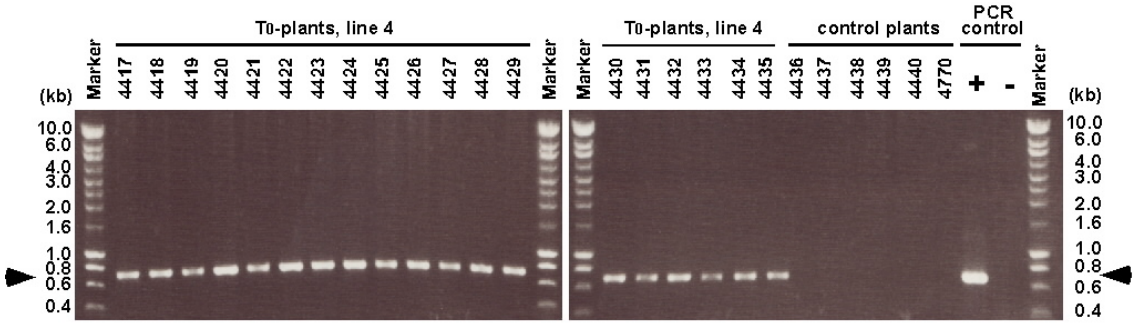

Fig.3.8 Genomic PCR assay on regenerated plantlets (T_0) to screen for the presence of pK225/ZMM6 constructs (line 4). The control plants were BASTA resistant, containing the empty vector. The PCR positive control sample contained pK225/ZMM6, the negative PCR control contained no DNA. The arrow head indicates the transgene specific 0.7 kb fragment

As to pK225/aZMM6, the PCR screening of the T_0-generation from the MPI involved in total 20 plants, of which 2 were PCR negative (=10.0%). Since most of the T_0 plants were male sterile they were crossed back using pollen of the wild-type line A69Y+. Out of 56 T_1-plants, 32 were negative (57.7%), whereas of 197 T_2-plants from back crosses of T_1-plants to A69Y+, 100 plants were negative (50.8%).

As to pK225/ZMM6, the PCR screening of the T_0-generation from the MPI involved in total 86 plants, of which 9 were PCR negative (=10.5%). The PCR screen on plants of the T_1 generation was combined with plants obtained from Hoechst AgrEvo. In total 465 T_1 plants were screened, and from the T_2 generation 10 plants. In the T_1-plants, 285 were PCR-negative, and 5 T_2 plants from a T_1-plant, back crossed to A69Y+, were negative. The high numbers of PCR negative plants (61.3% and 50.0%) stem from the out-segregation event of the transgene in a portion of the daughter plants (see below), as well as from a number of plants obtained from Hoechst of which the Basta resistant parent plant did not have the transgene, yielding only PCR negative daughter plants.

In order to check for independent transgenic lines, a Southern analysis was performed with the PCR positive plants derived from different calli and transformation experiments (fig.3.9). The genomic DNA was digested with *Eco*RV, a methylation insensitive restriction endonuclease, that

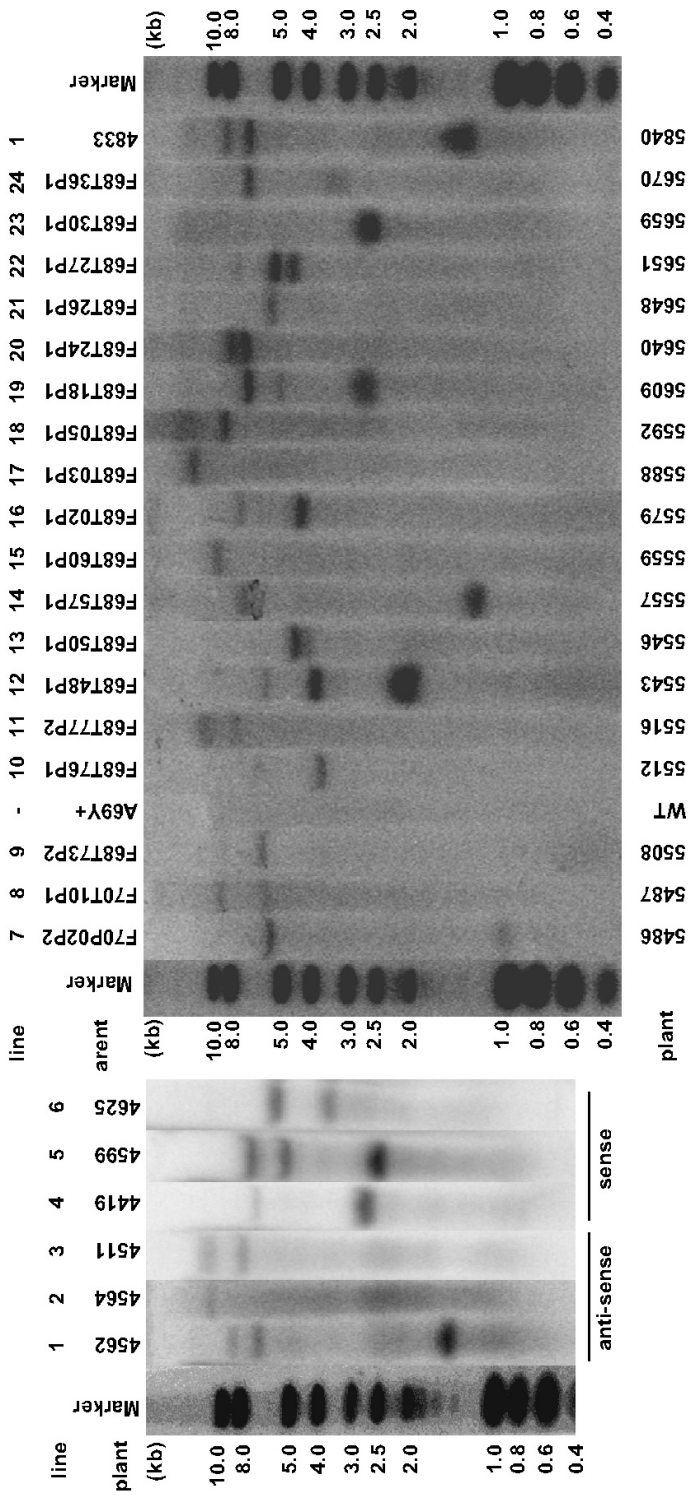

Fig.3.9. Southern blot analysis of maize plants transformed with a sense and anti-sense construct of *ZMM6*. Lines 1-3 contain pK225/aZMM6, lines 4-24 contain pK225/ZMM6. Lines 1-6 are T_0-plants (MPI), lines 7-24 are T_1-plants (Hoechst). The marker fragments are indicated in kilobases (kb). Plant numbers correspond to greenhouse numbers, parental labels show construct (F), independent transformation experiment (T), and parent plant number (P). As a control, wild-type A69Y+ DNA is included. Both sense and anti-sense lines contain only one to three copies of the construct.

does not cut within the sense and anti-sense cassettes, but only once within the pK225 vector. As a probe the CaMV 35S promoter, amplified out of pRT104 by primers CMV1 and CMV2, was random prime labeled and hybridized to the blots.

In all samples hybridizing bands were observed, indicating that the plant was transformed, except for the wild-type A69+ plant. The different integration sites in the genome were reflected by different sizes of the fragments containing the constructs. Both the sense and antisense lines showed only one to three fragments per genome, corresponding to the inserted copies of the transgene. The T_0-plants with pK225/aZMM6 construct from the MPI were derived from 4 calli (no.2, 6, 9 & 10) from the same transformation experiment (not shown). Plants from calli no.2 and 6 had the same *Eco*RV restriction pattern, and were hence derived from the same integration event (line 1). Calli no. 9 and 10 led to line 3 and 2 respectively. Similar holds for plants with the pK225/ZMM6 construct from the MPI. They were derived from 5 calli (no.1, 3, 4, 5 & 6) from one transformation experiment (not shown). Three calli (no.4, 5 & 6) were probably derived from the same integration event (line 6). Line 4 and 5 were obtained from calli no.3 and 1 respectively. The Hoechst T_1-plants were derived from independent transformation events, and hence originated from different integration events into the genome, as shown by Southern analysis. In total, 3 independent anti-sense lines were obtained, and 21 sense lines.

The inheritance of the transgenes was investigated by PCR screening for the transgene of populations of sibling plants of the T_2 and T_3 generation. All parent plants were back crossed with wild-type pollen, except one that was selfed. Per family, the line, parent, copy number, number of siblings and the number of transgenic (PCR positive) siblings was indicated (table3.6).

Even though the Southern suggested multiple copies per genome, the transgenes behaved as a single locus. The siblings out of a back crossed parent showed in about 50% of the cases the presence of the transgene, fitting to a 1:1 segregation pattern. Furthermore, in the population of siblings derived from the selfed parent, in 75% of the offspring the transgene was present. This correlates well with a segregation pattern 3:1 ratio, indicating the transgene was inherited as a single locus. This suggests that in case multiple copies were integrated into the genome it occured at only one locus.

Table 3.6. Inheritance of the transgene in transgenic plants. Independent lines are compared, in T2 and T3. Abbr.: pos.=number of positive plants per family, sibl.=number of siblings in the family, Tn= generation (n).

line	parent	copy	pos.	sibl.	Tn
1	4826	3	17	37	T2
1	4834x4831	3	21	28	T3
2	4563	1	4	10	T2
4	4484	2	2	5	T2
4	4744	2	5	10	T3
7	F70T10P2	1	5	10	T2
13	F68T50P1	2	5	10	T2
14	F68T57P1	2	5	9	T2
15	F68T60P1	1	3	10	T2
17	F68T03P1	1	6	10	T2
19	F68T18P1	3	5	10	T2

3.2.2 Phenotypic analysis of transgenic *ZMM6* plants

Transgenic plants harbouring the pK225/aZMM6 anti-sense construct were analyzed morphologically and compared to plants of the wild-type lines T232+, A69Y+ and B73+, control plants harbouring the empty vector, and plants transformed with the control pK225/aZMM15 contruct and p35SAcS/GCM5::*GUS* construct.

A qualitive analysis of the tassel showed that the transformed plants displayed various branching phenotypes as compared to the controls. For an overview, the main tassel phenotypes are schematically depicted in figure 3.10.

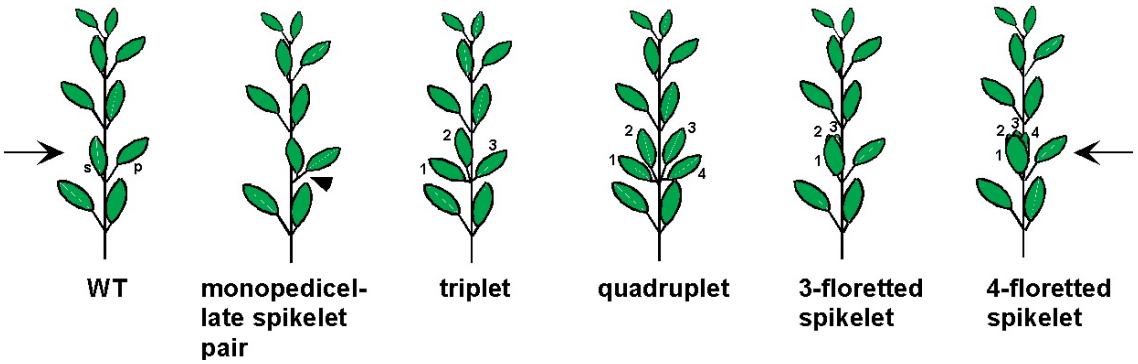

Fig.3.10. Schematic representation of branching phenotypes in transgenic tassels. Morphologically distinct structures are displayed between the two arrows. Drawn left is a wild-type pair (WT) having a sessile (s) and a pedicellate (p) spikelet. In a monopedicellate spikelet pair the two spikelets are placed on the same pedicel (arrowhead). A triplet or a quadruplet consists of three or four spikelets joined together, respectively. A 3-floretted or 4 floretted spikelet holds three or four florets, respectively, enclosed by the same number of glumes.

As an example male phenotypic spikelets of plant 5107 (anti-sense, line 2) are shown. On the inflorescence level, two spikelets were observed to have emerged from the same pedicel, designated a ´monopedicellate spikelet pair´. The extra spikelet on the pedicel, defined by the appearance of two glumes surrounding each other, could be empty (fig.3.11B), or even harbouring the wild-type number of floral organs (fig.3.11C). The position of the two spikelets of a monopedicellate spikelet pair varied, too. The extra spikelet could arise from the base of the pedicel, close to the rachis of the inflorescence (fig.3.11B), to the end of the pedicel, sharing the whole pedicel with its partner (fig.3.11C).

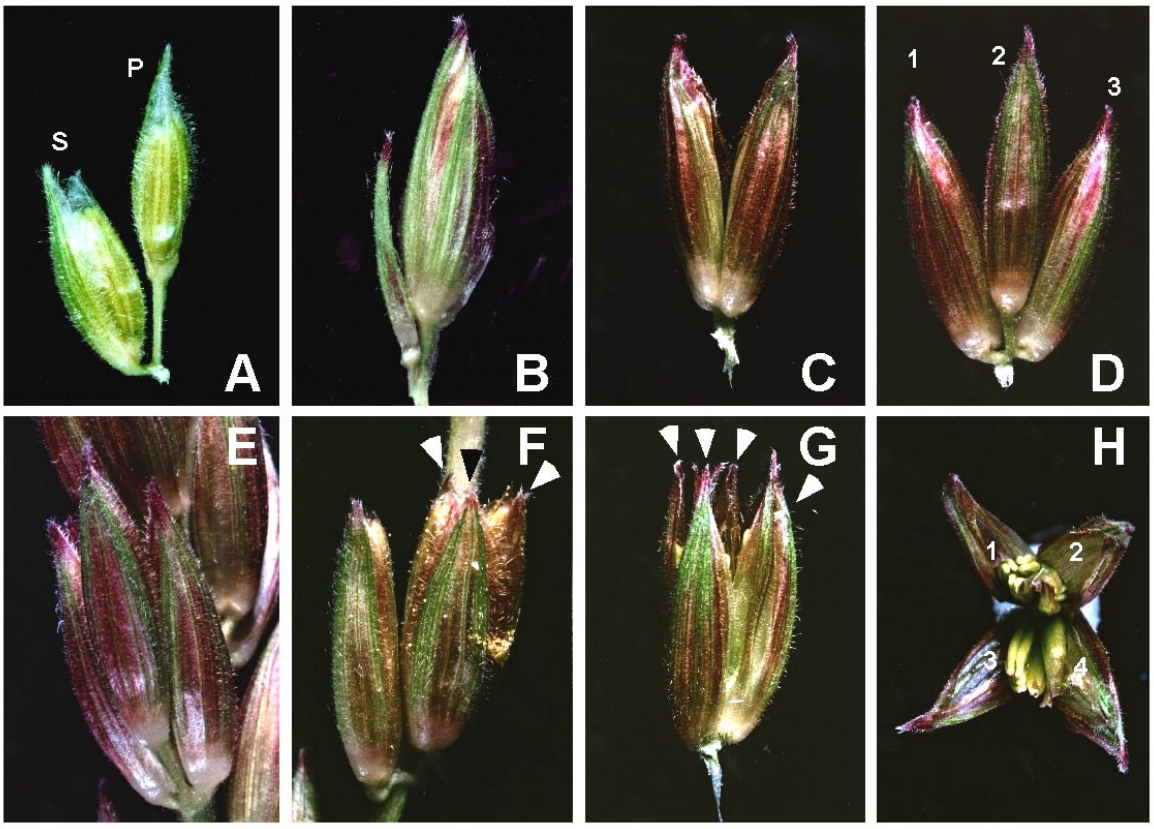

Fig.3.11. Functional analysis of *ZMM6* in pK225/aZM6 transformed plants. Phenotypic male spikelets of plant 5107 (line 2) are shown, compared to a wild-type spikelet pair of line B73+. **A**. Wild-type spikelet pair. **B.** Monopedicellate spikelet pair, in which the latterally formed empty spikelet has its own stem. **C.** Monopedicellate spikelet pair in which the extra spikelet, containing the wild-type number of floral organs, is formed at the edge of the pedicel. **D.** Monopedicellate triplet. Three spikelets branch off of only one pedicel. **E.** Triplet ´pair´ of spikelets arising from one point on the rachis. **F.** Spikelet pair in which the pedicellate spikelet has three glumes and three florets. **G.** Perfectly doubled spikelet harbouring four florets and glumes (side view). **H.** Doubled spikelet, showing four glumes enwrapping four florets (top view). s=sessile spikelet; p=pedicellate spikelet.

The wild-type pair of spikelets were also found to be converted into triplets, consisting out of three spikelets grouped together as a unit. In the wild-type this morphology was only observed at the base of the main inflorescence stem, where *ZMM6* is expressed at a lower level (Cacharrón, 1994), but hardly in the rest of the inflorescence (see below). In the transgenic plants, the position of the spikelets in the triplets could differ. The three spikelets could be placed onto the same pedicel, leading to a monopedicellate triplet (fig.3.11D). Alternatively, the three spikelets could have their own pedicel, the pedicels fused together at the inflorescence stem like in the wild-type situation (fig.3.11E).

On the spikelet level, spikelets were seen to contain up to four florets rather than two, as in the wild-type. In case the spikelet had three florets, these were surrounded by three glumes (fig.3.11F). The four floretted-spikelets contained also four glumes (fig.3.11G,H). The higher number of floral organs could lead to perfectly doubled spikelets (fig.3.11H), however, spikelets were also observed to lack some of the floret organs in the supernumerary florets, leading to a range of phenotypes from the wild-type two-floretted spikelet, via imperfect to perfect three-floretted spikelets until imperfect to perfect four-floretted spikelets. More florets per spikelets were not observed, contrary to the *ids1* phenotype (Chuck *et al*., 1998). Furthermore, an increase in floret numbers coincided with a proportional increase in glumes. An out-growth of the rachilla or spikelet axis was not observed. All the (extra) florets were positioned at the same horizontal plane at the base of the spikelet, next to each other, instead of on top of each other as in *ids1*.

In plants containing the construct pK225/ZMM6 for over-expression of *ZMM6*, the same range of phenotypes could be observed in the tassel on the inflorescence level (fig.3.12). The shown phenotypes of monopedicellate spikelet pairs (fig.3.12A), triplets including a pedicellate monopedicellate spikelet pair (fig.3.12B), a monopedicellate triplet (fig.3.12C), and a triplet directly branching off of the rachis (fig.3.12D) were most prominent. On the spikelet level, spikelets were found to contain up to four per spikelets (fig.3.12E,F), like in plants transformed with the anti-sense construct.

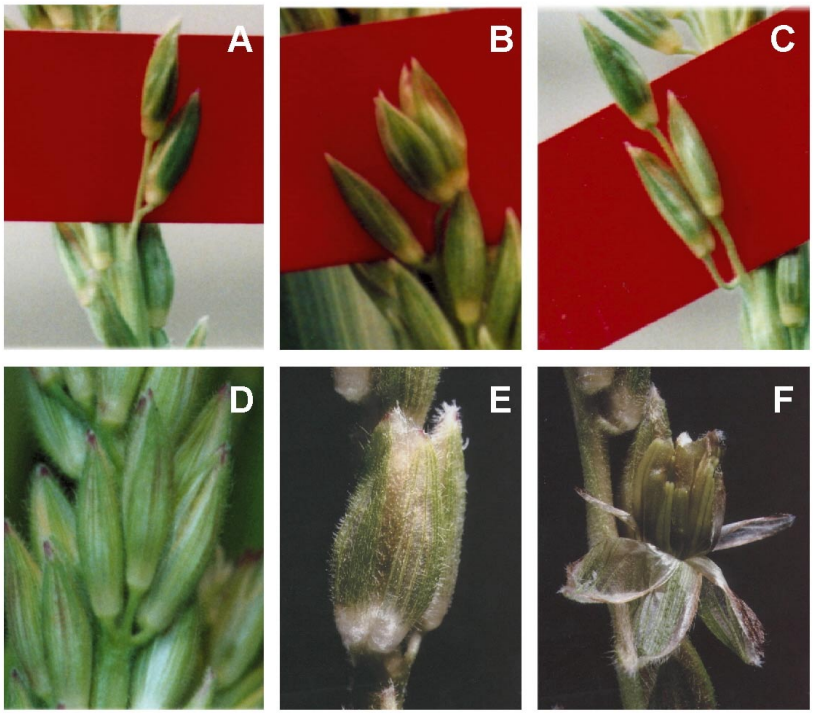

Fig.3.12. Functional analysis of transgenic tassels containing the sense pK225/ZMM6 construct. **A**,**C**, plant 4535, line 6, **B**, plant 4540, line 6, **D**, 4746, line 4, **E-F**, plant 7013, line 23. **A**. Monopedicellate spikelet pair. **B**. Triplet, formed ot of a pedicellate spikelet converted to a monopedicellate spikelet pair. **C**. Monopedicellate triplet. **D**. Triplet directly branched off the rachis. **E**. Spikelet having 4 glumes, filled with 3 to 4 florets. **F**. Glumes folded open showing the imperfectly doubled number of florets within the spikelet.

In a very few cases, spikelets were clearly grouped per four instead of the wild-type two spikelets per pair (fig.3.13), so-called ´quadruplets´. Their arrangement onto the rachis however, was different, due to the distinct site of branching of the spikelets on the pedicel. In the first example, the extra spikelets were braching off of the main pedicel (belonging to the actual pedicellate spikelet), thereby converting this pedicel into a small side branch of the main inflorescence stem. In the second quadruplet, the four spikelets have each retained their own pedicel, creating a losely organized panicle. Only at the base of the quadruplet the pedicels were fused before branching off of the rachis. The last quadruplet was different from the second, in that the pedicels of the two pedicellate spikelets in the middle were fused to give rise to a monopedicellate spikelet pair within the quadruplet.

In the examined tassels, more spikelets were grouped per four, that were directly branching off of the rachis, without any sign of fusion of the pedicels. These doubled spikelet pairs could therefore not be distinguished from pairs of spikelets that were placed directly next to each other. All three combinations were observed, -groups of four spikelets that had the sessile or the pedicellate

spikelets touching each other, and groups in which one sessile and one pedicellate spikelet were surrounded by one pedicellate and one sessile spikelet.

Fig.3.13. Distinct quadruplets of tassels of plants transformed with sense and anti-sense constructs. In one quadruplet (*left*) the extra spikelets were sideways branching off of the pedicel of the pedicellate spikelet In an other quadruplet (*middle*), having four pedicellate spikelets, the pedicels were only fused at the base of the quadruplet near the branching-off point from the rachis. The third quadruplet (*right*) displayed a total fusion of the pedicels of the spikelets that were formed in the middle. (*left*, plant 6022, anti-sense, line 1; *middle & right*, plant 4454, sense, line 4).

In the female inflorescences of transgenic plants similar branching phenotypes were observed. Since the plants transformed with sense construct displayed the same phenotypes as the ones having the anti-sense construct, they are presented together (fig.3.14). Compared to transformed control plants, most transgenic plants looked wild-type, showing a pair-wise arrangement of only the upper florets within the spikelets (fig.3.14A). Due to the restricted space on the ear rachis, corresponding to a predetermined number of florets or kernels, extra produced primordia that distorted the linear arrangement were more easily recognized. Single branching events on the spikelet pair primordial level gave rise to isolated triplets (fig.3.14B). Depending of the strength of the extra branching events, a range of triplet related phenotypes was observed. Pairs of spikelets could be converted into a stretch of triplets (fig.3.14C), or even into quadruplets via a triplet intermediate (fig.3.14D). Also isolated quadruplets were produced (fig.3.14E), after which the spikelet pair meristem resumed its wild-type activity by forming pairs of spikelets again. This finding re-enforced the observed quadruplet phenotype of the tassel where the spikelets are more losely organized in the inflorescence. Analogous to the tassel phenotype, ear spikelets were found to have an extra floret (fig.3.14F), the so-called pistillate florets. In pistillate florets the extra floret is formed at the position of the lower floret, because of an absence of abortion. The lower floret was of opposite orientation to the upper one, and upon fertilization this led to a ´reverse

germ orientation´ phenotype, in which the embryo is pointing to the base of the inflorescence in contrast to embryo´s in the wild-type kernels (fig.3.14G,H).

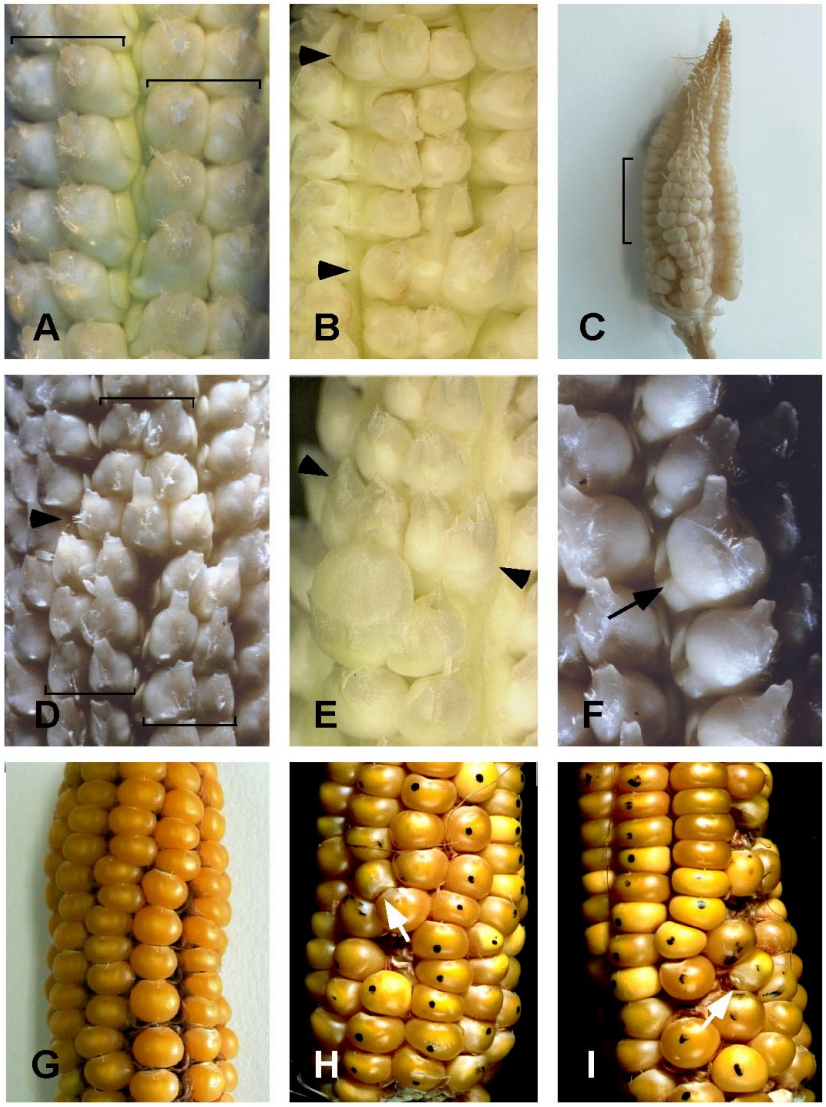

Fig.3.14. Functional analysis of *ZMM6* in female inflorescences of sense and anti-sense transformed plants. **A.** Plant 4627, line 1, showing a wild-type like arrangement of pair-wise aligned spikelets. **B.** An isolated triplet among spikelet pairs (plant 6011, line 1). **C.** Stretch of triplets (plant 5674, line 24) **D.** Conversion of a paired alignment of spikelets via a triplet into quadruplets, re-organized in double pairs of spikelets (plant 7004, line 18). **E.** Single quadruplet within a row of paired spikelets (plant 6020, line 1). **F.** Pistillate floret having an extra, lower, floret per spikelet, pointing downwards (plant 6993, line 18) **G.** Wildtype ear (A69Y+) showing an ordened rowing of pairwise aligned kernels. **H.** Kernel on plant 5721 (line 1) showing a reverse germ orientation after seed set in the lower floret of a pistillate floret spikelet. **I.** Kernel on plant 5721 (line 1) showing a reversely oriented kernel. Due to the pressure of neighbouring kernels the downward direction of the kernel may be a bit off-set.

Transgenic *ZMM6* plants also displayed a phenotype within the florets (fig.3.15). Floral organ numbers were often increased. As displayed in fig 3.15B and C, florets revealing four stamina

were present, compared to the wild-type number of three stamina per floret (fig.3.15A). Also paleas or palea–like structures could be observed in spikelets showing a branching phenotype as described above. Some of the organs were found to be intermediate to lodicules and paleas, suggesting the lodicules were converted into paleas (fig.3.15D-F).

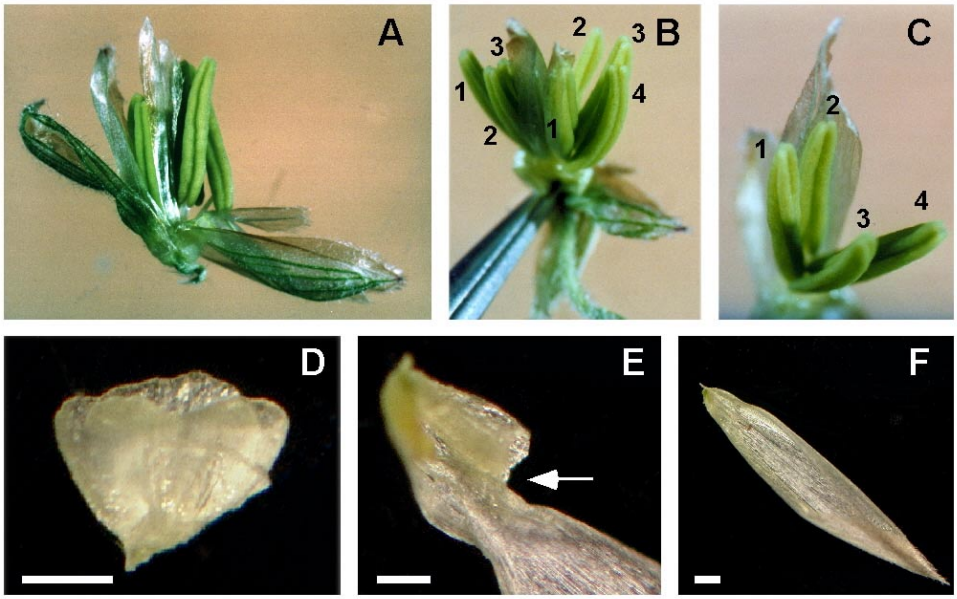

Fig.3.15. Floral organ phenotypes of *ZMM6* transformed plants. **A.** Wild-type HE98+ spikelet having two florets with each 3 stamina (control plant with empty vector). **B-C**, plant 4593 (line 2), **D-F**, plant 5723 (line 1) **B.** Spikelet in which one floret is wildtype-like, the second floret contains 4 stamina. Numbers indicate stamina. **C.** Close-up of a floret with four stamina **D.** Wildtype-like lodicule. **E.** Lodicule partially converted into palea (close-up). Arrow indicates the transition point. **F.** Wildtype-like palea. (**D-E**, bar= 0.5mm; **F**, bar = 1mm).

3.2.3 Expression analysis of *ZMM6* transcripts in transgenic plants

Plants transformed with a sense construct of *ZMM6* displayed the same phenotype as anti-sense transgenic plants. This suggested that the observed phenotypes are caused by a loss of function of *ZMM6*. In order to find a correlation between the transgenic phenotype and a difference (i.e. an expected decrease) in the *ZMM6* transcript level, Northern analyses were performed (Fig.3.16).

Total RNA from phenotypic (lane 1-3 and 7-12) and wildtype-like spikelets (lane 4-5) from transgenic *ZMM6* sense plants were compared with total RNA from wild-type lines B73+ (lane 6) and T232+ (lane 13) (Fig3.16). In several cases (plant 6446, 6475, 6502) the amount of *ZMM6* mRNA was not significantly changed from the amount in wild-type plants. Furthermore, transgenic *ZMM6* plants could either have a reduced (plants 6333 and 6338) or an increased

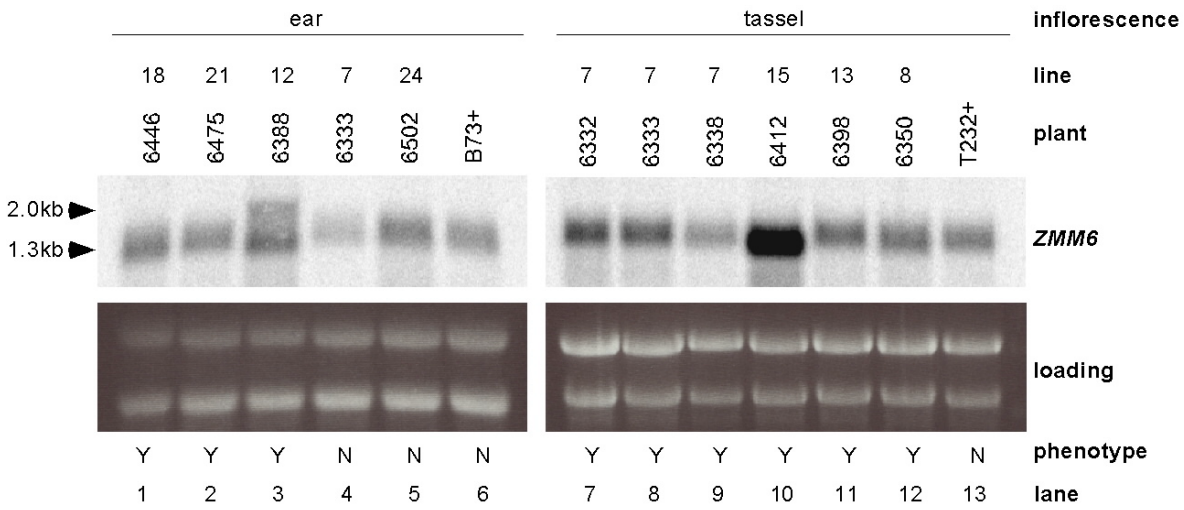

Fig.3.16. Northern analysis of *ZMM6* expression in *ZMM6* sense construct transformed plants. The blot contains total RNA from ear (lane 1-6) and tassel (lane 7-13). The transgenic plants from independent lines (lane1-5, 7-12) are compared with wild-type lines B73+ (lane 6) and T232+ (lane 13). Phenotypic spikelets (lane 1-3, lane 7-12) are compared with wild-type or wildtype-like spikelets (4-6, 13). The blot was hybridized with a 3´-specific probe of *ZMM6*. Apparent transcript size is shown on the left side.

amount of *ZMM6* transcript (plants 6388, 6332, 6333 and 6142). The up- or down regulation was independent from the line to which the plant belonged. Interestingly, in different samples of plant 6333 (of phenotypic (lane 8) versus non-phenotypic material (lane 4)) altered levels of *ZMM6* transcript were formed. Additionally, comparisons of *ZMM6* expression of plant 6332 and 6333 (lane 7, 8) with plant 6338 (lane 9) show that there is variation within lines, apart from variation among lines (see above). Furthermore, in plant 6388 also an abberant *ZMM6* transcript was detected. A total co-suppression of the endogenous *ZMM6* gene in the transgenic plants was not observed, neither in phenotypic, nor in wild-type looking spikelets. Taken together, in the plants transformed with the sense construct, a correlation between the phenotypes of the transgenic spikelets and a difference in the level of *ZMM6* transcript as compared to the wild-type was not observed. Similar results were obtained with the anti-sense plants (data not shown).

3.2.4 Quantitative and statistical analysis of phenotypic characters in *ZMM6* plants

In order to provide evidence that the observed morphologically distinct characters in the inflorescences of the *ZMM6* transformed plants are consistent transgenic phenotypes rather than just morphological random fluctuations, a quantitative and subsequent statistical analysis was performed in which only transgenic plants were compared to (transgene negative) control plants.

Plants obtained from the transformation experiments that were transgene-negative as shown by PCR or Southern analysis (see above) were discarded. Because of possible effects on the morphology by methylation of the endogenous gene, due to the presence of the transgene in the parental generation or callus during the regeneration period, these plants were excluded from quantitative and statistical analysis.

As the transgenic tassels and ears displayed different but related phenotypes due to the similar but not identical inflorescence morphology, their phenotypic categories differed accordingly. Since in some wild-type male inflorescences triplets and monopedicellate spikelet pairs were found at the base of the inflorescence, it was distinguished between these characters appearing at the base and elsewhere in the inflorescence, -the basal and non-basal groups, respectively (App.7.4)-. The base of the inflorescence is defined here as 2 cm above the upper most side branch or, in case of absence of side branches, 2 cm above the lower most spikelet pair. The more indeterminate nature of the basal spikelet pairs in the wild-type is explained by Irish (1998) who states that the more apically placed spikelet pair meristems directly produce the spikelet pairs, whereas the spikelet pair meristems at the base of the tassel can first grow out to give rise to side branches. Intermediately positioned spikelet pair meristems may occasionnally form monopedicellate spikelet pairs and triplets. The characters analyzed in the tassel were non-basal triplets, non-basal monopedicellate spikelet pairs, 3-floretted spikelets, and 4-floretted spikelets. In the ears the groups consisted of triplets, quadruplets and pistillate floretted spikelets.

The total numbers of wild-type spikelets and the total number of morphologically distinct spikelets were counted, and divided by each other to obtain the phenotypic ratio of the different characters (App7.4 and App.7.5). The quantitative data suggested that the observed branching phenotypes displayed a non-normal distribution. This is indicated by the fact that the mean of the ratios of the characters have the same order of magnitude as the standard deviation, or could even be lower than the standard deviation of that mean (App7.6).

Therefore, the non-parametric Mann-Whitney (U)-test was used as described in §2.2.20 for comparing the groups of transgenic plants to the groups of control plants with respect to the above mentioned characters. The resulting asymptotic significances of the U-tests, indicating the chance that the members between the compared group are the same, are displayed in table 3.7.

Table 3.7. Statistical evaluation of morphologically distinct branching characters of *ZMM6* sense and anti-sense transformed T_0-plants in the tassel using the Mann-Whitney (U)-test. Shown are the asymptotic significances that indicate the chance that members of the groups are the same. The test compares the phenotypic ratios of a character of individual T_0-plants in a group of transgenic plants, compared to those of the control plants. The original data used for the test are displayed in App.7.4. The groups used for comparison are numbered (U2(x,y)), where x is the control group, compared to the transgenic group y. Column N(x,y) indicates the total number of compared plants in group x and y, respectively. Abbrev.: NBmono= non-basal monopedicellate spikelets; NBtrplt= non-basal triplets; 3-floret= 3-floretted spikelets; 4-floret= 4-floretted spikelets, aZMM6=anti-sense plants, ZMM6=sense plants.

compared groups (U2(x,y))	NBmono	NBtrplt	3-floret	4-floret	groups (x-y)	N(x,y)
U2(1,3)	0	0	0.003	0	controls,To-aZMM6,To	36, 17
U2(1,4)	0.001	0.002	0.045	0.047	controls,To-ZMM6,To	36, 57

The asymptotic significances of the tests for each of the four individually evaluated characters were between 0 and 0.05. This means that these tested characters differed statistically in a significant way between the control group and the transgenic group of plants. Hence, these can be considered mutant phenotypes, due to the presence of the transgene in those plants. Similar results were obtained after more extensive statistical evaluation of these phenotypes in groups of transgenic plants in a different generation or background (e.g. Hoechst plants) (App.7.7). Similar results for the evaluated characters were seen in the female inflorescence, albeit with a lower frequency of the phenotypes (see below). The three characters (triplets, quadruplets and pistillate floretted spikelets) were therefore grouped together as a single trait. Comparison against the control plants indicated that the branching was also affected in the ear, due to the presence of the *ZMM6* transgene (App.7.7).

In order to show the frequency with which the phenotypes occurred, the phenotypic means of groups of sense and anti-sense transformed *ZMM6* T_0-plants in the tassel were compared to the controls and graphically displayed (fig.3.17). The phenotypic ratios of phenotypes of an affected spikelet pair meristem (SPM) were grouped together (non-basal monopedicellate spikelet pairs and non-basal triplets), as well as those of a defect in the spikelet meristem (SM) (3-floretted and 4-floretted spikelets).

The anti-sense lines showed in general to be more affected in the spikelet pair meristem than the sense lines. Similar results were obtained in the female inflorescence (data not shown; App.7.5).

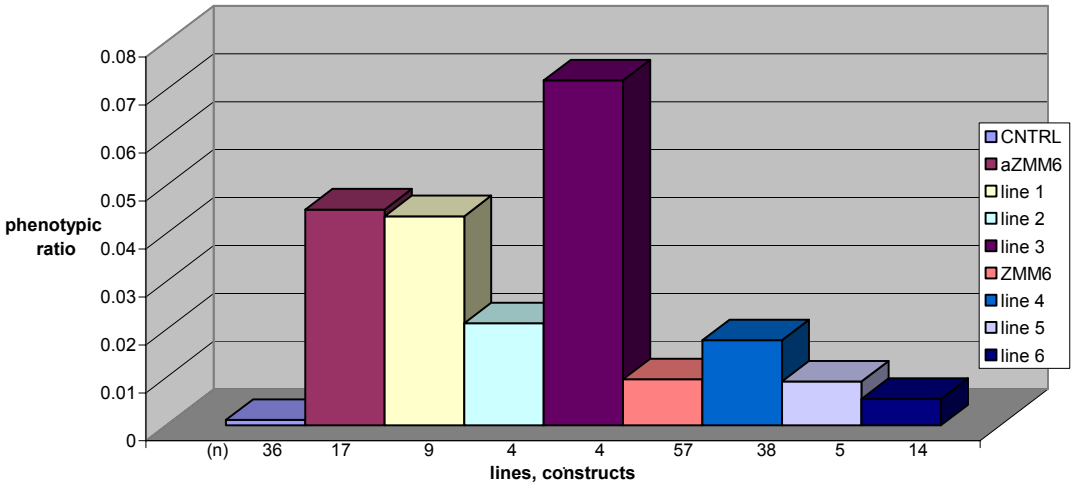

Fig.3.17. Phenotypic ratios of non-basal monopedicellate spikelet pairs and triplets in the T_0-generation in the tassel. Columns represent the T_0-control group (CNTRL), total antisense (*aZMM6*) and sense (*ZMM6*) transformed plants, and lines within the latter two groups. The total number of plants per column is indicated under the columns by (n).

Also with respect to the phenotypes on the spikelet meristem level, the anti-sense lines showed a stronger phenotype than the sense lines (fig.3.18). Furthermore, there is a tendency that the branching phenotypes derived from a defect in the spikelet pair meristem occur more frequently than the spikelet meristem phenotypes. In the ear, similar results were observed **(**data not shown; App.7.5).

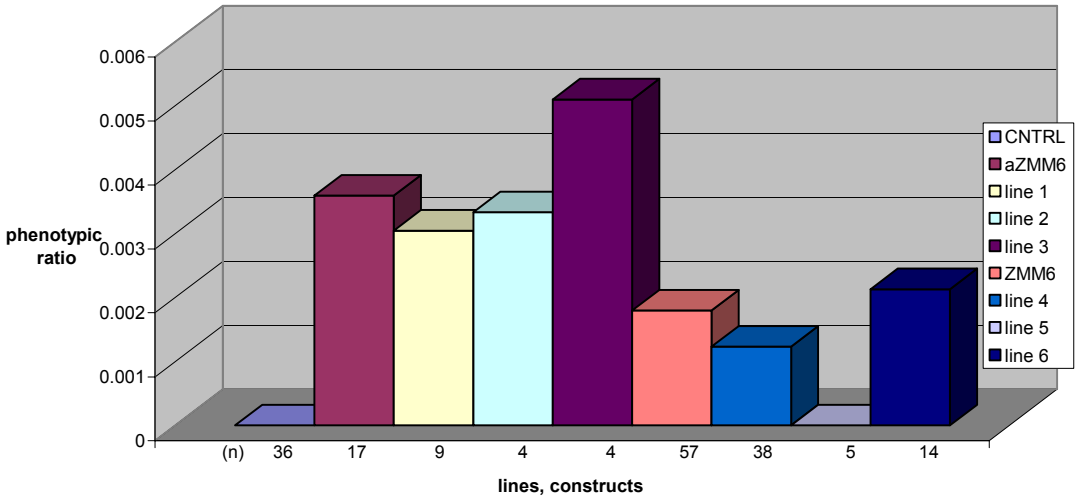

Fig.3.18. Phenotypic ratios in the tassel of 3-floretted and 4-floretted spikelets in the T_0-generation. Columns represent the T_0-control group (CNTRL), total antisense (*aZMM6*) and sense(*ZMM6*) transformed plants, and lines within the latter two groups. The total number of plants per column is indicated under the columns by (n).

In total, the above mentioned phenotypes were present -in tassel and/or ear- in 18 lines out of the total 24 lines (=75%) (App.7.4 and App.7.5). These are the lines 1, 2, 3, 4, 5, 6, 7, 8, 12, 13, 14, 15, 18, 19, 21, 22, 23 and 24. The phenotypes were present over four generations (T_0-T_3).

3.2.5 Regeneration and analysis of transgenic *ZMM8* plants

To screen for the presence of the transgene, a PCR on genomic DNA from young leaves was performed. The primer pairs only span an amplifiable region in the transgene, not in the genomic DNA of the wild-type plants. One of the primers in the pair anneals to the ORF of the gene, the other either to the CaMV 35S promoter (sense construct) or the *NOS*-terminator (anti-sense construct). For the sense construct the primers CMV1 and WD54 were used (App.7.1), rendering a 0.8kb fragment, and a fragment from the anti-sense construct was amplified by PLA2 and WD54, resulting in a 0.4kb sized fragment, as is examplified by figure 3.19, presenting a genomic PCR of pK225/ZMM8 and pK225/aZMM8 transgenic plantlets of the T_1 generation.

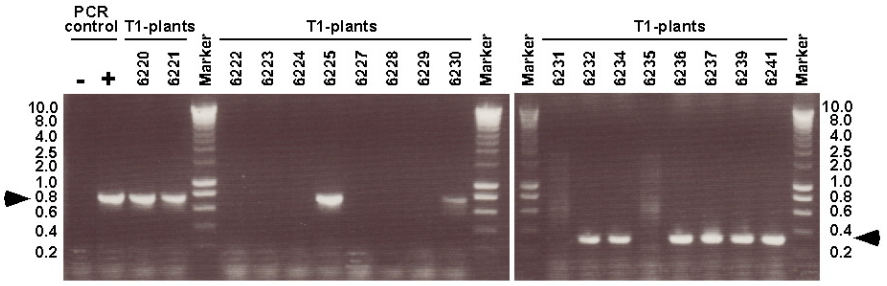

Fig.3.19 Genomic PCR assay on plantlets (T_1) to screen for the presence of pK225/ZMM8 and pK225/aZMM8 constructs. The PCR negative control sample contains no DNA, the PCR positive control sample contains pK225/ZMM8. Plants 6221-6230 are screened for the pK225/ZMM8 sense construct, whereas plants 6231-6241 are screened for the pK225/aZMM8 anti-sense construct. The positive sense plants show a transgene specific 0.8kb fragment, the positive anti-sense plants show a transgene specific 0.4kb fragment.

As to pK225/aZMM8, the PCR screening of the T_0-generation from the MPI involved a total of 69 plants, of which 11 were PCR negative (=15.9%). Since most of the T_0 plants were male sterile they were crossed back using pollen of the wild-type line A69Y+. Out of 90 T_1-plants, 49 were negative (54.4%), whereas from 17 T_2-plants from a back crosses of T_1-plants to A69Y+, 8 plants were negative (47.1%).

As to pK225/ZMM8, the PCR screening of the T_0-generation from the MPI involved in total 16 plants, of which 2 were PCR negative (=12.5%). The PCR screen on plants of the T_1 generation was combined with plants obtained from Hoechst AgrEvo. In total 180 T_1 plants were screened, of which 124 were negative (68.9%), and from the17 T_2-plants, 9 were not positive (52.9%). The high numbers plants without a transgene stem from the out-segregation event of the transgene, and as well as from T_1-plants obtained from Hoechst of which the parent plant (T_0) was negative. Out of three crosses between an anti-sense construct containing mother and a sense construct containing father, 22 plants segregated for the transgenes. Five plants did not contain any transgene, 9 contained the antisense, 2 contained the sense construct, and 6 contained both.

In order to check for independent transgenic lines, a Southern analysis was performed with the PCR positive plants derived from different calli and transformation experiments. The genomic DNA was digested with *Eco*RV, a methylation insensitive restriction endonuclease, that cuts once within the sense cassettes, and once within the pK225 vector, rendering a 1.5kb fragment. The CaMV 35S promoter was used as a probe. It was amplified out of pRT104 by primers CMV1 and CMV2, and subsequently random prime labeled and hybridized to the blots (fig.3.20).

In all samples hybridizing bands were observed, indicating that every plant was transformed, except for the control plant (6047). The different integration sites in the genome were reflected by different sizes of the fragments containing the constructs. The sense lines had one to five fragments per genome, corresponding to one to four copies, because the internal *Eco*RV fragment does not contribute to the copy number. The antisense lines showed two to four fragments directly corresponding to the inserted copies of the transgene. The T_0-plants with pK225/aZMM8 construct from the MPI were derived from 4 calli (no.2, 7, 8 & 9 (data not shown) for lines 1, 2, 3 & 4) originating from independent transformation events within one transformation experiment, as displayed by the different *Eco*RV restriction digestion pattern. The MPI T_0-plants with the sense construct regenerated from calli no.2/1, 6 and 2/2 (data not shown) revealed distinct *Eco*RV restriction patterns, and were hence derived from independent integration events (line 5, 6 & 7). The Hoechst T_1-plants were derived from independent transformation events, forming independent lines (lines 8-29), as shown by Southern analysis. In total, 4 different anti-sense lines were obtained, and 25 sense lines. The sense lines showed the *Eco*RV internal fragment of 1.5kb, except from the lines 5, 14, 23 and 26. In these lines the construct may have integrated only partially into the genome, or the sequence of integration lies within the 1.5kb *Eco*RV fragment.

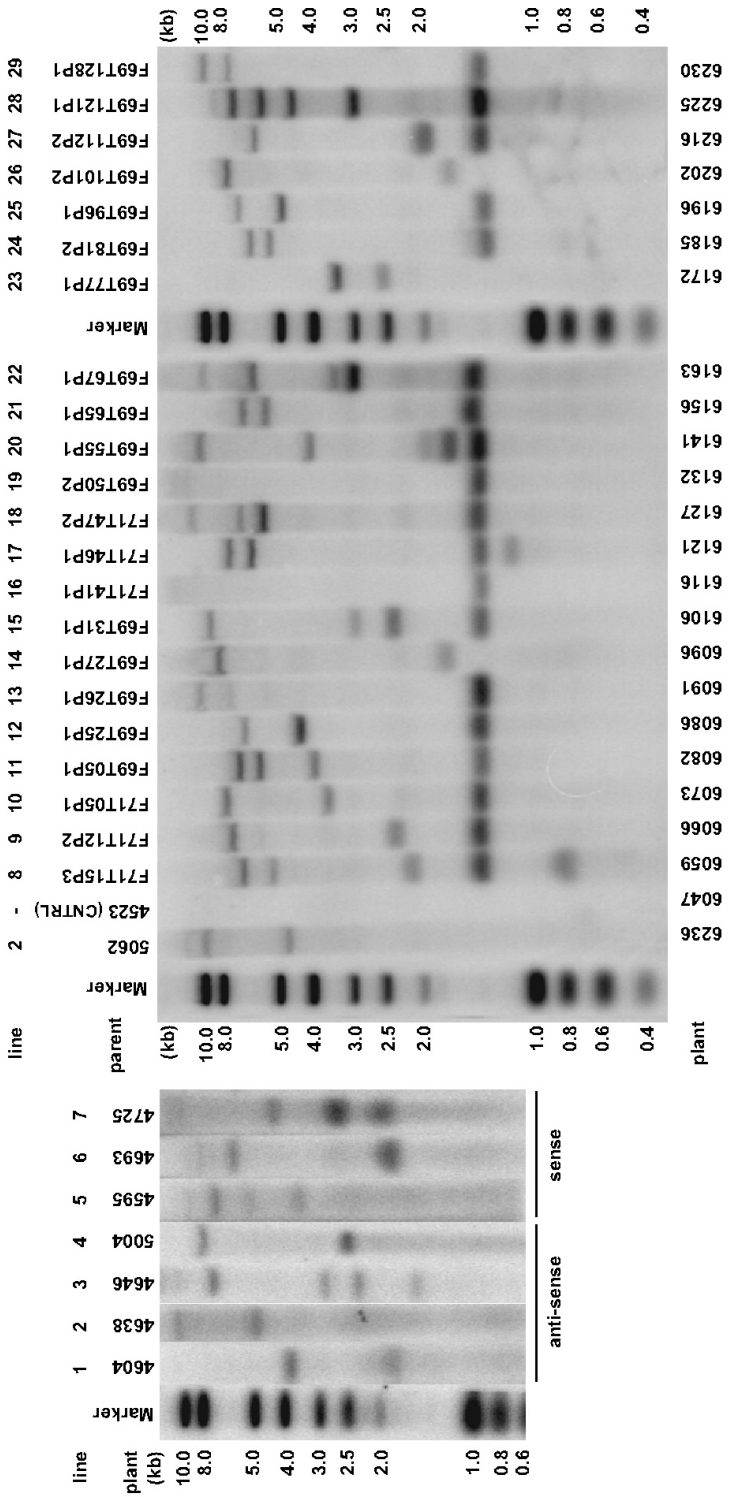

Fig.3.20. Southern blot analysis of maize plants transformed with a sense or anti-sense construct of *ZMM8*. Lines 1-4 contain pK225/aZMM8, lines 5-29 contain pK225/ZMM8. Lines 1-7 are T_0-plants (MPI), lines 8-29 are T_1-plants (Hoechst). The marker fragments are indicated in kilobases (kb). Plant numbers correspond to greenhouse numbers, parental labels show construct (F), independent transformation experiment (T), and parent plant number (P). As a control, the DNA of a non-transformed HE89 plant (6047) is included. The fragment number of the transgenes ranges from 1 to 5. In most of the sense lines, the 1.5kb *Eco*RV fragment of the construct pK225/ZMM8 is present.

The inheritance of the transgenes was investigated by PCR screening of populations of sibling plants of the T_2. All parent plants were back crossed with wild-type pollen. Per family, the line, parent, copy number, number of siblings, the number of transgenic (PCR positive) siblings and the generation was indicated (table3.8).

Table 3.8. Inheritance of the *ZMM8* transgene in transgenic T_2-plants of different lines with sense and anti-sense constructs. The presence of the transgenes occurs in about 50% of the offspring, independent of the copy number. Abbr.: pos.= number of positive plants per family, sibl.=number of siblings in the family, Tn= generation (n).

line	parent	copy	pos.	sibl.	Tn
2	4521	2	4	8	T2
2	4517	2	5	11	T2
4	4527	2	7	14	T2
4	4996	2	11	19	T2
8	F69T15P3	4	4	10	T2
9	F71T12P1	2	6	10	T2
11	F71T05P1	3	2	5	T2
12	F69T25P1	2	9	15	T2

Although the Southern showed multiple copies per transgenic line, the transgenes behaved as a single locus. The offspring of the back crossed parents showed in all lines about 50% of the cases the presence of the transgene, fitting to 1:1 segregation pattern, indicating the transgenes were inherited as a single locus. This suggests that when multiple copies were integrated into the genome the integration occurred at one locus only.

3.2.6 Phenotypic analysis of transgenic *ZMM8* plants

Transgenic plants harbouring the sense or anti-sense construct were analyzed morphologically and compared to plants of the wild-type lines T232+, A69Y+ and B73+, control plants harbouring the empty vector, and plants transformed with the control pK225/aZMM15 contruct and p35SAcS/GCM5::*GUS* construct. A qualitive analysis of the tassel showed that plants transformed with the sense construct, as well as plants having the anti-sense construct displayed a similar branching phenotype as compared to the controls (fig.3.21). The phenotypes were similar to the *ZMM6* transformed plants, suggesting these two *AGL2*-like genes function highly similarly, although their expression patterns are different.

ZMM8 transformed plants showed in the male inflorescence branching phenotypes originating from a malfunctioning spikelet pair meristem, like monopedicellate spikelet pairs (fig.3.21B),

triplets (fig.3.21C), including combinations stemming from spikelets that were differently positioned along the length of only one pedicel, and combinations among these. Linked to a spikelet meristem defect were spikelets having three glumes, yet, encompassing two wild-type florets (fig.3.21D), spikelets having three perfectly developed florets within three glumes (fig.3.21E,F), up to perfectly developed four-floretted spikelets (fig.3.21H) and intermediate branching variations between the latter (fig.3.21G).

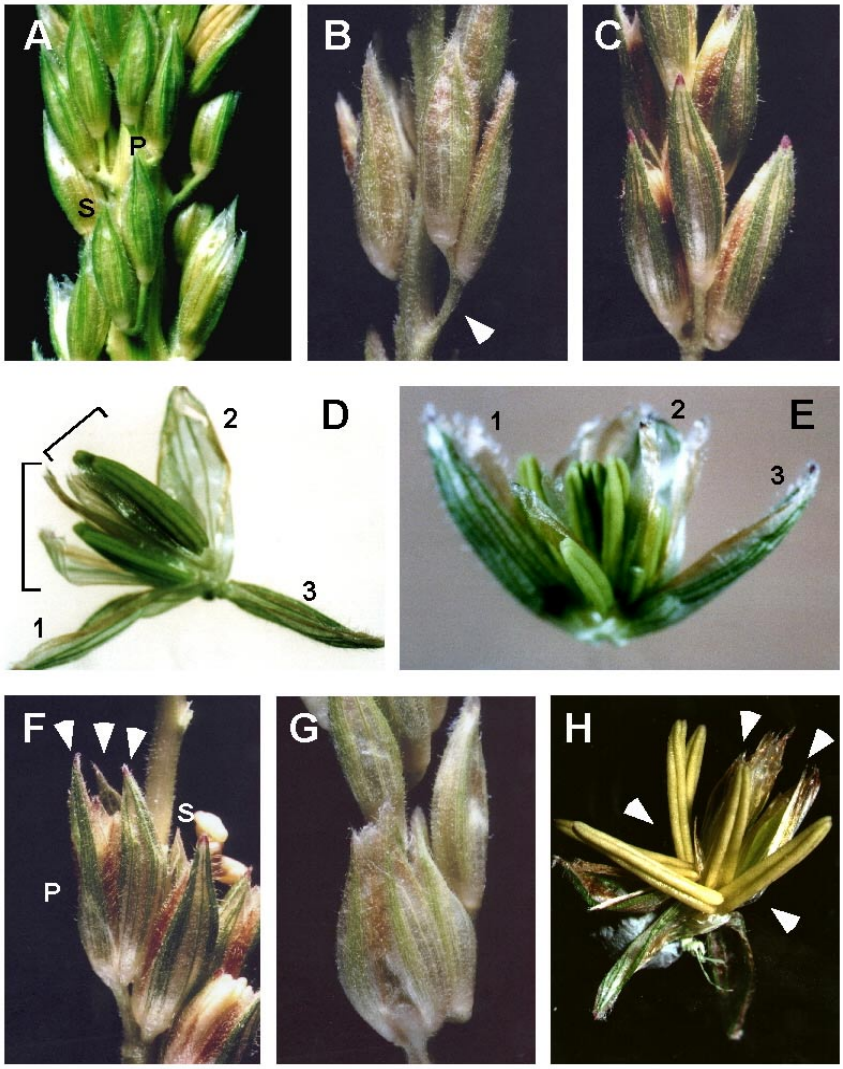

Fig.3.21. Functional analysis of transgenic plants with sense and anti-sense *ZMM8* constructs. **A.** Wild-type rachis section, showing a pair of spikelets consisting of a sessile and a pedicellate one (line B73+). **B.** Monopedicellate spikelet pair in which the pedicel of the sessile spikelet is fused to the pedicellate spikelet´s pedicel (plant 6883, line 4 x line 25). **C.** Triplet formation of three spikelets (plant 6922, line 3). **D.** Three glumed spikelet with two wild-type florets (plant 4517, line 2). **E.** Three-glumed spikelet surrounding three wild-type florets. Plant 4517, line 2). **F.** Three floretted spikelet as the pedicellate one of a pair of spikelets (plant 6909, line 25). **G.** Intemediate branched spikelet, having between three and four florets (plant 6883, line 4 x line 25). **H.** Perfectly developed four-floretted spikelet (plant 4801, line 1).

In the female inflorescences of plants transformed with sense and anti-sense constructs of *ZMM8*, branching phenotypes were observed analogous to the tassel phenotypes, and to the ones in inflorescences of plants transformed with *ZMM6* constructs (fig.3.22). Although most of the spikelets were organized in a wild-type-like fashion (fig.3.22A), transgenic plants were found to contain spikelets that were arranged per three (fig.3.22B). Astronger phenotype, interfering with the regular wild-type rowing, was seen in spikelet pairs that were converted via triplets into quadruplets (fig.3.22C). Singularly organized quadruplets were also observed (fig.3.22D). Extra florets within the female spikelets, the pistillate floret-spikelets, were sometimes grouped together, and sometimes found independently placed (fig.3.22E). Upon fertilization of these lower florets, that were reversely positioned to upper floret within the spikelet, a kernel developed with the embryo facing the ground (`RGO`-phenotype) (fig.3.22F).

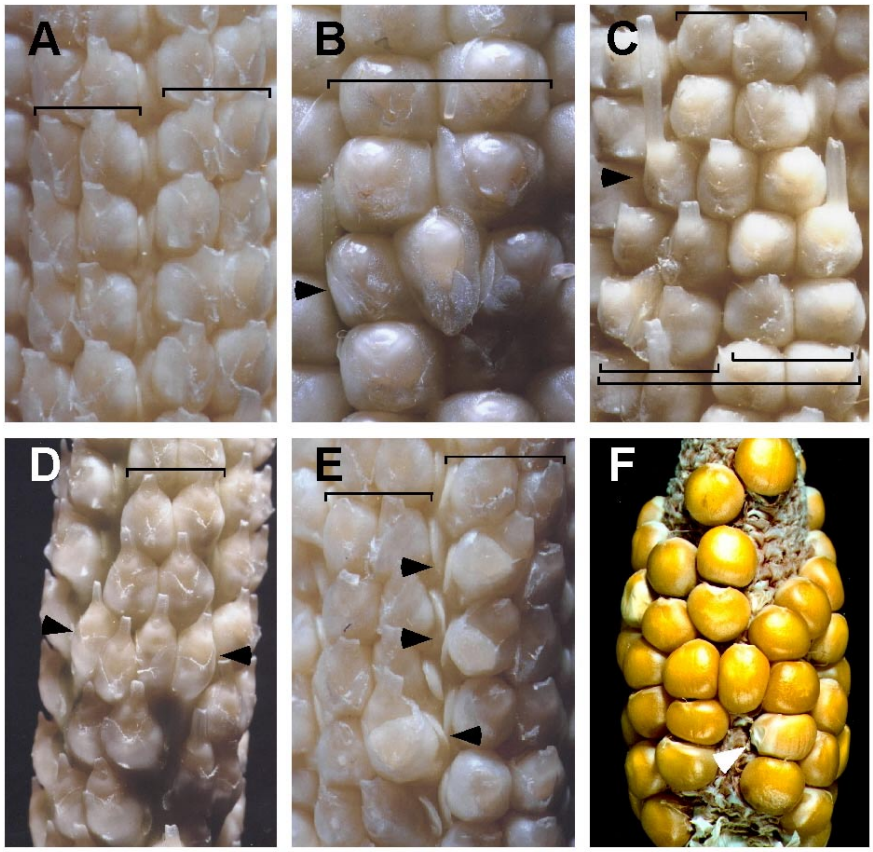

Fig.3.22. Functional analysis of *ZMM8* in female inflorescences of sense and anti-sense transformed plants. **A.** Wild-type rowing of spikelet pairs (plant 6981, line 20). **B.** Triplet formation disrupting the regular rowing of spikelets (plant 6982, line 20). **C.** Conversion of a single row of paired spikelets into two rows of spikelets (quadruplets) via a triplet intermediate (plant 6931, line 1). **D.** Quadruplet formed by a single defective branching event (plant 6923, line 3). **E.** Pistillate floret spikelets having, in addition to the wild-type upper floret, an oppositely oriented lower floret (plant 6985, line 20). **F.** Reverse germ orientation phenotype in a fertilized pistillate floret. The embryo is directed to the base of the inflorescence (plant 4817, line2).

Within the florets, similar phenotypes were observed as in plants transformed with *ZMM6* constructs. The floral organ numbers of lodicules (not shown), stamina (fig.3.23A,B) and paleas (not shown) could be increased, and lodicules were sometimes partially converted into paleas (fig.3.23D).

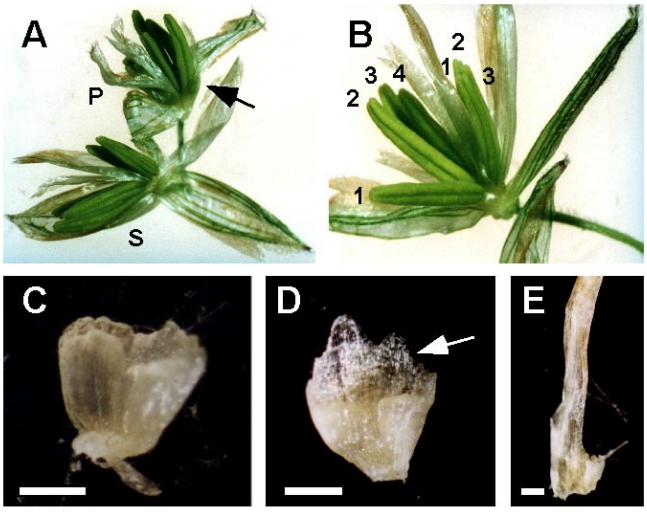

Fig.3.23. Floret phenotypes of *ZMM8* transformed plants. **A.** Spikelet pair with each spikelet having two florets. One of the four florets has 4 stamina (arrow) (plant 4518, line 2). **B.** Close-up of a floret with 4 stamina (plant 4518, line 2). **C.** Wild-type lodicule (plant 4643, line 3). **D.** lodicule, partially converted into palea, ´paleatic´ tissue at its outer rim (arrow) (plant 4643, line 3). **E.** Lodicule partially converted into palea (plant 4643, line 3). Bar=0.5mm; p=pedicellate spikelet; s=sessile spikelet.

3.2.7 Quantitative and statistical analysis of phenotypic characters in *ZMM8* plants

The analyses of the *ZMM8* transcript in transgenic plants yielded similar results as described for *ZMM6* (see §3.2.3). Since a correlation between the observed phenotypes and a change in expression could not be observed (data not shown), the presence of the transgene was causally related to the changes in morphology via a statistical analysis. The quantitative and subsequent statistical analysis was performed with only transgenic plants, compared to negative control plants, analogous to the analysis of *ZMM6* transformed plants (see §3.2.4).

The morphologically distinct branching traits were counted per inflorescence, as well as the total number of spikelets. In the tassel all non-basal triplets, non-basal monopedicellate spikelet pairs, 3-floretted spikelets, and 4-floretted spikelets were counted. In the ears triplets, quadruplets and pistillate floretted spikelets formed the distinct classes. In order to avoid abberations in phenotypic quantities that merely stem from differences in inflorescence size, the total numbers

of morphologically distinct spikelets were divided by those of the wild-type spikelets to obtain the phenotypic ratios (App7.4 and App.7.5). Analyses of the means of the traits showed they had the same order of magnitude as, or were lower than the standard deviation (App7.6). The branching phenotypes were therefore not normal distributed over the inflorescences.

The ratios of the individual traits in the transgenic plants were tested against those in control plants (table 3.9). The statistical test for this was the Mann-Whitney (U)-test (§2.2.20). The outcome of the test is the asymptotic significance. This states the chance event that the evaluated trait is the same between the control and transgenic batch of plants.

Table 3.9. Statistical evaluation of morphologically distinct branching characters of *ZMM8* sense and anti-sense transformed T_0-plants in the tassel using the Mann-Whitney (U)-test. Shown are the asymptotic significances that indicate the chance that members of the groups are the same. The test compares the phenotypic ratios of a character of individual T_0-plants in a group of transgenic plants, compared to those of the control plants. The original data used for the test are displayed in App.7.4. The groups used for comparison are numbered (U2(x,y)), where x is the control group, compared to the transgenic group y. Column N(x,y) indicates the total number of compared plants in group x and y, respectively. Abbrev.: NBmono= non-basal monopedicellate spikelets; NBtrplt= non-basal triplets; 3-floret= 3-floretted spikelets; 4-floret= 4-floretted spikelets, aZMM8=anti-sense plants, ZMM8=sense plants.

compared groups (U2(x,y))	NBmono	NBtrplt	3-floret	4-floret	groups (x-y)	N(x,y)
U2(1,8)	0	0	0	0	controls,To-aZMM8,To	36, 33
U2(1,9)	0.01	0.03	0.001	0.01	controls,To-ZMM8,To	36, 11

The asymptotic significances of the tests for each of the four individually evaluated characters were between 0 and 0.05, indicating that the trait was significantly different between the control plants and the transgenic plants. The altered morphology of these spikelets can therefore be considered as mutant phenotypes, due to the presence of the transgene in those plants. Similar results were obtained after further statistical evaluation of the phenotypes in other groups of transgenic *ZMM8* plants in a different generation or background, including the female inflorescence (App.7.7). As the occurrence of the phenotypes in the ear was much lower than in the tassel, the three characters (triplets, quadruplets and pistillate floretted spikelets) were joined and tested as a single branching trait. The results showed that the presence of the *ZMM8* transgene also cause an increase of branching in the ear .

Furthermore, the occurrence of the phenotypes were analyzed in the tassel in the T_0-plants. The phenotypic means of groups of sense and anti-sense transformed *ZMM8* T_0-plants were compared to the controls (fig.3.24). The phenotypic ratios of the non-basal monopedicellate spikelet pairs and non-basal triplets were put together as an indication of the expressivity of the phenotype in

the spikelet pair meristem (SPM). Similarly, the values of the 3-floretted and 4-floretted spikelets were joined to indicate the occurrence of a loss of function in the spikelet meristem (SM) (fig.3.25).

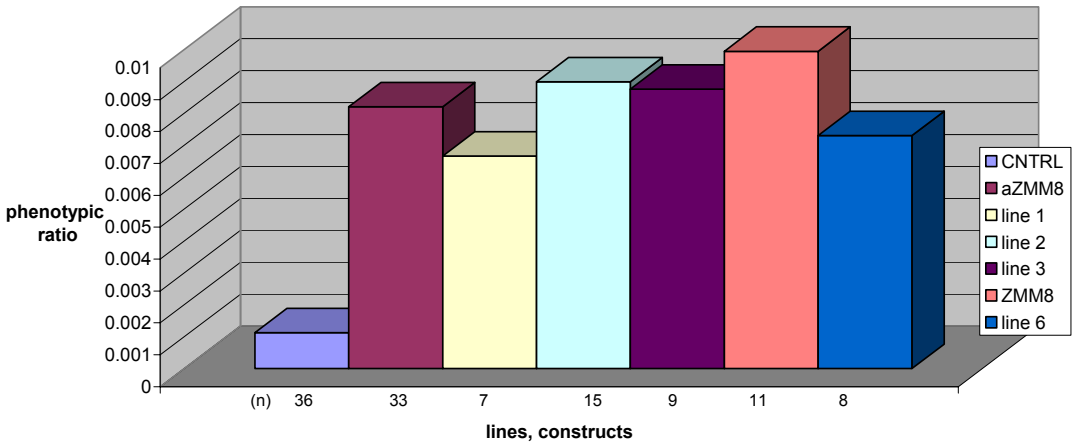

Fig.3.24. Phenotypic ratios of non-basal monopedicellate spikelet pairs and triplets in the T_0-generation in the tassel. Columns represent the T_0-control group (CNTRL), total antisense (*aZMM8*) and sense (*ZMM8*) transformed plants, and lines within the latter two groups. The total number of plants per column is indicated by (n).

The sense and anti-sense lines showed that the phenotypes occurred with approximately equal frequency. However, due to the low number of sense *ZMM8* T_0-plants and the non-normal distribution of the phenotypes, this result must be considered with caution.

The 3- and 4-floretted spikelets were more frequently occuring in the anti-sense lines than in the sense lines. The latter phenotypic ratios equalled those of the loss of SPM-function, unlike the situation for the *ZMM6* gene. This was also observed in the ear (SPM ratio = 0.0015 vs. SM ratio= 0.0012 (data not shown); App7.5).The *ZMM8* phenotypes were in both male or female inflorescences observed in 22 lines out of the total 29 lines (=76%) (App.7.4 and App.7.5). These are the lines 1, 2, 3, 4, 5, 6, 7, 8, 12, 13, 14, 16, 17, 18, 20, 21, 22, 24, 25, 26, 28 and 29. The phenotypes were present over three generations (T_0-T_2).

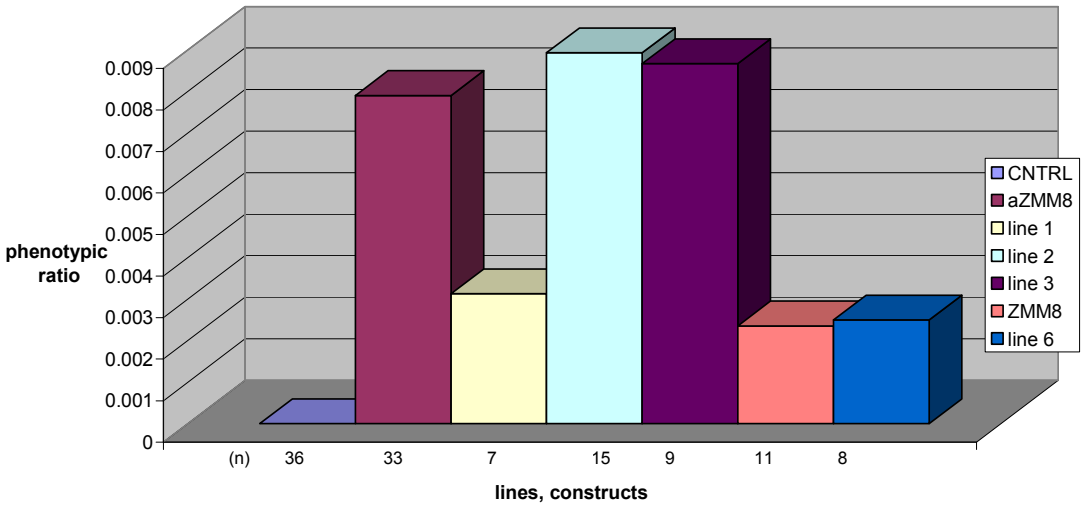

Fig.3.25. Phenotypic ratios in the tassel of 3-floretted and 4-floretted spikelets in the T_0-generation. Columns represent the T_0-control group (CNTRL), total antisense (*aZMM8*) and sense(*ZMM8*) transformed plants, and lines within the latter two groups. The total number of plants per column is indicated by (n).

4 DISCUSSION

4.1 Maize *AGL2*-like genes contain large regulatory sequences

The genomic library screen for the isolation of *AGL2*-like genes from maize delivered genomic clones to several previously isolated MADS-box gene cDNAs, due to the mixed probe and the non-stringent screening conditions. The latter made it inefficient to isolate clones of specific genes directly, though it allowed for the isolation of new genes, like the clone λDASHII-wd33. In particular, genes can be isolated that will escape the regular cDNA screens, due to their low expression level or their expression during a restricted phase of development (Foster & Twell, 1996).

The non-stringent hybridization conditions still rendered a large amount of *AGL2*-like genes to be isolated. Upon examination, the genomic inserts often contained a partial sequence of the *AGL2*-like MADS-box genes. In order to lower the chance of isolating partial genomic clones, a large insert genomic library could be screened, like YAC or BAC libraries, available from various maize varieties (Edwards *et al*, 1992; O`Sullivan *et al*, 2001; http://hbz.tamu.edu; http://genome.clemson.edu). Whereas in the phage λ replacement vector the insert size measures between nine and 25kb on average, BAC´s can have an average insert size of around 100-150kb, making it more likely to contain the whole genomic locus of a gene.

The extended size of maize *AGL2*-like genes is mainly caused by the large size of the introns and the extra intron I7, compared to the genomic *AG*-like *ZMM1* gene. This has also been reported for *OsMADS1* from rice (*Oryza sativa*) (Jeon *et al*., 2000), that is closely related to *ZMM8* and *ZMM14* (Cacharrón *et al*., 1999). Compared to the situation in *Arabidopsis*, an extra seventh intron as in *AGL9* has also been reported in the *AGL2*-like gene *AGL3* by Huang and co-workers (1995), though the other two *AGL2*-like Arabidopsis genes *AGL2* and *AGL4* (Ma *et al.*, 1991) contain the same number of introns and exons as *ZMM1*. The first intron in particular is very large in size in the maize *AGL2*-like genes, yet, it is of different size per gene. Intragenic sequences, such as the large second intron in the *Arabidopsis* MADS-box gene *Agamous* have been implicated in the specific spacial regulation (Sieburth & Meyerowitz, 1997). Additionally, downstream sequences such as the 3`UTR have also been shown to be necessary for the proper transcript expression during development (Larkin *et al*., 1993). As the various maize *AGL2*-like genes are expressed each in a different but highly specific manner during development, it is likely

to suggest that, apart from promoter and 3´UTR sequences, these different but impressively large introns may be directly involved in the establishment of their extraordinary expression patterns.

As to the coding regions, the amino-terminal location of the MADS-box and the remaining IKC-domain structure has been well conserved within the *AGL2*-like genes, like in most of the plant MADS-box genes. In addition, even the size of three exons (E4, E5 and E6) has been conserved beyond the divergence of monocots and dicots. The strong structural conservation and sequence similarity may reflect similarities in function. However, the exact functional signification of the similarities and differences in sequence and structure can only be determined after an experimental characterization of the respective genes.

4.2 Unraveling *AGL2*-like gene function in maize

The classical method to elucidate gene function is to describe a (natural or induced) mutant, and to isolate the corresponding gene via map-based cloning. The isolation of several mutant alleles, or a complementation via transformation of the mutant with the genomic locus of the wild-type will prove that the isolated gene codes for the mutation. Unfortunately, in most crop plant species like maize, this remains impossible to do due to the excessive size of the genome, and, until recently, a lack of libraries that saturate the genome with contigs. As this method starts with a phenotype (of the mutant), and progresses towards the gene, it is called ´forward genetics´. A modern ´forward genetics´ approach is via T-DNA or transposon tagging. The tagged gene is then isolated out of a population, segregating for the trait, via recovery of a co-segregating fragment of an RFLP obtained by hybridizing the plant DNA to a probe of the T-DNA or transposon. In the case that a gene is available, but not a mutant, the gene function can be revealed (´reverse genetics´) by isolating a mutant from a population of tagged plants, by screening via PCR, based on sequence information of the target gene and the T-DNA or transposon, or by regenerating plants transformed with the gene of interest.

Due to the limited number of functional analyses done on *AGL2*-like genes, it was decided to unravel the function of some of the members of this subfamily of MADS-box genes in maize. Screening of a tagged insertional population owned by Pioneer Hybrid (http://www.pioneer.com) was in conflict with the patent rules of the project due to the participation of agrochemical and plant breeding companies. Furthermore, a lack of candidate mutations that, besides a correlation

of the map positions, showed a phenotype fitting to the expression pattern of the genes suggested a transgenic approach. Plants were transformed with sense and antisense constructs, in order to bring about co-suppression or ectopic expression phenotypes, or phenotypes due to an antisense inhibition of the endogenous transcript.

The sense and antisense lines showed a similar phenotype. This suggests that the plants show a transgene-induced loss of function phenotype. For antisense lines it has been described that the antisense RNA may inhibit the endogenous (i.e. sense) transcript by annealing to this complementary strand and thereby rendering it non-functional (Rothstein *et al*., 1987; Hamilton *et al*., 1990; Dougherty & Parks, 1995). The sense-lines may have led to a co-suppression effect of the endogenous gene (Que & Jorgensen, 1998, and refs. therein). Co-suppression is a phenomenon in which sometimes the transcription of the target gene is reduced, due to the presence of a transgene, that is also silenced (Napoli *et al*., 1990; Meyer & Heidmann, 1994). This can explain the loss of function phenotypes in the *AGL2*-like genes transformed plants that show a lower level of expression. However, the majority of the maize plants examinated did not display a (total) loss of transcript. There is evidence that in sense plants displaying a phenotype the expression of a transgene can deregulate the control of the endogenous target gene, leading to elevated levels of the transgene and endogene mRNA (Metzlaff *et al*., 1997). Van Blokland and colleagues (1994) have shown via run-on transcription tests in isolated nuclei that loss of the endogenous gene function is actually not associated with reduced transcription. Hence, co-suppression is presently recognized as a post-transcriptional gene silencing (PTGS) event (Baulcombe, 1999). The loss of gene function has furthermore been linked to sequence specific degradation of the transcript (van Eldik *et al*, 1998; Baulcombe, 2001). This seems to occur via double stranded RNA intermediates (dsRNA), that are created by RNA-dependent RNA polymerase (RdRP) of the plant (Wassenegger & Pélissier, 1998). The dsRNA is subsequently degraded by RNases. The nature of the RNAs that can induce the formation of dsRNA, leading to PTGS, is believed to include normal transcripts (above a certain `threshold` level), as well as antisense and abberant transcripts (Lindbo *et al*, 1993; Baulcombe, 1996; Metzlaff *et al* 1997; Wassenegger & Pélissier, 1998). Abberant transcripts of the transgenes of *ZMM6* and *ZMM8* have also been observed in phenotypic plants (this work). Abberant transcripts usually stem from a single complex locus with multiple rearranged copies of the transgene (Iyer *et al*, 2000 and refs. therein). Direct DNA transfer methods, like PEG-mediated plant transformation used here, are especially prone to generate complex rearranged transgenes, compared to *Agrobacterium*-mediated transformation (Rossi *et al*., 1996; Iyer *et al*, 2000).

The transgenic approach to investigate *AGL2*-like gene function showed a low occurrence of the phenotypes. This might be because different silencing approaches (sense vs. antisense) were not always effective at inducing silencing. Alternatively, other genes that have the same function might (partially) complement the transgenic phenotype - e.g. *ZMM6* in *ZMM8* transformed plants, or *vice versa*-. As sense and antisense silencing might be traced back to the same mechanism of PTGS, co-expression of sense and antisense mRNA was persued by crossing the single transformed plants, as described by Waterhouse and colleagues (1998) (see fig.3.21B,G). Some of these plants showed a higher frequency of the phenotype than the average single transformed plants (data not shown), yet the phenotypes did not become present in all the spikelets of the inflorescences. This suggests that the phenotype of either gene (*ZMM6* or *ZMM8*) can (at least partially) be complemented by the other, or even by yet other ones than the two mentioned. It is noticed here that especially the many maize *AGL2*-like genes come into question for such a function, as is suggested by the candidate gene/mutant couples *Ts6*/*ZMM24*, *ifa1*/*ZMM14*. In addition to crossing sense and antisense lines, silencing can be more efficiently induced via co-expression of sense and antisense transgenes by co-localization of the ORFs on the same construct or by a single transcript that has self-complementarity (Waterhouse *et al*, 1998; Smith *et al*, 2000).

Silencing in plants has been described as a naturally occurring host defense mechanism against virusses and transposable elements (Baulcombe, 2001). Recently, it has been suggested that RNA silencing is also involved in regulating genes required for normal growth and development (Jacobsen *et al*., 1999). Mutation of the *CARPEL FACTORY* gene in *Arabidopsis* leads to defective flowers, apart from a defect in silencing. The gene is a homolog of *DICER*, an RNA-cleaving enzyme in *Drosophila melanogaster* (Bernstein *et al*, 2001). When silencing is a natural feature of plants during development, PTGS might be induced by mutant genes that are over expressing their (full length) transcript, above the `threshold´ level (see above). It is postulated here, that the *AGL2*-like candidate gene *ZMM24* to the dominantly inheriting and *ZMM24* over-expressing mutant *Ts6*, might actually show a silencing phenotype, due to deregulation of transcription of *ZMM24*. This would be in agreement with the phenotypes of other *AGL2*-like genes showing also a decreased degree of determinacy (see below).

4.3 *ZMM6* confers meristem determinacy on different meristem levels

Expression analysis shows *ZMM6* distinguishes between the sessile and pedicellate spikelet of a pair of spikelets. The phenotypic analysis of the transgenic plants at this level of reproductive development showed a higher degree of branching, leading to ´triplet´or even ´quadruplet´ formation in contrast to the wild-type arrangement of paired spikelets. The phenotype correlates therefore well with the early site of expression of the gene. However, it must be pointed out that although the expression of *ZMM6* makes a distinction between the two spikelets out of a pair, that may be molecularly tagged as having different identities, it has previously not been specifically linked with the identity of either the sessile or the pedicellate nature of the spikelets.

There is evidence that the two spikelets have different identities, since some mutations only affect one type of spikelet, but not the other. Maize plants carrying the dominant mutation *Suppressor of sessile spikelets1* (*Sos1*) lack the sessile spikelet in ear and tassel due to the inhibition of branching of the spikelet pair meristem (Doebley *et al*., 1995). Remarkably, in the wild ancestor of maize, teosinte (*Z.m*. ssp. *parviglumis*) both spikelet primordia are formed initially, but then the pedicellate spikelet is specifically aborted, leaving only the sessile spikelets to develop to maturity (Doebley *et al*., 1995). In the mutant *Tasselseed6* (*Ts6*) the development of the pedicellate spikelet is affected (Irish, 1997). The spikelet pair meristem produces the sessile spikelet, but stays indeterminate after its conversion to the pedicellate spikelet meristem, producing supernumerary floret primordia, thereby becoming a sort of branch. The development of *Ts6* inflorescences resembles those of *ramosa2* (*ra2*) in which the pedicellate spikelet meristem becomes indeterminate (Neuffer *et al*., 1997). None of these mutants however, is allelic to *ZMM6* (Neuffer *et al*., 1997). Furthermore, a different spikelet identity may be revealed by the different speed of development. The sessile spikelet is initiated before the pedicellate one, but the latter develops in advance (Cheng *et al*., 1983).

ZMM6 expression has been shown to be consistently present in only one spikelet of a pair of spikelets, however, the position of that spikelet initial within the pair was placed at random at the inflorescence (Cacharrón & Theißen, pers. comm.). This correlates well with the random orientation of the position of the pedicellate spikelet (or sessile spikelet) within the pair of spikelets with respect to their arrangement to the main inflorescence stem. Morphologically the two spikelets look nearly identical, and can only be identified in the male inflorescence by the

length of the pedicel. As the spikelets in the female inflorescence are not positioned on a stalk, they can not be distinguished morphologically.

The phenotypic analysis at the spikelet pair level of tassel and ear shows the same structures arise, irrespective of the morphological detectability of their identity. This suggests that the disruption of the wild-type *ZMM6* function specifies the degree of branching (i.e. the level of determinacy of the respective meristem), rather than the identity of the primordium. If *ZMM6* would be involved in specifying one of the two particular spikelet identities, this would be displayed in the transgenic plants by an occurrence of spikelet pairs only containing either pedicellate or sessile spikelets. Alternatively, it would be shown by an absence of one of the spikelet types, depending on which identity is specified. The latter would indicate a function in meristem initiation as well, a function that has been stated for the co-localized mutant *barren inflorescence2* (*bif2*) (McSteen & Hake, 2001). Both scenarios, however, have not been observed. In contrast, the higher degree of branching of the spikelet pair meristem led to an array of different triplets and quadruplets, in which different numbers of sessile and pedicellate spikelets were arranged together, and positioned at random with respect to each other. This is additional evidence for a decrease in determinacy of the spikelet pair meristem, irrespective of the identity the newly created spikelets adopt. Also, although *ZMM6* expression initially distinguishes between the two spikelets, later on throughout development the expression is turned on in both primordia. Again this suggests that *ZMM6* does not have a function in the specification of identity.

An identity-specifying scenario should also not lead to a phenotype within the spikelet, as *ZMM6* is expressed equally in both floret meristems, and therefore, does not distinguish between either one of them. The fact that the upper and lower floret might have a different identity, is shown by the specific abortion of only the lower floret in the female spikelets (Cheng *et al*, 1983), or by mutants that affect the development of one of the two florets. In the mutant *reverse germ orientation1* (*rgo1*) (Sachan & Sarkar, 1978) as well in the double mutant *pistillate florets* (*pi1pi2*) (Neuffer *et al*., 1997) the lower floret can develop due to an absence of abortion. Furthermore, in wildtype plants the two florets may have adopted different identities for they develop at a different speed, similarly to the sessile and pedicellate spikelet (Cheng *et al*., 1983). The upper floret develops in advance of the lower one, although it is initiated later.

If *ZMM6* would also have a function within the spikelet primordium in establishing the level of determinacy of the floret meristems, one would expect a phenotype upon interfering with the *ZMM6* expression. In the *ZMM6* transformed plants such a phenotype was observed, in tassel and ear. The spikelet meristem produced a higher number of floret primordia in transgenic plants, supporting the idea that *ZMM6* is involved in conferring meristem determinacy to both the floret meristems, irrespective of their identity. In the female inflorescence up to two florets developed. It could not be distinguished whether the proliferation of the spikelet meristem interfered with the actual abortion process of the lower floret, or that the extra floret was produced after abortion of the first initiated lower floret, thereby nullifying the effect of the abortion. However, the number of initiated glumes (see below) in the phenotypic spikelets was not increased in contrast to the tassel, where three to four florets developed within three to four glumes. This suggests that the developing lower floret in the ear arose due to an inhibition of the abortion, that in turn was accompanied with, or even caused by a more indeterminate spikelet meristem.

Within the floret the *ZMM6* transgenic plants showed a phenotype, too, correlated to its expression in the floral organ primordia. Here the number of floral organs (stamina, lodicules, paleae) was increased, and in addition evidence was found that lodicules were changed into paleae. So, on the flower level, there is a function for *ZMM6* to specify the identity, apart from a function in maintaining determinacy. This observation strongly resembles the dual functions described for *AG* in *Arabidopsis* (Yanofsky *et al*., 1990; Mizukami & Ma, 1995) (see below).

4.4 *ZMM8* functions to confer meristem determinacy

The transgene-induced silencing phenotypes of *ZMM8* are highly similar to those caused by the transgene *ZMM6*. This suggests that the genes work directly together to regulate inflorescence branching, or that they work in parallel developmental pathways bringing about the same developmental process. Alternatively, both genes might be affected simultaneously in the transgenic plants. The latter assumption would also explain the *ZMM8* phenotypes of triplets and quadruplets, fitting to a disruption of the spikelet pair meristem, eventhough the gene does not seem to be expressed there in the wild-type (Cacharrón, 1994; Cacharrón *et al*, 1999). Interestingly, MADS-box genes have previously been reported to be specifically expressed in the tisssues that they affect (Theißen & Saedler, 1995). Therefore, it is postulated here, that expression might in addition be present at the spikelet pair meristem, but under the detection

level. Circumstantial evidence for this comes from the candidate *ZMM14* gene -the partner gene of *ZMM8*- to the *ifa1* mutation (see also below; §4.7). Although *ZMM14* is similarly expressed as *ZMM8* (Cacharrón *et al*, 1999), *ifa1* is also affected in the spikelet pair meristem (McSteen *et al*, 2000).

In what way does *ZMM8* function? The *ZMM8* expression pattern shows it distinguishes between the two florets within a spikelet (Cacharrón *et al*., 1999). These have been termed upper and lower floret (Cheng *et al*., 1983) due to their fixed position on the female inflorescence, although in the male inflorescence such a distinction as to the place within the spikelet can not be made. This led to the interpretation of the floret primordia as lateral initials of the rachilla by Bonnet (1953), later supported by Chuck and colleagues (1998) based on phenotypic analysis of the mutant *ids1*, having multiple florets distichously placed onto the extended spikelet axis. In contrast to *ZMM6* that is initially only expressed in one spikelet meristem of a pair, but at a later developmental stage in both, *ZMM8* expression continues to be visible in only the upper floret meristem, never in the lower, nor in both. This led to the hypothesis that *ZMM8* might confer upper floret identity to the respective meristem (Cacharrón *et al*, 1998). A disruption in the *ZMM8* function would accordingly lead to an absence of upper floret identity, and hence possibly to an abortion of the upper floret, in addition to the abortion of the lower floret in the ear. This however has not been observed. In both the tassel and ear the number of florets per spikelet increased, suggesting the transgene-induced silencing of *ZMM8* leads to a decrease in determinacy of the (upper) floret meristems, having for a longer period of time spikelet meristem identity and thereby producing more than the wild-type number of florets. As this function does not seem to be linked to a identity of upper floret, one can hypothesize that *ZMM3*, a highly related *AGL2*-like gene with opposite expression pattern in only the lower floret, might confer determinacy to the lower floret (Cacharrón, 1994). Inhibition of *ZMM3* function would accordingly lead to a similar phenotype of an increased number of florets, as has been seen in *rgo1* (Sachan & Sarkar, 1978; Kaplinsky *et al*, 1999).

Within the floret the *ZMM8* transgenic plants showed a similar phenotype as the *ZMM6* plants, correlated to the expression in the floral organs. On the floret level, *ZMM8* contribute*s* to specify the identity, apart from a function in maintaining determinacy.

4.5 How can these phenotypes be explained in the present-day model of inflorescence and flower development?

The question whether the upper floret differs developmentally from the lower one remains elusive. The same holds for the developmental origins of the sessile and pedicellate spikelet. Clear is that the compound maize inflorescences are generated from the inflorescence meristem in a step by step process (Cheng *et al*., 1983; Irish, 1997, Irish, 1998). The inflorescence meristem produces lateral initials, the spikelet pair meristems, from which two initials arise, the spikelet meristems. These in turn give rise to each two floret meristems, which form the floral organs. The step-wise initiation of the four different meristem types in the inflorescence is accompanied by an increasingly higher level of determinacy in the respective meristem types (Irish, 1997). The establishment of the structures is unidirectional, indicating there are factors necessary that specify the determinacy in order to prevent a reversion of the identity of one meristem into a previous, less determinate one (Battey & Lindon, 1990; Okamura *et al*, 1996; Venglat & Sawhney, 1996; Irish, 1997). The sequential initiation of reproductive meristems is a clearly defined process in which the size of the meristems is specified by timing the on-set of the respective determinacy level. This potentially regulates the number of initials that the meristem subsequently produces, and therefore it is important determinant of the inflorescence architecture (Sundberg & Orr, 1996; Kerstetter, *et al*, 1997; Chuck *et al*, 1998). Treatments that influence or interfere with the expression of homeotic genes, including *AGL2*-like MADS-box genes like *TM5*, have been shown to bring about a back transformation of one meristem type into another, or to lead to alterations in meristem number and identity (Battey & Lindon, 1990; Okamura *et al*, 1996; Lozano *et al*., 1998) Furthermore, specifically in MADS-box gene mutant backgrounds reversal of meristem determinacy has been accomplished (Mizukami & Ma, 1997; Okamura *et al*, 1997).

The four reproductive meristems have a different identity as well as a different degree of determinacy. Irish (1998) makes these two aspects fuse by reasoning that the different meristem types accomplish a higher determinacy because of a change in identity: a spikelet pair meristem, after initiating a single spikelet meristem, *becomes* a spikelet meristem itself. Similarly, each spikelet meristem *becomes* a floret meristem after initiating a single floret meristem. This model was established based on analysis of two inflorescence mutants, *ts4* and *Ts6*, that are impaired, or delayed in acquiring determinacy in either the spikelet meristem (*ts4*) or floret meristem (*Ts6*) level, respectively. Chuck and co-workers (1998) challenged this view on inflorescence development based on mutant analysis of *ids1* by stating that all floral meristems are derived laterally from the spikelet meristem. An intermediate model was proposed by Irish (1998) that

combines the lateral branching model with the identity convertion model. After the spikelet meristem laterally initiates two floral meristems, the second floral meristem recruits almost all of the cells of the spikelet meristem, leaving only a small non-functional zone behind retaining the fate of the spikelet meristem. The incorporation of cells of the spikelet meristem by the laterally formed second floret meristem should constitute the change of identity by which directly the increased level of determinacy is achieved.

In the *Arabidopsis* mutant *terminal flower1* (*tfl1*), and the *Anthirrhinum* counterpart *centroradialis* (*cen*), the apex of the main inflorescence meristem looses its indeterminacy, and changes identity to a terminal flower meristem (Shannon & Meeks-Wagner, 1991; Bradley *et al*., 1996). This determinate meristem develops faster, like the upper floret within a spikelet in maize, and like the pedicellate spikelet within a pair of spikelets. Therefore, the conferring of determinacy seems to give the respective meristems a more `terminal-like´character. Recently, homologous genes in the grasses *Lolium perenne* (*LpTFL1*) and rice (*FDR1* and *FDR2*) have been isolated, indicating that a similar mechanism may act in maize as well (Jensen *et al*., 2001; acc. no. AAD42896, AAD42895).

ZMM6 and *ZMM8* seem to function on the last three reproductive meristem levels as deteminacy genes. Transgenic plants show deficiencies in the transition of one spikelet meristem to another, leading to a prolonged period of indeterminacy of the former meristem. Thereby they create ´branching´ phenotypes on those meristem levels, resembling back transformations of one meristem type to another. The terminal state of the lastly formed meristem at the respective levels of inflorescence development is not inhibited, but eventually adopted. Therefore, the two genes do not distinguish between the different identities of the primordia on the different meristem levels (sessile vs. pedicellate and upper vs. lower), but merely act before or independent from these identities. Furthermore, their phenotypes look similar to mutants that also have been implicated in inflorescence development by a loss of determinacy of the respective meristems (see below).

4.6 More florets, more glumes

In the transgenic *ZMM6* and *ZMM8* plants, a higher number of florets was accompanied by a proportional increase in the number of glumes. The spikelet meristems that are delayed in determinacy, first create extra glumes, and in the axils of those the extra florets develop. Finally determinacy is bestowed upon the last meristem, rendering it terminal, and abolishing the outgrowth of the spikelet apex. In contrast, the extra florets in the spikelets of *ids1* do not have a subtending glume (Chuck *et al*., 1998) (fig.4.1). This suggests the conferring of indeterminacy in *ids1* is occurring at a later time point, namely *after* the initiation of the glumes by the spikelet meristem, or is accomplished via a different mode of action. This is suggested from the fact that, the meristem continues to stay indeterminate from that point on, producing floral meristems repetitively, without the conversion of the ultimate one to a terminal position.

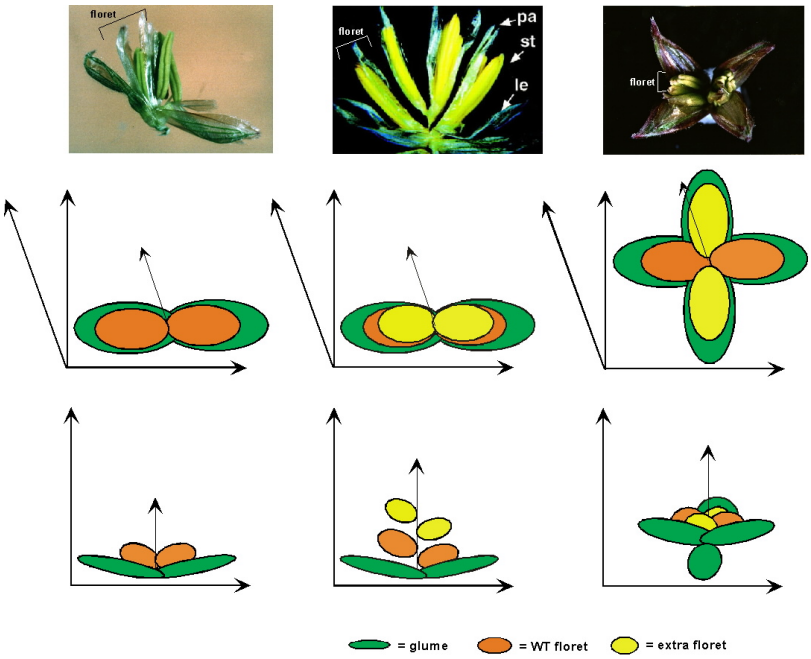

Fig.4.1. Phenotypic spikelets of *ids1* compared to those of antisense *ZMM6* transgene silenced plants. Column one showns the wildtype, column two shows *ids1*, and column three shows *ZMM6* transgenic plants. In row one the phenotypes are displayed, in row two and three the phenotypes are schematically represented from a top view and a side view, respectively.The *ids1* spikelets have only two glumes containing between 5 to 10 florets, distichously positioned on the extended spikelet apex. The spikelets of *ZMM6* or *ZMM8* transgenic plants only have 3 to 4 florets, each subtended by a glume. The rachilla is not visible. Phenotypes are compared to wildtype. (*ids1* photo from Chuck *et al*., 1998).

According to the phytomer hypothesis (Lyndon, 1998; Koppstech *et al*., 1998; Irish, 1998), plants develop modularly via the repeated production of morphological units (phytomers) by their apical meristems. The above-ground part of the plant, including the inflorescence is derived from phytomeres initiated by the shoot apical meristem (SAM). The phytomer consists out of a leaf,

node, internode and axillary bud, that are modified, after the transition to flowering, into bract, node, part of the inflorescence stem (internode) and flower (axillary bud), produced by the inflorescence meristem. Differences in morphology between the shoot and inflorescence apex are based on the extended or compressed nature of the compounds within the phytomere, as well as the suppression of those (Irish, 1998; Long & Barton, 2000). If the same developmental modular organization is applicable to maize, one could interprete the glume as a bract subtending the floral meristem, that develops in its axil, fitting to part of the rachilla (internode), that is not grown out. In this model the genes *ZMM6* and *ZMM8* act to confer determinacy on the total meristem, so that a whole new phytomer can be formed. However, due to the compressed nature of the phytomer that is not affected by the genes, the extra units develop in close proximity to each other. In contrast to this, in the *ids1* mutant, the compressed nature is affected, leading to an extension of the rachilla. Furthermore, the *ids1* spikelet meristem is initially only partially indeterminate, as it is not capable of forming extra glumes.

On the inflorescence level, the phytomers might consist of spikelet pair primordium as the axillary bud, connected to a part of the rachis (internode) and subtended by a cryptic bract. The presence of a cryptic bract is revealed by the mutation *tasselsheath1* (*tsh1*), in which the suppression of the structure is undone (Neuffer *et al*, 1997; McSteen & Hake, 2001). Similar observations have been made for *Arabidopsis* in which the initiation of a suppressed bract subtending each flower is suggested (Long & Barton, 2000).

4.7 Inflorescence branching mutants

Several branching mutants in maize show similar phenotypes to those of the putative transgene induced silenced plants. The *ramosa* mutants (*ra1*/*2*/*3*), characterized by the outgrowth of branches at the base of the female inflorescence, reveal a higher degree of branching at the spikelet pair and spikelet primordia (Neuffer *et al*., 1997). *ra1* was recently isolated and was found to encode a small protein with a single EPF-type zinc finger motif (Vollbrecht & Martienssen, 2002).

Indeterminate floral apex1 (*ifa1*) is affected in spikelet pair-, spikelet-, and floret meristem determinacy, too (Laudencia-Chinguanco & Hake, 1998; McSteen *et al*, 2000). In addition to this, a mass of pistillate material develops in the center of the floret, indicating the floral meristem

continues to proliferate (http://www.agron.missouri.edu/db_images/Variation/33laudencia.jpg). *ZMM14* maps within a 6 cM chromosomal interval to *ifa1*, in a duplicated region of the genome to which the partner gene *ZMM8* maps (Cacharrón *et al*., 1999). Both genes show expression in only the upper floret, with *ZMM14* having a stronger expression in the developing carpel. These data make *ZMM14* a likely candidate for *ifa1* (Cacharrón *et al*., 1999). Furthermore, the (unmapped) double mutant *pistillate florets* (*pi1pi2*) might also be a candidate for the genes *ZMM8* and *ZMM14* (Neuffer *et al*., 1997; Huelsen & Gillis, 1929). The mutant tassel shows a loss of determinacy of the spikelet pair-, spikelet- and floret meristem. In the ear, the two floret primordia produced in every spikelet develop both to maturity. Independent double mutant accessions with the same phenotype were described by Lorenzoni and colleagues (1971) and Micu and colleagues (1983).

Another mutant, *branched silkless1* (*bd1*) shows the formation of branches in the ear (Kempton, 1934; Colombo *et al*, 1998). The spikelet meristem remains indeterminate, forming more florets than the wildtype. Furthermore, the floral meristem is more indeterminate, creating occasionally four stamina instead of three, and giving rise to sterile florets consisting of ´glume-like´ structures. These glume-like structures do not assume the typical shape of glumes, but instead are two-lobed at the top, reminiscent of paleas. *Bd1* has recently been cloned and shown to encode a new member of the *AP2*-like genes (Chuck *et al*., 2002)

Reverse germ orientation1 (*rgo1*) also displays similar branching phenotypes in tassel and ear on the different meristem levels (http://www.agron.missouri.edu/mnl/72/33kaplinsky.html; http://mtm.cshl.org/cgi-perl/image.cgi?name=6659.1.JPG&class=Image; Sachan & Sarkar, 1978; Kaplinsky *et al*, 1999). In homozygous *rgo1* plants the phenotype of a supernumerary florets is present in all spikelets. Its map position to bin 9.04 (chromosome 9; App.7.9) makes it a candidate mutation to the *AGL2*-like MADS-box gene *ZMM3* (9S034; table 3.5). Interestingly, *ZMM3* transcript is present in the lower floret of the spikelet (Cacharrón, 1994).

Furthermore, at the tip of inflorescences of the *ts4* mutant, spikelet meristems fail to form out of spikelet pair meristems, that are turned into branches (Irish, 1997). In the more basal regions of the inflorescence, *ts4* resembles *Ts6* plants, having wildtype sessile spikelets but pedicellate spikelets in which more florets are produced (Irish, 1997). Interestingly, *ts4* has been suggested to encode a mutation in the *AG*-like MADS-box gene *ZAG2* due to the correlation in their map positions (http://www.agron.missouri.edu:80/cgi-bin/sybgw_mdb/mdb3/Map/258947) (Veit *et*

al., 1993). Combined with the previously mentioned *AGL2*-like *ZMM24* candidate gene to the *Ts6* mutation, it seems likely to suggest that determinacy is at least in part bestowed upon the meristems by MADS-box genes, some of which belonging to the *AGL2*-like subfamily. The fact that also *AG*-like genes in maize can contribute of conferring determinacy is examplified and discussed below for *ZAG1* (§4.9).

Isolation of the genes causing the above mentioned mutant phenotypes will help clarifying in what way the inflorescences of maize develop. Furthermore, the respective mutants can be used to investigate the function of *ZMM6* and *ZMM8* further. By performing *in situ* hybridization experiment on mutant inflorescences, one might find out whether the wildtype pattern of expression is altered. Alternatively, one might cross the transgenes into a mutant background. When *ZMM6* or *ZMM8* are part of a different pathway regulating inflorescence development, one would expect the phenotype to be additive. When the mutant acts before *ZMM6* or *ZMM8* in the same pathway, the double mutant plants would have the same phenotype as the mutant (epistatic interaction). In case that the genes are in the same pathway as the mutant, the phenotype should be stronger.

4.8 Functional equivalency of *AGL2*-like MADS-box genes

The functional analysis of *leafy hull sterile1* (*lhs1*) rice plants (*Oryza sativa*) showed that *OsMADS1* is mutated (Jeon *et al*., 2000). This *AGL2*-like gene is the closest relative of *ZMM8* and *ZMM14* in this related grass species. The map position of *OsMADS1* (on chrosome 3) suggests it is located in a syntenic region to *ZMM8* and *ZMM14* (Cacharrón *et al*, 1999). Although expressed in only palea, lemma and carpel (Chung *et al*., 1994), the phenotype of *lhs1* plants strongly resembles the loss of function phenotype of *ZMM8* plants (fig.4.2). Spikelets in rice, containing only one floret, are bisexual, with a lemma, a palea, two lodicules, six stamina, and a carpel. Mutant *lhs1* florets often have eight stamina, pointing to a higher indeterminacy, and the lodicules show a transition to palea-like structures. In strongly affected florets, all floral organs are converted into ´leafy´ organs, resembling paleas. The spikelet meristem is more indeterminate, sometimes producing an extra floret, on a grown out rachilla. The fact that the *lhs1* and *ZMM8* phenotypes are not identical may point to a divergence in function during evolution.

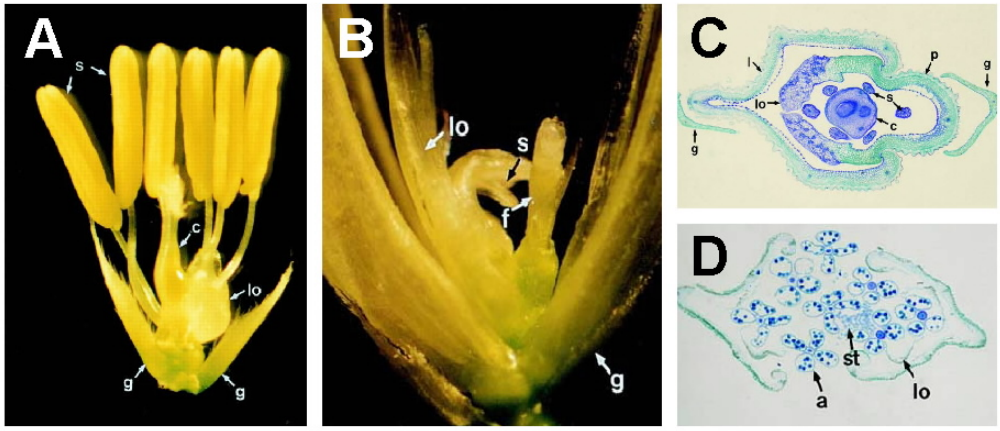

Fig.4.2. Phenotypes of the rice *AGL2*-like gene *OsMADS1* in mutant *leafy hull sterile1* (*lhs1)* plants.
A. Wildtype rice spikelets have only one floret. The spikelet consists of two glumes, two lodicules, six stamens and a carpel. The lemma and palea have been removed. **B.** *lhs1* spikelet shows a lodicule that has converted into a palea, a stamen that is partially converted into a palea and an extra floret primordium sprouting from the elongated rachilla. **C.** Cross section through a wildtype spikelet. **D.** *lhs1* spikelet (glumes are not visible). The spikelet contains eight stamens, and the lodicules have converted into paleas. g= glume, lo=lodicule, s/st= stamen, c= carpel, f= floret, l= lemma, p= palea, a= anther. (Figure modified after Jeon *et al*., 2000)

The complete conversion of floral organs into paleas leading to a reiterated pattern of paleas in rice compared to maize suggests there may be additional factors in maize such as *ZMM6*, that (at least partially) substitute for a total loss of *ZMM8* function as seen in rice. In maize there might be a larger number of genes, causing similar, eg. *ZMM6*, yet not identical phenotypes, eg. *ifa1*, that may encode *ZMM14*, the partner gene in maize. However, neither in *lhs1* nor in the *ZMM8* plants an increased number of carpels or ovaries has been seen. In another grass species, barley (*Hordeum vulgare*), the *AGL2*-like gene *BM7* is presently the most related gene from a phylogenetic perspective (this work; Schmitz *et al*., 2000). *BM7* is closely linked to the classical mutant *multi ovary1* (*mo1*) (Castiglione *et al.*, 1998), that produces a conversion of lodicules into palea-like structures, and a supernumerary number of ovaries in the center of the florets (Soule *et al*., 1995; Schmitz *et al*., 2000), reminiscent of the *ifa1* phenotype (Laudencia-Chingcuanco & Hake, 1998). Isolation of the loci causing the mutations of *ifa1* and *mo1* might ultimately shed light on their functional and evolutionary relationships. In addition, it must be added that the reiterated pattern of paleae in *lhs1* strongly resembles the *bd1* phenotype in maize, so that it can not be excluded that in maize *BD1* partially complements this part of the phenotype in *ZMM8* silenced plants, and hence is partially functionally redundant to it. This aspect of the phenotype could be revealed by analyzing *bd1*/*bd1*/35S::*ZMM8* plants.

In the eudicot *Arabidopsis* three *AGL2*-like genes (*SEP1/2/3*) have been functionally analyzed (Pelaz *et al*., 2000). Initially, no mutant phenotype was reported for each single homozygous mutant locus. Due to their relatedness as shown from phylogenetic analyses, a high level of functional redundancy was expected. Therefore, the single mutant loci were crossed, and the triple mutant was analyzed. The floral organs in the three inner whorls of the triple mutant had converted into sepals. Additionally, the meristem had become more indeterminate, producing a higher number of floral organs. Therefore, *SEPALLATA* genes do not only specify organ identity in the inner whorls of a flower by assisting B- and C-function genes (the E-function, Theißen & Saedler, 2001). They also participate in conferring determinacy to the floral meristem (Pelaz *et al*, 2000). This phenotype strongly resembles the *lhs1* phenotype of a reiterated pattern of paleas. Analysis of the *SEP3* gene by means of antisense plants showed a partial conversion of petals to sepals, and in addition, the occurrence of axillary flowers within a flower (Pelaz *et al*., 2001). These phenotypes correlate well with those for *ZMM6* and *ZMM8* in maize. Since Ambrose and co-workers (2000) stated that monocot lodicules and paleas are homologous to eudicot petals and sepals, the sepaloid petals are homologous to the observed palea-like lodicules. The production of an axillary flower places the subtending sepal in the position normally occupied by a bract. This resembles the formation of an extra floret, subtended by a glume in maize. Furthermore, Prof. M. Yanofsky kindly provided seeds, that are segregating for the *SEP* triple mutant phenotype (fig.4.3). The plants were analyzed morphologically and via PCR to identify the T-DNA or transposon insertions (as described in Pelaz *et al*., 2000) (Deleu & Theißen, unpubl. results). Plants with up to three mutant *sep* alleles displayed neither homeotic changes, nor a loss of determinacy (fig.4.3B), resembling wildtype plants (fig.4.3A). Double mutant plants (*sep1sep3*) showed a range of flowers with sepaloid petals (fig.4.3C). Plants having only one wildtype allele (*SEP2*) had a determinate flower consisting of a higher number of sepals encircling a carpel on a pedicel (fig.4.4D). The triple mutant was as described by Pelaz and colleagues (2000) (fig.4.3E), however, in a later stage the floral meristems did not scenesce, but continued to proliferate after having produced the reiterated pattern of sepals (fig.4.3F). The flower-like structure was transformed into an inflorescence, in which the flowers consisted of a reiteration of sepals, that in turn were converted into a similar inflorescence (fig.4.3G). The sepal-like organs were thereby turned into bracts. Therefore, in addition to the organ identity function (Pelaz *et al*., 2000), the *SEP* genes function to build the inflorescence by conferring determinacy to floral meristems. Knocking out these genes leads to an endless proliferation of the inflorescence meristem that is not able to form flowers.

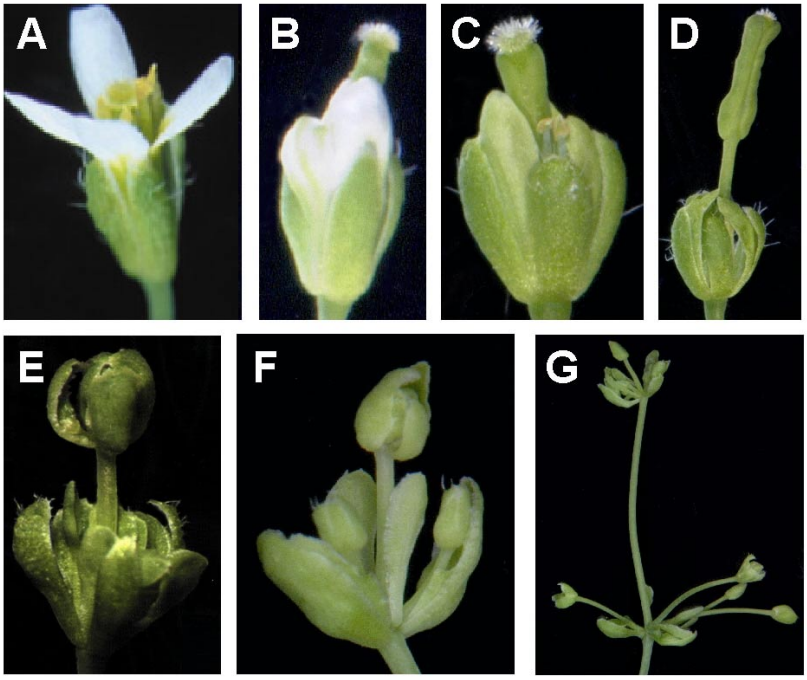

Fig.4.3. Functional analysis of *Arabidopsis AGL2*-like genes *SEPALLATA1-3* in a segregating population. **A.** Wildtype flower. **B.** *SEP1SEP1/SEP2sep2/sep3sep3* flower. The *SEPALLATA* genes function redundantly, displaying no phenotype when up to three *SEP* alleles are mutated. **C.** *sep1sep1/SEP2SEP2/sep3sep3* flower, showing sepaloid petals. **D.** *sep1sep1/SEP2sep2/sep3sep3* flower. If five *SEP* alleles are knocked out, the flower consists of a higher number of sepaloid petals and petals completely converted into sepals, surrounding a terminal pedicellate carpel. **E.** In case all 6 *SEP* alleles are mutated, the inner 3 whorl organs are first converted into sepals (Pelaz *et al*, 2000). **F.** Triple *sep1/2/3* mutant, a few days later than shown in E. The axillary primordia of the 'sepals'/bract-like structures are grown out into flower-like structures. **G.** At a later stage, the `flower` reiterates the production of flower-like structures, thereby transforming into an inflorescence. (fig.4.3A,E, modified after Pelaz *et al*, 2000).

In the eudicot *Petunia* co-suppression of the *AGL2*-like gene *FBP2* caused similar phenotypes as described in *Arabidopsis* (Angenent *et al*., 1994). Plants formed sepaloid petals, suggesting the floral organ identity function has been conserved. Furthermore, some plants produced stronger phenotypes including a reiterated pattern of sepals, and the formation of an inflorescence-like structure sprouting from the axils of the carpels. These latter phenotypes point to a conserved function in conferring determinacy as well.

Antisense inhibition of *TM5*, the tomato ortholog of *SEP3* and *FBP2*, caused sepaloid petals, and more inner floral organs were formed, suggesting similar functions to the above mentioned *AGL2*-like genes have been conserved.

In the dicot *Gerbera*, 2 *AGL2*-like genes, *GRCD1* and *GRCD2*, have been functionally analyzed by means of antisense inhibition of transformed plants (Kotilainen *et al*., 1999; Kotilainen *et al*.,

2000). *GRCD1* is expressed in the all four floral whorls but is only involved in specifying the identity of the third whorl organs (Kotilainen *et al*., 2000). This suggests that there may be other *AGL2*-like genes in *Gerbera* that co-specify the organ identity in the remaining whorls, together with the B- and C-function genes. This in turn points to a diversification of the floral organ identity specifying functions of *Gerbera AGL2*-like genes. Furthermore, the *GRCD2* is expressed in the outer two regions of the capitulum (i.e. inflorescence in *Compositae*), and showed upon antisense inhibition a proliferation of those inflorescence parts, thereby producing supernumerary wildtype trans- and ray florets (Kotilainen *et al*., 1999; Prof. T. Teeri, pers. comm.). In addition, a loss of organ identity specification in the third whorl was observed. Therefore, *GRCD2* is not only specifying identity, but is also conferring determinacy to those inflorescence regions in which it is expressed. Thus, it is likely to suggest that during evolution the *AGL2*-like ancestral genes within the monophyletic taxonomic clade of the *Compositae*, underwent a functional diversification as to the determinacy function and the floral organ identity specifying function.

4.9 Diversification in function: conferring floral organ identity versus determinacy

A similar diversification has been suggested for the two duplicate *AG*-like maize genes *ZAG1* and *ZMM2* by Mena and colleagues (1998). A transposon-induced mutation in *ZAG1* (*zag1-mum1*) was isolated and characterized. *ZAG1* is phylogenetically closely related to the *Arabidopsis AG* gene, that is required for specifying reproductive organ identity and for floral determinacy (Yanofsky *et al*, 1990). *ZAG1* mutants exhibited only a loss of determinacy, forming two or more silks in whorl four. The identity of the inner two whorl floret organs remained largely unaffected. The authors state that *ZAG1* is expressed as a C-function gene like *AG* in carpels and stamina, but expression is stronger in the carpels, supporting the phenotype. That *zag1-mum1* plant had wildtype floral organs pointing to redundancy in organ specification. The duplicate gene of *ZAG1*, *ZMM2* is expressed in the stamina and in carpels as well, but expression is stronger in the stamina. This suggests *ZMM2* itself may participate in regulating the development of the reproductive organs. Because of extensive sequence similarity between *ZAG1* and *ZMM2*, it is likely that they share some redundancy in function, and yet, evolved partially distinct roles in floret development, unlike *AGAMOUS*.

Similar partially distinct expression patterns are described for the duplicate *AGL2*-like genes *ZMM8* and *ZMM14* (Cacharrón *et al*., 1999). The stronger expression of *ZMM14* in the carpel

may point to a partial diversification in function, not redundant with *ZMM8´*s function. The co-localization of *ZMM14* with *ifa1*, suggesting that *ZMM14* is mutated in this mutant, is supported by the distinct phenotype of a mass of pistillate tissue in the center of the floret of *ifa1* plants (Laudencia-Chingcuanco & Hake, 1998), not present in *ZMM8* transgenic plants. The suggestion that *ifa1* might encode a mutant allele of *ZMM14* is further supported by the following data. Fan and co-workers (1997) showed that AGAMOUS interacts with (among others) the AGL2-like proteins SEP1, SEP2 and SEP3. Similarly, protein interactions between the *Antirrhinum* AG-like PLE and *AGL2*-like DEFH200 and DEFH72 proteins were also revealed (Davies *et al*., 1996). In analogy to this, one could expect *ZAG1* to interact with maize *AGL2*-like genes, such as *ZMM14*. Crossing of *ifa1* to *zag1-mum1* plants led to an increase in the phenotype in double mutant *ifa1*/*zag1* plants by developing ectopic inflorescences in the center of the floret (Laudencia-Chingcuanco & Hake, 1998). Therefore, ZAG1 and IFA1 interact and function partially redundantly. The cloning of the gene causing the *ifa1* mutant phenotype might finally resolve whether homologous functional interactions exist between *AG*-like and *AGL2*-like genes in maize versus *Arabidopsis*, and in addition to this, whether similar diversifications among *AG*-like and *AGL2*-like genes regulating flower development have been conserved after the divergence of the monocots and eudicots.

4.10 Separability of function in conferring floral organ identity and conferring determinacy

Strong alleles of *AG*, like *ag-1*, always show two functions, specification of reproductive organ identity and controlling floral meristem determinacy (Yanofsky *et al*., 1990). However, weak *AG* alleles like *ag-4* are reminiscent of the indeterminate floral phenotype of *zag1-mum1* plants (Sieburth *et al*, 1995; Mizukami & Ma, 1995). Mizukami and Ma (1995) show that the two functions can also be separated in *Arabidopsis*. They analyzed antisense inhibited *ag* plants and observed three different classes of mutant flowers: flowers phenocopying the *ag-1* flowers; indeterminate flowers containing partially converted reproductive organs; and flowers having wildtype floral organs enclosing an indeterminate floral meristem inside the fourth whorl carpels. The existence of the third class of flowers indicates that *AG* function can be perturbed to affect only floral meristem determinacy, but not floral organ identity. Furthermore, Mizukami and Ma show that the different phenotypes correlate with a proportional reduction in *AG* expression, stating that the maintenance of a determinate floral meristem requires a higher level of AG activity than the specification of the stamen and carpel identity.

Similar phenotypic observations have been made in the *ZMM6* and *ZMM8* transgenic plants in maize. A large number of phenotypic spikelets had wildtype florets, yet a higher number of them. Similarly, phenotypic spikelet ´pairs´ showed wildtype spikelets, yet a higher number arranged together than the wildtype sessile and pedicellate. In addition, a loss of organ identity has not been observed without loss of determinacy, whereas loss of determinacy can be seen without homeotic conversions. These finding suggest a similar mode of action to confer organ identity and to maintain determinacy by maize *AGL2*-like genes as *AG* does in *Arabidopsis*, either directly, or indirectly by interacting with *AG* homologs.

4.11 Determinacy uncovered: lessons from *Arabidopsis*

Indeterminacy is achieved by the ongoing activity of meristems, that continue to initiate new primordia. The indeterminate apical meristem must contain a pluripotent stem-cell population, that can replenish those regions from which cells have been recruited to establish lateral (determinate) organs, as well as maintain the pool of stem-cells required for further growth (Lenhard & Laux, 1999). The imposition of determinacy of a meristem can therefore be seen as the inhibition of stem-cell division in that meristem.

WUSCHEL (WUS), a homeodomain protein, functions to maintain the integrity of the central zone in apical meristems by conferring stem-cell identity in *Arabidopsis* (Mayer *et al*, 1998). CLAVATA3 (CLV3) acts as a secreted ligand to CLAVATA1 (CLV1), a Leucine-rich receptor kinase, activating a signal transduction cascade that restricts the size of the central zone (Brand *et al*., 2000; Trotochaud *et al*., 2000). The *CLV* signalling pathway leads to the repression of the transcription of *WUS*, starting a self-regulatory feedback loop that controls the stem-cell population in the center of the meristem (Schoof *et al*, 2000).

After the transition to flowering the shoot apical meristem (SAM) is converted into the inflorescence meristem, that finally produces lateral determinate floral meristems. In *Arabidopsis*, *LEAFY* (*LFY*) is a master regulator of the floral initiation process, conferring a floral fate to meristematic cells (Schultz & Haughn, 1991; Weigel *et al*., 1992). Floral identity brings about determinacy once the initiation of floral organs has been completed, due to the actions of *AG* (Yanofsky *et al*., 1990). LFY has been shown to directly activate *AG* transcription (Busch *et al*.,

1999). Furthermore, WUS activates *AG* as well, and in turn, AG represses *WUS* expression, dependent of LFY (Lenhard *et al*, 2001; Lohmann *et al*., 2001). Therefore, LFY is a flower specific factor involved in the negative regulation of the floral meristem stem-cell population by AG, leading to the determinate nature of flowers.

Whether a similar pathway conferring determinacy also functions in monocots like maize awaits the isolation, functional characterization and interaction of the individual components of this model (i.e. the functional equivalent genes) in maize.

5 SUMMARY

5.1 Summary (english)

This thesis describes a molecular and functional analysis of *AGL2*-like MADS-box genes in maize and discusses their involvement in the grass inflorescence architecture. MADS-box genes are homeotic selector genes that are usually transcribed in those tissues whose identity they specify. The complex maize inflorescence is composed of paired spikelets, that have each two florets. Previous research indicated that maize *AGL2*-like MADS-box genes have extraordinary expression patterns, suggesting they have been recruited to establish novel positional information not found within eudicot inflorescences. Screening of a genomic library resulted in the isolation of several genomic clones of *AGL2*-like MADS-box genes, including a new member of the subfamily. Comparison of the genomic sequences of members of the *AGL2*-like gene subfamily showed that some of the introns were much larger than those of *AGL2*-like MADS-box genes in other species like *Arabidopsis*, pointing to a role in the diversification of the expression patterns during evolution. The genes *ZMM6* and *ZMM8* were chosen for further functional characterization based on previous expression analysis. *ZMM6* distinguishes between the spikelets of a pair and becomes subsequently expressed in both florets of both spikelets. *ZMM8* is expressed in the upper floret in both spikelets of a pair. A transgenic approach in maize led to loss of function phenotypes that are complex in nature, due to the action of the genes on several of the reproductive meristems. The *ZMM6* and *ZMM8* transformed plants showed the same complex phenotypes, indicating that the genes function alike. These transgenic phenotypes consist of a higher number of spikelets per `pair´. Their point of branching could be at the inflorescence stem, directly below the base of the spikelet, or at an intermediate position. Furthermore, the number of florets per spikelet was increased, proportional to the number of glumes. Also a higher number of floret organs was seen. Finally, lodicules were observed that were converted into paleas, indicating a change in floret organ identity.

These data suggest that the investigated genes are involved in conferring determinacy to different kinds of meristems in the maize inflorescences, as well as in specifying organ identity. It is further discussed how these phenotypes relate to the function of *AGL2*-like MADS-box genes in other plants, as well as what can be learned concerning the development of the inflorescences.

5.2 Zusammenfassung (German)

Dieser Doktorarbeit beschreibt eine molekulare und funktionelle Analyse von *AGL2*-ähnlichen MADS-box Genen in Mais und diskutiert ihre Beteiligung an der Ausprägung der Infloreszenz Architektur bei Gräser. MADS-box Gene sind homeotische Selektor Gene, die normalerweise in den Geweben transkribiert werden, deren Identität sie spezifizieren. Die komplizierte Mais Infloreszenz besteht aus gepaarten Ährchen, die zwei Blüten haben. Vorherige Untersuchungen haben gezeigt, daß Mais *AGL2*-ähnlichen MADS-box Gene außergewöhnliche Expressionsmuster haben. Dieser Befund legt nahe, daß sie rekrutiert worden sind, um neue positionelle Informationen zu etablieren, die in Eudikotylen Pflanzen nicht vorhanden ist. Durchmusterung einer genomischen Bank resultierte in der Isolation mehrerer genomischen Klone von *AGL2*-ähnlicher Genen, sowie einem neuen Mitglied der Subfamilie. Vergleiche der genomischen Sequenzen der Mitglieder der *AGL2*-ähnliche Gen Subfamilie haben gezeigt, daß einige Intronen viel großer sind als die der *AGL2*-ähnlichen MADS-box Gene in anderen Species wie *Arabidopsis*. Dies deutet auf eine Rolle in die Diversifizierung der Expressionsmuster während der Evolution. Die Gene *ZMM6* und *ZMM8* sind ausgewählt worden für eine nähere funktionelle Characterisierung, basierend auf vorhandenen Expressionsanalysen. *ZMM6* unterscheidet zwischen die Ährchen einer Paar und wird später exprimiert in beider Blüten in beider Ährchen. *ZMM8* ist exprimiert in der oberen Blüte beider Ährchen eines Paares. Ein transgener Versuch in Mais führte zu komplexen `loss-of-function` Phenotypen, weil die Gene in mehreren Reproduktionsmeristemen aktiv sind. *ZMM6* und *ZMM8* transformierte Pflanzen zeigten dieselben Phenotypen, was nahelegt, daß die Genen ähnliche Funktionen haben. Die Phenotypen zeigen ein höhere Zahl der Ährchen pro `Paar`. Ihr Verastungspunkt könnte am Infloreszenzstamm sein, direkt unten der Boden eines Ährchen, oder an einer zwischengestellten Lage. Weiterhin war die Zahl der Blüte pro Ärchen erhöht, proportional zur Zahl der Hüllspelzen. Ein erhöhte Anzahl an Blütenorgane ist auch gesehen worden. Weiterhin wurden Schwellkörper beobachtet, die umgewandelt waren zu Vorspelzen, was eine Veränderung der Blütenorganidentität andeutet.

Diese Daten legen nahe, daß die untersuchte Gene involviert sind bei der Vermittlung der Determinanz der verschiedenen Meristemtypen in der Maisinfloreszenz und in der Spezification der Blütenorganidentität. Weiterhin ist diskutiert worden wie sich diese Phenotypen zu der Funktion der *AGL2*-ähnlichen Gene in anderen Pflanzen verhalten und was man von der Infloreszenzentwicklung lernen kann.

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Maize Database	http://agron.missouri.edu
MADS-box gene database	http://www.mpiz-koeln.mpg.de/mads/
Brook Haven National Laboratory (BNL)	http://burr.bio.bnl.gov/acemaz.html
Pioneer Hybrid	http://www.pioneer.com
pollinations protocols	http://www.agron.missouri.edu/IMP/WEB/pollen.htm.
Blast at NCBI	http://www.ncbi.nlm.nih.gov
ifa1 phenotype	http://www.agron.missouri.edu/db_images/Variation/33laudencia.jpg
rgo1 phenotype	http://www.agron.missouri.edu/mnl/72/33kaplinsky.html
rgo1 phenotype	http://mtm.cshl.org/cgi-perl/image.cgi?name=6659.1.JPG&class=Image
ts4/*ZAG2* loci	http://www.agron.missouri.edu:80/cgi-bin/sybgw_mdb/mdb3/Map/258947

7 APPENDICES

Appendix 7.1. Primer sequences.

A601:	CCA GCA CGC AGA GAA TTC CAA AAA CAC
A602:	CAG TAG TTC GAT CCC ATG GCA ACC ACG
A801:	ACA AAA CAC TCT AGA GAT ACC GCA GCT CC
A802:	GCA GGG AAG GAT CAT TGC CAT GGT AC
AJ31:	CAT GTA TGG ACA GAA TTC TTG ACC GGT ACG
AJ32:	CAG GTG GCT AAG CAT CCA TGG TGG TAG
$CER_{(RQVT)}2$:	CA(A/G) CA(A/G) GTG AC(G/C) TTC T(G/C)C AA(A/G) CG
CMV1:	CTT GCA TGC CTG CAG GTC AAC ATG G
CMV2:	TAG AGG AAG GGT CTT GCG AAG G
LDL1:	CGG AAT TAA CCC TCA CTA AAG GGA ACG AAT TCG
LDR1:	CGT AAT ACG ACT CAC TAT AGG GCG AAG AAT TCG
M601:	AAG TCG GCG CCG ACC ATG GGG AG
M602:	ATT CGG AAT TCC GCA GTA GTT CG
M801:	CAA GCA GAA GCT TGG CCA GAT GGG TCC
M802:	GCA TAC CGC AGG ATC CTA TAT GCA TGC
PLA2:	GCT TGC ATG CCT GCA GGT CAC TGG
PT059:	TCG TTC GTT TCG CAC CTG CTGC
P018:	GAC TCG AGT CGA CAT CG
P038:	GAT CAA G(A/C)G (G/C)AT CGA GAA
WD01:	GCC TGC TCA AGA AGG CGT ACG AGC
WD02:	AAC TCG TAG AGC TTG CCG CGG TTG G
WD06:	CTC AAA GAT CCA TCT TCA GGG TAG CCA TGC
WD09:	GCG CAG TAG TTC GAT CTC ATG GCA ACC
WD32:	CTG CGC TCT GCC TTG CTT TCC TGC
WD34:	GTT CTC GAT CCG CTT GAG CTC GAC C
WD54:	CTC TGG TCC TCA ACT TCA AGT AGT CCT GG
WD56:	CCG CAT ACC GCA GGA AAC TAT ATG CAT GC
WD57:	CCA TCC AGA CCG ACC GTG CTC GT
W121:	GAG TTG CAG GAT CTC AAC AAA GAC CTA AGG
W124:	GTG AAG CAA ATA AGT GTC TTC G
W126:	CAC GTG CCT TCA GTT TGA GGT ACT CAT TGC
W128:	CTG AAG TTC TGT CAG TTG ATC AAC CAT GTG T
W167:	AGC TCG AGC AGC TGG AGA ACC AGA TAG
W171:	CGA GGG AGC TTT TAG AGC AAG AGA GGA C
W257:	GCA CGA GTT GAG GTT TTA CAA CAC TCG C
W263:	GTC CAT AGT ATG GTA TGT AGG TTG CAG AGG
W268:	TCT TCA GGG TAG CCA TGT TGG CAT GAA G
W258:	GGA CAA GGG TTG AAT TCC TCC AAA CTA CAC
W270:	GTA GGC CTG CTG ATG GTA CCC GAA C
W618:	AGC AGC CAA CGC AAG TAC GCG ACC
W619:	CCA GCT CGC AAC AGC GAC CAA AGC
W620:	PO_4-ACC AGC CC-NH_2
W621:	GTA ATA CGA CTC ACT ATA GGG CAC GCG TGG TCG ACG GCC CGG GCT GGT
W622:	GTA ATA CGA CTC ACT ATA GGG C
W623:	ACT ATA GGG CAC GCG TGG T
W762:	CTG CTG TGC ATC AAC TTT ATT TTG GGT CAT CAG
W753:	AGC TAG AGG GAC GCA AAA CAA GCT AGC
W756:	TCT TGG GGA GGA TCT GGG TCC ACT TAG
W785:	CCT TCC CGC GCC CCA TGG TGC
W812:	GGA AGC TGG GCG AGT TTG AGG CAG

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W813:        AAG CAC TGG CTA AGA CAT CCA TGC AGG
W816:        GAA CTA GGT TTG TCG GTT TGT TTG GGA AAC ACG
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Appendix 7.2. Sequence of genomic clone λDASHII-wd33

Sequence of partial genomic clone λDASHII-wd33 of *AGL2*-like gene *WD33*, containing the promoter, MADS-box and 3 kb of the first intron. The nucleotide sequences coding for the conceptual amino acid sequences are displayed in bold.

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   1  AGTCGACCGC GGACTGTTGG ATCTGGCGTG TGCGGCTCTG ATTAGATAGG
  51  AGATACCCTT TCGCTCGGTT GAATTGCATC CATCGATGTT CAATCGGGCG
 101  ATCCGCGATG CTTTGACCAC CATAAGATCT GAGCCATCCA TATGCAATCC
 151  TGGGGCTCCT ACGCAATACC GGTTCATTAT TGCGCTCGAT CTGATCCCAC
 201  CGTCGGTTTC GGATCTGATG GCCGGGGGTT AGACGATACC CCTTCGTGAC
 251  AGCAATTACA CTAAAGAGTC CCTCTGTTTA TCTAAAAACA ACCCGTCGTC
 301  CACCTCTACA GTGCCCTGAG TCTTAGGTTT AGTTGCGCCG AGGCCCCTGA
 351  TCTTTCTGAA AATAGACGCC CAGTCCAGAA CTTATTTAAA ATGAATAAAT
 401  GAATTAGTAA ATGCATAGAA AACTGTTTTG ACATGAAACT GGTAAAATGC
 451  ATAGAAAATT CTAGAAACTG GTAAAATGCA TAGAAAATTC ATTTTAGCTC
 501  CAAATTCATC CATTCTAATT TCTAAAATTT TGTAATATTA TTGTCTATCA
 551  TTTAGTGTCT CTGTTTTGAC ATGAAAACAA TAAGAAAATT AATTTCTCAT
 601  TTAATCATAT TTCAAGCACA TTAAACCTTT GGAAATTCAT AATTCAAAAT
 651  CCATAACTCC AAAATTAACG ATTCCTGTTC CTAGGTTCTT ATTTTAATGT
 701  GTAGATTTTT ATTGTATATT TTATTTACCT GTTTGGTGTG ATGTTAATTT
 751  TCGCTATACT ATGTATGTAT TGTGTTGATG CGAGTAGACG AGCAAGCTAC
 801  TGTAGATCCT GAGGTTCAGC AGGTAGAAGT TGCTGAGCAG GAGCTCATTG
 851  AAGGCAAGTT GTGCACTTGA TCACTTACTT TTTCCAGCCA TGTTCATATT
 901  AATTATAATG ACTGCATAGG TTAATTTTGA TGGGATCCAA TAGGTTACCC
 951  TAGTATTGGT TATCTTTATA CCTTGTTTAC CTCTGAAATT TTTTTGGGTA
1001  GTATCTGCTA TTGCTTTATG TGGATTTGGG TATGGAGATA CTTTATTCAT
1051  GATTATACTT TTATTATCAT TTTAGTATTA CTGTTCATGT TAAGATCATT
1101  ATGTTAATGG GAACATGGAG CGACCACCCG GGAAAACAGT GCTACCACAA
1151  GGGTTTAATG GGACGCCCTT GGCTGATTAA CTAGGAAAGC TAGTGGATGA
1201  CTTCCTTACC CGAAAGGGGC AAGGGCAGTA GGGGAGTGGT CAGTGTAGGG
1251  AGGTCCTTGG GTTGATTTTG CTGCGATGGT GGTCAGGCGA GGGATTCCTG
1301  CACTGGAGCT TCCTATAAAC TGTAGCGGGA TTTCTGAAGC TAGTGGAACT
1351  TTGTAAAGGC CTCATAGTGT TACCCTGCCT CGCCTCCTTG GTAGAGGTGT
1401  ATGGGATTGG CCGTCTCTTG GCAGATGGGT AACATGACTT GTGGGTAAAG
1451  ATGTGCAACC TCTGCATAGT GTAAAACTGG TATACTAGTC GTGCTCACGG
1501  TCATGAGCAG CTCAGACCCT CACATGATTA ATCTATGTAA TTAAAACTCA
1551  ATTTGACATT TGCATCACAT TTGGGATTAT TTTATTATTA CTTTTCTTTA
1601  TTATTATTAA GGTTTGGTAT TTACTTACAC TTAGTAATTG CTAATAAAAC
1651  TTTGACCAAC TTATAAAAGC AATGCTTAGC TTCAGCCTAT ATTTTATTGA
1701  TCAGCCTTAC ACTTCACATG AACTCCCACC TTTGGTGAGT TCATGCACAT
1751  TATTCCCCAC GACTTGTTGA GCTATGAACG TATGTGAGCT CACTCTTGCT
1801  GTCTCACACC CCCCACAGGA GAAGAACAAG TGGTCGAAGA GGAGCCGCCT
1851  AACACTGAGG CTTTCGACTT GATCTAGGTG GCGTCTCCCA GTCAGCTTTG
1901  TGGCGCCAGG GAATAATATT TAGTTCGCTT TATTTTATCA TTTATTTTTG
1951  TAAGACTTCC GCTATGTAAT AAGTACATTA TGATATTTAT GACATTTATC
2001  TCTATGCACT CCGTTATTAT GTGTGTTGTT CTTCCTTGAC GCATATATGA
2051  GATGCACCCG GATTTGCTCC TTAAATCCGA GTGTGACAAC CCTACACCCT
2101  TACGTATACG TCGTCCCGGT TCTACCTACG TATATGTTAC ACGCTGCACG
2151  GCGCATGCTC AGCTGAGCTG CAAATTAAAG GCATATATAG GCAATTAGTA
2201  CAAGTAATAG TAGCGACGAC TAGCGAGAGA GAAAGCAGTG CTACTGCTAC
2251  GACTACTCAG CTCGTAGTTA GTAGAATGCT AGATAGAAGG TGACGAGAGA
2301  GAGGGGGAGA GGAGATTCTT GATAGGGATG AAAACGGTCG GAAACGGTCG
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2351  GTAAACACTA AAACCATTAC CGTTTTCGTA TTTTTTATCG GAAACAAAAT
2401  CGAAATCGGT AACTCCGGAA ACGGAAACGA TATCGGTATT TCGGAAACAT
2451  CGGAAACGAA AATTTGGTGC GGAAAATACA CCGGTAACGG TCGAAATCTA
2501  AAATACGATC GGTAAACATA TCAAACTTCA TAATACAAAA AAGTTGACAA
2551  AAGATCACAA GTTCACAATT CATAATATAA CAAGTTCACA ATATAACAAG
2601  TTCACAAGGA TCACAAATTT ATAATATAAA AAATTTACAA AGATCACAAG
2651  TTCACAATAT AACAAGTTCA CAGTAAAACA AGTTCATAAA GATTACAAGT
2701  TCACAGGCTC AAAGTTCACA ATTCAGAATA TCCACTCGAT CTAGCTGACA
2751  TGGCATGCTT ACTTATGAAA TATTTTTTTC TCACATAAAG AGTTTTTCAC
2801  AACCTCACAG GAAAACCATA AAACCTAGTG TGTGAAAAAG ACCAAATCGT
2851  TAATCTCAAC TGCCTTCCAA CACCATCTAT CCCAAAAAAT CTTAAGGCAT
2901  CCAGAAAATA CAAAAATAAG TGATCCTTAT CTAAAGACGT ACAGGCGATG
2951  CGAAAAATCA CATGCTGGAA AATTCCAAAA AATTTCGAGA CACAATTCCG
3001  AAAATTTCCG AGACACAAAT CCGGTAATTT TCGACAAAAC CGGTAACTGA
3051  AGGAAACGGT CGGTAAAACA CCACGCCGAT TCCGATACCG ATTCCGATAG
3101  AAAATTCCGA AAACTTATTT CGTTTTCGAA AAATACCGTT ACCGGTGAAT
3151  CCGATCGAAA AAATTCGAAA TCGGTTTCCA GAATTTCGAA AAATTCCGAA
3201  ACTGTTTTCA TCCTTAATTC TTGATCTAGT TGAGTGCTAA CGATCGAGGA
3251  GAGCAGTGGA GGAACTGAGC TAGCTAGGAA GGAGAATCGA AGAAAAAGTA
3301  GGAGATGGGG CGCGACAAGG TGGAGCTGAA GCGGATCGAG AACAAGATCA
          M  G    R  D  K  V    E  L  K    R  I  E    N  K  I  S
3351  GCCGGCAGGT GACGTTCGCC AAGCGCCGGA ACGAGCTGCT CAAGAAGGCG
        R  Q  V    T  F  A    K  R  R  N    E  L  L    K  K  A
3401  TACGATCTAT CGGTGCTCTG CGACGCCGAG GTCGCCCTCA TCATCATCTC
      Y  D  L  S    V  L  C    D  A  E    V  A  L  I    I  I  S
3451  CAGCTGTGCC CGCCTCTTCG AGTTCTCCAC CTCCTCCTCG TGGTACACAT
       S  C  A    R  L  F  E    F  S  T    S  S
3501  GCTGCTCCGC CCCACCTGTG CCTTACATAC CCAGACGATC TGGTTTTGAA
3551  CATGAACGTT GAACGCATCT TCTGTTTGTC ATGCATTTTT CTACGGTGTA
3601  CGTTACTCCT CTTAGCGCTA GTTTGACAAA CTTTTTTTCT AAGGGATTTT
3651  TATTTTCTTG AAAAATTAGT TTATTTTCTT TGGAAAATGA AAATATCTTA
3701  AAAAACTGGA GTTGCCCTTT CTGCCTCTAA TAATTATTCC CTATGTTTAT
3751  TTATTTCTCT TGCTTTGCTT GGACTTTACT GTTCCATTAA GACACAGTCT
3801  CTCTCTGTGG AAAATGGCAT CTGCTCTAGC TAGACTCTAC TGTTCCATTG
3851  GGGAACTGAT CAGCGTGTTG TCGCTCTCTC TCTCTACGTC TCTCAAACAT
3901  GTTAAAAGAA CTTTTAAGAT AATAACTTAA TAGTAAAATG TCGAATAATT
3951  AGTAGCCACG CGTTCATAAC CCGGTCGTAT TTAAACATGT TAAAAGAACT
4001  TTTAAGATAA TACTATAATA TATATCTTTT TATTTATTTG TTGCTTGGTA
4051  TGAATATTTT TAGATGTGTC TTTAGTTTAT ATATTGCACT ACCGGAATCG
4101  CGTTCTTTGT CGAGTGTCTA AGACACTCGG CAAAGACTAT TTTACACTCG
4151  GCAAAGGCTT TGCTGAGTAT AACACTCGGC AAAGAAAAAC ACTCGGCAAA
4201  TTAAGAATTG AAAAAAAATA AAAAAAACAG TAAAATAATT TTTTAAATTA
4251  TAGGAACAAC TCTCCACAAT AGGAACAACT CTCCACAACC TACCCATTAT
4301  CTTACCCATT GCCCTATCAT ATTCACTATT ATTTGAATCA AATTTATATA
4351  TTGTGAATGG TGAGATTGGA ACTCGCAACC TCTCTCGCGT GCATACCCTT
4401  CTAACCACTC ACTACTACAC CTATGTTTAT ATTACGTTTT TATTCCCCAT
4451  GTACTATAAC AAACCGAGAG TATTTGATTA TTTAAGGCAC TAAATGAGTT
4501  TATTTGAAAA TGTAACCAAC TATAAAGTTG CATAACGTTT TAAGATCTAC
4551  AAGTTTTATT TTAATAGTTT CTACATCCGA GACCGTTCAC AAATTTTATA
4601  CATCTCTCTC TTAGTTTCAT AAACTACGAG AGAGATATAG GTTTTATGAA
4651  CAAATTTATT TTTATTTTGT CATATGAATA AATGTTGAAT ATATAAATTG
4701  TACATCATAA TGAGTTATAC AAATTTGTAG TTGAAAACTT TTTCATTTAA
4751  ATTAATTTAC TGCTTTAAAA TGTGATTTTT AAATTGTCTT TGCCTAGTGT
4801  TGGAAAAAAG CACCCGGCAA AGAGCTCTTT GCCGAGTGTC AACAAAAAAC
4851  ACTCGATGGC CCAGAATATG ATCCGAGCAC CGAGCAGATC GACCCCGATG
4901  TGCTTATGAA GGTCGGAGGA AGCAAGAGAC ATGGGCGGTA CTGGATTGCC
4951  GACAGGGAAA TTGACTCGTC CTCCACTCCC ACTCTGTCTT AGGTGCGAGC
5001  AAGGAGCACG GGCTCGAGTC CAACCATACG ACCTCGGCAC GATAGCTCAC
5051  AACATCACAT ACAGCAACTC GAGGTTAGTG CTTCTGTAAC TCGTCCTTAC
5101  TTGAGTTATA TACCTTCTCT TTGAGTTACT ATAACATTGG CTTGTAATAT
5151  TACAGACCCA ACTAGAAGAG ATGGAGGCGA GGATGATGGC GGAGCGGGTG
5201  GCTCGCGAGG CAGATCATCA GAGGATGACA GAGATGTTTC AATACATGCA
5251  GAGCCTTGGC GCCGCACAGG GTTTCGCTCA GCCACCTCCA TTGTTCCCTA
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5301  CAATGGACCC TGCTCTATTC CATACTCCTG TGAGTATCAA AATTGTAGTT
5351  AGATGTTGGT AATGCATCTG GTATAACTCA TGCAATCTCT TCTCTGTGCA
5401  GGGCCAATCT GGGGCGGCAT CCAACAACCC TCATGAAGTG TCCAGCCCAA
5451  CGCAGCACCA GTCCAACCGC CCACCTCAAT GAGTTTAGTG TTTAGACTAC
5501  AAAACTTATG TTGAATACTT ATGTCAGAGC TTGTGAACTT GTGTTGATAC
5551  TTCTGAACTT GTGTTCATAC TGTTTGTGTT GAGAACTTCT GAACTTATGT
5601  TTGTATATTA ACGTTTGTGT TGGCTATATA TGTCTGTGAT GATATGTGAT
5651  GTATATATGT GATATCTGTG AAATATCTTT TGTTTGTTTG GATGGAATAG
5701  GAAAAACAAA TAAAAAAGGT GTGTACTGGT CTCTTTGCCG AGTGACACAC
5751  TCGGCAAAGA GGTGCTTTGC CGAGTGTCAG GACCATAGCA CTCGGCAAAG
5801  AACCAAGACC TGGGCACCGG TACAGGTTCT TTGCCGAGTG TAGTGGCTCT
5851  GACACTCGGC AAAGAGGCCT TCTTTGCCGA GTGCCATCCA GAAGCACTCG
5901  GCAAAGAACC TGACATAGGG ACCCCGCTGA CGGATTCTTT GCCGAGTGTT
5951  GGCCGGCAGA CACTCGGCAA AGATAACTAC TTTGCCGAGT GTCACCTAAG
6001  ACACTCGGCA AAGACGCCGT CTCCGTCACC CGGCGCCGTG ACGGCTGCTT
6051  TTCTTTGTCG AGTGTCCGAG AAAAAGTACT CGGCAAAGAA TGCTTTGTCG
6101  ATGCACTGTG TGCCGAGCCC TCTTTGCCGA GTGCGACACT CGGCAAAGAG
6151  TTTGCCGAGT GTTTTTAAGG CTTTGCCGAG TGCTTCAGGC ACCGGCAAAG
6201  TCGTCGATTC CGGTAGTGTT GTTTAGAAGA TTAAATCTCA TCTATGATTC
6251  TCAGTGATTG AGAGAAAAGT TAAGTTAATT TGCAATTTCT CTCTCAATCC
6301  CTAGGGGGGA TTTAAGTTCT CAAACTATCC CTAATTATCG CATTTTTCCA
6351  GTGGGATTTC ATGTAAGGCT AGCTATTCTC TTCATATATG TCTGGCTCGC
6401  ACACGTACGT CATAGCCTCA TACATACATA GCTCCTTTAT GTATTCTTCT
6451  TTTAGTTGTG TTTGGGGAAA TATTGGTTGT CATGTATTTT ATCATCTCAT
6501  TGAACGCTTA ATTTTTCCTC TTTTGTTTTC CGCGAGCAAG ACAGCGAGTT
6551  GATCAGTAAC TTCAGTTACA TACGTTTTGC GTAAAGGGGC AGTCAATAGA
6601  AGATGACACA CAAATGATCT GGATCCGAAT TCTTCGCCCT ATAGTGAGTC
6651  GTATTACG
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Appendix 7.3. Genomic sequences of loci of *AGL2*-like genes.

The nucleotide sequences coding for the conceptual amino acid sequences are displayed in bold. The MADS-box is displayed in black, the I-region is displayed in red, the K-box is displayed in blue and the C-region is displayed in green.

A. Genomic sequence of Arabidopsis *AGL2*-like gene *AGL9*, obtained from the database at NCBI (http://www.ncbi.nlm.nih.gov) (acc. no. AC002396, chromosome I, BAC F2I6). The cDNA sequence (acc.no. AF015552) used for comparison was published by Mandel & Yanofsky (1998).

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   1  ACTGATCAAA GGGTTTATGA AAAACACTAA CTTCTTATCC TCTAATTGCG
  51  ATTACCCATA GACGAAACCA ATAAAAAAGC AATGGAGAAC TAGAGCACAG
 101  TCACTACAAG AAATACCCTA TAAAAGTACC GACCTGCACC GATGAGGATG
 151  GTGAGCTTCC CGAGCGGAAG AGCCATGGCT AGAGACGAGC TTATACGGCG
 201  AAGAACTAAG ATGGCAAACG AATCCGCGTG AGAATATCTA AGAGAGTATT
 251  GGTAAGAGAG AGCTGCAGGA ACGTACCGGT GAAACAGAGG CGTTTTTTGG
 301  GACGATGAAG TGAGGCAGCG AGAGAGATAC GACGTGCGAC TATATTGTTC
 351  GCTTGTTGAG GCAACAAAAC AGAGTTGCTT CTAAAACCCG AACCGAAATG
 401  TCCGGTCTGA TTCGGTCTAA ATCACGATTA GGTTCGTTTT AAAACCTAGG
 451  AGGCAATAAC CGGACGGATC ATAAATTCAT AATAGAGACA GACAAATTGG
 501  TCCATTATTA AAATCACTTG GGCATTTGGG GATGATTCAA ATGCCCAAGT
 551  TTTCTCAAAT TTGGACGATT CATTCACCTA AGACATACTT GAGCAACAAC
 601  AAAGTGAAGT CCACTGTCAT ATCTTATGTC TCAAAAAGTA TTGAAATGTG
 651  TCAATTGATA TTGGAGAGGC ACACTAGCTA AGGGATTATT CAATCAATTT
 701  CCAGCAATTT AATTAAACTT ATTTGTAGTG AAAGTGGGAA GATAAAAGAT
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 751  CTCACCCTCA CATGTTCAAA AAAAAAAGTT GAAAATGGAA GTAATTCAAC
 801  ATGTAGCATA GAGCCCAAAT ATGTCTCATT TTTTTAATCC ATATAATCTC
 851  AAATCCTCTT ACTTACTTCT AAACATATGG TTCCCATAAT CATAACAATG
 901  CTATGTTAAC ATGGCCGGTT CTAAAGGAAG CCAAGTGCAG CAACTGCCTT
 951  ACGCCTCTAC GTGTTAAAAT GAAAATGAAG ACCACTGACC ACTTCTATTA
1001  AAGCTTCATT CACTAGTGTA TAATTACACA TTTTTTTAAG GATTTATGAG
1051  TAGTGATTGA GGCCCATATG TTTGTATGTT TGTTTTTCTT ACTATATCAT
1101  TACTTGACTA TAAGAGTTGG TTTCCTATTC CATTCTCTTT TCTAACAGCC
1151  TATATATGTA AAAATCTAAG CAAAATTTCT TGTCAAGAGG ATGATTGTAC
1201  ATTTGTACTT GGTTATCTCG CCCCGGCCCA AAACATACCT AAGGCCAGGT
1251  GCTATATCCT CAACCTGCTT TGGCATTCAT CAATCTACGA ACTTTGGCGT
1301  GAAACGGTGA CAAGATTAAC AAGATTCACT CTCAACTACG ATGTTCTACT
1351  ATCTCAAATC TTTAAAAAAG TGGATCAAAC TGTCAAAAGT CTAGTTCGAT
1401  GGACTAGCTT CAACACTCCT CCAAATCTAG TTCGATGGAC TATATATTCT
1451  CTTCTGATGC TATCCTTATC TTGGATTAGG CATCTAAACT ATGGTTTTAA
1501  TGGTGTCATG AGGTTTTACA ACTTACAAGG ATGAAAGTTA TTTACTCCCA
1551  GTCACTATCT TAATCAAATG ACAAAATGTT AACTAGTTTG AGTGCTTATA
1601  TATTAGTTAT GAATCTGAAA TTTATTAGTG TGTACATAAG TGATACAACA
1651  CTTAAATAAC ATCTACATGA GTTTTTAAAT AACATAATAA TCCATTATAG
1701  TAGTTTACGG CATAAGGTAT GAACCAAATT TTTCATTGCA CGCTGAAAAG
1751  TGAAAACCTT TAAAATGCAT AATGACTAAG AGTCTATGAC AACAGTAACT
1801  TACTATATAT TAGAGGAGGG GTGAAAAAAA AAGTAGAGAG ACTGGTCCAA
1851  AAACTTAACC CCACTCAATA AACCCAGACG TGACTTGTTT GACGATAACT
1901  CCATCTTTCT ATTTTGGGTA ACGAGGTCCC CTTCCCATTA CGTCTTGACG
1951  TGGACCCTGT CCGTCTATTT TTAGCAGATT AATCCAACGG TTCTTATTCT
2001  TTCTTCGACC CTTCACGACA TTGCCTCAAA GCCGTCCGAT TCTCATCTCA
2051  CGCCCAATGG ACCACATATA TCACCAGTAC TCCGCAACTT AGCTGTCGTG
2101  TAGGATTTCA CGTGGCATTT ATTTGTTCTA GTTTGTAGTG CAAACATTGC
2151  AAGTTGATAT GGTCCCCTAT CGATCACCGT CGTCTCTTTA GCTTCACATC
2201  GAGATTCTTC TTTCTTTCCT ACGTGTAATA GCATTTTTGA TTTTGAGAAT
2251  TTCTTTAGAA CCGTTGGATC TCTCATCGTT GGTTGATCCA TCCATCCAAA
2301  TGGGACCTGT GTGTGCTCCA TCCAGGGCAT ATGATCCCAA AGCCAAAAGA
2351  GTATTTCCAA GTGCTTTCTT TCTTTCTTTC TTTCTTTCTT ACTAACCTTT
2401  TTTTTTCTTA TGCTTTAGAC TAAGAAATTT ATTCGGCCAT ATCCACTTTT
2451  ACGAATATAC TTCTTACAAG ATCTAGATTT TTTTGAGTTA ATTCGGTGTA
2501  TATAACATTG GCATAGACTG CAATTAAGTA AATGATAATG TGATAATGAT
2551  GCGATGTGTT GTTATCAAGT AGTATAATAT AGATGGCTAA ATAGGTAAAC
2601  AAAATTACGT GTTATATGTA CACAATTAGG TAGAACCGTA GAAATTAAAC
2651  TGAATAAAAC CTTTATTAAT TTTAAAATTA TATGGTACAG ATAAATACGG
2701  AAAAACATTC ACGCTTTACG TAACAATTAA GTGGAAAGTA AAATTATCCC
2751  AAAAATATTT ATATCACATC ATTGTTATAT TTCTAAGTTT TTTTTATTTC
2801  TCTAATGGTA TATGTTTTAC AGATTGTTTT TTGGGAAAAT TCTTAAAGAG
2851  ACTTGAAGAA TGTTTTTTTT TTATTTTCTT GAAATGTTTG ACACTTGAAA
2901  CCGTTTAAAA ACTAAATATA GTATATATCA TTGTTGGTCT CATACCTTGT
2951  AATTCACCAC ATATATTATC AATGGGGAAG ATTTGAAAAT TTTTGGGGGA
3001  TCACAAAACG AAGGAAAGAG TACAAAAAGA GAAGGAAAAG ATAGAAGATA
3051  TATGTTTTTA ACTTCATTGG TATGACATCA ATAAATAAAT AGTTGAATGT
3101  ACTTTAGTTT CTCTTTTGGT TTAATGCACA TCATCTCGAT CAATTGTCAT
3151  CATCTTACAT TGAATTATAC GACCAGATCT GATAACAAGT GAATTCGTAC
3201  TTGCCCTTCC CTTTCTTCTC ATACGTCCTT CTAACTAATT TTGATTGTAA
3251  CTTATAATTA TATAACCATA TTTAATTTTA TTTTATCTAA AACCAATTGA
3301  AGCAAATTAA AATATCATAA ATCTTGAGTC CCACATGAAG ACAATATATA
3351  AAACTCGTGC AAATTTGCTT AAAATGCTTC TATGAGACCA TGACCAAGTG
3401  AGATTAATAA GCGATTCAAT GTGCAAATCA AAAGAGAAAA GAAGCTAATG
3451  GGTTTAAATA TAACCAAACA GAATAATAAT GCTATGTTTA GTTTTTCTAA
3501  TTGAATCATA CCTTTGTGTC CATCACCTAC TTACCGGTCA GAATAAAGCA
3551  ATTACGTCTG CAACCAAAAA GCACTAAGAC TTTCGGTCAG ACATGATCTC
3601  TAACATCGGA CGAACCCTAA GATAACCAAA ATAAACTATA TCTTATATTC
3651  AAATCTCTGT TTATTTTATC CATTTATGTT TTCTTTCTTT CCCATAATTT
3701  TTTTTGTGTC TCATCAGACT CTCTTACCAA ACTGAATTTA TCAACATGGT
3751  TTTTTTTTTG GCCACATCAA AATGGTGGTT TATAAAGTAG ACTAATACAA
3801  AAGACATTTC TGTTAATTTC ACTAACAAAA ATAATCTTAG CAGTACTATA
3851  GATTGGAAAA GGAAAAGCAA ATCTAGCAGT AAGATTTATC AAAACTAGCA
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3901  GTAAGAGTTT TAGATATCAT GAAAACATCA CAAACGAGTA GTGTTTTACT
3951  TTACATTTTT AACCAATCAC AAGGGTAGTT CCGTAAGTTG GGAAAATCGT
4001  ACGAGGCTTC ACCTAGTTAA GGTTAGGTCA CATGATTCCC TGAACTCGAT
4051  TTTATAAGTA AAAAAGAAAA ATTTATAAAA TCAAAATTTT TTATATAAAA
4101  AAATCAGGTG GATTTATCAG ACCCTACCAT CGAGATGTCG ACACGTGTCC
4151  AAACTCATTC ATTGCCCTAC TATTTTCTGT TTAGGGTTGC AATCACTCAT
4201  CGCACACGCG CCATCTCCAC CTTCCATTAT TAATCTCTCA TTTTCAACAT
4251  CACACTCTTA CGAATCATAC GATTTTAATA TCTCTGTCTC TCTCAACGTA
4301  TTAAATAAAA ATGGTTTTAA ATGTTAGGGT TTTTTGTAGG ATTTTCAATT
4351  ATTAATCTCT ATAATTCGAT GAACTAAGTA AAAAAGCATC AAACTTTCTT
4401  GGCAGAATCA CATTTTTCTC TAAACTAAAT ATGGACTGAA ATTGAAAAAT
4451  TAAACCACTA GCTAGAATAA AGTGTTGGTG AGAGTGGAAC TCTAATTTCT
4501  CTCCTTTACT AATTATGTAT AAACACAAAA ATGCACCAAA TTTTTAGGTT
4551  TGAAAATATC TAAGCATGGA TAGGGTAATT AACATTTTTT CTTTCAATTT
4601  TGCAATATTT GAATAAATCC TATGAGGGTC TTTGGTACAC AATAATTGGA
4651  GGGTATATAG TTGAGTCTGA GAGTATATTA GAAAGAGAAT ATTTCAAGTA
4701  ATGAAGCTGA CATGTTTATA TGTACTTTGA GAGAAGTGTT GTGAGATTTG
4751  TACAAATGTA TATGTACACT TTAAAAAGCA ATATAAGATA GATAAAAAAA
4801  ATATAAAGAA AAAAAGAAAG AAAGAAAGAA AGAAAGAGAG AGGCTCATAT
4851  ATATATAGAA TTGCTTGCAA GGAAAGAGAG AGAGAGAGAT TGAGATATCT
4901  TTTGGGAGAG GAGAAAGAAA AAGAAAATGG GAAGAGGGAG AGTAGAATTG
                                    M  G   R  G  R   V  E  L
4951  AAGAGGATAG AGAACAAGAT CAATAGGCAA GTGACGTTTG CAAAGAGAAG
      K  R  I  E   N  K  I   N  R  Q   V  T  F  A   K  R  R
5001  GAATGGTCTT TTGAAGAAAG CATACGAGCT TTCAGTTCTA TGTGATGCAG
       N  G  L   L  K  K  A   Y  E  L   S  V  L   C  D  A  E
5051  AAGTTGCTCT CATCATCTTC TCAAATAGAG GAAAGCTGTA CGAGTTTTGC
        V  A  L   I  I  F   S  N  R  G   K  L  Y   E  F  C
5101  AGTAGTTCGA GGTATATATC TACTTTTGTA TATATATTAC TTATAACATA
      S  S  S  S
5151  AACATTTTAT ATACATATTA AGTAACACAA AAATGTCTTG TATGTATGGG
5201  TCTCTCTGTG ATGTGTTGTT GTGTCGTACG TACGTGTTCT ATCATATCCT
5251  TTTAAAAGAA GCAAAGAGGA AAAAAAATTT GGGATACCCC AAATCTGTAT
5301  CATTTTATAA CAAGTTTGCT TTTTTGATGT TCTTTTGTGT TTCTCTTTGA
5351  TTTCCATTTT TGTTTTTGAT TTTTTTTCTA TTTCTCTTTA CATCTATCAA
5401  AGTTTTTTTT CTTATATTTT ATTGCTTATT TGTTTGTCTA CTTAATTCAC
5451  ATTATCTGAG AGAAGAACAA TCTATCTGAT ATGAAATTAG GGTTAATTTC
5501  TCTTGTGAGT ACTCTTTAAT TCACATAAGC TTAAAGTTTC CACCTTTTGA
5551  TTCTGGGGGT CGTCCAATTC GATCAAATCA CTCAATTTTG TTGTCAGATT
5601  GATATAAGTT CATAGGGGGA TATTGTTTCC ACGACAATCC ATTTTAGTAA
5651  CCCTTAGGGG TTTCCAATTT TGGGTTTTGA ATTGACGCTA ATGTCAAATT
5701  CATCTAAAGT CCGTTGGATA TGTATACTTG GGGATGGGAT TCATCCTTTT
5751  TTCTGGGTTC TTTAGATCTT CTCTTAAAAG ACTAACAGAT TTTGTTGTAA
5801  ACCCTAGGAA ACAGTTAAAA ATCCCATTTT TAAAAACATG TTTTGAACTT
5851  GATGAGTAAG ATTAATGGAA GAAATGATGT TTTTGTGTGG TGTGAAGCAT
                                                        M
5901  GCTTCGGACA CTGGAGAGGT ACCAAAAGTG TAACTATGGA GCACCAGAAC
       L  R  T   L  E  R  Y   Q  K  C   N  Y  G   A  P  E  P
5951  CCAATGTGCC TTCAAGAGAG GCCTTAGCAG TTGTACCCAA TTCTCTTCTC
        N  V  P   S  R  E   A  L  A  V
6001  TTTCTTCTAA TTACCTTAAT TAATTACTCT CAATTTTTAC TTTGATTTTT
6051  AGAGTCAAAT GATTAATGTT ATAATTTGTC ATATACTTCA GGAACTTAGT
                                                    E  L  S
6101  AGCCAGCAGG AGTATCTCAA GCTTAAGGAG CGTTATGACG CCTTACAAAG
      S  Q  Q  E   Y  L  K   L  K  E   R  Y  D  A   L  Q  R
6151  AACCCAAAGG TAAACTAATT AGCTTCTTCA GCTACCTTCA GAGAGTGTTT
       T  Q  R
6201  GTTTTTTTAG TAGATTTTTT TGATGGTTTT GATGTTGAAA TAGGAATCTG
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6251  TTGGGAGAAG ATCTTGGACC TCTAAGTACA AAGGAGCTTG AGTCACTTGA
      L  G  E  D   L  G  P   L  S  T   K  E  L  E   S  L  E
6301  GAGACAGCTT GATTCTTCCT TGAAGCAGAT CAGAGCTCTC AGGGTACTAC
       R  Q  L   D  S  S  L   K  Q  I   R  A  L   R
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6351  TTTGTTCATC AATATCTTTA TACACTGATC TATTTCCATA GTAAGATTAA
6401  ATTTGGTGTT TAATTCTGCA GACACAGTTT ATGCTTGACC AGCTCAACGA
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6451  TCTTCAGAGT AAGGTAAATA AAGAAACACT CATTCTCCTC TCTAAATTCC
       L  Q  S   K
6501  TCATCTAAAA GTAATGTAAC CAAGAAAACA CAAATATTTG GAGCAGGAAC
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6551  GCATGCTGAC TGAGACAAAT AAAACTCTAA GACTAAGGGT AATTAATATA
        M  L  T   E  T  N   K  T  L  R   L  R
6601  CATTCTCATA TCACCAAATT AATGCATCAC TAAATTTGGT TATAATGTGT
6651  GTGTGTATAT ACATATGTGA CAGTTAGCTG ATGGGTATCA GATGCCACTC
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6701  CAGCTGAACC CTAACCAAGA AGAGGTTGAT CACTACGGTC GTCATCATCA
      Q  L  N  P   N  Q  E   E  V  D   H  Y  G  R   H  H  H
6751  TCAACAACAA CAACACTCCC AAGCTTTCTT CCAGCCTTTG GAATGTGAAC
       Q  Q  Q   Q  H  S  Q   A  F  F   Q  P  L   E  C  E  P
6801  CCATTCTTCA GATCGGGTAA CTTTAGACTA GTATAACCAA TTTGATTTGA
        I  L  Q   I  G
6851  GTTCTATTAT AAGCTTTTCT TAAGAAAGTA TCTCAAACTA CTAAATTTTA
6901  TGGAGCAGGT ATCAGGGGCA GCAAGATGGA ATGGGAGCAG GACCAAGTGT
                Y   Q  G  Q   Q  D  G   M  G  A  G   P  S  V
6951  GAATAATTAC ATGTTGGGTT GGTTACCTTA TGACACCAAC TCTATTTGAA
       N  N  Y   M  L  G  W   L  P  Y   D  T  N   S  I  *
7001  TCTTTCTCAC TTAATTAATC TCTCTTTTTT TTGACATTTT TAAGATGATG
7051  TTTCTATTTT ATTACCTCTC TCACGTTTTC TGTCTTGTGT GCATGTGTCT
7101  GTGTAATGTT TATTGCCCTT CTATTATTCA ATGATTTCTC GACAATTTTG
7151  CTTCCTATTT TTACCCATTA CTCCTAAACT TCCTGATCCA GTTTCTTTTA
7201  AAATAACTCC CATTTTATGC ATGTTATCTA ACCAATTCTC TTAACTATGA
7251  TTTATGGTAC GATATAACTC ACAGTCTCAC ACTATCTATT TGGTGTTTTT
7301  TTGTTTGAGT CTTGAGAAGG GACCGCTTGT TTATCTCTCT TGTTAAAGAG
7351  CAACTCACTG GCCACTGCTT ATGTATCTGT AGGCCCCACC TATATCATTT
7401  TGGCTATATC TATACTTTTG TAGAGGGAGT ATTACTATAG AGAAGAAGAT
7451  AAATTTGGTT CTAATATATC TTGCAGGTAG TTGATATTCT CAATTATCAT
7501  GAAGATTTGA TAGACAAGTT TATCAGATAC CTTAAACATA GGTTTAAGAT
7551  CTCAATTGAA ATGTGAATTC ACCCGACGAT TAGAGTTACG ATCTAAGGAA
7601  GCGTTTCTTG AATTTTGAGT TTGTTTGATC AAGAGTAGAA TGCTTTTCTA
7651  TTACTAAGGT TGTTAATGCT TATATTCCAT GACCAAGGCC AAGAGAACAA
7701  ACAAAAACAT GGTGCCTCTT GATGTATAGT AATGGCTCTT AATGGTCATA
7751  TACAGAGAAA AAAAGATTAA TGTCGTTGCA CAAGCTTGAA GTTACTTACT
7801  CCTCGTCTTC CTCATTAGTG TCTTCGTCTT CCTCATCCTC ATCGCTCCCA
7851  ATATAGGGCT TCATCTACTT GAAAACCAAA TGCTCATGCA GTGGAAAAAG
7901  ATAACAGAGG TTCAAATTAA GGCAAACAAA ACTACAAGTG AGAAAGGGAA
7951  ACTACAAGTG GTAAGATGTA ATGTTTTGAC TCAAAACCAG ATCAGACAAT
8001  GAAAAAAAGT ATTGATACAA AAAGTCCATC CGGAAGCATA ATTACCGCTT
8051  GCAGGATGTC ATCAGAGATG TCTGTTAGTC GGCCAATGGC ATAGATGGTG
8101  AGCGGACCAG AGTAGCGTAA ATCCTCTAAA TACTGTCTAA AAGCCGGACC
8151  GACCCGACAA GGATCACAGT CAAGGGGAAT AGGACACCTA TTGATATCCC
8201  AAAAGACTGT TGTTACAGCC ACATCATCCT TGTCCAACTG GGTAGCCCAA
8251  AGGGAAACTA GTTGTGGTAA GAGCTTGTTT GACTCAAAAA ATGGCTAACT
8301  AGGATGATGC TGAATTACCA TCTGTTCATG TTTTTGACTA GAGAGATGGG
8351  TAGTGAAATT TTCAAAGCCT TTGCAAAACG CCTGTGGGAC CTGTTTCAGA
8401  AAAAGACTTA AAAGACTTGA GACTCAAGGA AAATAATATC CATTATATAA
8451  AGATGACAAC AAATATTAAC GGAAGTAGGA GTGATTGAGA ACGATTCTAG
8501  TAGAAGAGAC GGCTCGCAGG ACGTCGTTTA TAATAGGCCA ATGGCAGAGA
8551  TAGTGAGAGG ACCGGAGTAG CCTAAATTCT TTAAATGTCG TTTGATACAC
8601  GGACCAACTA GACGAGCATC ATACTCAGAG GGAACCGGAC ACGTCTTGAT
8651  ATCCCAGAAG ACCGATGTTA CGGCCTTAGC TTGCTGCCGC GTTGCCTTCA
8701  TCATCATCTT CTCCTTTTAA TCTATAACGG AAATCAAACA TCAGATAAAG
8751  CATTCGAAAA GATAGATTGA CACAGGTTAA ATCATCCACT TCAGAGAAAA
8801  AGAGAGGGAC ATGGCCGTAA ACAATGAGAT AAGGATCGGC CTAATGTTTA
8851  TAATGGGCTT GCGTTTAATG GGCCTACAGT TTCTTGAATC AGCCTTATGC
8901  ATGAGTCCTA GTATTTTATC AACTTTTTTT TTTCATCTTT CTTTAGTTAC
8951  AATAGATTTA AAGTGTTTTT TGTTAATGCC ATTGCAAAAT TTGGTAACTG
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 9001  TTTATAACAT TGTTCCTCAC TTCAAAATTT AAAGCACCAT TAATAAAAGC
 9051  TATACATATA ATTATAACTT GGGTTTTGTG CAAAAAAAAC AAACAAATTA
 9101  ACCTTTCATT TTAAATAAAT GCAATTCAAT ACCGCAATAT CAAAAGTAAC
 9151  CCGTATAACC TTTATTCGTG TATAGATTTT AGAAACAGTA TAAGTCAAAT
 9201  TATCAAAACT ATGTTGTTTT AAGCATTTTA AAAATAAGAA TAATAATAAT
 9251  GTTGAAGGGT GGATTTGAAC CCATGAACTA TAGAACAAAC CAAAGCATGC
 9301  ATAACCACAT GCGCCGAACA AACCAAAAAC TCATGGCTTT GTTAAACATA
 9351  TAAAAATATT CGAATAAAAA ATGTGGGGAA CTTGTTACCA GTTTTGGTTC
 9401  TTTTTGGAGC CATTTTTTTC AACACAGATA TTGTTAAGGA GTTTCAGGTA
 9451  AAACTGTATA TTATGCAGGG AACCACAGTA GGCTATAATG AAAGTCACAC
 9501  TGTGAAGTTA GCAGACAAGT TTTTACTTAA AGATGTGAGT TGTGATCTTT
 9551  TTGATGTAAG TCTTGATGTA TATGTTGACA AATTATATAA GTTTGTATTG
 9601  CATATTCTAT GACTTACGAA GTTTCTATGC AAGAAAAGCC GGGAGAAAAT
 9651  TTCCGTCAAG TAACTAAGAG ATCGTAATTC TTGTCTGAAG AACAACCCTT
 9701  TTTTATTATT TGAGTTTAGG TTGCCAACAG TGAACAAAGG GACGAGATAC
 9751  CATATGACAA ATATCCTCTA ACGCCATTTC AACAGTTAAT CAACAGTGTC
 9801  GGCTATATGC ATGTGCTAAC AATGCACAAG AACATTGTCA CCATCCCGTG
 9851  AATATGAATA TTAATGATTA TGAACGAGTT TGTAGAGTTC CAAGAGGAAG
 9901  GTACTACCTT CTCATACTCA TTGATCATAT ATTTTGTTTC TTGTTTGTTT
 9951  TAGTAACTAG GGTTATTCGG ATTGTTTTTC AAAATAATAG TAATATGTCA
10001  ACTATATTTA TAAAAAAAAA AACTAAATAA CTTTTGTACA ATTGATCATT
10051  TTTTAAATAT ATCATAAAGA TTCATC
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B. Genomic sequence of maize *AGL2*-like gene *B9_20*, obtained from genomic clone λDASHII-14-2-1, kindly provided by Dr. Zh. Meng.

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    1  TCGGATCAAT TCATCTCCTT ACTGTTTTTC ATTCTACAAA TTATATATGT
   51  TCATACCTGC TGTTTTATTT ATAGTCATAA ATTTATCCAA TCGGTTTTGT
  101  CGTATTATAT CATGACGTGT CTGATGCCTG ATCATACTAG TAATATTGTT
  151  GTCTTAATTT TACATTCATG CTGTTTATGT TGCTATCTGT TTATTTTCGG
  201  TTGTTCCTTA ATAATACATG TTCCATGTTT ATTTGCTTAT TATATTTATA
  251  TGGTTCATAT GTTTCATGTT CTCTTGATCC ATATTGTTAT GGATATATTT
  301  GAGATAATGA TTTCTATGAT TAAACATATT TTATATGTCA TCATCATAAT
  351  GTTAATTTAT GGAATTAAAA TAATACGGAA AATGCCTATA TTTCTAACAA
  401  TTTAGTCCTT TTTGCCAAAC ACTAGGACTA AAATATTGAC TAAAATGATT
  451  TAGTCTTTAG TCATTCACAT ATGTGCTAAA ATGGACTAAA CCATATTAAT
  501  TTCATATTTA CCCCTTATTT AGTTCAATTG TACTAATAGC AGGAGAATGT
  551  TAAAGACCAT TTTAGTCTTC TTAGGAGTCA TTTAGTATAT TCTTACTATT
  601  TTTAGCCCTA AAAACAAACA TGTTAGAAAC TAAACTTTAG TCCCCTAACT
  651  AAACTTTAGT CTCTAGACTA AAGAAACCAA ACATGACCAA AGCCCAGAAC
  701  TCCACACATG CATCATCTTT ACACTTATTG CTTGCACACA TGCATGCCGC
  751  GAGTCTGGAA CAAGAACATC CCGGATCGAT CCCTAGGCTG AAACGGATCA
  801  TATATATAGC GTCTCCTTTC TGGAAAAAGG CGATGCAAAA CGGATCGATG
  851  AGCTCTCACC ACGTCACCCG TGTGTGCTCA TGAGGAGCTC CGTCCTGTCT
  901  TCGCGAACTA TGGCAATGCA TGGCGACCGA CCTCTTCCGA ATCAGAACGG
  951  CATGTCTGCA GGCTCGATCG CTGCAAGCAT GCATGCCCGT TGGAAGACGC
 1001  CGGAACGAAA ACGGGTGGAG CTCGAATCGA TCGATGGAGG AAAGAGGAAA
 1051  GCAGCGCGCC GAGCCTAGCA GCCATGGCAG TTAGCAGGGG AGGGCCTCTA
 1101  GCGGCAGGGG GCTTTGCCCA AAACCACGAC AAGCCGAGGT TGCATCCCAG
 1151  AAAAGCCCAC GCCCTTTCAG CCTTTCTCTC CTTTTTCTCT CTCTAGCATC
 1201  GACCTGCTGT CGAGTCCTCA CTGGTCAATT CAGCTGAGCG ATGAGAGAGA
 1251  TGGAGTGACG GGAGGCTGAC ACATGGCCGG GTATTGAGGC GCGGAATTGA
 1301  ATTTGATTAA ATAACGCAAA AAGGTGGCGT GCCAGGACGG AGGACAGAAA
 1351  GCAATCACGA GACGGGAGGG GTTGGCCTTT CGCCTCTAGG GTTCCGGGTC
 1401  GCCCACCACC TTACCGGAAA TGGCAATTGC GGGACGCGCC CCCAATCACT
 1451  CACCACCCCT TTCGTCTCGC CCTTTTAACC CAATCCCCTC TCCACGACTC
 1501  CATCCACCCA TCTCCTGAGT CCTGCCTG***CG CTCGCCTCCT TTCTTCCCCC***
 1551  ***CCGGCCCCTT GGCTTTTGCT TGGTTGCATC GGGCCGGTCG AGCCGGAGAG***
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1601  GCCGGAAAAG CTAGCTAGCT ACCAGCTCTC CGGTGTGGTA GTACGTCTGC
1651  CTGCAGCTGG GGTTAGCTGC AAGGGTTGGG AGCCATGGGG AGGGGTCGGG
                                        M  G   R  G  R  V
1701  TGGAGCTCAA GCGGATCGAG AACAAGATCA ACCGCCAGGT CACCTTCGCC
        E  L  K   R  I  E   N  K  I  N   R  Q  V   T  F  A
1751  AAGCGCCGCA ACGGCCTGCT CAAGAAGGCG TACGAGCTCT CCGTGCTCTG
      K  R  R  N   G  L  L   K  K  A   Y  E  L  S   V  L  C
1801  CGACGCCGAA GTCGCGCTCA TCATCTTCTC CAACCGCGGC AAGCTCTACG
       D  A  E   V  A  L  I   I  F  S   N  R  G   K  L  Y  E
1851  AGTTCTGCAG CGGACAGAGG TATACGCACG CATGCGTGTG CGAACACCAC
        F  C  S   G  Q  S
1901  TCACGCACAT ATACATGGAC ACATGGTACA TGTGTGTGTT CTGTGGCAGT
1951  TGCCGGAAGG CCGCAGATCC TCTCTCGGAT CTGACATCCG GGGAAGCGCG
2001  GGGTGGTGGC GGGAGCTTAG ACGGTTTTTG TGCTTGTTTA TAGGGGCGTA
2051  GTTTCACGGG GATCTGGGTA CCACAGGGTG GATCCGCGCA CGTGCGAGGC
2101  CAAGCTCGTC GTTTCTCATT TCTGGTGATC TGTGATTCGT TTTTTAGGGT
2151  TTAATCTGGG AGTAGAAATT AAGCCTGATG GATTTCTTTC CGAATTAGTT
2201  AGGGTTCTGA ATTGCTCCTT GGCTTGTGAA GAATGGTGAT TTTAGCAGAT
2251  CTGGTCAATT TTCGTTTGTT CCTGGGGTTG TGGAGTACTT GTATTATTGA
2301  TGGATCGATT GGTCTAGAGA TGGCGTGAAT CCCCAGTTAA ATTAATCGCT
2351  ATTTGTATAT GCATAAAAAG CTACAAAATT CGGGTGAGCA AAGGGCGAGG
2401  ACTAGGGCCC TCTATCGAAT TTGTCGTGTT GCACGTACAT GTAAGCTTTA
2451  ATTTCACCCG GGATCGAATC CGGCCGTTTT TCTCAGATTT TTCATTTGCA
2501  TGTTTTGGTC TCATATTTGG CAAGCTTAGC TTAGAGGCTC AGTTCAAGCA
2551  TGAATGGACG TCACGTGTTG CTTATTACTA GCTGATCTTC GAATGCAATC
2601  TCTTTGAAAA TAAAAATGAA TTTTGTTACT GCCAAATAAA TGAGATTTAG
2651  TACACGTTAT AAACCTTCTC TATCTATCCC TGCGTCCCTC CCACGGTCAA
2701  AATTAGGAAT ACACTTTTAC TGATATCATT ATGAAAAGAT CTCAGTTGCA
2751  TTTGCTTCAT ATGGAGTAGT GTATATATAA TATTTTTATC AAGTACCCAT
2801  ATCATTGTTC ATGATCCGAA AAGGGCATTA TATATATGAC AATCTGTTTT
2851  AATTAAATGA CTCAAAAAAC CATTGAAATC AACCCATTAA TTAGCAATGT
2901  TTGTCTAGAT CAAGTAAGCC CTCACACAAT TAAAATTAAA CCGTTAGCAT
2951  TGTTGTCTAG ATCAATTAAC CCAATCAGAT TTTTTCACTA ACCTTTTTCA
3001  TTTTTGTGGA CAGAAAATTA AATACAATCA CATAACTTAT TACTGGGAAG
3051  ACTATATAAG ATCAAACCAT ATGGGTGGTT AATTTGTTTA TATATGGTAA
3101  CGTCTTAAAA TTTTTGTATT GCCTAGGAAG ATCTCTTAAT TTCCACAATG
3151  TTCTGTAACG TAACTAGATA ACAGCATTGT GTCCAACTCT CTTGCTGTTT
3201  GTCTAAGGCT TGTGTCAGTA TTAGTTTTGT TGGGATGCTG AAAATTTTCA
3251  GTTAATGATT CCATGATCCA GCAAGTGCGG CAAGCATTTA ATATTTTATA
3301  AATTTATTAG TTTTAAATTT GGAATTAAAT TCTCCTTGCA ATTTGCAAGT
3351  GTGTAGAAAT ACTAGATTAT ATTAAGGTTT TCTTTACTCC AAGATTTGTT
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3601  ACCTTGTAAA CAGGATTCCT AGGACTGCCC TGGAGGTATA AACTAGAGTA
3651  CAATCAAGTG TCAGGTTTCA AATGTCATTC GTTGTGTCCC ACTGACTCCA
3701  CCTTTCTGAT CGAACTGTCC TTTCATTTCA CTGCATTTCA ATCCCCTTGC
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4001  TTAGTCGGGC TAGTCGGTAA TTAGTACACG ATTTGCTGGA CGACTCGACC
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4101  AGTCGTCCGA CTTATGTGTC CTGGTCGTCC CGGCTAGGGT TTAGTTATTG
4151  AGCCTTTTTT AGCCCATGTA CAGTCACAGC ACGTCTCCTC TCCTCTCCTA
4201  TTCTAACCTA CCCGCCAACA GTCTCTGGCT CTTTGCACGT CTCCTCTCCT
4251  CTCCTCTCTT GCACTGCCCC TGCAGTCCTG GACTTTGATG TCGACGGATA
4301  CACCGACGGG CGGCGGCTAC AGCACCCTAC GTCCCTGCCC CTTCTCCTGG
4351  AGTCCTGCAG CCTGCTGCAC TACTGCAGTC TCTAATTTAT ATAATGTATA
4401  CATGTATATT TATATATATA CCTATATAAG TATAGCTACT AGTGTAGGAC
4451  GACCAGGGAC CGACTAGGAC CGACTAGGGG TCGACTAGTC GCCCTAGTCG
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4551  ACTAGGCGAC TTGAAATCAT TGCTCACGTC TTTTTTCTTT CTGAAAGTCT
4601  AAAGCACATG TTGTTAGGGC AACTGACACT CCCGTGGTAT TGAGGGAAAT
4651  TAAATACTCT ATATATGGTT TTGCTGGGTC AAGACATAAT TTTTCTATTA
4701  TTTTGTTTGT TACAATGTAT CGTAAGCTTA AATCAGGAAT TTTCTGTGTT
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4801  ATAGGAAGAT ATAGTAAAAC TAGCGATGCG CTATAAGATT TGTTTCGTCG
4851  ACAAGCATAT GCTAATACGT ATGCATATTA TTATCCTTGT TGACAAAATT
4901  TAGTTGCATA TTATTATCCT TGTTGACAAT GCTTCATCTT CTCCATGAAC
4951  CACATATTTT CTGTGGTCTG CTAACTATCC ATGCTAATTT TGAACTTGTA
5001  GCATCACCAA AACACTTGAG AGGTATGAAA AAAACAGTTA TGGAGGACCA
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5051  GATACTGCTG TACAGAACAA GGAGAACGAG GTAATCTATC TAAGCTCGAC
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5101  ACATTTATTT ACCTGAATCA ATGTCAGGAG TTCTCTTCTG TTTTACTAGT
5151  ATTATATTTC CTCATACAAA GATTCTAGTA CTCATTGGTT TGATGAAAAC
5201  TTAAATCCAT ATATTGATGT TTAAAAAATT AGTTTCATGA TTCAACATCT
5251  CTTTGTGAAA ATTTAAAAGA TACAGTGGTT TTGTTCTCGT ACAGTTATCA
5301  CATATAATTA AAAAGATTCT AGAACTCTAA TTTGGAAAAT ACAGTTATTA
5351  TAGAAGCTAC ATCTTCGTTT TGTTCAAGTG AACATCTTAT TCTGTTTGTG
5401  TAGGGCTGGC AATTACAGAA GCTACATTTT CGTTTTGTTA TTTCCTGTTT
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5501  ATAATTCCTA TGTTGTAGAA AACACAAGTT TACATTGAGC ATGTGAACTG
5551  ATATTCCATT CCCGTCACTA TTGCCCAGCC ACTATTTGCT TCAAAAGCAT
5601  TTAATGCTCA CTTTTTTTCA ATATATTTTG GTGGACTGAA GTACCTCTTA
5651  TTTTGCAGTT AGTCCAGAGC AGTCGTAATG AGTACCTCAA GCTGAAAGCG
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5701  AGGGTGGATA ATTTACAGAG GACTCAGAGG CAAGTTGACT CTACGTTTGA
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5751  GTTGTATCTT CGCAACAAAA GGAATCTCTC TTGCCCGATC CATATTGTCC
5801  ATTAGAAAAA TTGCTACACA TGTTTGGACA ATTTCATCAA AATAAATTTG
5851  TGGAACTGTC ATTTCCAGTG TATGAACTAT TAGGTACCAG GTAAATGACA
5901  TCCCTTTTCA GGGAAAAATG TTTCTGTATT GTTTGTTTGC CCTACTTTGG
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6051  TTTGAATTAG ATATCATTTA TGTACACAAA ATGAATCCAG AAACCATAAT
6101  TTAATCTCAA CGAAGTTAAA CTAAGTTTCT TAATGGAATG TGTAATGCTT
6151  AGCTAAGATG CACATATTTG ATTTTCGCTT TATTCACAAT TACAGGAATT
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6201  TGCTTGGTGA AGATCTGGGG TCACTTGGTG TCAAAGAGCT TGAGCAGCTT
        L  G  E   D  L  G   S  L  G  V   K  E  L   E  Q  L
6251  GAGAAGCAAC TTGATTCATC CTTAAGGCAC ATAAGATCCA CAAGGGAATT
      E  K  Q  L   D  S  S   L  R  H   I  R  S  T   R
6301  CATGAGAAAC CATTTTCTAA TGCGTTATTT TTTTGGATGA AGCTAGTGTT
6351  GATAACACAG TTCATATTTC TAATTAACAA GCCATTTTGA ATATAGAGTA
6401  CTCTATATAT AATTGTGATC GTCACACTAG TATATAAAAG CTCTATACTG
6451  TTAGAGGTAA ACCTATTTGC ACTGGCGTTT TTTTACAATC GTCAGTACTA
6501  GAGGTCAGTA GAAATAATTT TTTCTACAAA CGTGTAACTG AAAACCTCCA
6551  CTGGCAGTTG AACTAAGAAA ACCGTAGTGA AACTCGAATT CCATAGGCGG
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6651  AGGTTTAAAA ATAGCAAAAA GATATTTTTA TTAGGAGGGA GGTTCTCAAC
6701  TACGTATAGT ATAGTACTCC TATTTATAGT ATATATGACT TTAATTTGTA
6751  ATGGGACTCC TGTTACCTTT AGAAGGTAAG ATCTTATAGA AATCATAACC
6801  CTTGGTTTAT TATAAAAAAT AAACAATCAA ATGATATAAT AAGCAAATTT
6851  CTAAAATCGC ACCATCCCTC AAGAAATAAA TATCTTTTGC CCCCTCCTTA
6901  TTTGCCCGAC ATGATCAAGC ATGGACGGAG CCAGAAAAAA GTTTAGAAGG
6951  GACTGATCTA AACTGATGCA GTATAGAAGC CTCTTCTATA TTATATTGTG
7001  CACATTTTAG ATTAGGAGAG GCTCTAGTAA GACTACGTGG GATCCGAAGC
7051  GATAAGGGAT CTCGAGCCTC GCCTGCCCCA CCGCTCGATC CGTCTCTGTG
7101  ATCAGGTAAA GAGAATCGTT TGGTATACAC GATGAAATTG TACACGCACG
7151  TGAGTAATTT TTTTATCTTC GCATTGGCTT GAGTAAGTTT GAGTAGTGAA
7201  GCGTCAAGAG CATAATAAAA CTACCAAAGC CATTTTTTCT ACCCCCAAAG
7251  TTGTGATCAG AACGGTGAAG AATTGCACCA CTTGTATCCT TTCGTTAGAT
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7301  CTAAACTAGG GTTGTATATA TAGAGTAGTT AGGTATTTTT TTTCAAAGTC
7351  GCAAGATCTG ATGTAATTTT TTCTTCACGT CATAAAAATG TTGTGAATCC
7401  CGTCAGTTTT TTTTCCAAAA ATGCTACACT TAAACTTTTT TTATTACAAA
7451  TAAATAATTT GAAGAAATAC ACTTACATTT TATACCGTAT GTCACGACAT
7501  CACGTTACAT TAAATAGGAA AGTTGGATTA TACATGTACG TTCGATCTAA
7551  TGGAAGATAG TTTCCCTAGC TATTTCTCCT CCTACCACCA AGGAAGTCAT
7601  AAACATCTTC ATAATCTACT CCAAATCTTG ATTCCCTGGC AAGATTTTAA
7651  AAAATGTTCC CCCACGATTC TCTATCTTAA CTCCCAACAC TGGCCCAACC
7701  ACGCGGAAAT ATACACATGT ATATTGATTG GCCAAACTAA AGACATAATG
7751  ATGTGGGGAA GAATAACTGG AACATGGTTC TCAATGCAAA TATACAAGTG
7801  AGAAAGAAAA TATATAAGCA ATCATAGTGA TTTGGATATA GTCCATGATT
7851  TCTGATACAA AATTGTTATA TATATTTTAG GAGTGGGATT TTGAAAACTA
7901  TTGGAGAAGC TCAACCAATA ATCTCCCAAT TAAGTTTTTT AGCCACTTGA
7951  AAAACTTACT GATTTACCAA TTGATTTTTG GAAATATTAA AGAGTCCAAC
8001  ATATGAAACT TAACATATGG ATTAACAGTC CATAGATATT TGAGAGTCCA
8051  TCTATTGTAA TTCATCTCTA ATATTTTCTG TGACTAATGC ACCTTAATTA
8101  GGTAGGTCTG GAATTTGAAA TATTTTGAAT GATGAGATTC ATTTCATATA
8151  AAAAAACTCG ACAAACGGGG GTATGAGAGC CTCATACATT ATATTAAGAA
8201  GACTTTCTCA TGCAAGTCGA GAAAACCCTC GAACACCTAT CACACCCATA
8251  CACAATGGCA CCATAAATCA TATGAGAAAC GACTGTGGTC GGGACCGGGC
8301  CTTAGACCCA TGCTTTGGCG TGGTACTGAC GAGGAGATTT CTTTAACCAC
8351  AACCTGAAAT TCGCTCCCAT GGGAAGTCAA CTCCAGGATC TGAGTAGTGC
8401  TACTCAGACC ACCTAACTAA CTCAGCTATA ACCCTTTCGC ACATACATTT
8451  CATATAGGTA TGAACGATTT TGTATTTTGT ATCTTTCAAA TGCTAATCAA
8501  GGAAATTTCT GATTTCGCAG ACACAACATA TGCTTGATCA GCTCACTGAT
                            T  Q  H  M    L  D  Q    L  T  D
8551  CTTCAGAGGA GGGTATGCTA TTAATATGAT AGAATTTATA AATATTTCTA
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8601  CATGTAGATA TCTTTTTGGA TGATTTCTTC CTAGATAAGA GCATATGTAC
8651  TGTTCTAACT AATTAATGAT ATCGCTAAAC CATGTCCAGG AGCAAATGCT
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8701  GTGTGAAGCA AATAAGTGCC TTAGAAGAAA GGTATGCATG CATAATGCTC
       C  E  A    N  K  C  L    R  R  K
8751  CTAACCATGT CCCTTAAATG CTGCATATAC ATATTTTACC CGGTCTTATT
8801  TAGATACTCG AGAAAACATC TTATTATTTT GTTTTGATGT CATGTTTTGA
8851  GTTAAAATAA ATGGAATATA TCAATAAAGC AGCATTGAGC TCTTCAAGTT
8901  GGAAGATCTG TTTTGATGCA TATGCTCGTT GCACACATAT AATCTGCGTA
8951  TATATGTTTG CGAATACATG TGCAATGAAC ACATTAAATT GAGTTTCCAT
9001  GAGCACAAAT ATACACTAGT ACTGGTTCAT GTTAAACAGT TGAACCAGCT
9051  ATCACTTATA TATGCTGTCT CCAGCAGCAG CTACGACCCT ATCACGTCCC
9101  CAATCTCATC TATTATTTCA AACTTTACTC TATATATAAA TAGTATAATA
9151  TACAGTACAA ATTCTTATTT TACATGATCT GTTGCGGATA ATACTTCTCT
9201  ATGCATGGTG TTTGTCTTAA TATATCCGCC ACAGGATGTT TTGCATAAGG
9251  AATACTGTTG TTCAACTGTG GCAGCACAAC TGTAATTCAT ATATTAACCT
9301  CTCAAAATAT ATATTGTGCA TATTGTTACA TTTGACTGCT ATTACAGTAT
9351  GCATGTGATA ACAGTATTTA CCAACTAAAT GAGTTTTAAG GGGGGTTTCT
9401  GAGATCAATG AGGTTTCTTG TTTCGTATAA AAAAATTAAT GTGGTTTCTT
9451  CACGTGATTG TTTTGCAAAT AAAAAGCTGG AGGAGACCAG CAACCAGGTG
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9501  CATGGCCAAG TGTGGGAGCA CGGTGCCAAC TTACTCGGCT ACGAGAGGCA
      H  G  Q  V    W  E  H    G  A  N    L  L  G  Y    E  R  H
9551  CTCTCCTCAA CAGCAGGCCC CATCACATGT TGGCAATGGT TTGTTCTTTC
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9601  ATCCCCTGGA AGCTGCAGCA GAGCCAACCC TGCAGATCGG GTATGTCATC
        P  L  E    A  A  A    E  P  T  L    Q  I  G
9651  ATATGCCTCC AGATGCATCA TGTGATTCGC TATATCGTAC ATAAAGCGAG
9701  CCTGTGCATC GATCTGATCA TGTCTTGTTG TTGTGTTTGG CATATGCATG
9751  TTTGCAGGTT CGCTCCTGAG CATATGAATA ACTTCATGCC AACATGGCTA
             F    A  P  E    H  M  N  N    F  M  P    T  W  L
9801  CCCTGAAGAT GGATCTTGAG GCAAAGGAAA TAAATAAAGA TGCGGTGGCA
      P  *
9851  GCATCATACT CTATGCAAGC TAAGAGTGAC AGCTGTGTTT CAGTCACATA
9901  TATGATCTAG TCTGTGTGTC AATGTGTCGA AAAACATGTC TGTGATTTTG
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 9951  TTATATGAAT GTAACGCGCA ATGAAACATA CAGGACTAGC TGCTTGCTTG
10001  ACGATGCGTT TGCGTGTATC CGTATAAATT AAAGGCTATA AGCCACATCG
10051  TATATGCGTA CTGCACATGC GTGCATTATC TAAAGGGCGT GATTGAAAGC
10101  CTGCATAGAT TCTAGGTTAG GTTCCACGGG TGCAGGTAGC GTAATGTTTG
10151  GATGCCTGTA TTGGTCTTTG GACCAAGCTG GATTCATGGA ACTAGAAAAA
10201  AATATAAAAA TTTTGACTTT TTTGAAATTT AAACACACTC AATCTACGTT
10251  GATTCAGAGC AAAACGAAAG TCATAAAATA GACGTGGGGA TCAGGGTTGA
10301  GCGTATCTAG TGGAGCCAAG TTGAGTTGGT GCAGGCACAC AAATCAAGAA
10351  AGCGTTTTAT TTATATTTTT TGTTATATCC TTTTAATTGA TATATCTAAT
10401  TAATAAATCG ATTGCACTTT CGTGTTTACA AAACTAAACT ACTACACTTA
10451  CTATATAACA TATTATATAT TTATTTATTC ATCTTGTTAA CCCAGTAAAC
10501  CAAACATTGT TTCCTCTCCT AGCCTCGCAT GCATATGGCC AAACCAATCA
10551  CATTTGTATG TGTACCAAAT CAATATGGCT CAACATGGTT CGGCTCTGTT
10601  GAGCACTAAA AAGAGCACCG GACGGTCCGC GCCTGTGGTT CGGATGGTCC
10651  GCGGAAGCGC AGAACAGATT AGGGTTCCGA GTTTCTTGCT ATGTTTATTG
10701  GCGAGAATCT CGGGATTAGC TCGGAATTTT GTTGGTAACG GGTCCAGCCC
10751  CCTCCTCTAT CAATCAATCG AACCCTCAAT CAATATAATT TACATTTATT
10801  CTTAGTAGTA GTTCTAGTCT AGTTCTAGGT TAGTCTTCCA ATCTCCAAAA
10851  TCTTCGCTTC TCTTCGACTC TACGTCGATT AGAGGAGTCT ATGTCGGCCT
10901  GCCGAGCCTA GACATCTCCT AGGATCTCTC CTCCCCGACG GGGTCCCTCC
10951  CGGGAGCGAG ATCCAGGCGC CGCTGGCGAC TTCCGCCGCC CTGCGCACGC
11001  GCGGACCGTC TTGCCCCAGG GCGCGGACCG GCAGGCAGGA AATCCTAGCC
11051  CTACGCCAGG CCGCGGACCG TTGGCCCCTG TGCAGAGAGC ACCGCCATGG
11101  TTCGTGTTGA GTGATTGGCG CCCGAAAAAG GTGTCAACAT ACTTTTGGCG
11151  ACTCCGCTGG GGACAACACA TCTAGACCCA TCAAATCGGC CCTCAATGGC
11201  CGGTTCAAGG GATAGTTCTG AAGTCTCCCC CAGCAACATT ATAGAGCCGA
11251  CTTGGGAAAC CTTGCTGGCT CACGAACAGC TCCAGTTCGA GGAGCACAAG
11301  GAGCAGTTGA TTCAAGAGGC AAAGGCGAAA TTCCTGGCTA ACTTCAAAGT
11351  GGACAGGAAC AATAAAGTCG TCTGACAACG GGCGATGGAT CCGGCTTCGC
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C. Genomic sequence of maize *AGL2*-like gene *ZMM3* (clone λDASHII-wd10).

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    1  GGATCCACAT GGTCCCGTTG TGTAGACATG ATCGTTTCCT CACGCGGTTA
   51  AGCGTAAAAT GAGGCACGAA GCTCTGATAC CAATTGAAAG TAGCCTAGAG
  101  GGGGGGTGAA TAGGCTACAC CTGAAAATTT TCACTAAAAA CTTCGAAATA
  151  GGTTAAATCA AAGTTGCACG GGTGCAAACC AGTTCAGTCG ATTTTAATCC
  201  CAACTGAACA AGTTTGAACC CACTCAACTT GAATTTAGTA ATCTATTGAA
  251  CAAATTCGAA GTAGTAAAGA AAACAGCTAA AGATTTGCCC TACACAAGAA
  301  CTACTTGAAT GAATAATATG AACCAACCAC CGAATATGAA AGCTTAACAA
  351  GAACACACAA GAACACGCGA TATATCCCGA GGTTCGGCAA CCACCACAAA
  401  GGTGTCCTAC TCCTCGTTGA GGAACCCACA AAGGGCCGGG TCTTTTCCAA
  451  CCCTAATCCT CCACAAGCCG ACCACAAAGG TCAAGGCAAT CTCTTCTCAA
  501  ATCTGCTCAA GGAGCGGGTG ATACAAACTT CTTGGGGTCG TCCACAAATT
  551  TGGAGACTCC CAAGCAACCT CTAACCGTCA AGGAACACGA GGTTCCAAGA
  601  GTAACAAATC CGCACACGGT CAAGTTTGCA ACGAGCTCAA GAACAAGAGA
  651  AAGGGAGAAT CAAGATGAAA TTGACAGCGA GTTCGATCGA GTTCACCTCA
  701  CACCAAGGGT CCTTCAAATG ATTGAAGGAG ATGCGATTGC GGGTGTGAGA
  751  GGTGAAGTGA ATGCTCTTGT TTGAGGGTTG GTCAGCCAAA GATTCGTGGG
  801  AGAGGTAGAA GTAAATGAGA GAGAGAGTGT GAGGGGGGTA TATAAGGGAT
  851  CCCCCAAAAG CTGGCAGCCG TTGGGAGAAA GAGGGCAAAA ACCGGTTGAA
  901  CCGGTTTTCA AACCGGTTGA ACCGGTTTCT ACCAGGGGGA TCCCGGTCCA
  951  CTGGTCTACT CAGCCGGACA CTGGACCGGT TTCAAAACCG GCTGAGTAGG
 1001  GTCAAAATTC GGCTCAACCG GTTTCAGAAA AATATCTGCT CCGACTTTTC
 1051  TGACAGCTCT GACTGTCAGA CAGGTCAGAG CAGAGGTGGC AGGATGAACA
 1101  GTACCAAAAC CGGTTGAACC GGTTTTGGGA CCGGTTGAAC CGGTTTTGGG
 1151  GGCTGAAACC AAACTTTGAG AATTTTGAAG AGAACTTAGG TGGAGACTTT
 1201  GTGGGAAGTG AAATTTGTTT TTCTCAAGAG CATTCATGAT CTGGGAAAAA
 1251  TGATCTCAAG GAGTTTTCAA AAGAATTGAA CTTGGCTCGT TTCACAACTT
 1301  ACTTAACCGT CGCGGATCCC TCTTAATTGT ACGGCGATTC CTATGACTCA
 1351  AGAATTATAA ACTTAGTACC GCCGACGATT CATAGCACTT GGAGCACGCC
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1401  ATTTGATGTG GAATTTTAAA TCTGCTGTGC TTTATACTTT TACGCTCGTA
1451  GGATCTGTGC TTCTCCTGTC TGTAAAAATA CTGTGTACAC ACTAGGAGCA
1501  AACTTGTTAG AATCTTAGTT TGTTTTGTCA TTAATCACCA AAACCCTCAA
1551  TTAGGGTTGA TTGCACTTAC AATGTGACAT TGCCAACTAG AATTATGTAG
1601  GTGAGCTCCA TCTAACTCGA CCCCTTAATT GCTTAATTAA AATCTTTGTA
1651  AGGCATTTGT GATAAATAGA GGGATGCAAT CTTAGCTTGG CCATTTAGTT
1701  TATTTTTATA TTTGTTTTGG TTAGCATGCG TGATTAGATT TTAGAAAGGA
1751  ATATTCATCC TCTCTAGTCC ATCATCTTGA TCATACACGC TTCCATCACC
1801  CGCGGTCGGC GGCTCTCGCC ACCTCACATG TCACCCACCT CTTGCTGCCA
1851  TCCCCTTGAC CCCATCCTCC CTTAATCATA GGGTAGCCAT CAACCACTTT
1901  ATCGTGATGC CCCACTGCCA ACCTTTTCTA ACCGTTGTCT CACACTGCCC
1951  CTATGGATAG CGCTCCACCT AGGGTGCTAA TGGATCTCGA TCAAAATATT
2001  TATTCACAAT TGGTTTGGGC CATTAATTAC TTTTAGTTCA AAAAGAATAG
2051  AAATGATGTC AAATCCTAAT TCGATTTGAT CCTTAAATCT TATAGCGTAA
2101  AATTTAGAGC CTGTTACCAC CCCTGGCTCC ACCGCTCCAT GGTACCGTTT
2151  GCTCGTCCTC CACCACCTAT GTGTCACGAC CACCGCCTCT TAAACCCTAA
2201  TCCTACACCA TCCGCTGAGT GAATCTTCTG AAATCCAAAC CCTACTCTTA
2251  CCGACTCTTT TTATTCTTCT CTGTGAGAGG CAGATGGGTC ACATGAGTAT
2301  ATGAACACAC GACACATAAA AGTTTTCGCG TGATTATATG ATAAACATAC
2351  TGAATGAAGT TTCATGTGAA AAATCGCTAT GTTTTGTTTT TCTTTCTTAA
2401  GCCAAGACAA GTGTGCATAT GATTCTATTT GATCTTGTCA CCAGCCAAAA
2451  CCAAATTAAG GTGTCGTTTG GTTCTAGAGA TTAATCTTTA GTCCCTGGTT
2501  TTTAGTCCTG TTTAGTCCTT TTTCGCCAAA CAGAAGTACT AAAATATAGA
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2601  AAGCACTTTT ATTCCTCTTC TTGCCCTTCG TCTATTGCTC TCTCTCTCTC
2651  TCCGCGATGT CTTAAACATA CAAGGGGTAT TTAAGTCTTT TTTTATAATG
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2951  AATCAAAGGT ATAATATAGT GGTTGCTAAT ATATGATCCA TAACTCTGTT
3001  GTAGTATGTC AACCAAAGTT TAAAAAAACA TAATATAAGA TAAAATACTC
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3101  ACGTTGATAT CTAAACCTCA CTCGTTTATG ATCCATCTTA TCATCTAGCT
3151  GGGCAGCTAT CGATTGCCGT CGACCATTTG ACTTGCCTCT TCAATAGCCC
3201  CACAACCAAA GTCCAGAGAA GGTGATCACG CGAAACAACA GAATGAGCGC
3251  CAAGAGTCCA ACAACATCAA TCACCAGAAC CACATGTCGC AACCGGGACA
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3401  TGAATTCATG GACTAAGATA ATTTATAAAG AACTAGAATT CTTATTCCAT
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3951  GGCCGATGGA CAGGTGAACA GAGACCGGCC GGAAGGGGAG AGGAGAGGAT
4001  GGGAGAAAGA GAGAGACTTT CACTATAAAC AGACAGACTT GCGTTGCGAG
4051  AGAGGAGACG GAGAGTCAGA G***ACCGAGAGA GGGACGATAG AAGGCATAGG***
4101  ***AGCTAGGAGC GATGACCGTA CGCGCATATA CTAGGAGCAG CAAATTAAAG***
4151  ***GCATATATAG GCAAATAGTA GTAGTAGCGA GTAGCGACGG GCGAGAGAGA***
4201  ***AAGCAGTGCT ACTGCTACGA CTACTCCTTA GCTCGTAGCC GGGTAGAAGG***
4251  ***TGACGAGAGA GAGAGAGAGA AAGGGGGAGA GAGATCTTGA TCTAGTTCTA***
4301  ***GTAGACTGCT AGCAGTGAAC AGGCCCGGTG GAGGAGCTGA GGTAGCTGGG***
4351  ***AAGGAGAAGG AAGGAATCGA AGAAAAAGGC AGCAAAACAA GCAGG***ATGGG
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4401  GCGCGGCAAG GTGGAGCTGA AGCGGATCGA GAACAAGATC AGCCGGCAGG
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      S  V  L  C   D  A  E   V  A  L   I  I  F  S   S  R  G
4551  CCGCCTCTTC GAGTTCTCCA CCTCCTCATG GTACACGCGC GCGCTCTGGC
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4601  CACCTGCTGC GCGGCCTTAT TAGCCCTCCG CTCCTCCCTT CTTTTAATTT
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6651  TTGATCCTCA ATGCTTGCTT TCCTCAACAG CATCTACAAG ACGCTGGAGC
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6701  GATACCGCAG CTGCAGCTTT GCATCCGAAG CATCAGCTCC ACTAGAGGCT
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6751  GAATTAGTAA TCTTATTACC GATTTCTTCA CACTACTTTT AGTGAGTGTG
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7151  AATGAAATAA TAATGCTGGC AAGAGGTACT GATGGACATA TAACACTTTT
7201  GCAACTGAGT GTGTATAATA TATCTACTAT TTAGTTTTTA CACTTCGATC
7251  TTGTGTTTCA TGTGCTAGCT AGCTGTGTAA TGTCAGCATG CACGTATGCT
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7451  TTATGTATGT TCACCAAGAC ACATTTCTGT ATTTTAATAA TTACGATTTT
7501  ACTCAAGCTA ATTCTTAGAC CTCCTTTTCT TGTCCAACAG AATAATTATC
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7551  AGGAGTACTT GAAGTTAAAG ACAAGAGTTG AGTTCTTACA AACAACTCAG
        E  Y  L   K  L  K   T  R  V  E   F  L  Q   T  T  Q
7601  AGGTAAGAGA CATGCATTAC TTTACTGTCA GTGAACTATA ATGGTGGAAA
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7651  ATTGGAATAT ATATATCTAT AACAAGTGGC AGTCTAATTA GGTTTGGTTA
7701  TCTTAATCTG CAGAAATCTA CTTGGTGAGG ACTTGGGTCC ACTTAGCGTG
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7751  AAGGAGTTAG AGCAACTTGA GAACCAAATT GAGATATCTC TCAAGCATAT
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7801  CCGATCATCA AAGGTAGAGC CAATGAAAAT TTAGTAACAA GCAATAAACA
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7901  AGAACCAGCA GATGCTCGAC CAGCTCTTTG ATCTCAAGCG CAAGGTGATC
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7951  AAATCATTTT CATTTTTTTT TTGCTCGCTT TTGGGCTCTC TTCCTAAAAG
8001  ACTCCCGAAA TACTAATCAA CGGTGAGGTT TTGCAGGAAC AACAACTGCA
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9801  AACATATAAT GTCTTAAATA CCGTGAGTTG GATGAAATGA CTTAAATCCA
9851  ATCCTGCAAT GCATGAATAA CTTGGACACA ATATAAAAAT ATCTTAGAAA
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9951  CTTAGATGTA AATAAAATGT CTTAAATACT ATGAGTTATA GCGATGTAAC
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10051  ATCTTTTTTA TGTTTGGTTA GTTCAACTAT GAATTTACAC AAGTATTGTA
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10151  TCATTTCAAG TCTTCTATCA CTGTACCAGA TAGAAGAAAC TAGTGAAGAA
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10201  AATGTGCTGC GACTGTCTAG CCAGGATATT GGGTGTAGTG GATCTAGTGG
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10351  CCACTAGCAA ATGGTCGTTA AAAATTCAAT ATATATTGCT TACTTTGTAT
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10551  TTATCAATGG AGTATTTAAA AACTATAGTA CCTGCAAAAA AAAATATATC
10601  CTTCAGACTA TCTGATCCTT TTACTCTCCT ATGTAGGTAT CAAGCTTACA
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10651  TGGACCACCT GAACAATGAT TAAGTTGCTT CTTTGTGCGC TGTGTGCTCT
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12801  TTTTTTTGCA TGCGTGCAGT GAAAACGAAA ATATCATCCA AAATTTTCGC
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12901  AAGTGAAAAC CCCATGGATG CGTCTCCCAT ATTTTTCGAA ACTGCCATAA
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13451  CCTATATTGT GGAGGAAATA AAGCATTAAA TACGATTTTT TTATTTCCAT
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13601  ATCTAAACTG TGTAGCATAA TTAGTATCAG TATATGTCAT AAATTGGTCC
13651  TGACGAGTCT AGCTGCATGT CTGTTGCATC GATTCTATAT TTATGGAGGC
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13951  TATATTTGGA GCCTTGTACT TCCAATAATT AGAGGAAGAC CTAGTTTAAT
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14201  ACTAAGAATA TAATACTAGA AATGCAACAA TGCTAAGACA ATACTTCAAA
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14301  GTAAAGCAAG GTTTAGAAGA CTCCTCCAAT TTTTCCCGAG GTATCGAAGA
14351  GTCGGCACTC TCCACTAGTC CTCGTTGGAG CACCCGCACA AGGGTATCGC
14401  TCCCCCTTGG TCCTTGCAAG AACCAAGTGC TCACTACGAG ATGATCCTTT
14451  GCCACTCCGG CGCGATGGAT CC
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D. Genomic sequence of maize *AGL2*-like gene *ZMM6*.

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  201  AGTGCAGCAT TGTAAAAATG AGTTCGCAAA GGAATTACAT CTGATCATGC
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  301  TGGCAGTAGA TCCCGCCAAT TTTACCCTTT TGTAATTTTG TAATTTACTT
  351  TCCTGTATGC TTCTAACTCT GTCCTTTTAA AATTTTAATA AAATTTCAGT
  401  AGGGGCTTGC CCCTCCTGTC CTATAAAAAA AACCTCATGC TGAGCTGTCA
  451  ACCCACACAT CTTTATTTAT AGGTGCGCTG CAACAGAGGC CCATCAGTCT
  501  CTTGTTGGGC TGGACGCCTC CATCAAGGCG CGAGGTCAAG GGCACAGTTA
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 1101  GGCGCGCGTA CAGTACAGCT CGGCACCCAA CAGCTAGTAG TACGCACGTT
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1301  CCTACGCCTG CGGCGTGCAG CATGCGCGCG GATCGTAGTC CCCGGTCGAT
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2301  CATCGCTTTC CTCTCGTTAT TTAGCCCTCC GTTCCCGACC CTCATCCTCC
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2651  AAGCTCTACG AGTTCTCCAG CACGCAGAGG TATACACGCG CGCGCGCATG
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6601  TTCCATTTGA TGAGCCTATA CTTCTTATGT CCTTCCCCTT GCCAATGTTG
6651  GCTTTATTTT CATTCCTCTG TGTTAAGTTT GCACTCAATA TTTTTCAATG
6701  ACATGTGTGA ATTTTCTAGA TATTAGCTGC TGTATGCCTG GGTTTTCATG
6751  CCTTTCCTAT CTGCAGCATG CCAAAAACAC TTGAAAAGTA CCAAAAATGC
                        M   P  K  T  L   E  K  Y   Q  K  C
6801  AGTTTTGCAG GGCCTGAAAC AGCACTCCAG AATAGAGAGA ATGAGGTAAG
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6851  ATCTTTTTCT CTAGTTAAGA CAAGCGTGGA CAAGTTTTTT GCAAATATCT
6901  ATAGCATCGA TGTATACTCC GAAAAACATT AATCAACAGC TACTTTGCTA
6951  CTAAGATGTT TCATCTACGA AATCACGTTG TCTAAATAAA TGTTATCTTC
7001  GAAATATAAT GGTTTGGATT TGTCATGTGT GAAACATGTA CATATATTTA
7051  TTATTAAAAA AAAATCAAAA GTGCCTCCTG TACAAGGCTA TTCGAGGAGA
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7251  TAAATAGACC CAAACCAAGC AAATAAAGGA ACACAATAAT TGTCTCACTA
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7351  ATTTCATGCG AGGTATAAAA TAATGCATGT GAAACCAGTA AATATTGTCA
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7451  ATCATTAGCT GTATTATCTT TACATTCAGA AATCAGAACT CTAGTAGTAG
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7651  GTGTGGTCTT TTGCAAGCAA GGAAATATAT ATAAGCATCT CTATTCTTCT
7701  GTTACAATTT AAATTATGTT TTTCTAGTGA CAATTATGAG GCCTAATATA
7751  GAGATCATCA TTGTGATCTC AATATAGTTT GTTGAACAAG GCATTCCTGT
7801  TTATGCTAAA CATACATAAT GACATTTTTT CAGCAACTGA AAAGTAGCCG
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7851  CAATGAGTAC CTCAAACTGA AGGCACGTGT TGATAATTTA CAGCGGACTC
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8001  GCTTAAAGCA AAATAGCATG GCTTATTTAT TCATCTCAAA TCAGTAAATT
8051  CATGAAGCAA CATTGAATTT AAATTGAAAT AGTGCAAAGC TAGGAATACC
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8201  ATATATCGTA ACTTTGCAAT ATAAAAACAA ATGAAATGTG GAATGTGCTA
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8351  AATATGTCGT CTGTATAACA TTTCTAGTCT GCATCATATA TTTTTATTTT
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8501  TTTACTAACA ATCCCCTCAG GAACTTGCTT GGTGAAGATC TTGAGTCATT
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       G  I  K    E  L  E  H    L  E  K    Q  L  D    S  S  L  K
8601  AGCACATAAG ATCTACAAGG GTACTGAAAA GTGCTAACAC CATAAACGAA
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8751  CTGATTTCTT AACTACAATC CTTTTCTGCT AGACTATGAT AATATGAGTT
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8851  TCTGAGTATA TGTTCTGACC CAGTTCTTTT CCTTCTCTTT TTCTCCTGTA
8901  GACACAACAC ATGGTTGATC AACTGACAGA ACTTCAGAAA AAAGTATGCT
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8951  ATTCTTCATG ATTTAACAAA AATAATATTG GTGACTTCAC TCAAGCAACT
9001  TTACTTATTC AATTTGCAGG AACAAATGTT TTGTGAAGCA AATAAGTGTC
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9201  CAGTTTCCTC ATAAATTTCT GAGTTTGAAT AGGGATGCAC ATTTCTATAC
9251  ATACGGGATC ATAATTAACC ACATATAGGG CTGGTTTGGT GACAAACGAA
9301  TTGGAGGGGA TCTCCAATTC CCTTGTCACC AAACCAGGCC ATAAGGTCTC
9351  AGGCAGTAAG GATTATTTAC CTTCTTCATT GTTTTGAGAT GTGCAGCTGG
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9401  AGGAGAGCAA CCAGGTTATA TGGCAGCATG CGTGGGAGCA AAGCGAGCGG
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      H  S  E  V    Q  P  Q    Q  L  N    G  N  N  F    F  H  P
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9551  TATTTTCCGT GAATCTCTCT TCCTACCCTA AATATTGTGT AATACTATTT
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 9651  TTATCATATA GAAATGATCT TCAAACTAAA TGGTGTTTTC TTTTAACTAG
 9701  ATTAATTTTA CCCTATGCTC GTTTGATCGT CCTATACCAA CGTACACGAC
 9751  CATCAAAATG ATACCTTGTC CTATTATTTT CCTGACGGAA GAAAGGCTAA
 9801  ATCACATGCA TGGTATGTCA CAAACTAGAG AACACTGTGA TTGTAAATTG
 9851  TTAAATCTTT AACTACAAAT TAAACAAGAC CAATGTAGTA CTGAACTACT
 9901  GATGACAAGT TCTTATCACA GAGTAGCTCT TTGTAATGAA ATTTAAAAGC
 9951  TGATTCGTTC CTCCATTTGT GCAATTTGTT TGCAGGTATC CTTCAGAGGC
                                          Y  P   S  E  A
10001  ACTGACTAGC TCATGCATGA CCACCTTCCT GCCCCCGTGG TTGCCATGAG
        L  T  S   S  C  M  T   T  F  L   P  P  W  L   P  *
10051  ATCGAACTAC TGCGCAATTC CGAATTAATA AGCATGGCCG CATGCAGCTG
10101  CCTGTGCTAT ACGTTGTGCT TGGAGTGTAC TTTAAGGTTC TTGCATGGCA
10151  AGTATGTGTG TAACATTTGT GATGAACATG GAAACTATCC ACTTAATTCT
10201  CGAATAATCA ATGAACTGGG TAAGATTGTA ACATAGTGCT TTATGCCTGA
10251  AACATTAATT TAGGCTGCGT GTGAATGATG GAGGTTGTGT TCCGCGTGTT
10301  CAGTTTACTA GTAACAGCAG TTTAGGGTTG GTGAGGAAAG TGATAAATTT
10351  AAGCAGTTTT CTAGTAACAG CAGTTTAATT AGAGCTGGAG CTGCCACGTC
10401  ACGCATTGAT CCCATGCTAT GGACCCACAC CTGGCCTAGG ATTTTGAAAC
10451  CGTCACTGGT CGACGTAGAA TTTCTTATTA TGCCTATATT AGTCTTCCCA
10501  GCCTCAAATT CTTGGTTTCT CTTTAGCTCT ACGTCGATTA GAAGTATCTA
10551  GGATGGACTG CCAGCCCTAG ACAACACCTA AGTTCTCTCC TCTCCCGATG
10601  GGTCCCTCCT GGGAGCGAGA TTCAGGCGTT GGCGAACTCC GCCGCCCCTG
10651  CGCACGCGCG GACCATCCGG CCTATAGGCG TGGACTGTCT GGCTCGTTAG
10701  GCAGGGAAAT CGAGCCCCGC GCCAAGTCGC AGACCGTCTG GCCCCTGGTC
10751  GCGGACCGTC CGTGCATGTG AAGAGAGCAC CGCCGCCGGT TCTCAATGCA
10801  GTAATTAATT GACGTCCGGA TCGACGCTAA CACACTTTTT GGCGACTCCG
10851  CTGGGGACAT CACATATAGA CCTATCAGAT CGACCCCCAA TGGCCGGTTC
10901  AAGGGATAAC TCTGACATCT CCCCAACAAT ATCATAGAGC CGACTTGAGA
10951  AACCTTGTTG GCTGACGAAC AACTCCAGTT CGAGGAGCAC AAGGAGCAGC
11001  TGATCCAAGA AGCAAAGGTG AAGTTCCTGG CCAACTTCAA AGTGGACAGG
11051  AACAACAAGG TTGTTCGGCA ACGGGCAACT GATCTGGCTT CTCTCCGACC
11101  CACAACGGAT ACCCCCAATG TAAGTAACAC CAACGAGCTC CAATCTCTTA
11151  AAGTTTACAT AGATGAACAG CGAGAGCAAA TGCAACATAT CGTAGGGGTA
11201  TACAAAAGGA TTATAAAAGG CTAGTGCGTG CGTTTGATAA ATCTACCACT
11251  GCAAATTTTC CTTCGCACGA GGTTAAGTTG GGGGGAAACA CGCGTGATTC
11301  ATCGTCCACA GGTTGTCACG ACCAGTCTTA ATGGGATGCC GATAGACACG
11351  TACCCTAGGC AACCACA
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E. Genomic sequence of maize *AGL2*-like gene *ZMM14*.

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  101  AAAATCTTCG TTGTTATGCT ATTCTTTGGT TTATCATGTA TTCATATTTC
  151  CTTAAACTAC TATGACTGGA GTGCATGTTT CCATGACAGA TTGCAACTTT
  201  TTTCTGTTTG AGGTGATTTG AGCCCTATTT GGATTATATT CTGCTAAGCT
  251  ATTTTCAAAT TTTAACGATC TAGCTGAGTT AGTTAAGTGG TCTGAGTAGC
  301  ACTCCTCTGG TCATTGATTC GACTCCCCGT GGGAGCGGAT GAGGTTAATA
  351  AAAGTTACTC GCTGGTTCTC TTAGTCGGCA TGGGTGAACC AACCTATGGT
  401  GGGCGGGCCC TCGTGTAAGG GGCTGGTCGT GTGGATGAGG TCTCAAAGCA
  451  TGGGTCAAGG TCCGAACCAT TGGGGGCGGC ATCTCCATGT ATGAGGGCGC
  501  CAGCTTTTGT GGTTTTTTTT TTGGCCAGGC TCCGGTTGAG TTCTTATTGA
  551  AATGCTGTGG GGGCGGTCTT TCCCCCAGCC AAGTTTTTTT TGTTTAGTTT
  601  TTTTCTCGGT TCCCTTTCAG ATGGAGGAAC AAAAAAGGGA CCAAAGAAAC
  651  AATCTGGTGG TTCGAAATCG AAGACAGCTG ATGCTGATAT TACGGTTGCA
  701  AAATTGGATA TTAGAGTGGG CCTTATCAGG AAAGCAGAGA AGCATCCAGA
  751  TGCTGATTCC CTTTACGTGG AGGAGATTGA TGTTGGAGAG GACACACCAA
  801  GAACAGTGGT CAGTGGCCTC GTCAAATTCA TACCTCTCGA AGAAATGCAG
  851  GTTGCCCTTT CTAAGCATCA TTTTGATCTT TCTAGATACT GTCTTTTGAT
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1001  CAATGGTTTT GGCTGCATCG AATGAGGACC ACACGAAGGT GAGTCATCTA
1051  TATTAACCTT CAAATACAGT TAACTTCACA CCGAACCATT TTTAGCTCTC
1101  AATTGGAAAC CATCAATGTA AGACAGCACA GTACCAGTCA TTTTGATTTC
1151  CGACTACGCT TGTAAGGTTG AGTTGGTGGA GCCACCCGAA TCTGCTGCCG
1201  TGGGCGAGCG TGTTACCTTT GCTGGGTACT CGGGGGAGCC TGAGGCTTCC
1251  CTCAGTGGCA AGAGCAAGAC GTGGGAGAAG CTGGCCGCCG AGCTGCACAG
1301  TAACGGTGAG CTCGTGGCGT GCTACAAAGA TGTGCCCTTC ACAACCTCGG
1351  CTGGAATCTG CAAGGTCAAG ACAATAGCAA ACGGAGAGAT TCGCTAGGCA
1401  CATCTCCGAA CTGCAGCCAG CTGACCCAAT CTGGGAAGTG CTAAACTTTA
1451  GCATCATGAA CCTAAACAAG CCTAAAGCCT TTTATGTTTG AAAAGTCGTG
1501  TACAAGACAA ACTCTCCGTG AAGAGATGCT TTTTTTTTAC TTACTCTACT
1551  AGAGACTCCT AAAAAATACC GATATTAATT GGGGTTATAC ATGTCCTAAG
1601  CATAGATCAA ATATAGGTAG CACTCGCTGA TAGTTTAGTA AAACACAATT
1651  ATTATGTTTA CAGAAAATAC GATTATTTGG CAAATTTAAA TAATGAAAAA
1701  CTTATAAAAT AATATATAAT ACATATTTTA ATAACGTAAT ATACCAAAAA
1751  TCGATAAACT ACAAATACAT ACTTAAAAAT ATAAAAAATA ATCGATATAT
1801  AAATAACTAA AGTACTTAAA CTCAATATGA TTAGTTATAT TGAAATTAGT
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2101  AGCTGGTAGC AGTTGCTCGG GGATCTAGGA CGGGGGTCCT GTGATGCAAC
2151  AGCGAGTCAG ACTGCGTCTG CGTCCAGCCA AGTACTTCCC CCGCCCCGCC
2201  TACGCAAAAA GAGAAGAACA CGCGGCGCCA ACAAGCTTAA GTACTCGTCT
2251  CTTTTGTTCC TTTACCACCT TTTCTCCATA TGATTAAAGA GGAGAGCTCC
2301  CTTCCGGCAT CCAGCCTAAC GCGCGCGATG TACTGTGCCG TGCATCTAAG
2351  ACACAGTTAC CTACAGTACT ACTTTGAGAA GGCCTGCATG CAAGTATGTT
2401  AGAGAACGGA AAGAGACATA TTTACTTTGC AGGTGTCCTT TTTGCGCGAG
2451  ATTGCATGGT GTTTTATTTG AAAAGCACGC CGGACTTGTA AACGGATCCC
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4001  AAATAGCGTA GGGCATACAG TCTGCGGCTG CAGATTTTTT TTTTGTTTCG
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4251  AACGCGTGCC TGGGCGACAA GCGGGCGAGA GGGAGGCGAG GCGCTGATGA
4301  TGGTGGGTGC GCGGGCAAGA GCAAGTTGGC GCGATCTGAT TGGTCCATGT
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 7751  AATGTTTATG GTCAAACTTC AATTTTACAA CCTTTGACTA TCGAAGGTTT
 7801  TACTAGCTAG ATCAAATCAT GAAATAGTTT GTGAATATTT ATTTTGAATT
 7851  TTACTTTCAT GATACTATAT AAGTTTACTA GATTTTTCTA ATACATTTTA
 7901  ATATATAACA TTTATGGTCA AAGTTATATT TTATAGACCA TATAGGAAAT
 7951  GTCGTCATTC AAACAATCAA GCGTTTTCAA CATTATGTAT TTAAGTGCAT
 8001  GTAGGGAGTT TGATCAATTC GGTTGTAGAA GTGTTGACAT AAGTAATAAA
 8051  ACAATGCATA AAAGGAAAAA ACATAACATA ATAATTTACC TATTAATACC
 8101  GGTGTGACAT GGAAATATGT ATATATGTAC AACTTTATCC ACACAAGATA
 8151  GTAATATATA GTATATAGAG AGCTTTGTAC CATCAATACA TCTTCATATT
 8201  TTATTGTAAT TCTCCTATGA TTGTTAAATT ATTGTGTGGA GCTAGCCACA
 8251  AAGCATAGTT AATATGTGTA TAAAAATATT ATATGCAACC ACTATAGATA
 8301  GGGCTGATAA TGGGCTATAA ATTTTGCACT ATAAGATTGA AGGATCGAAG
 8351  CGGGTTAGGA TCGAGCTCTA TTTCTAGTCA TTTTTGAGCT ATAATTTATT
 8401  TAGGGCCTTG ACATTTTGTG GAGAACTATT TGGATCACAA TCCATTACCA
 8451  CCCCTAACTA TAGATGATAG GAATTCAAAA AGATCTTTGA AAAATCATGC
 8501  TTTACGTTGT AAGATGTAAT TTATAAATTT AGATAATAGA GTAAGCATGC
 8551  ATATTTGTAC AACTATTGTA ATTAATTGTT TAAGGCAATG TTGTGGTTAT
 8601  AGGCCATGTG GTGGCATATA TTATGTCAAC AATGGAGCTT GACCAAACAA
 8651  GGGGAGCAGT CTATATAATT GAGGAGTAAA CACTAAAATA TCACTATTTA
 8701  GACAATGGGG ATCACATTGA GAAAGGGTGG CTATGGGCTA GCTAGTTTGG
 8751  TCTTAACCAA GATCCTCCCC TGTAATTAGG TGTGTGGTGC TGACTAACTG
 8801  ACATCCTAGT GGCACTAATT ATTAGAGTTC TTTGTTTCAA CTTTGTTTTT
 8851  CACATGTTAG AAAATACAAT TATCCAAAAT CTCTTTAACT ATATGTTTTT
 8901  AATGTGGACG ACCCACAACC TTAGAGCGTC ATGCATTAGA ATATACAAAA
 8951  GGAACTCCAA ACTAACTAAT GGATTCTAGT ACACCCGCCA TATTATATTT
 9001  GATATTGCAT GGTCTTCCAC ATCTGAAAGG TGACTTAATT ATTTAAACAC
 9051  GGGACTACAT GGACATAAGC ATAAATGTTG TATTACTATG ATTAGAGTAA
 9101  TAAACAACCA AATAATTTGT TTTATTCTAA GAATCATCTA AAAATATTTA
 9151  TTCTAGTTTC CAACAAAAAC AAATAAATTG GTTTCCAAAG TAAAGTATAT
 9201  TGTTTTGTTT TTGCTAGCAT CCTCAATTCC TCAGGTATGT TTTTGCTACA
 9251  TAAATTATAT TACGATTGAG GTCTCAAGAA TTTTGAGAAG ATACTTTAGC
 9301  ATATCTAAGA CTTTAAAATA GATGGCAGCT ATCGATCTAT ATGACTGTTT
 9351  GAAGAAGAAA AAGTTCATAT AGTTATATTA ACTAATCATG CTATGGATCG
 9401  GGTTACATTA CCTTATTTAC TTCCACTCAA TATTAAATAT ACAATTTGTA
 9451  AACACAAGAC AATCCATCGG TTAGGTGTTT AATTGAAACA CCACAAATCT
 9501  AATGGGTAAA TTAAATGCAT TTTGTCTAAA TATGCATTAT AGATTCATGG
 9551  ATAGATTTAG CAGACATGAA ATCTTGACAT AAATCTCATG GAAGAGTTAT
 9601  TAGTCTCCGG AGTTTCTAGA CCTAGTAGTT TTCTAAAATT ACAATAGAGC
 9651  TCAATAGTAC AGATATTTAC CTTTGGATTC ATTCATGGAT AAAAGGCTGC
 9701  TAACACGTAG TGGAGTTCTC TGGTGAGTTT CAGTCCAATA TATGTAGGTT
 9751  CTTTAGTGGA GATTGTAGTC ATTTTCTTAG ATATTTTTAT GAACTATATA
 9801  CCATTATTCC ATTACCATGG TCTTTCACAA GTAAATTCGT TATAATATTT
 9851  GTGTGGCATA CAATACATCA TTTGTGCTTT TACATTCAGT ATTTAAGTAT
 9901  TTTATGCTAT GATACTAAAA GTTCAATTCA CTGGTAGTCT GGTACTAAAC
 9951  CTTCAATTCA CAATACAACG AAACGTCATT GCCAGTTTGC CACTCATGGC
10001  CTATGGCCCT ATGCTATAAC ATCGGTTGCT GTGCTGCACT TGCAAGTACG
10051  TAGGAGTATA TGAACCCTCA ACTTAGTTCC TATATTTGCG TTTCCACTAC
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10101  CAGTCTACCA TGCAAGCAGC GTTTCCACTA CCATGCAATG ATGCAATGCC
10151  TCTCTCTCTC TCACCCCTTG GTTAATGGTG CTATATCAGC CCTATGACAT
10201  GCTAACATGC ACAAGATCAC ACATTTTCTG TTTTAGTTTC ACACCCAAAT
10251  TTGGACCATG ATGGCCCCCC GGGAGATATA TGGTCTCTCT TTTTTGTCCT
10301  GTAAAGGGGT GCAACCTATT TTTCTATAGC TGTCAATGGC ATAAATAGAT
10351  AAAAGGATTA TCTATGAACA TAGACACATG ATGAGAAGAT TAAAGAGAAG
10401  GGAAAAAAGG TGAATGGACG AAGCCCTTGT AGTTTGTAAA TCAGTCTAAG
10451  TTGCAAACAC TCTCTCTGCA TGCGTTACAT AGTTGCATTT AGCACCCCCG
10501  GCCCAGCATC TCTCACTCTG CTGCAATCTC ACAAGTCACA CCCAAGAGTG
10551  GCACAAAAAG GAACGAGTTT CCAAATATAT AATAACTAAC TATATATTTC
10601  AATTATTGTG CAAGGGTATT TGCAACTTAG ACTCAAACCT AGGATTTATC
10651  ATATATAAGC GTACATTTAC ATTACACACG GGAGGTGTGA ATGGACTGAA
10701  CTATGAAACT ATAAGCCTAT TCCCTATTCT TGTATCGTGT GGTGTCAAAC
10751  ACGCCATGTC TTCTAAAGAG CAAATGCTAC TTACAATTTA CAACATATAG
10801  TACAATATGG CATTATTTCT TAGAAATGGT GCAACAATTA ATCGTTTTAT
10851  GAATGACAGT GGCCGGCCTT GAAATCAAAG AATGTTTGTT TGTGCTACTA
10901  GCTGTCGTTT TTCAAATGCT GTCATTGATC ACTTTTGCCT TGTGCCAACA
10951  GCATGTACAA AACACTCGAG AGATACCGCA GCTCCAACTA CTCACAAGAA
         M  Y  K   T  L  E   R  Y  R  S   S  N  Y   S  Q  E
11001  GTAAAAACTC CACTGGACAC TGAAGTAAGT GTAATGAGTT CTTGACATCC
       V  K  T  P   L  D  T   E
11051  TTTTTCGACG TAACAGTGCA TTTGTAAATT TATTTCTGTG AGCACATAAA
11101  TCACCTGTAA TATCTTATAT AAACAAGAAT ATTGTAGCAA GCTAATAAAA
11151  TACTCCCTCT GTTTCGTTTT AGTTGTCGCT GTATAGTGCA AAATTGAACT
11201  ATTCAGCGAC AACTAAAAAG AAACGAAGGG AATATGTTGC TTGCTTCCAG
11251  AGCCACCAAT CGCTCAAAAA ATGAACAAAC CAGCGATCTG CTTTATCATC
11301  GGTCAAGTGA TTATATTCAT GTATTTTCCA AGCTAACTGA TACCATACCT
11351  TATGTTTAAA TTTCTGTTTT TTGAATTCAG GTAGATTTAT TTTTCGCATC
11401  ATAAACACTC CAACAAGTGT GAAGACATCC TTGCTCAGTA TTGTCTATAC
11451  CAGGGATAGA CGTATAGCCG TATAGGATTG GCCTAGCAGC AGGATCGAAT
11501  ATAGTCATAA AGAACCGTAG GAACATATAT ATAAGGTTAT TCTATAATAA
11551  TCTGAATTAC TATAAAGCAA ATATTTCCTA TAATCTAGCT AATTGGTTTA
11601  GATGGCATTG AAGTATCTTG TCCTATCCGA GTTTATGTTT TTAGATAGAC
11651  TTGTGCGGGT GCTCGTACCT CCCCGTTTGT AGACACAAAT GTATGTGTGT
11701  TTTCGTGCTG GACATGTGCT AGTTTATGGG TGCAAGTCAT TGTCTAAAAA
11751  ATGTTTATGA ATGTTGTATA TGGACACATA TACTCTCTCT GTTTCAAAAT
11801  ATAATTTATT TAGACTAAAC ATATATATTC ATCTATTAAC TTATGAATGT
11851  AGTTTATATG TATTTTTATA TTTATTATCA TTTATTTGAA TGTGGACTGA
11901  AAAAACAGGA CTATAAAGAA CTATATTTTA GAACTGAGAG AGTATATGTT
11951  TGCGATGTTT CTACGTGAAT GCGCAAGTAT GCACAAATTG TGCTATCAGA
12001  AAAGAAACTA TTCTTTTTGT TAACAGACGT AGTTAAAATT AGAGCATAGA
12051  ACCGTGGGCA GCAGCACTCC GGCCAGTGGC ATAGTAAAAT TAGAGAAATT
12101  TGACTCAAAG TAATTAGAAA TGTTCACATT TTGGAAAGGA GGAAGGTCCT
12151  AATTGCACTA TAGCCAATAA TTAACTGCTA AAATAAATTG TAGACATTCA
12201  AACAGCTCAA CCAATAATTC AGTATTAGCT AGCCTTTTAG TAAAGTCGTC
12251  AATAGCTAGC CAACTATTTG TTAACCAGCT AATTCTACTA CTAGTTTTTT
12301  ATTGAGCTAA CTAGCTCTCT AGTGCATTCA AATAGGCCGA AGTATATCTC
12351  CAGCGCTGTA GAGATTCACT TAGTTGGGCT TGGGTTGGTC GTTGGTTTGA
12401  TTGTCATCAA TGAGTAAACG AAGGCGCGAA AGTGAACATT TTTTACATGT
12451  CAGCTTAGAA TTTCTCTCTC TATAGAATAG AATAAACTAG AAGCATAACT
12501  TCTTTGTTGC TGTGGATCTT TTTTTTTCAA AATTAAAACC ATTGCACATG
12551  TTCTTGTAAT TCTCATATAC TCTCTTTGTC CCAAATTAAA ATTCGTTTTT
12601  AAAAACTAAT AGATTCATAC CATACTTGAT TTATATGTTT TATATATGTG
12651  TTTAGATTTA TCATCATCTA TTTGGATATA GACATAAAAA TTGAGAGCTA
12701  AAGCGAATAC TATTTTAGAA TGGGGAGTAT TAAAAATAGT GTTGGACTTA
12751  GTAGAGGTGA TGCAGATGTC TTGCATTCAT AGGTTTAAAG TTACTGGACA
12801  CGTGGTATCC GTGGAAGATG GTATGGATAG TTAGTGAGAA ATGATGTAGT
12851  CATGTAGAGC TTGTATGCCG AGATAGATTT TATAGTAGAG TTTAAAAATC
12901  TAAGATAACA TATATAAATA TATATTGCAA TGAAGATGGG GGCTCAAATT
12951  AGCTACCATT TGTGGCCCTG ATATCAACCT GACAGGCTGA GCCTTTATTT
13001  TGGTTTGCAA GTAAGAGACT GGGCTGGGGG CTGGACAACT GAGCCAGATA
13051  TAAACATACC TATCTTCCTC TGGCGGAGCC CATCATATAC AATTCCGGCC
13101  CATCTCTCTT ATCAAAGTAA TGGATGCGTG CTGCTGATTT TGTGGTAAAA
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13151  TTTTAAGATT TTATTTGTAT CTTTTTTAAC CTATAGCATG CATCGTGAGG
13201  ATCCATGTAC AAGCTTTGTT GAAGGTCTTA ACTTAGTAAT ATTAAGCTCC
13251  ACTCGGTTTC TACTGATTTC TAAAGTAGTT CAGTGGTACT GGATTCTTTT
13301  TTTATATAAA AAAGTAGACG CTATCTTCTA TTTTCTTTAG AACTAGGTTT
13351  GTCGGTTTGT TTGGCAAACA CGATCTATGT ACTGACCTGA ACTTTGCGTA
13401  TTCCCTTTTT CCAAGCAGGA TCTCACGTTG ACTTACTCTG TTTCGTTGCG
13451  CTCTTTCTTT TCAAAACTAG ATTAAATACC AGGATTATCT CAAGTTGAGG
                             I  K  Y  Q    D  Y  L    K  L  R
13501  ACAAGGGTTG AATTCCTCCA AACTACACAA AGGTATTCTT CAAAATGGCA
       T  R  V  E    F  L  Q    T  T  Q    R
13551  CTGAGCTACT GATTGCTTGT CTGAGGTGCA CAACAATTGC TAATGAGCCA
13601  ATGATGTATG ACATCTACCA CCAATTCATT TCTGCAGAAA TATTCTTGGG
                                                N    I  L  G
13651  GAGGATCTGG GTCCACTTAG CATGAAGGAG CTCGAGCAGC TGGAGGACCA
       E  D  L  G    P  L  S    M  K  E    L  E  Q  L    E  D  Q
13701  AATAGAGATA TCTCTGAAGC ATATCAGCTC AAGAAAGGTA GAACACCTGA
        I  E  I    S  L  K  H    I  S  S    R  K
13751  CTGCGTGGCT TACATATTTC TTCAGTGCGC TGTTTTGGCT CGCAATTGAC
13801  AAGGGCTGAA ATGTTTGCAG AATCAAATGC TACTTGATCA GCTCTTTGAC
                             N  Q  M  L    L  D  Q    L  F  D
13851  CTCAAAAGCA AGGTAGCAAT GACGAGGTCC TCTTACCTTT TGCCTAGTTT
       L  K  S  K
13901  TCTTTCTGCT CCATGCATCC GTACCAAAAC CTTGTAGTGC CGAGCTAACG
13951  GTTATGTTTT TGCAGGAACA AGAATTGCTG GATCTCAATA AAGACCTAAG
                        E  Q    E  L  L    D  L  N  K    D  L  R
14001  GAAGCAGGTA ATGCAATGGC CTCCAAACAT CTCTGTGTAT ACATACGTAC
        K  Q
14051  GTACCTTGAT GTTGAGTTGT TTACACGCAT ACGTTTCTTA GTTCTTACTG
14101  TACTCTCCAT GAGAACTTGG AACAGCTTTT AAACCTTTTT TTTATATAAA
14151  AACTTTTAAA CTGATGCTAT ATTTGTCTCT GTTCCGACAT GCCTGCTTGA
14201  CGTAAACTTA CTGGTCCGTT ACTGATTTCT TGCAAGTGGC AGCTGCAAGA
                                                   L  Q  E
14251  AACAAGGCCA GAGAACGCGC TCCGTGTCTC CTGGGAGGAA GGTGGGCACA
        T  R  P    E  N  A  L    R  V  S    W  E  E    G  G  H  S
14301  GTGGCGCAAG TGGGAATGTT CTTGATCCTT ATCAGGGGCT CCTTCAGCAC
         G  A  S    G  N  V    L  D  P  Y    Q  G  L    L  Q  H
14351  CTGGACAACG ATCCTTCCCT GCAGTTCGGG TAAGTTTTTC CAGGTGACAA
       L  D  N  D    P  S  L    Q  F  G
14401  TGACTATGAT AGATAGAACA AAATATGCAT AGATGAAAAC ACACACAACT
14451  ATATATTACC TGAACTTAAG CAATCACAAT CGCATCGGAG CCAGCCTCGC
14501  ATGGACGAGT CCGGAGTCCA TGACACCATC ACAATTTCCT GTTGAGAAAT
14551  AGAATCCACA TTCAGCACTT GAGCTTTAGC ATGCTTTCAT CATATCTTAG
14601  CCGCGTTAAT GAGTGTGTGT GTGTGTTCGT CCTGGGCAAA GGTACCATCA
                                                      Y  H  H
14651  TCAAGCCTAC ATGGATCAGC TGAACAACGA GGACCTGGTG GACCCGAATG
        Q  A  Y    M  D  Q  L    N  N  E    D  L  V    D  P  N  E
14701  AGCATGGTCG ATCTGGATGG ATATGAAATG CTCTGATGAC CCAAAATAAA
         H  G  R    S  G  W    I  *
14751  GTGATGCACA GCAGGGATTC ACCCTGCAGG ACGCGAGACT ATGTATTGAA
14801  GCTAAGACCT CGTGTGTGTT GTGTTGTGTG CGAAGCTTGG AACTTTATAA
14851  ATCGTTGTCA TGCCCATGGC TTTCTATTTC TTTCTCGGAG CTGCGC
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Appendix 7.4. Branching characters in the tassel.

Displayed are the branching characters of the tassels in control and transgenic plants. The analyzed plants are grouped per control group, construct, gene, generation and line, as indicated by the number in columns U1 and U2. The numbers indicate the assignment of plants into this group for statistical analysis by the Mann-Whitney (U)-test.

U1= U-test grouping of control plants only; U2= U-test grouping of transgenic and control plants.
Abbrev.: gr.h.no.= greenhouse number, constr.=construct, Tn= generation, spklts= spikelets, mN= non-basal monopedicellate spikelet pair, mB= basal monopedicellate spikelet pair, tN= non-basal triplet, tB= basal triplet, 3flr= 3-floretted spikelet, 4flr=4-floretted spikelet, sng=single unpaired spikelet, monoNB= phenotypic ratio of mN, monoB= phenotypic ratio of mB, trpltNB= phenotypic ratio of tN, trpltB= phenotypic ratio of tB, 3-floret= phenotypic ratio of 3flr, 4-floret= phenotypic ratio of 4flr, single= phenotypic ratio of sng, branching= sum of the monoNB, monoB, trpltNB, trpltB, 3-floret, 4-floret and single, empty v.=empty vector pK225, GCN5= p35SAcS/GCM5::*GUS*, aZM15= pK225/aZMM15, aZMM6= pK225/aZMM6, ZM6= pK225/ZMM6, aZMM8= pK225/aZMM8, ZMM8= pK225/ZMM8.

U1	U2	gr.h.no.	constr.	Tn	spklts	mN	mB	tN	tB	3flr	4flr	sng	monoNB	monoB	trpltNB	trpltB	3-floret	4-floret	single	branching	line
1	1	4436	empty v.	T0	240	0	0	0	1	0	0	0	0	0	0	0.00417	0	0	0	0.004167	
1	1	4437	empty v.	T0	250	0	0	0	1	0	0	0	0	0	0	0.004	0	0	0	0.004	
1	1	4438	empty v.	T0	316	0	0	0	2	0	0	0	0	0	0	0.00633	0	0	0	0.006329	
1	1	4439	empty v.	T0	172	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1	1	4440	empty v.	T0	328	0	0	0	1	0	0	0	0	0	0	0.00305	0	0	0	0.003049	
1	1	4770	empty v.	T0	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1	1	4771	empty v.	T0	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1	1	4772	empty v.	T0	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	1		GCN5	T0	236	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	1		GCN5	T0	388	0	0	0	0	0	0	2	0	0	0	0	0	0	0.00515	0.005155	
2	1		GCN5	T0	220	0	1	0	1	0	0	0	0	0.00455	0	0.00455	0	0	0	0.009091	
2	1		GCN5	T0	218	0	0	1	1	0	0	1	0	0	0.00459	0.00459	0	0	0.00459	0.013761	
2	1		GCN5	T0	290	0	0	0	0	0	0	1	0	0	0	0	0	0	0.00345	0.003448	
2	1		GCN5	T0	308	0	0	1	0	0	0	2	0	0	0.00325	0	0	0	0.00649	0.00974	
2	1		GCN5	T0	386	0	0	1	1	0	0	0	0	0	0.00259	0.00259	0	0	0	0.005181	
2	1		GCN5	T0	292	0	2	0	0	0	0	2	0	0.0069	0	0	0	0	0.0069	0.013793	
2	1		GCN5	T0	230	0	1	1	2	0	0	3	0	0.00435	0.00435	0.0087	0	0	0.01304	0.030435	
2	1		GCN5	T0	132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	1		GCN5	T0	174	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	1		GCN5	T0	190	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	1		GCN5	T0	108	0	1	0	1	0	0	0	0	0.00926	0	0.00926	0	0	0	0.018519	
2	1		GCN5	T0	234	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	1		GCN5	T0	446	0	1	0	2	0	0	0	0	0.00224	0	0.00448	0	0	0	0.006726	
2	1		GCN5	T0	238	0	0	0	2	0	0	2	0	0	0	0.0084	0	0	0.0084	0.016807	
2	1		GCN5	T0	308	0	0	2	3	0	0	0	0	0	0.00649	0.00974	0	0	0	0.016234	
2	1		GCN5	T0	296	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	1		GCN5	T0	272	0	0	1	0	0	0	0	0	0	0.00368	0	0	0	0	0.003676	
2	1		GCN5	T0	198	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	1	4525	aZM15	T0	406	0	0	1	1	0	0	0	0	0	0.00246	0.00246	0	0	0	0.004926	1
3	1	4526	aZM15	T0	206	0	0	0	2	0	0	0	0	0	0	0.00971	0	0	0	0.009709	1
3	1	4579	aZM15	T0	452	0	1	0	0	0	0	0	0	0.00221	0	0	0	0	0	0.002212	1
3	1	4583	aZM15	T0	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
3	1	4735	aZM15	T0	196	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
3	1	4760	aZM15	T0	360	0	0	2	1	0	0	0	0	0	0.00556	0.00278	0	0	0	0.008333	1
3	1	4548	aZM15	T0	316	0	0	0	2	0	0	0	0	0	0	0.00633	0	0	0	0.006329	2
3	1	4523	aZM15	T0	394	0	0	3	1	0	0	2	0	0	0.00761	0.00254	0	0	0.00508	0.015228	4
4	2	4975	aZM15	T1	436	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
4	2	4984	aZM15	T1	786	0	1	2	4	0	0	1	0	0.00127	0.00254	0.00509	0	0	0.00127	0.010178	2
4	2	4989	aZM15	T1	638	0	0	2	2	0	0	0	0	0	0.00313	0.00313	0	0	0	0.00627	2
4	2	4990	aZM15	T1	760	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
4	2	4992	aZM15	T1	702	0	0	0	0	0	0	2	0	0	0	0	0	0	0.00285	0.002849	2
4	2	4976	aZM15	T1	662	0	0	0	2	0	0	2	0	0	0	0.00302	0	0	0.00302	0.006042	2
4	2	4980	aZM15	T1	520	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
4	2	4983	aZM15	T1	686	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
4	2	4987	aZM15	T1	578	0	0	0	0	0	0	2	0	0	0	0	0	0	0.00346	0.00346	2
4	2	4954	aZM15	T1	712	0	0	1	0	0	0	2	0	0	0.0014	0	0	0	0.00281	0.004213	4
4	2	4956	aZM15	T1	674	0	0	2	3	0	0	0	0	0	0.00297	0.00445	0	0	0	0.007418	4
4	2	4959	aZM15	T1	720	0	0	3	1	0	0	0	0	0	0.00417	0.00139	0	0	0	0.005556	4
4	2	4973	aZM15	T1	580	0	0	2	2	0	0	1	0	0	0.00345	0.00345	0	0	0.00172	0.008621	4
4	2	5011	aZM15	T1	522	0	0	0	0	0	0	1	0	0	0	0	0	0	0.00192	0.001916	4
4	2	5012	aZM15	T1	624	0	0	0	1	0	0	0	0	0	0	0.0016	0	0	0	0.001603	4
4	2	5013	aZM15	T1	684	0	0	1	1	0	0	0	0	0	0.00146	0.00146	0	0	0	0.002924	4
	3	4542	aZMM6	T0	395	12	5	5	0	4	2	0	0.03038	0.01266	0.01266	0	0.01013	0.00506	0	0.070886	1
	3	4506	aZMM6	T0	132	4	2	0	0	0	0	0	0.0303	0.01515	0	0	0	0	0	0.045455	1
	3	4508	aZMM6	T0	106	5	1	2	0	0	0	0	0.04717	0.00943	0.01887	0	0	0	0	0.075472	1
	3	4509	aZMM6	T0	164	2	3	1	0	0	0	1	0.0122	0.01829	0.0061	0	0	0	0.0061	0.042683	1
	3	4510	aZMM6	T0	274	2	3	5	3	0	0	2	0.0073	0.01095	0.01825	0.01095	0	0	0.0073	0.054745	1
	3	4562	aZMM6	T0	340	9	3	5	3	0	1	3	0.02647	0.00882	0.01471	0.00882	0	0.00294	0.00882	0.070588	1
	3	4627	aZMM6	T0	290	4	0	10	0	0	0	0	0.01379	0	0.03448	0	0	0	0	0.048276	1
	3	4706	aZMM6	T0	41	3	2	0	0	0	0	0	0.07317	0.04878	0	0	0	0	0	0.121954	1
	3	4728	aZMM6	T0	108	2	0	3	1	0	1	1	0.01852	0	0.02778	0.00926	0	0.00926	0.00926	0.074074	1
	3	4563	aZMM6	T0	390	2	3	16	6	2	2	0	0.00513	0.00769	0.04103	0.01538	0.00513	0.00513	0	0.079487	2
	3	4565	aZMM6	T0	326	3	2	4	2	1	0	0	0.0092	0.00613	0.01227	0.00613	0.00307	0	0	0.03681	2
	3	4709	aZMM6	T0	106	0	0	1	0	0	0	0	0	0	0.00943	0	0	0	0	0.009434	2
	3	4778	aZMM6	T0	248	0	0	2	1	0	0	0	0	0	0.00806	0.00403	0	0	0	0.012097	2
	3	4511	aZMM6	T0	232	31	0	5	0	0	1	0	0.13362	0	0.02155	0	0	0.00431	0	0.159483	3
	3	4707	aZMM6	T0	84	1	1	1	1	0	0	1	0.0119	0.0119	0.0119	0.0119	0	0	0.0119	0.059524	3
	3	4719	aZMM6	T0	192	10	2	6	2	0	1	2	0.05208	0.01042	0.03125	0.01042	0	0.00521	0.01042	0.119792	3
	3	4758	aZMM6	T0	276	8	2	3	2	3	0	7	0.01449	0.00362	0.01087	0.00362	0.01087	0	0.02536	0.068841	3

U1	U2	gr.h.no.	constr.	Tn	spklts	mN	mB	tN	tB	3flr	4flr	sng	monoNB	monoB	tpltNB	tpltB	3-floret	4-floret	single	branching	line
	4	4418	ZMM6	T0	152	0	0	0	2	0	0	2	0	0	0	0.01316	0	0	0.01316	0.026316	4
	4	4419	ZMM6	T0	110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4420	ZMM6	T0	246	0	3	4	3	0	0	3	0	0.0122	0.01626	0.0122	0	0	0.0122	0.052846	4
	4	4421	ZMM6	T0	156	2	2	2	2	0	1	0	0.01282	0.01282	0.01282	0.01282	0	0.00641	0	0.057692	4
	4	4423	ZMM6	T0	198	1	1	1	3	0	0	0	0.00505	0.00505	0.00505	0.01515	0	0	0	0.030303	4
	4	4424	ZMM6	T0	118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4425	ZMM6	T0	102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4427	ZMM6	T0	88	0	0	1	0	0	0	0	0	0	0.01136	0	0	0	0	0.011364	4
	4	4428	ZMM6	T0	98	2	0	4	1	0	0	0	0.02041	0	0.04082	0.0102	0	0	0	0.071429	4
	4	4429	ZMM6	T0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4431	ZMM6	T0	114	1	1	2	1	0	0	0	0.00877	0.00877	0.01754	0.00877	0	0	0	0.04386	4
	4	4432	ZMM6	T0	62	0	0	3	1	0	0	0	0	0	0.04839	0.01613	0	0	0	0.064516	4
	4	4434	ZMM6	T0	72	0	0	1	0	0	0	0	0	0	0.01389	0	0	0	0	0.013889	4
	4	4435	ZMM6	T0	42	0	2	0	2	0	0	0	0	0.04762	0	0.04762	0	0	0	0.095238	4
	4	4453	ZMM6	T0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4455	ZMM6	T0	40	0	0	0	1	0	0	0	0	0	0	0.025	0	0	0	0.025	4
	4	4456	ZMM6	T0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4457	ZMM6	T0	38	2	1	6	2	0	0	0	0.05263	0.02632	0.15789	0.05263	0	0	0	0.289473	4
	4	4460	ZMM6	T0	148	0	0	1	0	0	0	1	0	0	0.00676	0	0	0	0.00676	0.013514	4
	4	4461	ZMM6	T0	116	0	0	1	0	0	0	3	0	0	0.00862	0	0	0	0.02586	0.034483	4
	4	4462	ZMM6	T0	78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4463	ZMM6	T0	90	0	0	1	0	0	0	0	0	0	0.01111	0	0	0	0	0.011111	4
	4	4464	ZMM6	T0	242	1	3	2	3	1	1	3	0.00413	0.0124	0.00826	0.0124	0.00413	0.00413	0.0124	0.057851	4
	4	4479	ZMM6	T0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4480	ZMM6	T0	126	0	1	2	0	0	0	0	0	0.00794	0.01587	0	0	0	0	0.02381	4
	4	4481	ZMM6	T0	82	0	0	0	1	0	0	0	0	0	0	0.0122	0	0	0	0.012195	4
	4	4482	ZMM6	T0	192	0	0	0	0	0	0	2	0	0	0	0	0	0	0.01042	0.010417	4
	4	4485	ZMM6	T0	120	0	0	0	0	0	0	1	0	0	0	0	0	0	0.00833	0.008333	4
	4	4486	ZMM6	T0	86	0	0	0	0	0	0	1	0	0	0	0	0	0	0.00116	0.001163	4
	4	4532	ZMM6	T0	128	1	0	1	2	0	0	0	0.00781	0	0.00781	0.01563	0	0	0	0.03125	4
	4	4533	ZMM6	T0	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4534	ZMM6	T0	58	0	0	1	0	0	0	1	0	0	0.01724	0	0	0	0.01724	0.034483	4
	4	4535	ZMM6	T0	106	4	2	7	2	1	0	4	0.03774	0.01887	0.06604	0.01887	0.00943	0	0.03774	0.188679	4
	4	4536	ZMM6	T0	80	0	0	0	1	0	0	0	0	0	0	0.0125	0	0	0	0.0125	4
	4	4537	ZMM6	T0	88	0	0	1	2	1	0	2	0	0	0.01136	0.00727	0.01136	0	0.00727	0.037273	4
	4	4538	ZMM6	T0	178	0	0	0	0	0	2	0	0	0	0	0	0	0.01124	0	0.011236	4
	4	4539	ZMM6	T0	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	4	4540	ZMM6	T0	102	3	0	5	2	0	0	2	0	0	0.04902	0.01961	0	0	0.01961	0.088235	4
	4	4593	ZMM6	T0	174	1	0	1	0	0	0	0	0.00575	0	0.00575	0	0	0	0	0.011494	5
	4	4594	ZMM6	T0	224	0	1	0	1	0	0	1	0	0.00446	0	0.00446	0	0	0.00446	0.013393	5
	4	4598	ZMM6	T0	128	0	2	0	3	0	0	2	0	0.01563	0	0.02344	0	0	0.01563	0.054688	5
	4	4599	ZMM6	T0	242	1	1	1	1	0	0	1	0.00413	0.00413	0.00413	0.00413	0	0	0.00413	0.020661	5
	4	4842	ZMM6	T0	116	1	0	2	0	0	0	0	0.00862	0	0.01724	0	0	0	0	0.025862	5
	4	4561	ZMM6	T0	250	0	0	0	0	0	0	2	0	0	0	0	0	0	0.008	0.008	6
	4	4685	ZMM6	T0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
	4	4688	ZMM6	T0	166	0	0	0	0	0	0	2	0	0	0	0	0	0	0.01205	0.012048	6
	4	4724	ZMM6	T0	178	1	0	0	1	0	0	1	0.00562	0	0	0.00562	0	0	0.00562	0.016854	6
	4	4739	ZMM6	T0	166	0	0	1	0	0	1	0	0	0	0.00602	0	0	0.00602	0	0.012048	6
	4	4740	ZMM6	T0	136	0	1	0	0	0	0	4	0	0.00735	0	0	0	0	0.02941	0.036765	6
	4	4762	ZMM6	T0	170	0	0	0	1	0	0	0	0	0	0	0.00588	0	0	0	0.005882	6
	4	4765	ZMM6	T0	192	0	0	7	3	0	0	0	0	0	0.03646	0.01563	0	0	0	0.052083	6
	4	4843	ZMM6	T0	104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
	4	4843b	ZMM6	T0	308	1	1	2	2	2	2	0	0.00325	0.00325	0.00649	0.00649	0.00649	0.00649	0	0.032468	6
	4	4844	ZMM6	T0	152	0	0	1	2	1	0	0	0	0	0.00658	0.01316	0.00658	0	0	0.026316	6
	4	4845	ZMM6	T0	236	1	0	1	2	1	0	0	0.00424	0	0.00424	0.00847	0.00424	0	0	0.021186	6
	4	4853	ZMM6	T0	244	0	1	1	3	0	0	2	0	0.0041	0.0041	0.0123	0	0	0.0082	0.028689	6
	4	4854	ZMM6	T0	210	0	0	0	0	0	0	1	0	0	0	0	0	0	0.00476	0.004762	6
	5	4826	aZMM6	T1	728	0	0	3	0	0	0	0	0	0	0.00412	0	0	0	0	0.004121	1
	5	4830	aZMM6	T1	788	0	1	3	0	0	1	0	0	0.00127	0.00381	0	0	0.00127	0	0.006345	1
	5	4831	aZMM6	T1	794	2	0	8	0	0	1	0	0.00252	0	0.01008	0	0	0.00126	0	0.013854	1
	5	4833	aZMM6	T1	684	0	0	14	0	4	2	0	0	0	0.02047	0	0.00585	0.00292	0	0.02924	1
	5	4834	aZMM6	T1	738	0	0	5	2	1	1	1	0	0	0.00678	0.00271	0.00136	0.00136	0.00136	0.01355	1
	5	4835	aZMM6	T1	764	0	0	4	2	0	0	0	0	0	0.00524	0.00262	0	0	0	0.007853	1
	5	5111	aZMM6	T1	510	0	0	0	3	0	0	2	0	0	0	0.00588	0	0	0.00392	0.009804	1
	5	5115	aZMM6	T1	586	0	0	5	1	1	2	1	0	0	0.00853	0.00171	0.00171	0.00341	0.00171	0.017065	1
	5	5116	aZMM6	T1	736	0	0	9	0	1	3	0	0	0	0.01223	0	0.00136	0.00408	0	0.017663	1
	5	5117	aZMM6	T1	596	0	0	10	1	2	1	0	0	0	0.01678	0.00168	0.00336	0.00168	0	0.02349	1
	5	5118	aZMM6	T1	754	2	0	6	2	0	1	0	0.00265	0	0.00796	0.00265	0	0.00133	0	0.014589	1
	5	5824	aZMM6	T1	186	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	5	5844	aZMM6	T1	188	0	0	0	0	0	0	7	0	0	0	0	0	0	0.03723	0.037234	1
	5	5093	aZMM6	T1	440	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	5	5098	aZMM6	T1	542	0	0	2	0	0	0	0	0	0	0.00369	0	0	0	0	0.00369	2
	5	5099	aZMM6	T1	628	0	0	2	0	0	0	0	0	0	0.00318	0	0	0	0	0.003185	2
	5	5101	aZMM6	T1	526	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

U1	U2	gr.h.no.	constr.	Tn	spklts	mN	mB	tN	tB	3flr	4flr	sng	monoNB	monoB	tpltNB	tpltB	3-floret	4-floret	single	branching	line
5		5102	aZMM6	T1	618	0	0	0	2	0	0	0	0	0	0	0.00324	0	0	0	0.003236	2
5		5121	aZMM6	T1	582	0	0	5	2	0	0	0	0	0	0.00859	0.00344	0	0	0	0.012027	2
5		5122	aZMM6	T1	614	0	0	6	1	2	0	0	0	0	0.00977	0.00163	0.00326	0	0	0.014658	2
5		6033	aZMM6	T1	343	0	0	2	3	0	1	0	0	0	0.00583	0.00875	0	0.00292	0	0.017493	2
6		4745	ZMM6	T1	402	0	0	11	4	0	4	0	0	0	0.02736	0.00995	0	0.00995	0	0.047264	4
6		4746	ZMM6	T1	528	0	0	14	0	2	4	7	0	0	0.02652	0	0.00379	0.00758	0.01326	0.051136	4
6		4747	ZMM6	T1	520	1	0	1	0	0	0	1	0.00192	0	0.00192	0	0	0	0.00192	0.005769	4
6		4799	ZMM6	T1	632	0	0	7	3	0	0	2	0	0	0.01108	0.00475	0	0	0.00316	0.018987	4
6		4887	ZMM6	T1	504	0	0	4	2	0	0	0	0	0	0.00794	0.00397	0	0	0	0.011905	4
6		4890	ZMM6	T1	588	0	0	3	1	0	0	0	0	0	0.0051	0.0017	0	0	0	0.006803	4
6		5682	ZMM6	T1	502	0	0	2	0	1	0	1	0	0	0.00398	0	0.00199	0	0.00199	0.007968	6
7		6350	ZMM6	T1	308	0	0	1	2	0	1	2	0	0	0.00325	0.00649	0	0.00325	0.00649	0.019481	8
7		6399	ZMM6	T1	312	0	0	2	0	2	0	0	0	0	0.00641	0	0.00641	0	0	0.012821	13
7		6402	ZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
7		6403	ZMM6	T1	236	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
7		6432	ZMM6	T1	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17
7		5670	ZMM6	T1	208	0	0	5	0	0	0	0	0	0	0.02404	0	0	0	0	0.024038	24
7		5671	ZMM6	T1	304	0	0	2	0	2	2	1	0	0	0.00658	0	0.00658	0.00658	0.00329	0.023026	24
7		5673	ZMM6	T1	290	0	0	4	3	0	1	1	0	0	0.01379	0.01034	0	0.00345	0.00345	0.031034	24
7		5674	ZMM6	T1	425	0	0	4	0	1	1	0	0	0	0.00941	0	0.00235	0.00235	0	0.014118	24
8		4513	aZMM8	T0	212	1	1	0	2	0	0	0	0.00472	0.00472	0	0.00943	0	0	0	0.018868	1
8		4514	aZMM8	T0	270	1	2	6	1	0	0	3	0.0037	0.00741	0.02222	0.0037	0	0	0.01111	0.048148	1
8		4603	aZMM8	T0	338	2	4	2	2	5	1	2	0.00592	0.11834	0.00592	0.00592	0.01479	0.00296	0.00592	0.159763	1
8		4604	aZMM8	T0	240	0	2	1	3	1	0	1	0	0.00833	0.00417	0.0125	0.00417	0	0.00417	0.033333	1
8		4631	aZMM8	T0	198	0	1	0	2	0	0	0	0	0.00505	0	0.0101	0	0	0	0.015152	1
8		4779	aZMM8	T0	128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
8		4861	aZMM8	T0	208	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
8		4516	aZMM8	T0	196	0	2	0	1	0	0	7	0	0.0102	0	0.0051	0	0	0.03571	0.05102	2
8		4517	aZMM8	T0	267	0	0	0	2	3	3	0	0	0	0	0.00749	0.01124	0.01124	0	0.029963	2
8		4518	aZMM8	T0	246	1	1	2	1	1	0	0	0.00407	0.00407	0.00813	0.00407	0.00407	0	0	0.02439	2
8		4520	aZMM8	T0	164	0	0	1	3	3	0	3	0	0	0.0061	0.01829	0.01829	0	0.01829	0.060976	2
8		4521	aZMM8	T0	282	0	0	3	1	0	0	0	0	0	0.01064	0.00355	0	0	0	0.014185	2
8		4544	aZMM8	T0	284	0	0	5	2	2	4	0	0	0	0.01761	0.00704	0.00704	0.01408	0	0.045775	2
8		4545	aZMM8	T0	250	0	0	1	3	3	1	0	0	0	0.004	0.012	0.012	0.004	0	0.032	2
8		4546	aZMM8	T0	404	0	0	2	3	3	7	0	0	0	0.00495	0.00743	0.00743	0.01733	0	0.037129	2
8		4574	aZMM8	T0	190	2	0	2	0	1	0	1	0.01053	0	0.01053	0	0.00526	0	0.00526	0.031579	2
8		4612	aZMM8	T0	252	0	0	0	2	1	0	0	0	0	0	0.00794	0.00397	0	0	0.011905	2
8		4613	aZMM8	T0	222	2	3	4	3	3	1	0	0.00901	0.01351	0.01802	0.01351	0.01351	0.0045	0	0.072072	2
8		4638	aZMM8	T0	146	0	0	2	0	0	0	0	0	0	0.0137	0	0	0	0	0.013699	2
8		4710	aZMM8	T0	182	0	0	1	0	0	0	0	0	0	0.00549	0	0	0	0	0.005495	2
8		4733	aZMM8	T0	116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
8		4759	aZMM8	T0	250	0	0	3	1	0	0	0	0	0	0.012	0.004	0	0	0	0.016	2
8		4767	aZMM8	T0	138	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
8		4768	aZMM8	T0	204	0	0	3	1	0	0	0	0	0	0.01471	0.0049	0	0	0	0.019608	3
8		4769	aZMM8	T0	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
8		4640	aZMM8	T0	234	1	3	1	4	3	1	0	0.00427	0.01282	0.00427	0.01709	0.01282	0.00427	0	0.055556	3
8		4642	aZMM8	T0	262	0	0	2	3	0	0	0	0	0	0.00763	0.01145	0	0	0	0.019084	3
8		4643	aZMM8	T0	358	1	1	5	2	3	3	1	0.00279	0.00279	0.01397	0.00559	0.00838	0.00838	0.00279	0.044693	3
8		4646	aZMM8	T0	212	0	0	0	0	0	5	0	0	0	0	0	0	0.02358	0	0.023585	3
8		4647	aZMM8	T0	208	2	0	0	0	3	0	4	0.00962	0	0	0	0.01442	0	0.01923	0.043269	3
8		4712	aZMM8	T0	326	0	0	7	0	1	1	0	0	0	0.02147	0	0.00307	0.00307	0	0.027607	3
8		4615	aZMM8	T0	286	1	0	2	2	3	0	1	0.0035	0	0.00699	0.00699	0.01049	0	0.0035	0.031469	4
8		4616	aZMM8	T0	366	0	1	0	2	4	2	2	0	0.00273	0	0.00546	0.01093	0.00546	0.00546	0.030055	4
9		4595	ZMM8	T0	190	0	0	1	0	0	0	2	0	0	0.00526	0	0	0	0.01053	0.015789	5
9		4596	ZMM8	T0	340	0	0	1	2	1	1	0	0	0	0.00294	0.00588	0.00294	0.00294	0	0.014706	5
9		4690	ZMM8	T0	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
9		4693	ZMM8	T0	156	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
9		4695	ZMM8	T0	210	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
9		4766	ZMM8	T0	148	3	0	1	0	1	1	0	0.02027	0	0.00676	0	0.00676	0.00676	0	0.040541	6
9		4847	ZMM8	T0	154	0	0	4	0	1	0	0	0	0	0.02597	0	0.00649	0	0	0.032468	6
9		4856	ZMM8	T0	184	0	0	1	0	0	0	3	0	0	0.00543	0	0	0	0.0163	0.021739	6
9		4858	ZMM8	T0	114	0	0	0	0	0	0	2	0	0	0	0	0	0	0.01754	0.017544	6
9		4859	ZMM8	T0	148	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
9		4725	ZMM8	T0	164	4	3	3	0	0	0	0	0.02439	0.01829	0.01829	0	0	0	0	0.060976	7
10		4801	aZMM8	T1	450	0	0	1	0	0	0	0	0	0	0.00222	0	0	0	0	0.002222	1
10		4802	aZMM8	T1	616	0	0	1	0	0	0	0	0	0	0.00162	0	0	0	0	0.001623	1
10		4804	aZMM8	T1	612	0	0	1	0	0	0	0	0	0	0.00163	0	0	0	0	0.001634	1
10		4806	aZMM8	T1	708	0	0	0	0	0	0	2	0	0	0	0	0	0	0.00282	0.002825	1
10		4817	aZMM8	T1	838	3	0	6	0	3	3	0	0.00358	0	0.00716	0	0.00358	0.00358	0	0.0179	2
10		4822	aZMM8	T1	726	0	0	4	0	0	0	0	0	0	0.00551	0	0	0	0	0.00551	2
10		4823	aZMM8	T1	688	0	0	3	0	2	0	0	0	0	0.00436	0	0.00291	0	0	0.007267	2
10		4824	aZMM8	T1	650	1	0	2	0	2	1	0	0.00154	0	0.00308	0	0.00308	0.00154	0	0.009231	2
10		5042	aZMM8	T1	410	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

U1	U2	gr.h.no.	constr.	Tn	spklts	mN	mB	tN	tB	3flr	4flr	sng	monoNB	monoB	tpltNB	tpltB	3-floret	4-floret	single	branching	line
	10	5051	aZMM8	T1	538	3	1	8	0	1	0	3	0.00558	0.00186	0.01487	0	0.00186	0	0.00558	0.02974	2
	10	5054	aZMM8	T1	354	0	1	1	0	0	0	2	0	0.00282	0.00282	0	0	0	0.0565	0.062147	2
	10	5056	aZMM8	T1	550	0	0	2	0	0	0	0	0	0	0.00364	0	0	0	0	0.003636	2
	10	5062	aZMM8	T1	644	0	0	1	1	0	0	0	0	0	0.00155	0.00155	0	0	0	0.003106	2
	10	5064	aZMM8	T1	530	0	0	1	0	0	0	0	0	0	0.00189	0	0	0	0	0.001887	2
	10	5068	aZMM8	T1	740	0	0	16	2	2	0	2	0	0	0.02162	0.0027	0.0027	0	0.0027	0.02973	3
	10	5070	aZMM8	T1	756	0	1	2	1	0	0	0	0	0.00132	0.00265	0.00132	0	0	0	0.005291	3
	10	5072	aZMM8	T1	704	0	0	13	1	4	0	0	0	0	0.01847	0.00142	0.00568	0	0	0.025568	3
	10	4937	aZMM8	T1	538	0	0	3	1	1	0	0	0	0	0.00558	0.00186	0.00186	0	0	0.009294	4
	10	4938	aZMM8	T1	676	0	1	7	2	2	2	0	0	0.00148	0.01036	0.00296	0.00296	0.00296	0	0.02071	4
	10	4942	aZMM8	T1	724	0	0	4	2	2	1	0	0	0	0.00552	0.00276	0.00276	0.00138	0	0.012431	4
	10	4943	aZMM8	T1	558	0	1	0	2	0	0	0	0	0.00179	0	0.00358	0	0	0	0.005376	4
	10	4996	aZMM8	T1	552	0	0	2	1	2	1	0	0	0	0.00361	0.00181	0.00361	0.00181	0	0.010843	4
	10	6234	aZMM8	T1	224	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	10	6243	aZMM8	T1	542	0	1	8	2	1	3	0	0	0.00185	0.01476	0.00369	0.00185	0.00554	0	0.027675	4
	11	5014	ZMM8	T1	560	0	0	6	0	1	0	0	0	0	0.01071	0	0.00179	0	0	0.0125	5
	11	5015	ZMM8	T1	462	0	0	3	0	0	1	0	0	0	0.00649	0	0	0.00216	0	0.008658	5
	12	6073	ZMM8	T1	390	0	1	0	3	0	1	0	0	0.00256	0	0.00769	0	0.00256	0	0.012821	8
	12	6084	ZMM8	T1	348	0	1	1	7	2	2	4	0	0.00287	0.00287	0.02011	0.00575	0.00575	0.01149	0.048851	8
	12	6066	ZMM8	T1	298	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
	12	6069	ZMM8	T1	376	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
	12	6086	ZMM8	T1	296	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
	12	6088	ZMM8	T1	226	0	0	0	2	0	0	0	0	0	0	0.00885	0	0	0	0.00885	12
	12	6091	ZMM8	T1	194	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
	12	6093	ZMM8	T1	292	0	0	1	8	1	2	0	0	0	0.00342	0.27397	0.00342	0.00685	0	0.287671	13
	12	6096	ZMM8	T1	306	0	2	0	3	0	0	0	0	0.00654	0	0.0098	0	0	0	0.01634	14
	12	6098	ZMM8	T1	272	0	0	1	0	0	0	0	0	0	0.00368	0	0	0	0	0.003676	14
	12	6099	ZMM8	T1	442	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
	12	6116	ZMM8	T1	354	1	0	4	2	0	3	0	0.00282	0	0.0113	0.00565	0	0.00847	0	0.028249	16
	12	6117	ZMM8	T1	334	0	0	4	4	0	1	3	0	0	0.01198	0.01198	0	0.00299	0.00898	0.035928	16
	12	6120	ZMM8	T1	308	0	0	1	1	0	0	3	0	0	0.00325	0.00325	0	0	0.00974	0.016234	16
	12	6121	ZMM8	T1	244	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17
	12	6142	ZMM8	T1	414	0	0	18	2	0	4	0	0	0	0.04348	0.00483	0	0.00966	0	0.057971	20
	12	6144	ZMM8	T1	470	0	0	12	3	3	4	1	0	0	0.02553	0.00638	0.00638	0.00851	0.00213	0.048936	20
	12	6200	ZMM8	T1	420	0	1	2	2	0	0	3	0	0.00238	0.00476	0.00476	0	0	0.00714	0.019048	25
	12	6204	ZMM8	T1	488	2	2	11	3	0	3	0	0.0041	0.0041	0.02254	0.00615	0	0.00615	0	0.043033	26
	12	6221	ZMM8	T1	378	0	0	5	1	3	1	0	0	0	0.01323	0.00265	0.00794	0.00265	0	0.026455	28

Appendix 7.5. Branching characters in the ear.

Displayed are the branching characters of the ears in control and transgenic plants. The analyzed plants are grouped per control group, construct, gene, generation and line, as indicated by the number in column U1. The numbers indicate the assignment of plants into a group used for statistical analysis by the Mann-Whitney (U)-test.

Abbrev.: trplt= triplet, pipi= pistillate spikelet, quad= quadruplet, triplet= phenotypic ratio of trpt, pistil= phenotypic ratio of pipi, quadr= phenotypic ratio of quad, singl= phenotypic ratio of sng, branch= sum of tripet, pistil, quadr and singl, pitriquad= sum of triplet, pistil and quadr. Remaining abbreviations as in App.7.4.

U1	gr.h.no.	constr.	Tn	spklt	trplt	pipi	quad	sng	triplet	pistil	quadr	singl	branch	pitriquad	line
1	4436a	empty v.	T0	90	0	0	0	0	0	0	0	0	0	0	
1	4436b	empty v.	T0	200	0	0	0	0	0	0	0	0	0	0	
1	4436c	empty v.	T0	150	0	0	0	0	0	0	0	0	0	0	
1	4437a	empty v.	T0	180	0	0	0	0	0	0	0	0	0	0	
1	4437b	empty v.	T0	240	0	0	0	0	0	0	0	0	0	0	
1	4438a	empty v.	T0	210	0	0	0	0	0	0	0	0	0	0	
1	4438b	empty v.	T0	150	0	0	0	0	0	0	0	0	0	0	
1	4439a	empty v.	T0	150	0	0	0	0	0	0	0	0	0	0	
1	4439b	empty v.	T0	112	0	0	0	0	0	0	0	0	0	0	
1	4440a	empty v.	T0	80	0	0	0	0	0	0	0	0	0	0	
1	4440b	empty v.	T0	96	0	0	0	0	0	0	0	0	0	0	

U1	gr.h.no.	constr.	Tn	spklt	trplt	pipi	quad	sng	triplet	pistil	quadr	singl	branch	pitriquad	line
2	6041	aZM15	T1	330	0	0	0	0	0	0	0	0	0	0	
2	6042a	aZM15	T1	180	0	0	0	0	0	0	0	0	0	0	
2	6042b	aZM15	T1	330	0	0	0	0	0	0	0	0	0	0	
2	6043	aZM15	T1	400	0	0	0	0	0	0	0	0	0	0	
2	6044	aZM15	T1	250	0	0	0	0	0	0	0	0	0	0	
2	6045	aZM15	T1	120	0	0	0	0	0	0	0	0	0	0	
2	6047	aZM15	T1	290	0	0	0	0	0	0	0	0	0	0	
2	6048	aZM15	T1	380	0	0	0	0	0	0	0	0	0	0	
2	6049	aZM15	T1	240	0	0	0	0	0	0	0	0	0	0	
2	6050	aZM15	T1	480	0	0	0	0	0	0	0	0	0	0	
3	5692	aZMM6	T1	96	0	0	0	0	0	0	0	0	0	0	1
3	5695	aZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	1
3	5698	aZMM6	T1	308	0	0	0	0	0	0	0	0	0	0	1
3	5699	aZMM6	T1	180	0	0	0	0	0	0	0	0	0	0	1
3	5704	aZMM6	T1	110	0	0	0	0	0	0	0	0	0	0	1
3	5708	aZMM6	T1	180	12	0	0	0	0.066667	0	0	0	0.0666667	0.0666667	1
3	5714	aZMM6	T1	180	0	0	0	0	0	0	0	0	0	0	1
3	5716	aZMM6	T1	156	2	0	0	0	0.012821	0	0	0	0.0128205	0.0128205	1
3	5719	aZMM6	T1	130	0	0	0	0	0	0	0	0	0	0	1
3	5721	aZMM6	T1	200	0	3	0	0	0	0.015	0	0	0.015	0.015	1
3	5723	aZMM6	T1	190	0	0	0	0	0	0	0	0	0	0	1
3	5726	aZMM6	T1	120	0	0	0	0	0	0	0	0	0	0	1
3	5729	aZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	1
3	5733	aZMM6	T1	140	5	6	0	0	0.035714	0.0428571	0	0	0.0785714	0.0785714	1
3	5734	aZMM6	T1	340	0	0	0	0	0	0	0	0	0	0	1
3	5735	aZMM6	T1	140	1	0	0	0	0.007143	0	0	0	0.0071429	0.0071429	1
3	5741	aZMM6	T1	190	0	0	0	0	0	0	0	0	0	0	1
3	5742	aZMM6	T1	280	0	0	0	0	0	0	0	0	0	0	1
3	5746	aZMM6	T1	110	0	0	0	0	0	0	0	0	0	0	1
3	5750	aZMM6	T1	55	0	0	0	0	0	0	0	0	0	0	1
3	5751	aZMM6	T1	240	0	0	0	0	0	0	0	0	0	0	1
3	5755	aZMM6	T1	190	0	0	0	0	0	0	0	0	0	0	1
3	5756	aZMM6	T1	300	0	0	0	0	0	0	0	0	0	0	1
3	5759	aZMM6	T1	72	0	0	0	0	0	0	0	0	0	0	1
3	5770	aZMM6	T1	150	0	0	0	0	0	0	0	0	0	0	1
3	5771	aZMM6	T1	100	0	0	0	0	0	0	0	0	0	0	1
3	5774	aZMM6	T1	130	3	0	0	0	0.023077	0	0	0	0.0230769	0.0230769	1
3	5780	aZMM6	T1	210	0	0	0	0	0	0	0	0	0	0	1
3	5784	aZMM6	T1	140	0	0	0	0	0	0	0	0	0	0	1
3	5786	aZMM6	T1	330	0	0	0	0	0	0	0	0	0	0	1
3	5787	aZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	1
3	5790	aZMM6	T1	220	0	0	0	0	0	0	0	0	0	0	1
3	5796	aZMM6	T1	260	0	0	0	0	0	0	0	0	0	0	1
3	5799	aZMM6	T1	240	0	3	0	0	0	0.0125	0	0	0.0125	0.0125	1
3	5800	aZMM6	T1	98	0	0	0	0	0	0	0	0	0	0	1
3	5803	aZMM6	T1	180	0	0	0	0	0	0	0	0	0	0	1
3	5806	aZMM6	T1	182	0	0	0	0	0	0	0	0	0	0	1
3	5808	aZMM6	T1	200	0	1	0	0	0	0.005	0	0	0.005	0.005	1
3	5821	aZMM6	T1	220	0	0	0	0	0	0	0	0	0	0	1
3	5828	aZMM6	T1	180	0	0	0	0	0	0	0	0	0	0	1
3	5831	aZMM6	T1	200	0	1	0	0	0	0.005	0	0	0.005	0.005	1
3	5833	aZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	1
3	5835	aZMM6	T1	310	0	0	0	0	0	0	0	0	0	0	1
3	5836	aZMM6	T1	350	0	1	0	0	0	0.0028571	0	0	0.0028571	0.0028571	1
3	5837	aZMM6	T1	90	0	0	0	0	0	0	0	0	0	0	1
3	5838	aZMM6	T1	290	0	0	0	0	0	0	0	0	0	0	1
3	5842	aZMM6	T1	300	0	0	0	0	0	0	0	0	0	0	1
3	5846	aZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	1
3	5847	aZMM6	T1	250	0	0	0	0	0	0	0	0	0	0	1
3	6025	aZMM6	T1	150	0	0	0	0	0	0	0	0	0	0	2
3	6026	aZMM6	T1	300	0	0	0	0	0	0	0	0	0	0	2
3	6029	aZMM6	T1	280	0	0	0	0	0	0	0	0	0	0	2
3	6030	aZMM6	T1	160	0	0	1	0	0	0	0.00625	0	0.00625	0.00625	2
3	6033	aZMM6	T1	400	0	0	0	0	0	0	0	0	0	0	2
3	6034	aZMM6	T1	320	0	0	0	0	0	0	0	0	0	0	2
3	6035	aZMM6	T1	440	0	0	0	0	0	0	0	0	0	0	2
4	5856	aZMM6	T2	220	0	0	0	0	0	0	0	0	0	0	1
4	5861	aZMM6	T2	380	0	0	0	0	0	0	0	0	0	0	1
4	5862	aZMM6	T2	280	0	0	0	0	0	0	0	0	0	0	1
4	5864	aZMM6	T2	370	0	0	0	0	0	0	0	0	0	0	1
4	5865	aZMM6	T2	170	0	0	0	0	0	0	0	0	0	0	1
4	6003	aZMM6	T2	260	0	0	0	0	0	0	0	0	0	0	1
4	6006	aZMM6	T2	350	0	0	0	0	0	0	0	0	0	0	1

U1	gr.h.no.	constr.	Tn	spklt	trplt	pipi	quad	sng	triplet	pistil	quadr	singl	branch	pitriquad	line
4	6008	aZMM6	T2	220	0	0	0	0	0	0	0	0	0	0	1
4	6010	aZMM6	T2	390	0	0	0	0	0	0	0	0	0	0	1
4	6011	aZMM6	T2	340	3	0	0	0	0.008824	0	0	0	0.0088235	0.0088235	1
4	6013	aZMM6	T2	320	0	0	0	0	0	0	0	0	0	0	1
4	6014	aZMM6	T2	280	0	0	0	0	0	0	0	0	0	0	1
4	6019	aZMM6	T2	250	1	0	0	0	0.004	0	0	0	0.004	0.004	1
4	6020	aZMM6	T2	260	6	0	1	0	0.023077	0	0.003846	0	0.0269231	0.0269231	1
5	4423	ZMM6	T0	120	0	0	0	0	0	0	0	0	0	0	4
5	4419	ZMM6	T0	80	0	0	0	0	0	0	0	0	0	0	4
5	4424	ZMM6	T0	128	0	0	0	0	0	0	0	0	0	0	4
5	4421	ZMM6	T0	128	0	1	0	0	0	0.0078125	0	0	0.0078125	0.0078125	4
5	4418	ZMM6	T0	120	0	0	0	0	0	0	0	0	0	0	4
5	4420	ZMM6	T0	112	0	0	0	0	0	0	0	0	0	0	4
5	4461	ZMM6	T0	112	0	0	0	0	0	0	0	0	0	0	4
5	4480	ZMM6	T0	112	0	0	0	0	0	0	0	0	0	0	4
5	4482	ZMM6	T0	140	0	0	0	0	0	0	0	0	0	0	4
5	4487	ZMM6	T0	160	0	0	0	1	0	0	0	0.00625	0.00625	0	4
5	4486	ZMM6	T0	128	0	0	0	0	0	0	0	0	0	0	4
6	5446	ZMM6	T1	180	0	0	0	0	0	0	0	0	0	0	6
6	5447	ZMM6	T1	240	0	0	0	0	0	0	0	0	0	0	6
6	5448	ZMM6	T1	150	0	0	0	0	0	0	0	0	0	0	6
6	5449	ZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	6
6	5450	ZMM6	T1	182	0	0	0	0	0	0	0	0	0	0	6
6	5463	ZMM6	T1	140	0	0	0	0	0	0	0	0	0	0	6
6	5468	ZMM6	T1	110	0	0	0	0	0	0	0	0	0	0	6
7	5477	ZMM6	T1	320	12	0	0	0	0.0375	0	0	0	0.0375	0.0375	7
7	5480	ZMM6	T1	410	0	0	0	0	0	0	0	0	0	0	7
7	5481	ZMM6	T1	220	0	0	0	0	0	0	0	0	0	0	7
7	5484	ZMM6	T1	260	0	0	0	0	0	0	0	0	0	0	7
7	5486	ZMM6	T1	240	0	0	0	0	0	0	0	0	0	0	7
7	6332	ZMM6	T1	640	0	0	0	0	0	0	0	0	0	0	7
7	6333	ZMM6	T1	640	0	0	0	0	0	0	0	0	0	0	7
7	6334	ZMM6	T1	560	2	0	0	0	0.003571	0	0	0	0.0035714	0.0035714	7
7	6335	ZMM6	T1	500	1	0	0	0	0.002	0	0	0	0.002	0.002	7
7	6336	ZMM6	T1	620	0	0	0	0	0	0	0	0	0	0	7
7	6337	ZMM6	T1	650	0	0	0	0	0	0	0	0	0	0	7
7	6338	ZMM6	T1	560	1	0	2	0	0.001786	0	0.003571	0	0.0053571	0.0053571	7
7	6339	ZMM6	T1	640	0	0	0	0	0	0	0	0	0	0	7
7	6340	ZMM6	T1	610	0	0	0	0	0	0	0	0	0	0	7
7	5490	ZMM6	T1	300	0	0	0	0	0	0	0	0	0	0	8
7	6342	ZMM6	T1	630	0	0	0	0	0	0	0	0	0	0	8
7	6344	ZMM6	T1	340	0	0	0	0	0	0	0	0	0	0	8
7	6348	ZMM6	T1	500	0	0	0	0	0	0	0	0	0	0	8
7	6349	ZMM6	T1	520	0	0	0	0	0	0	0	0	0	0	8
7	6350	ZMM6	T1	650	0	0	0	0	0	0	0	0	0	0	8
7	5508	ZMM6	T1	260	0	0	0	0	0	0	0	0	0	0	9
7	5543	ZMM6	T1	180	0	0	0	0	0	0	0	0	0	0	12
7	6382	ZMM6	T1	400	0	0	0	0	0	0	0	0	0	0	12
7	6383	ZMM6	T1	400	0	0	1	0	0	0	0.0025	0	0.0025	0.0025	12
7	6388	ZMM6	T1	480	3	0	2	0	0.00625	0	0.004167	0	0.0104167	0.0104167	12
7	5544	ZMM6	T1	150	0	0	0	0	0	0	0	0	0	0	13
7	5545	ZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	13
7	5546	ZMM6	T1	220	0	0	0	0	0	0	0	0	0	0	13
7	6391	ZMM6	T1	560	0	0	0	0	0	0	0	0	0	0	13
7	6396	ZMM6	T1	600	0	0	3	0	0	0	0.005	0	0.005	0.005	13
7	6398	ZMM6	T1	540	0	0	0	0	0	0	0	0	0	0	13
7	6399	ZMM6	T1	470	0	0	0	0	0	0	0	0	0	0	13
7	5554	ZMM6	T1	250	0	0	0	0	0	0	0	0	0	0	14
7	5556	ZMM6	T1	155	1	0	0	0	0.006452	0	0	0	0.0064516	0.0064516	14
7	6402	ZMM6	T1	340	0	11	0	0	0	0.0323529	0	0	0.0323529	0.0323529	14
7	6403	ZMM6	T1	360	0	0	0	0	0	0	0	0	0	0	14
7	6405	ZMM6	T1	430	0	0	5	0	0	0	0.011628	0	0.0116279	0.0116279	14
7	6409	ZMM6	T1	340	0	0	0	0	0	0	0	0	0	0	14
7	6410	ZMM6	T1	560	0	0	0	0	0	0	0	0	0	0	14
7	5562	ZMM6	T1	270	0	0	0	0	0	0	0	0	0	0	15
7	6412	ZMM6	T1	600	0	0	0	0	0	0	0	0	0	0	15
7	6413	ZMM6	T1	540	0	0	0	0	0	0	0	0	0	0	15
7	6418	ZMM6	T1	490	0	0	5	0	0	0	0.010204	0	0.0102041	0.0102041	15
7	5580	ZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	16
7	5582	ZMM6	T1	180	0	0	0	0	0	0	0	0	0	0	16
7	6428	ZMM6	T1	350	0	0	0	0	0	0	0	0	0	0	16
7	5588	ZMM6	T1	120	0	0	0	0	0	0	0	0	0	0	17
7	6431	ZMM6	T1	570	0	0	0	0	0	0	0	0	0	0	17
7	6432	ZMM6	T1	560	0	0	0	0	0	0	0	0	0	0	17

U1	gr.h.no.	constr.	Tn	spklt	trplt	pipi	quad	sng	triplet	pistil	quadr	singl	branch	pitriquad	line
7	6434	ZMM6	T1	460	0	0	0	0	0	0	0	0	0	0	17
7	6437	ZMM6	T1	470	0	0	0	0	0	0	0	0	0	0	17
7	6438	ZMM6	T1	400	0	0	0	0	0	0	0	0	0	0	17
7	6440	ZMM6	T1	400	0	0	0	0	0	0	0	0	0	0	17
7	5592	ZMM6	T1	100	0	0	0	0	0	0	0	0	0	0	18
7	5598	ZMM6	T1	270	0	0	0	0	0	0	0	0	0	0	18
7	6441	ZMM6	T1	460	0	0	0	0	0	0	0	0	0	0	18
7	6442	ZMM6	T1	540	0	0	0	0	0	0	0	0	0	0	18
7	6443	ZMM6	T1	400	0	0	0	0	0	0	0	0	0	0	18
7	6446	ZMM6	T1	530	0	1	0	0	0	0.0018868	0	0	0.0018868	0.0018868	18
7	5609	ZMM6	T1	155	0	0	0	0	0	0	0	0	0	0	19
7	5613	ZMM6	T1	280	0	0	0	0	0	0	0	0	0	0	19
7	5614	ZMM6	T1	170	0	0	0	0	0	0	0	0	0	0	19
7	5616	ZMM6	T1	130	0	0	0	0	0	0	0	0	0	0	19
7	5619	ZMM6	T1	160	0	0	0	0	0	0	0	0	0	0	19
7	5620	ZMM6	T1	180	0	0	0	0	0	0	0	0	0	0	19
7	5625	ZMM6	T1	220	0	0	0	0	0	0	0	0	0	0	19
7	5627	ZMM6	T1	280	0	0	0	0	0	0	0	0	0	0	19
7	5628	ZMM6	T1	250	0	0	0	0	0	0	0	0	0	0	19
7	6452	ZMM6	T1	450	0	0	1	0	0	0	0.002222	0	0.0022222	0.0022222	19
7	6453	ZMM6	T1	470	0	0	0	0	0	0	0	0	0	0	19
7	6454	ZMM6	T1	460	0	0	0	0	0	0	0	0	0	0	19
7	5640	ZMM6	T1	88	0	0	0	0	0	0	0	0	0	0	20
7	5641	ZMM6	T1	180	0	0	0	0	0	0	0	0	0	0	20
7	5643	ZMM6	T1	170	0	0	0	0	0	0	0	0	0	0	20
7	6461	ZMM6	T1	330	0	0	0	0	0	0	0	0	0	0	20
7	6464	ZMM6	T1	370	0	0	0	0	0	0	0	0	0	0	20
7	6465	ZMM6	T1	530	0	0	0	0	0	0	0	0	0	0	20
7	6466	ZMM6	T1	380	0	0	0	0	0	0	0	0	0	0	20
7	6467	ZMM6	T1	360	0	0	0	0	0	0	0	0	0	0	20
7	6468	ZMM6	T1	380	0	0	0	0	0	0	0	0	0	0	20
7	5649	ZMM6	T1	170	0	0	0	0	0	0	0	0	0	0	21
7	6471	ZMM6	T1	480	0	0	0	0	0	0	0	0	0	0	21
7	6472	ZMM6	T1	380	0	0	0	0	0	0	0	0	0	0	21
7	6475	ZMM6	T1	510	1	0	0	0	0.001961	0	0	0	0.0019608	0.0019608	21
7	6476	ZMM6	T1	570	0	0	0	0	0	0	0	0	0	0	21
7	6478	ZMM6	T1	520	0	0	0	0	0	0	0	0	0	0	21
7	6479	ZMM6	T1	510	0	0	0	0	0	0	0	0	0	0	21
7	6480	ZMM6	T1	290	0	0	0	0	0	0	0	0	0	0	21
7	5651	ZMM6	T1	200	0	0	0	0	0	0	0	0	0	0	22
7	6482	ZMM6	T1	460	0	0	0	0	0	0	0	0	0	0	22
7	6489	ZMM6	T1	420	1	0	0	0	0.002381	0	0	0	0.002381	0.002381	22
7	5659	ZMM6	T1	96	2	0	0	0	0.020833	0	0	0	0.0208333	0.0208333	23
7	6493	ZMM6	T1	470	3	0	0	0	0.006383	0	0	0	0.006383	0.006383	23
7	6494	ZMM6	T1	530	0	0	0	0	0	0	0	0	0	0	23
7	6496	ZMM6	T1	700	0	0	0	0	0	0	0	0	0	0	23
7	5673	ZMM6	T1	170	1	0	0	0	0.005882	0	0	0	0.0058824	0.0058824	24
7	6507	ZMM6	T1	640	0	0	0	0	0	0	0	0	0	0	24
7	6508	ZMM6	T1	670	1	0	0	0	0.001493	0	0	0	0.0014925	0.0014925	24
7	6510	ZMM6	T1	650	0	0	0	0	0	0	0	0	0	0	24
8	6248	aZMM8	T2	450	0	0	0	0	0	0	0	0	0	0	1
8	6236	aZMM8	T2	420	0	0	0	0	0	0	0	0	0	0	2
8	6239	aZMM8	T2	340	0	0	0	0	0	0	0	0	0	0	2
8	6232	aZMM8	T2	330	0	0	0	0	0	0	0	0	0	0	3
8	6241	aZMM8	T2	420	0	0	0	0	0	0	0	0	0	0	3
8	6243	aZMM8	T2	450	0	0	0	0	0	0	0	0	0	0	3
8	6245	aZMM8	T2	290	0	0	0	0	0	0	0	0	0	0	3
9	6059	ZMM8	T1	480	0	0	0	0	0	0	0	0	0	0	8
9	6060	ZMM8	T1	490	1	0	0	0	0.002041	0	0	0	0.0020408	0.0020408	8
9	6066	ZMM8	T1	330	0	0	0	0	0	0	0	0	0	0	9
9	6068	ZMM8	T1	490	0	0	0	0	0	0	0	0	0	0	9
9	6069	ZMM8	T1	440	0	0	0	0	0	0	0	0	0	0	9
9	6073	ZMM8	T1	490	0	0	0	0	0	0	0	0	0	0	10
9	6074	ZMM8	T1	450	0	0	0	0	0	0	0	0	0	0	10
9	6082	ZMM8	T1	400	0	0	0	0	0	0	0	0	0	0	11
9	6083	ZMM8	T1	550	0	0	0	0	0	0	0	0	0	0	11
9	6084	ZMM8	T1	410	0	0	0	0	0	0	0	0	0	0	11
9	6086	ZMM8	T1	480	0	7	0	0	0	0.0145833	0	0	0.0145833	0.0145833	12
9	6087	ZMM8	T1	480	0	0	0	0	0	0	0	0	0	0	12
9	6088	ZMM8	T1	480	3	0	0	0	0.00625	0	0	0	0.00625	0.00625	12
9	6582	ZMM8	T1	430	0	0	0	0	0	0	0	0	0	0	12
9	6584	ZMM8	T1	500	0	0	0	0	0	0	0	0	0	0	12

U1	gr.h.no.	constr.	Tn	spklt	trplt	pipi	quad	sng	triplet	pistil	quadr	singl	branch	pitriquad	line
9	6585	ZMM8	T1	540	0	0	0	0	0	0	0	0	0	0	12
9	6586	ZMM8	T1	600	0	0	0	0	0	0	0	0	0	0	12
9	6588	ZMM8	T1	620	0	0	0	0	0	0	0	0	0	0	12
9	6589	ZMM8	T1	570	0	0	0	0	0	0	0	0	0	0	12
9	6091	ZMM8	T1	460	0	0	0	0	0	0	0	0	0	0	13
9	6093	ZMM8	T1	440	0	5	0	0	0	0.0113636	0	0	0.0113636	0.0113636	13
9	6096	ZMM8	T1	430	0	0	0	0	0	0	0	0	0	0	14
9	6098	ZMM8	T1	420	1	0	0	0	0.002381	0	0	0	0.002381	0.002381	14
9	6099	ZMM8	T1	400	2	0	0	0	0.005	0	0	0	0.005	0.005	14
9	6106	ZMM8	T1	410	0	0	0	0	0	0	0	0	0	0	15
9	6116	ZMM8	T1	460	0	2	0	1	0	0.0043478	0	0.00217391	0.0065217	0.0043478	16
9	6117	ZMM8	T1	420	0	0	0	0	0	0	0	0	0	0	16
9	6120	ZMM8	T1	540	1	0	0	0	0.001852	0	0	0	0.0018519	0.0018519	16
9	6121	ZMM8	T1	330	1	0	0	0	0.00303	0	0	0	0.0030303	0.0030303	17
9	6122	ZMM8	T1	460	0	0	0	0	0	0	0	0	0	0	17
9	6124	ZMM8	T1	310	0	0	0	0	0	0	0	0	0	0	17
9	6125	ZMM8	T1	370	0	0	0	0	0	0	0	0	0	0	17
9	6127	ZMM8	T1	420	0	0	0	0	0	0	0	0	0	0	18
9	6129	ZMM8	T1	350	2	4	0	1	0.005714	0.0114286	0	0.00285714	0.02	0.0171429	18
9	6132	ZMM8	T1	400	0	0	0	0	0	0	0	0	0	0	19
9	6141	ZMM8	T1	380	1	0	0	0	0.026316	0	0	0	0.0263158	0.0263158	20
9	6142	ZMM8	T1	230	0	0	1	0	0	0	0.004348	0	0.0043478	0.0043478	20
9	6143	ZMM8	T1	480	0	6	0	0	0	0.0125	0	0	0.0125	0.0125	20
9	6144	ZMM8	T1	410	0	0	0	0	0	0	0	0	0	0	20
9	6145	ZMM8	T1	420	0	0	0	0	0	0	0	0	0	0	20
9	6156	ZMM8	T1	490	2	3	2	0	0.004082	0.0061224	0.004082	0	0.0142857	0.0142857	21
9	6157	ZMM8	T1	360	0	0	0	0	0	0	0	0	0	0	21
9	6163	ZMM8	T1	420	0	5	0	0	0	0.0119048	0	0	0.0119048	0.0119048	22
9	6164	ZMM8	T1	300	0	0	0	0	0	0	0	0	0	0	22
9	6172	ZMM8	T1	420	0	0	0	0	0	0	0	0	0	0	23
9	6173	ZMM8	T1	490	0	0	0	0	0	0	0	0	0	0	23
9	6174	ZMM8	T1	350	0	0	0	0	0	0	0	0	0	0	23
9	6185	ZMM8	T1	400	2	0	0	0	0.005	0	0	0	0.005	0.005	24
9	6196	ZMM8	T1	390	0	0	0	0	0	0	0	0	0	0	25
9	6198	ZMM8	T1	500	0	0	0	0	0	0	0	0	0	0	25
9	6199	ZMM8	T1	540	0	0	0	0	0	0	0	0	0	0	25
9	6200	ZMM8	T1	420	1	2	0	0	0.002381	0.0047619	0	0	0.0071429	0.0071429	25
9	6202	ZMM8	T1	390	0	0	0	0	0	0	0	0	0	0	26
9	6203	ZMM8	T1	500	0	0	0	0	0	0	0	0	0	0	26
9	6204	ZMM8	T1	520	1	0	0	0	0.001923	0	0	0	0.0019231	0.0019231	26
9	6216	ZMM8	T1	156	1	0	0	0	0.00641	0	0	0	0.0064103	0.0064103	27
9	6219	ZMM8	T1	480	3	0	0	0	0.006522	0	0	0	0.0065217	0.0065217	27
9	6220	ZMM8	T1	530	3	0	0	0	0.00566	0	0	0	0.0056604	0.0056604	27
9	6221	ZMM8	T1	480	0	0	0	0	0	0	0	0	0	0	28
9	6225	ZMM8	T1	490	0	0	0	0	0	0	0	0	0	0	28
9	6230	ZMM8	T1	570	0	0	4	0	0	0	0.007018	0	0.0070175	0.0070175	29

Appendix 7.6. Mean values and standard deviation of branching.

Displayed are the mean values and standard deviation of the branching phenotypes of *ZMM6* and *ZMM8* transgenic T_0-plants. The original data for the calculation of the mean and the standard deviation is shown in column `branching´ in App.7.4 and App.7.5. Group (Ux) indicates the number of the group in these appendices, from which data the mean and standard deviation is calculated. (n) is the number of plants in that group. Abbrev.: Stdev= standard deviation, CNTRL= control plants, hoechst= plant obtained from Hoechst (= var. HE89xA73+)(see §2.2.15).

A.		CNTRL	aZMM6	ZMM6	aZMM8	ZMM8
	Mean	0.0060236	0.0676234	0.0305554	0.0307993	0.0185238
tassel	Stdev	0.0070463	0.0383359	0.0471796	0.0298289	0.019716
branching	(n)	36	17	57	33	11
	group (U2)	1	3	4	8	9

B.		CNTRL	aZMM6	ZM6hoechst	aZMM8(T2)	ZM8hoechst
ear	Mean	0	0.0041944	0.0017174	0	0.0029845
branching	Stdev	0	0.0140513	0.0056755	0	0.0054553
	(n)	10	56	99	7	61
	group (U1)	2	3	7	8	9

Appendix 7.7. Statistical analysis of branching.

Mann-Whitney (U)-test results of comparison of the phenotypic ratios of traits in tassel (**A.**) and ear (**B.**) of transgenic plants versus control plants, and among groups of control plants. Data is obtained from App.7.4 for the tassel and from App.7.5 for the ear. Groups are classified as listed under column U1 and U2 (App.7.4) and under U1 (App.7.5). The indicated outcome of the test is the asymptotic significance (§2.2.20). Significant differences are highlighted in yellow ($0 \geq p \geq 0.05$). Comparison of group x versus group y is indicated as Uz(x,y), with z specifying the column in App.7.4 or App.7.5 that assigns the group number. N(x,y) indicates the number of plants in the tested group x and y. Abbreviations as in App.7.4 and App.7.5.

A: male inflorescences

compared groups (Uz(x,y))	monoNB	monoB	trpltNB	trpltB	3-floret	4-floret	single	groups (x-y)	N(x,y)
U1(1,2)	1	0.128	0.089	0.955	1	1	0.061	empty v.-GCN5	8, 20
U1(1,3)	1	0.285	0.118	0.855	1	1	1	empty v.-aZM15,To	8, 8
U1(3,4)	1	0.566	0.918	0.403	1	1	0.229	aZM15, T0-T1	8, 16
U1(1+2,3)	1	0.618	0.317	0.636	1	1	0.418	emptyv.&GCN5-aZM15,To	28, 8
U2(1,2)	1	0.27	0.403	0.535	1	1	0.386	emptv.&GCN5&aZM15,To-aZM15,T1	36,16
U2(1,3)	0	0	0	0.231	0.003	0	0.068	cntrls,To-aZM6,To	36, 17
U2(1,4)	0.001	0.182	0.002	0.127	0.045	0.047	0.072	cntrls,To-ZM6,To	36, 57
U2(1,5)	0.042	0.164	0	0.469	0.001	0	0.659	cntrls,To-aZM6,T1	36, 21
U2(1,6)	0.023	0.252	0.001	0.711	0.001	0.001	0.132	cntrls,To-ZM6,T1	36, 7
U2(1+2,3+4+5+6)	0.001	0.022	0	0.135	0.002	0	0.146	cntrls,To,T1-(a)ZM6, To,T1	52, 102
U2(1+2,3+5)	0	0.008	0	0.636	0	0	0.694	cntrls,To,T1-aZM6, To,T1	52, 38
U2(1+2,4+6)	0	0.107	0	0.078	0.009	0.014	0.077	cntrls,To,T1-ZM6, To,T1	52, 64
U2(1+2,3+4+5+6+7)	0	0.046	0	0.235	0.001	0	0.032	cntrls,To,T1-(a)ZM6,To,T1+Hoechst	52, 111
U2(1,3+4)	0	0.013	0	0.103	0.021	0.015	0.045	cntrls,To-(a)ZM6,To	36, 74
U2(1,8)	0	0.07	0	0.015	0	0	0.41	cntrls,To-aZM8,To	36, 33
U2(1,9)	0.01	0.626	0.03	0.064	0.001	0.01	0.477	cntrls,To-ZM8,To	36, 11
U2(1,10)	0.035	0.719	0	0.16	0	0.002	0.447	cntrls,To-aZM8,T1	36, 25
U2(1,11)	1	0.728	0.05	0.223	0	0	0.463	cntrls,To-ZM8,T1	36, 2
U2(1+2,8+9+10+11)	0	0.093	0	0.683	0	0	0.821	cntrls,To,T1-aZM8,ZM8, To,T1	52, 71
U2(1+2,8+10)	0	0.041	0	0.194	0	0	0.781	cntrls,To,T1-aZM8, To,T1	52, 58
U2(1+2,9+11)	0.004	0.646	0.005	0.015	0	0	0.976	cntrls,To,T1-ZM8, To,T1	52, 13
U2(1+2,8+9+10+11+12)	0.001	0.087	0	0.268	0	0	0.855	cntrls,To,T1-(a)ZM8,To,T1+Hoechst	52, 91
U2(1,8+9)	0.001	0.177	0	0.215	0	0	0.355	cntrls,To-(a)ZM8,To	36, 44

B: female inflorescences

compared groups (Uz (x,y))	triplet	pistillate flo	quad	single	pitriquad	groups (x-y)	N(x,y)
U1(1,2)	1	1	1	1	1	CNTRL, T0-T1	11,10
U1(1+2,3)	0.16	0.121	0.54	1	0.03	cntrl,T0,T1-aZM6,T1	21,56
U1(1+2,7)	0.095	0.513	0.211	1	0.03	cntrl,T0,T1-ZM6,T1hoechst	21,99
U1(1+2,6+7)	0.107	0.527	0.228	1	0.037	cntrl,T0,T1-ZM6,T1+hoechst	21, 106
U1(1+2,4)	0.029	1	0.221	1	0.029	cntrl,T0,T1-aZM6,T2	21,14
U1(1+2,3+4)	0.107	0.168	0.436	1	0.027	cntrl,T0,T1-aZM6,T1,T2	21,70
U1(1+2,3+6)	0.186	0.145	0.564	1	0.042	cntrl, T0,T1-(a)ZM6,T1	21,63
U1(1+2,3+6+7)	0.121	0.299	0.299	1	0.032	cntrl,T0,T1-(a)ZM6+hoechst,T0,T1	21, 162
U1(1+2,3+4+5+6+7)	0.111	0.299	0.299	0.734	0.031	cntrl,T0,T1-(a)ZM6+hoechst,T0,T1,T2	21, 187
U1(1+2,8)	1	1	1	1	1	cntrl,T0,T1-aZM8,T2	21,7
U1(1+2,9)	0.013	0.083	0.304	0.404	0.002	cntrl,T0,T1-ZM8,T1hoechst	21,61
U1(1+2,8+9)	0.02	0.102	0.33	0.429	0.003	cntrl,T0,T1-aZM8,T2+ZM8,T1hoechst	21,68

Appendix 7.8. Abbreviations.

A. thaliana	*Arabidopsis thaliana*
Amp	ampicillin
bp	base pair(s)
BSA	Bovine Serum Albumin
cDNA	complementary deoxyribonucleic acid
dATP	deoxyadenosinetriphosphate
dCTP	deoxycytosinetriphosphate
dGTP	deoxyguanosinetriphosphate
dTTP	deoxythymidinetriphosphate
dNTP	deoxynucleotide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DEPC	diethylpyrocarbonate
DMSO	dimethylsulfoxide
DTT	dithiothreitol
E. coli	*Escherichia coli*
EDTA	ethylene diamine tetra-acetic acid
EtBr	ethidiumbromide
EtOH	ethanol
IPTG	isopropylthio-β-0-galactopyranoside
kb	kilobase(s)
MOPS	3-(*N*-morpholino)-propanesulfonic acid
mRNA	messenger RNA
PCR	polymerase chain reaction
PEG	polyethylene glycol
RNA	ribonucleic acid
RNase	ribonuclease
SDS	sodium dodecyl sulphate
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
*Z.m.*ssp. *mays*	*Zea mays* subspecies *mays*

Appendix 7.9 Mutant map positions.

Mutant		map position	markers
bd1	*branched silkess1*	7L140	npi611a-umc35a
ids1	*indeterminate spikelet1*	1L255	bnlg504-bnl6.32
ifa1	*indeterminate inflorescence1*	1S048	umc115-umc11a
ra1	*ramosa1*	7L087	bnl15.40-umc136
ra2	*ramosa2*	2S030	cdo511-bnl8.35a
ra3	*ramosa3*	chrom3	bin4.00-4.11
rgo1	*reverse germ orientation1*	chrom9	bin9.04(-9.05)
Sos1	*Suppressor of sessile spikelet1*	4S025	umc277-bnl17.13b
tr1	*two ranked ear1*	2S052	bnl6.22a-bnl10.12a
ts4	*tasselseed4*	3S069	umc102-bnlg1108
Ts6	*Tasselseed6*	1L254	npi238-bnl8.29a

Appendix 7.10 Eidesstattliche Erklärung

Ich versichere, daß ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jeder Einzelfall als Entlehnung kenntlich gemacht habe; daß diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; das Sie – abgesehen von unten angegebenen Teilpublikationen – noch nicht veröffentlicht worden ist sowie, daß ich eine solche Veröffentlichung vor Abschluß des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Heinz Saedler betreut worden.

Köln, den 25.03.2002

.....

Wim Deleu

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Deleu, W., Cacharrón, J., Saedler, H. & Theißen, G., 2002. The maize MADS-box gene *ZMM6* affects inflorescence branching. Maize Genet. Conference Abstract 44: 41.

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Appendix 7.12 Lebenslauf

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