Characterisation of inflorescence development in *Zea mays* with four developmental mutants

A thesis presented in partial fulfilment of the

requirements for the degree of

Master of Science

in

Biological Science

At Massey University, Palmerston North, New Zealand

Murray Brown

2000

Ad endum

P. 9 The beginning of section 1.3.3: "Floricaula" should be spelt "FLORICAULA"

P. 11 paragraph 3. The logic of the regulation '*AP1* and *TFL1* negatively regulate *FCA*' contradicts previous statements, which suggest that *FCA* negatively regulates *TFL* and positively regulates *AP1*.

Page *et al.* (1999) State that:

- "Mutations involved in the *FCA* gene strongly delay the floral transition resulting in plants with much larger numbers of rosette leaves and coflorescences".
- "Mutations in the *TFL1* gene result in the conversion of the indeterminate apical meristem into a determinate floral meristem (Shannon and Meeks and Wagner, 1991).They can also cause an acceleration in flowering time, the extent differing in different alleles (Alvarez *et al.*, 1992)
- "In the LD cabinet conditions, the *ap1-1* mutant flowered earlier and with fewer rosette leaves than the L*er* parent (in agreement with (Schultz and Haughn, 1993)."

When *FCA* is combined in double mutants with *TFL1* and *AP1*:

- "the flowering time of the double mutants, *fca-1 tfl1-2* and *fca-4 tfl1-2* was later than the respective *fca* parents. Coflroescence and cauline leaf number on the main inflorescence of the double mutants was similar to the *fca* parent. The number of floral nodes produced was less than the *fca* parent but >six fold greater than the *tfl1-2* mutant. Production of the terminal flower was suppressed in the double mutants but did occur eventually in all cases....The late flowering phenotype was epistatic to the early flowering conferred by the *tfl1* alleles and the formation of the terminal flower was significantly delayed."
- "the *fca-1 ap1-1* double mutant plants flowered at a similar time to the *fca-1* parent, with a similar number of rosette leaves, but the *fca-4 ap1-1* plants flowered considerably later than *fca-4*. The characteristic determinate branched flowers previously described for *ap1-1* mutations(Bowman *et al.*, 1993) were present and these formed a dense mass of floral-like structures at the inflorescence apex in the late flowering backgrounds.....In summary, all *fca* alleles enhanced the *ap1* inflorescence phenotype but did not significantly affect floral development in the *ap1* flowers. As *ap1-1* is a strong allele this suggests that *FCA* functions to promote the formation of flowers in pathways that act redundantly with *AP1*. the early flowering phenotype of *ap1-1* mutations indicates that *AP1* functions as a repressor of the floral transition and epistasis of the late flowering phenotype of *fca-1* over the early flowering of *ap1-1* suggests that it acts via inhibiting FCA function.

Furthermore Page *et al.* (1999) state:

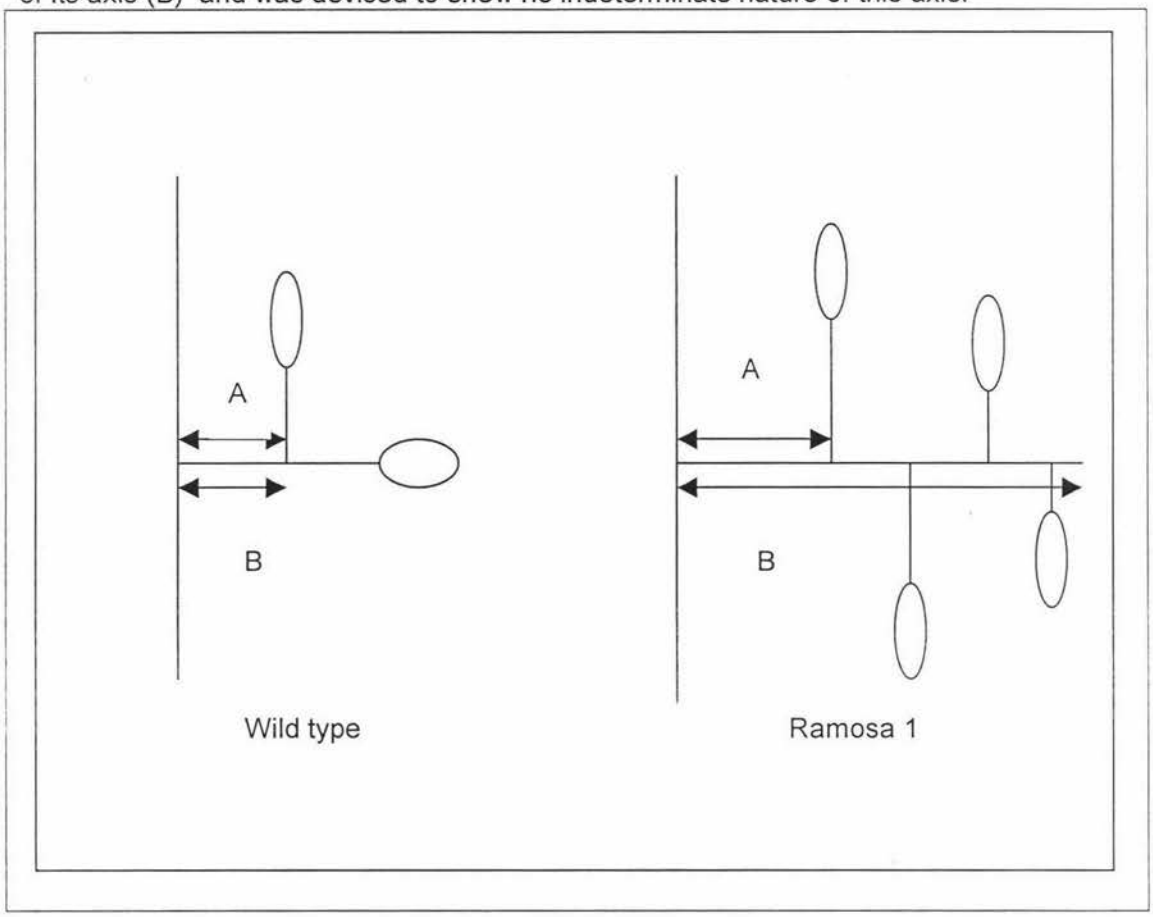
- "The results are consistent with *AP1*and *TFL1* negatively regulating *FCA* function..."

Therefore any suggestion that *FCA* negatively regulates *TFL* and positively regulates *AP1* is incorrect based on the double mutant analysis of Page *et al.* (1999) and any implications I have made should therefore be disregarded.

Evans (1940) should be Evans, M.W. and Grover, F.O. (1940)

P 33 and P34 the small unexplained numbers and octagons present in the figures are outliers with the number referring to the individual in that dataset which is the outlier.

P 41 Figure 3-19 refers to the distance from the central culm to the base of the first spikelet axis (A). Figure 3-20, however, refers to the distance from the central culm to tip of its axis (B) and was devised to show he indeterminate nature of this axis.



P 43 "*wild type* siblings" should be deleted from figure caption

P 67 The red in the pie charts is the mean percentage of the tassel that branched and the blue is the mean percentage of the tassel that is spikelet pairs.

P 73 The branches in fig. 3-51 and 3-52 are subtended by two glumes and arise in place of the upper floret with a single floret on the surface away from the cob. The branches produce two secondary structures similar to spikelet pairs along its flanks perpendicular to the axis of the inflorescence axis. These structures are most likely derived from the loss of determinacy of the spikelet meristem which then reverts to a developmental program similar to a branch meristem and produces spikelet pairs in an alternate phyllotaxy.

P 107 in response to the question weather the map location of the *ids1* mutant is known. The reference Chuck *et al.* (1998) did not give a map position nor did Neuffer *et al.* (1997). However, the Maize DB (http://www.agron.missouri.edu/) lists *ids1* locus as being on the long arm of chromosome 1 while *bd1* is located on chromosome 7 (Neuffer *et al.*, 1997).

A explanation on how to interpret boxplots has been included in Appendix1 on page 118

References:

Alverez, J., Guli, C.L., Yu, X.-H. and Smyth, D.R. (1992) terminal flower: a gene affecting inflorescence development in Arabidopsis thaliana. Plant Journal., **2**, 103-116

Arber, A. (1934) The Graminae: A study of cereals, bamboo and grass. J.Cramer (Weinheim)

Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M. and Symth, D.R. (1993) Control of flower development in Arabidopsis thaliana by APETALA1 and interacting genes. Development, **119**, 721-743.

Bradley, D., Carpenter, R., Copsey, L., Vincent, C., Rothstein, S. and Coen, E (1996) Control of inflorescence architecture in Antirrhinum. Nature, **379**(6568), 791-797

Chuck, G., Meeley, R. and Hake, S.(1998) The control of maize spikelet meristem fate by the APETALA2-like gene indeterminate spikelet1. Genes Dev **12**,1145-1154

Cutler, H.C. (1946) Races of maize in South America. Harv. Univ. Bot. Mus. Leafl. **12**, 291-294

Emerson, R.A., Beadle, G.W. and Fraser, A.C.(1935) A summary of linkage studies in maize. Cornell Univ. Agric. Exp. Sta. Mem. 180:1-83

Evans, M.W. and Grover, F.O. (1940) Developmental morphology of the growing point of the shoot and inflorescences in grasses. Journal of Agricultural Research **61**, 481-515

Kempton, J.H. (1934) Heritable characters in maize. XLVII. Branched silkless. Jour. Hered. **14**, 243-252

Neuffer, M.G., Coe, E.H., and Wessler, S.R. (1997) Mutants of maize. Cold Spring Harbour Laboratory Press.

Schultz, E.A. and Haughn, G.W. (1993) Genetic analysis of the floral initiation process (FLIP) in Arabidopsis. Development, **119**, 745-765

Shannon,S. and Meeks-Wagner, D.R. (1991)A mutation in the Arabidopsis TFL1 gene affects inflorescence meristem development. Plant Cell, **3**, 877-892

Weatherwax, P. (1916) Morphology of the flower of *Zea Mays*. Bulletin of the Torrey Botanical Club **43**, 127-143

Abstract

The genetic control of inflorescence development has been studied in great detail in the model dicotyledonous plants *Arabidopsis thaliana* and *Antirrhinum majus*. In contrast, little is known about the genetic regulation in monocotyledonous species. Using maize (*Zea mays*) as a model system, the phenotypes were documented for the *branched silkless1* (*bd1*) and *ramosa* (*ra1*, *ra2*, and *ra3*) inflorescence mutants that are characterised by abnormally branched ears. A comparison of the adult morphology and developing inflorescences using scanning electron microscopy in mutant and normal maize reveals that there are at least five reproductive meristems that can be identified in maize: the inflorescence meristem, the branch meristem, the spikelet pair meristem, the spikelet meristem, and the floret meristem. The abnormal branching in *bd1* and the three-*ramosa* mutations is the result of the failure to determine the fate of specific types of reproductive meristems in both tassels and ears. Both *RA1* and *RA3* are required for the determination of spikelet pair development in branch primordia. *RA2* is necessary for determinate growth in spikelet pair meristems. *BD1* is required determinate growth of spikelet meristems by specifying a determinate floral meristem identity. The classification of the different types of reproductive meristems and the genes that regulate their development is essential to understanding the genetic programs that underlie inflorescence morphogenesis in maize and other *Gramineae*.

Acknowledgements

I would like to thank:

- My boss Allan Hardacre for allowing me the time off work to finish experiments and write up.
- My supervisor, Dr Bruce Veit for his help and patience.
- Doug Hopcroft and Raymond Bennett for help with the SEM and processing the photographs.
- Rebecca Heaphy for her support and putting up with me when I was stressed.
- Finally, Mum and Dad, who support me no matter what happens and are always there for me.

Table of Contents

List of Figures

1 Introduction

A wide variety of inflorescence architectures exist in the plant kingdom (Coen and Nugent, 1994). A significant number of differences in structure and shape are discretely inherited and their regulation is governed by one or two genetic loci (Doebley, 1993; Gottleib, 1984). By contrast, other characters in plants, particularly those of dimensions, weight, and number (the classical components of agricultural yield), generally exhibit continuous variation with differences in their expression are usually governed by multiple gene systems (Gottlieb, 1984). Therefore, the relatively simple genetic mechanisms that are likely to determine inflorescence architecture offer unique insights into the regulation and evolution of plant morphology.

Genetics provides framework for the understanding of the series of events that occur during inflorescence morphogenesis. The study of mutants has been used widely to document the genetic requirement for the establishment of patterns in animal development. More recently, the same principles have begun to be applied to plant development and have been further extended by molecular cloning techniques.

The development of plants is quite unlike that of animals. Growth and differentiation in plants are normally initiated in meristems that occur at the apices of all shoots and roots (Esau, 1953). As a consequence, organogenesis may occur in different parts of the plant body in a largely autonomous manner, enabling them to respond independently to different environmental and developmental cues. This is a critical distinction between animals and plants as higher animals differentiate most organs during embryogenesis. Plants also have fewer organs, tissues, and cell types than animals. This is because much of plant metabolism is partitioned at the cellular and subcellular level rather than in spatially separated organs specialized for different functions (Gottlieb, 1984).

These and other developmental features of plants must be taken into account when considering the regulation and evolution of morphological characters. Gottliebs' (1984) hypothesis that discrete characters, such as presence vs. absence, determinate vs. indeterminate, changed structure, shape, or architectural orientation, are governed by relatively few genes has been well supported with studies on the evolution of maize inflorescences by Collins (1919), Rodgers (1950), Galinat (1983), Iltis (1983),

Doebley et al. (1990), Doebley and Stec (1991), Doebley (1993), Doebley and Stec (1993), Doeweiler et al. (1993), Meyerowitz (1994), Doebley (1997), Doebley et al. (1997), Dorweiler and Doebley (1997).

1.1 Maize as a model system to study inflorescence development

Although it is clear that plant development is guided to a large extent by information encoded in genes, describing how this is accomplished has been challenging. Maize is a model system that has several biological and genetic features that make it ideally suited to the study of the role of genes in plant development, which has been summarised by Sheridan (1988).

The comparatively large size of the developing and mature inflorescences make maize ideal for the study of morphogenesis, making dissections and sectioning studies relatively simple. Furthermore, the abundance of tissue provides ample material for biochemical and DNA analysis often without having to sacrifice the whole organism. The large size also facilitates analysis of the mature inflorescences as many of the structures are of sufficient size to be observed with the unaided eye.

Although the development of the maize inflorescence is a complex process, it can be divided into a sequence of morphologically distinct stages (Cheng et al., 1983). Moreover, because the inflorescence is a reiterated structure, with the oldest, most differentiated florets at its base, departures from the normal sequence of development can be easily discerned (Veit et al., 1991).

The long life cycle can be a disadvantage for experimental use that can be minimised with proper planning and facilities. The prolonged elaboration of meristems and primordia can be advantageous, however, providing large time intervals for the isolation of meristems at different stages of development.

Two further biological features that aid tremendously in the genetic study of inflorescence morphogenesis are the size of the ear and the monecious flower development. A normal, vigorous maize plant will produce an ear that will bear between 300 and 600 kernels. These may be cross- or self-pollinated in a single act, so that large populations can be obtained with minimal effort. Moreover, the separate

location of the functional male flowers and female flowers in the tassel and ear allow simple bagging of the ears to avoid contamination with stray pollen, thereby avoiding the need for emasculation.

Finally, over the last century, extensive genetic stocks and maps of maize have been constructed with translocation and other cytogenetic stocks. McClintock (1947) first discovered transposable elements in maize. Several systems have now been well characterised (Dellaporta and Moreno, 1994; Auger and Sheridan, 1994; Cone, 1994; Chomet, 1994) and will prove especially valuable in future work. These allow the simultaneous induction of new mutants at known as well as unknown loci affecting inflorescence morphogenesis and also enable the transposon tagging of these genes.

1.2 Inflorescence morphogenesis

The inflorescence architecture of maize is as rigorously inherited as any of its other feature and is therefore an issue of production of a phenotype. Unlike traits that are linked to a simple tangible object, like a pigment or a protein, morphogenesis is inherited as a reproducible succession of molecular and cellular processes occurring at a myriad of locations (Green, 1991) that generate the form of the plant over a period of time. However, optimal methods for linking morphogenesis to the genome are less obvious compared to when the phenotype is a more tangible object. A satisfactory explanation needs to meet five basic criteria.

1.2.1 Features of a satisfactory explanation of morphogenesis

Green (1991) proposed that there are five basic qualities required for a satisfactory explanation of morphogenesis, in which the mechanism of change is: (1) explicit; (2) confined to essential components; (3) sufficient; (4) efficient and (5) testable.

1. For each conversion, the "before" and "after" conditions should be fully specified, as well as the essential process itself for it to be considered explicit. At the molecular level this is not a problem. However, this does become a problem with morphogenesis, as many of the factors and tissue interactions are unknown.

2. Obviously, an explanation should be restricted to the necessary elements that when absent stops the process. However, there are many essential components with small effect that may not be restricted to the process of interest and may be ascribed a

low significance. In contrast, small changes in other components may elicit far more serious and un-correctable changes and are much more significant (Green, 1991). Therefore, a satisfactory explanation should contain a short list of only the most essential components.

3. For the mechanism to be sufficient, the interaction of all the essential components must account for the phenomenon or conversion in question (Green, 1991). When the developmental process is a single definable change, a simple explanation is sufficient. However, when intermediary stages or correlated processes are involved, the problem becomes more complex. To cope with this problem, more and more essential components can be used in the hope that a sufficient mechanism will become apparent. However, it is not necessary to know how each component interacts on a molecular level in order to have an operational (sufficient) understanding of the whole, provided that one knows the function of the components and how they are connected. Therefore, sufficient, but formal, explanations provide a good start to resolving morphogenic mechanisms (Green, 1991).

4. Among all explanations the simplest and briefest is usually preferred (Occam's razor). For morphogenesis however, a simple sequence is not enough to explain the complex interactions between components over time. Instead, the explanation has to have a generative or "breeding" character (Green, 1991). For example, at a molecular level, one gene may activate the expression of whole gene arrays in a localised pattern dependent upon interactions with other gene products distributed temporally and spatially, e.g. pattern formation in chick limbs (Wolpert, 1981).

5. It is necessary to test for validity among various sufficient explanations. In practice, such tests show that none of the proposed mechanisms are correct. Failures, however, typically point to better potentially sufficient solutions, and ultimately lead to a satisfying answer (Green, 1991).

There are two major ways of testing morphogenic mechanisms: (1) response to perturbation and (2) correlative data. These activities provide information on what are essential components to a given process and their possible time of action.

1.2.2 Tactics to study inflorescence morphogenesis

Green (1991) noted that the tactic of learning about a process by its response to perturbation has a long record of fruitfulness. In inflorescence development, the perturbation may be environmental (alteration of day length day length), physiological (hormonal application) or genetic (mutation). Genetic tests for essentiality can be extremely explicit, with precision down to single codons in cloned genes. However, sufficiency at a cellular and organ level, however, is another matter (Green, 1991). In order to understand the context a second method, collection of correlated data, is used.

In order to understand the context of gene action, it is necessary to correlate the changes of many distinctive features such as shoot form, RNA levels, and hormone levels between perturbed and unperturbed systems over time. Maize inflorescences provided an ideal model system; as a number of genes are known to influence inflorescence development and developing maize inflorescences have gradients of reiterated structures at morphologically distinct stages.

1.3 Current and proposed models of inflorescence development

The morphogenesis of maize inflorescences offers several insights into the evolution and determination of architecture in plants. However, there are no currently available models that cohesively draw together all the elements of inflorescence morphogenesis in maize. Several models, based on basic principles of plant genetics and development, have been developed that explain different aspects of inflorescence morphogenesis in maize and other model species. These are summarised in the following sections.

1.3.1 The phytomer as a unit of development

The structure of maize inflorescences can be described in terms of developmental modifications to a metameric unit, the "phytomer", which is basic throughout the entire plant (Galinat, 1959). Each manifestation contains the same essential elements, which in a vegetative form consists of the node, internode, a leaf, and axillary bud.

Maize inflorescences are of interest because they have several types of phytomer, which can be related to the different types of primordia formed during inflorescence

development. Each phytomer type represents a different phase of development, which is arranged in a temporal order, forming a developmental sequence (Alberch, 1985). Each phase is defined by the type, arrangement and number of organs produced and the structures produced during a specific phase retain the morphological and physiological features characteristic of that phase even after the shoot has entered a new phase (Poethig 1990).

1.3.2 Combinatorial model of shoot development

The combinatorial model implies that the development of plants is specified by a series of independently regulated, overlapping programs that modify the expression of a common set of processes required for shoot growth. Evidence that the morphology of the shoot is determined in a combinatorial fashion rather than from a series of mutually exclusive programmes comes from many different sources. The intermediate character of structures produced during phase transitions provides some of this evidence for this conclusion, as well as the aberrant combinations of traits from different phases of development that can be induced experimentally or genetically (Poethig 1990). In maize, for example, leaves produced during the transition from juvenile to adult growth have a combination of juvenile and adult cell types and express a variety of other traits in a quantitatively intermediate fashion (Poethig, 1990).

The very specific effects of many inflorescence mutants led Veit at al. (1993) to propose that inflorescence development can be treated as a modular process, with the mutant phenotypes defining the boundaries of component subprograms. Moreover, some of these processes proceed independently of each other given the limited effects of a particular mutant such as sex determination and primary branching (Veit et al., 1993). However, Irish (1997) reported that class II tassel seed mutations (*ts4* and *Ts6*) have altered branching patterns and feminisation of the tassels and therefore are involved in both the sexual determination and branching subprograms. It is still conceivably these two subprograms are independent, as they occur during chronologically separate phases of development, in different types of meristems. Analysis of double mutant phenotypes provide a further test as to how mutationally defined developmental processes might be related to each other.

Postlethwait and Nelson (1964) first characterized the development of maize through the use of the mutants, *Polytypic* (*Pt*) and *ramosa-1* (*ra1*), to trace developmental pathways in the maize inflorescence. To do this they noted changes in the meristematic activity beginning with the vegetative meristem and ending with the initiation of the ovule. From these morphological observations, several "switch points" at which deviation form normal development could occur, were identified. These "switch points" were also used by Bonnett (1940, 1948), Cheng et al. (1983) and Irish and Nelson (1991) to describe the development of maize inflorescences. Postlethwait and Nelson (1964) hypothesised that *wild type Ra1* allele is required for the successful transition from a branch meristem into spikelet pair meristem. The suggesting that development can be thought of as sequential and modular processes provides a useful conceptual framework for understanding mutant phenotypes (Veit et al. 1993).

How the activities of these separate developmental programs may interact is as yet unclear. It is possible that the programs are sequential where the completion of one program signals the initiation of the next along with other distinct conditions being meet. However, other genetic programs might be triggered independently of one another and proceed in parallel fashion. The normal pattern of development might therefore represent the combined output of these programs. Moreover, failure of one of these programs, results in altered patterns of growth. This type of explanation was used by Poethig (1990) to explain the profound changes in the inflorescences of *Teopod* (*Tp*) mutants. In this case, it is proposed that the *Tp* mutations indirectly affect inflorescence development by extending programs specifying juvenile patterns of development. This does not prevent reproductive development, instead alters the context in which gene expression occurs resulting in altered patterns of development. A similar explanation has been provided for the homeobox gene *knotted1* (*kn1*) (Freeling, 1992).

Irish and Nelson (1991) identified several sequential steps in the transition to flowering in maize inflorescences using culture experiments (Figure 1-1). These closely correlate with the switch points proposed by Postlethwait and Nelson (1964). Furthermore they reported that the determination of reproductive development occurs late in development after all the vegetative organs had been initiated. Each different

stage represents a transition from one phase of development to the next and can be characterised by the phyllotaxy and type of organs produced during each phase.

Poethig (1990) proposed that the initiation of the transition between phases is regulated by factors extrinsic to the meristem. In contrast, the ability of the meristem to respond to these factors and remain in a particular phase of development is intrinsically regulated. Moreover, Irish and Nelson (1991) reported that the different stages (Figure 1-1) represent transitions, which cannot occur in isolated meristems/immature tassels, but require input from other regions of the plant.

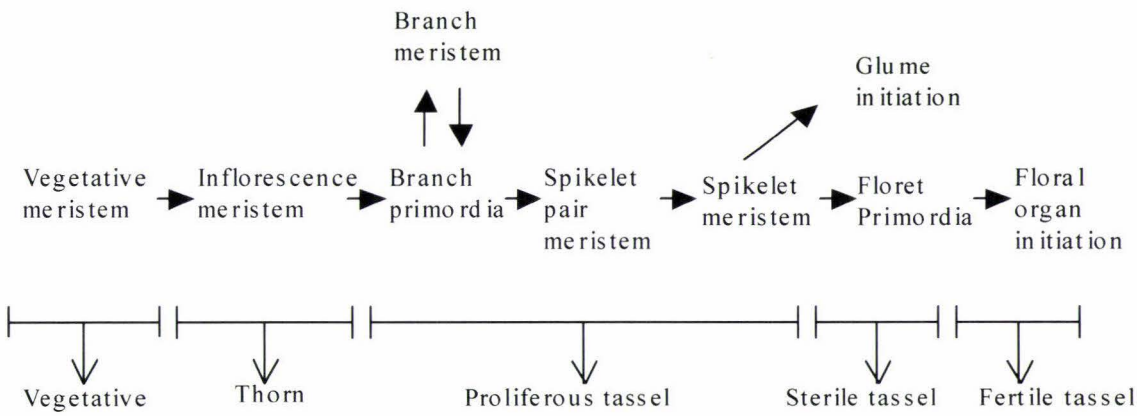


Figure 1-1 Summary of the steps in maize development

A schematic summary of the developmental phases of the transition from indeterminate, vegetative development to determinate, floral development, adapted from Irish and Nelson (1991). The upper portion represents the morphologically identifiable stages in tassel and ear development. Each vegetative meristem becomes elongated and initiates branch primordia. These branch primordia may develop into branches bearing spikelet pairs or directly into spikelet pairs. Spikelet pair meristems give rise to spikelets that initiate glumes and florets. Each floret initiates a lemma, palea and floral organs. The lower portion represents the type of development observed when meristems of those stages are cultured.

A further four key switch points have also been determined as being critical for the determination of architecture: (1) indeterminate to determinate growth of the meristem, (2) initiation of buds on the meristem, (3) identity of the initiated bud and (4) sterile to fertile shoots (florets) (Irish and Nelson, 1991). Consequently, genes affecting maize inflorescence morphology can be grouped into a list of essential components affecting inflorescence morphology according to the switch points

affected. Furthermore, double mutants can be used to determine the relationships between specific components that combine to form a component subprogram regulating inflorescence morphogenesis.

In addition, several studies on inflorescence development currently exist in other species for which several mutants have been cloned and their expression studied. Genetic and molecular studies show a high degree of conservation between two dicotyledonous species, *Antirrhinum* and *Arabidopsis* for the control of floral development (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994; Weigel, 1995). Recent studies also show that these genes, which can affect branching, may also be conserved between species as diverse tobacco (Kato et al., 1998; Amaya et al. 1999), tomato (Pnueli et al. 1998), petunia (Souer et al. 1998), *Brassica napus* (Mimida et al. 1999), rice (Kyozuka et al. 1998), and Eucalyptus (Southern et al. 1998). Several recent reviews on inflorescence development and the associated floral patterning are also available (Schmitz and Theres, 1999; Pineiro and Coupland, 1998; Pidkowich, Klenz and Haughn, 1999; Ma, 1998). The models derived from these studies offer some unique insights into how maize inflorescence development may be controlled at a genetic and molecular level.

1.3.3 Regulation of inflorescence development in *Antirrhinum*

Gene expression studies of *FLORICULAR* (*FLO*) and *CENTRORADIALIS* (*CEN*) suggest the architecture of *Antirrhinum* inflorescences reflects in part the way these genes are coordinated. In *FLO* mutants, flowers are replaced by continually branching indeterminate shoots, in contrast to the *cen* mutants that abruptly terminate in a flower. Bradley et al. (1996) propose that 1-2 days after floral induction; *FLO* expression, linked with the commitment to form flowers, is activated in the axillary meristems. In *wild type* plants, during the next few days, the central apex generates further axillary meristems, which express *FLO* and will give rise to flowers. However, no *CEN* expression was observed at this time. Instead, about day 6, *CEN* expression can be found in the sub apical region of the inflorescence meristem and is linked to the inhibition of *FLO* expression in the apical meristem. In *CEN* mutants, *FLO* expression is not inhibited in the inflorescence meristem, resulting in its commitment to floral development. Interestingly, expression of *FLO* in the axillary meristems is

not inhibited by *CEN*, either because *CEN* is spatially restricted or axillary meristems are not receptive to *CEN* expression.

According to this model, *CEN* and *FLO* regulate each other non-autonomously. First, induction of *FLO* in axillary meristems activates *CEN*, even though the areas of expression are localised and were not found to overlap, suggesting *FLO* must signal across cells to activate *CEN*. Secondly, *CEN* is largely expressed in the sub-apical region and does not extend to the outer cell layers of the apex, while *FLO* is expressed ectopically throughout the apical dome. Therefore *CEN* represses *FLO* in the *wild type* apex by signalling across cells. How the *CEN* and *FLO* proteins interact remains unclear, but their antagonistic interaction provides an interesting insight into how genes may interact to control branching.

1.3.4 Regulation of inflorescences development in *Arabidopsis*

The shape of *Arabidopsis* is typically that of a raceme. However, several mutants exist that alter the shape and elongation of these inflorescences. Komeda et al. (1998) reported that a morphological change in the tip of the inflorescences, changing a raceme to a corymb, was caused by failure of the pedicels to elongate in *CRM1-1* and *ERECTA-105* mutants. In the case of *CRM2-1*, the morphological difference was caused by the increase of floral buds in the inflorescence. Several other differences beside the shape of the inflorescences were also noted where other structures also showed a decrease in size. From these results, the authors concluded that the defects were caused by a decrease in cell number or inadequate cell size. The *CRM1-1* phenotype was related to inadequate cell sizes in both the pedicels and internodes, while the cells of *CRM2-1* plants were only reduced in the pedicels. The *ERECTA* mutants on the other hand appeared to be caused by a decrease in cell numbers. The further analysis of these genes and others offer unique insights into the possible mechanisms for altering inflorescence morphology through cell division and elongation.

The overall morphology of an *Arabidopsis* plant is also determined by the behaviour of meristems derived from the shoot apex, which either develops as a shoot or flower. These different fates require a separation between the function of the meristem identity genes and an antagonistic group of genes that includes *TERMINAL*

FLOWER1 (*TFL1*), a homologue of *CEN* (Kato et al. 1998). Like in *Antirrhinum*, the meristem identity genes such as *LEAFY* (*LFY*), *APETALA1* (*AP1*) and *CAULIFLOWER* (*CAL*) control the transition from inflorescence to a floral meristem by synergistically inhibiting *TFL1* in the floral meristems on the apex periphery. *TFL1* can inhibit the activity of meristem identity genes at the centre of the apex in two ways; first by delaying their upregulation, and second, by preventing the meristem from responding to *LFY* or *AP1*. These observations suggest that the *wild type* pattern of *TFL1* and floral meristem identity gene expression depends upon their relative timing of up regulation (Ratcliffe et al. 1999).

The *TFL1* locus is also interesting as it has a unified effect on the rate of progression through different developmental phases. Ectopic expression of *TFL1* in Arabidopsis plants results in the extension of the vegetative and reproductive phases and exhibit dramatic changes in their morphology. These results and others lead Ratcliffe et al. (1998) to suggest that *TFL1* participates in a common mechanism underlying major shoot apical phase transitions, rather than their being unrelated mechanisms that regulate each specific transition.

The genes controlling the timing of the major phase change form vegetative to floral development in Arabidopsis were also found to regulate the initiation of floral development. Page et al. (1999) reported that based on double mutant and transgenic expression studies that a gene controlling flowering time, *FCA*, promotes flowering in multiple pathways. One of these pathways leads to activation of *LFY* and *AP1* and another acting in parallel with *LFY* and *AP1*. The results are also consistent with *AP1* and *TFL1* negatively regulating *FCA* function. As a consequence, the *fca* alleles enhanced various aspects of the *lfy* phenotype, most notably being the large increase in the number of shoot-like structures on the inflorescence, while all *fca* alleles enhanced the *ap1* inflorescence phenotype but did not affect floral development of *ap1* flowers. The combination of the mutant alleles *FCA* and *EMBRYONIC FLOWER (EMF)*, a floral repression gene, confirmed that *FCA* is required for the early flowering of *emf* plants with the loss of *FCA* function delaying the formation of reproductive structures in *emf* individuals. Therefore, *EMF1* and *FCA* are likely to operate in different pathways.

The inheritance of the control of the transition form inflorescence to floral meristem has been found to be dependent on the synergistic interactions between *LEAFY/FLORICULAR* and *APETALA1/SQUAMOSA*. This lead Huijser et al. (1992) to propose that *SQUAMOSA* may have been involved in the evolution of racemose from cymose inflorescences. Furthermore, Alvarez et al. (1992) implicates *TERMINAL FLOWER1*, which controls the change form indeterminate to determinate growth in inflorescences, as also being involved in the evolution of these two inflorescence forms. Furthermore, the elaboration of plant form requires the activation of complex developmental pathways as a response to environmental or internal signals, such as *LEAFY/FLORICULAR* and *APETALA1/SQUAMOSA*. Such a response is more easily coordinated if relatively few regulatory genes activate the scores of downstream loci needed to produce a phenotype, than if all downstream loci were to respond individually to the signal. As a result of this hierarchical design, mutations to regulatory loci that articulate plant response to environmental and internal signals would be predisposed to produce major evolutionary shifts in morphology by relatively simple genetic changes (Doebley, 1993).

1.3.5 A model for maize inflorescence development

Although our knowledge of the genetic regulation of inflorescence development in dicotyledonous species has increased in the last few years, many features of grass inflorescences and their development make them distinct. Unlike *Arabidopsis* and *Antirrhinum*, which produce flower meristems directly on the flanks of inflorescence meristems, we know that maize undergoes multiple stages in the conversion form vegetative to floral development (Irish and Nelson, 1991; Irish, 1997).

As previously stated, a number of genes affecting inflorescence morphology have been identified with their mutant phenotypes characterised (Figure 1-2), each mutant defining a particular stage of inflorescence development. By arranging the different mutants in sequence based on the type of structure they affect, a basic program of essential components can be constructed (Figure 1-3).

Admittedly, this is a simplified program as maize inflorescences have two quite distinct morphologies. However, the patterns of development of the different meristems are essentially similar until selective abortion of the sexual organs occurs

(Cheng et al., 1991). Many of the quantitative characters like: mass, length and girth arise after the determination of the floral organ identity, though there are differences in early gene expression required for the establishment of tassel and ear inflorescence meristems. For example, *tasselless* (*tl1*) homozygotes fail to form tassels while ears are absent in *barren stalk* (*ba1, ba2,* and ba3), *barren stalk fastigate* (*baf1*) and *barren sterile* (*bs1*) homozygotes.

An essential difference between the morphology of ear and tassel is the presence of prominent branches on the latter. However, this can be explained by the timing of the *ramosa* (*ra1, ra2* and *ra3*). During the initiation of the first branch primordia on the tassel meristem, the *ramosa* family of gene products are not present in sufficient levels to determine the branch primordia, resulting in the expression of *few branches* (*Fbr1*) and *unbranched* (*ub1*) required for establishment of branch meristems. In the ear, the *ramosa* family of genes are either present much earlier or the branch primordia are competent to discern their presence much earlier in ear development.

One formal model to explain this difference focuses on the expression of the group of genes known as *ramosas* that regulate the ability of inflorescence meristems to produce branches by determining branch primordia to become spikelet pairs instead. In tassels, these *ramosa* genes are not expressed early in development resulting in the formation of branches from branch primordia that would otherwise form more determinate spikelet pairs. Once a defined number of branches have been produced, *RAMOSA* expression is triggered in the ear and tassel resulting in the replacement of branches with spikelet pairs instead.

Once the branch primordia have become committed to spikelet pair development, sequential gene expression is required for the morphogenesis of floret primordia that in turn produce floral organ primordia. Starting with the expression of *paired rows* (*pd1*) and *Suppressor of sessile spikelets* (*Sos1*) promoting the establishment of the two-spikelet meristems, which is determined by *tasselseed 4* (*ts4*). The spikelet primordia continue to develop initiating two floret primordia before themselves becoming determined by *branched silkless1* (*bd*), *indeterminate spikelet* (*ids1*) and *Tasselseed 6* (*Ts6*).

Figure 1-2 Mutants affecting inflorescence architecture in maize

Gene	Phenotype
Inflorescence meristem	
barren stalk 1 (*ba1*)	Ear shoots and most tassel branches absent
barren stalk 2 (*ba2*)	Like *ba1* but tassel more normal
barren stalk 3 (*ba3*)	No ear produced
barren stalk fastigate (*baf1*)	Ear shoots often absent, tassel branches erect
barren sterile (*bs1*)	Ear shoots absent; sterile
tasselless (tl1)	Tassel absent
Branch primordia	
Barren inflorescence (*Bif1*)	Spikelets absent from ear and tassel: branching reduced
barren inflorescence 2 (*bif2*)	Thin bare unbranched rachis replaces ear and tassel
unbranched (*ub1*)	Unbranched tassel
few branches (*Fbr1*)	Reduced branching in tassel, leaf bract replaces 2nd tassel bract
ramosa-1 (ra1)	Branches replace ear and tassel spikelet pairs
ramosa-2 (ra2)	Like *ra1* but branches abnormal
ramosa-3 (ra3)	Like *ra1*
paired rows (*pd1*)	Paired rows dominant over single spikelets of Teosinte
Suppressor of sessile spikelet (*Sos1*)	Decreased branching, single spikelets replace spikelet pairs
tasselseed 4 (*ts4*)	Extra branching in the tassel and ear, extra tassel florets, some feminised; double florets
Spikelet primordia	
branched silkless1 (bd1)	Florets converted to indeterminate branches
indeterminate spikelet (*ids1*)	Florets converted to indeterminate branches
Tasselseed 6 (*Ts6*)	Like *ts4*; double florets

To refine the model below, it is necessary to document the affect of individual null alleles on morphology and correlate this with changes in morphogenesis. Furthermore, double mutant analysis can be used to determine potential relationships between the different components in the pathway. This study focuses particularly on the phenotypes *ramosa* family of genes and *bd1*, which all have branch-like structures in the mature male and female inflorescences, and their relationships to each. From this it should be possible to either reject the above model or refine it further and hypothesize about the possible role of these genes in the evolution of maize and grass architecture.

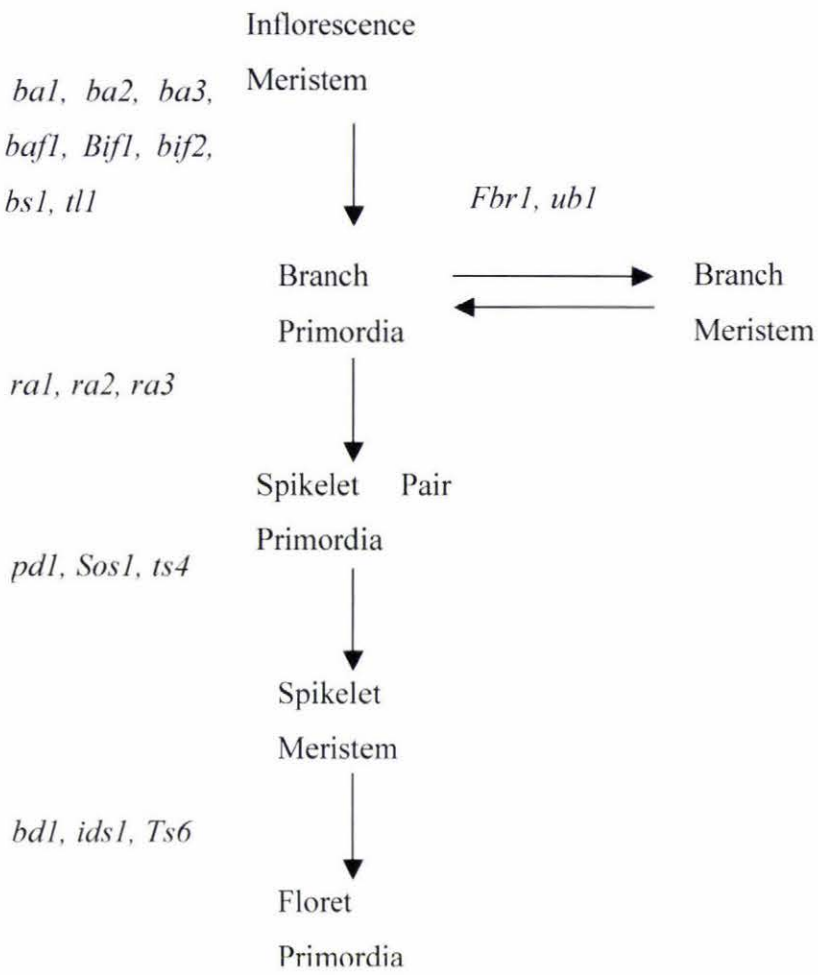


Figure 1-3 Model of timing of gene action relative to inflorescence morphogenesis.

Each group of genes is required for the correct transition between each morphogenic phase; either the mutants of these genes fail to determine the transition between developmental phases resulting in the continued elaboration of the meristem, e.g. *ra1* is required for the determination of branch primordia to become spikelet pair primordia; or are required for the establishment of the subordinate meristem, e.g. *Sos1* is required for the establishment of sessile spikelet meristems.

2 Materials and Methods

2.1 Materials

2.1.1 Plant Lines

Name	Source	ID number	Stock Centre ID Number	Map Location
Mutator	S. Hake [1]			
B73	Maize genetic stock Centre	47638		
Mo17	Maize genetic stock Centre	47846		
A188	Maize genetic stock Centre	47602		
branched silkless1	Maize genetic stock Centre	109446	7136I	7L-109
ramosa-1	Maize genetic stock Centre	14429	708A	7L-32
ramosa-2	Maize genetic stock Centre	14103	308E	3S-49
ramosa-3	Maize genetic stock Centre	96345	408J	4

[1] Sarah Hake; Plant Gene Expression Centre, Berkley California

2.2 Buffers and Solutions

2.2.1 0.1M Sorenson's buffer at pH 7.0

39ml of 0.2M NaH_2PO_4 was added to 61ml Na_2HPO_4 and made up to a total volume of 200ml with deionised water.

2.2.2 4% Paraformaldehyde

100 ml of fresh paraformaldehyde was made fresh each time required. 50ml of 0.1M Sorenson's buffer at pH 7.0 was added to 50ml MilliQ water to a final concentration of 0.05M. The pH of the solution was adjusted to the pH 11 with 1M NaOH and heated to 60 °C in the fumehood. 4g paraformaldehyde was added to the warmed solution, which was immediately place on ice. The solution is then stirred constantly in the fumehood on ice until the paraformaldehyde dissolved. Once the solution became clear, the pH was readjusted to 7.0 with H_2SO_4 while stirring constantly. The fresh solution was then keep on ice and used immediately.

2.2.3 Fixative

20ml of electron microscopic grade 25% gluteraldehyde (BDH) was added to 100ml of fresh 4% paraformaldehyde solution. A further 50ml of 0.1M Sorenson's buffer at pH 7.0 was added and the solution made up to a total volume of 200ml with deionised water.

2.3 Methods

2.3.1 Origin of mutant lines

Lines carrying the *Pn-bd1*[2], *ra1*, *ra2* and *ra3* alleles were originally obtained from Dr Bruce Veit, Massey University, who had already begun intergressing them into the inbred background B73. Introgression of the mutant alleles into B73 was continued, so that the material here had been backcrossed into B73 five (*Pn-bd*, *ra1* and *ra2*) or two (*ra3*) times. Several sibling lines of *Pn-bd*::*mu* were also obtained from Dr Bruce

---

[2] Papyrescent glume architecture (*Pn*) is a dominant allele that was co-segregating with *bd1* in the *bd1* stocks obtained

Veit, Massey University. These stocks were derived from individuals identified from a large-scale non-targeted mutagenesis screen with the aim of inducing transposon-tagged mutations. Plants homozygous for *Pn-bd::mu* were identified in the F2 population and crossed to *Pn-bd*/+ to generate populations that were segregating 1:1 (*Pn-bd*/*Pn-bd::mu* : *Pn-bd*/+) intended for DNA co-segregation analysis for DNA cloning.

2.3.2 Maintenance of genetic stocks

As *bd1* and *ra1*ears are sterile, stocks were maintained as heterozygotes. By backcrossing pollen from a male parent homozygous for *bd1* and *ra1* onto B73 ears, heterozygous stocks were generated. These stocks were then self-pollinated the following generation to generate populations segregating homozygous recessives 1:3 (*Pn-bd1*/ *Pn-bd1* or *ra1*/ *ra1*: *wild type*) for backcrossing and morphological analysis. To generate seed for developmental analysis, pollen from homozygous recessive parents was crossed to the heterozygous stocks; the resultant population segregated 1:1 (*Pn-bd1*/ *Pn-bd1*: *Pn-bd1*/+ or *ra1*/ *ra1*: *ra1*/ +). To check that the development of the heterozygote was similar to the homozygote, families segregating 1:3 (*Pn-bd1*/ *Pn-bd1* or *ra1*/ *ra1*: *wild type*) were also analysed.

Both *ra2* and *ra3* were maintained as heterozygotes and homozygotes stocks as homozygous plants were able to produce viable seed. Pollen from plants homozygous for *ra2* and *ra3* were backcrossed to B73 ears and self-pollinated the following generation to generate populations segregating 1:3 (*ra2*: *wild type*) or 1:2:1 (*ra3*/*ra3*: *ra3*/+: +/+) as appropriate. These segregating populations were also used for backcrossing, developmental and morphological analysis. Sibling populations homozygous for *ra2* and *ra3* were also used for developmental analysis.

Several double mutant stocks were generated. The *ra1*/*ra1* *ra2*/*ra2* double mutant combination was made by crossing pollen from *ra1*/*ra1* males to *ra2*/*ra2* females to make the *ra1*/+ *ra2*/+ heterozygote that was self-pollinated the subsequent generation. Crossing pollen from *ra1*/*ra1*males to females heterozygous for *Pn-bd* made the *bd*/*bd* *ra1*/*ra1* double mutant combination. These were self-pollinated the following generation and only the seed from individuals expressing the *Pn* phenotype (*Pn-bd*/+ *ra1*/+) were harvested and used the subsequent generation. The *bd*/*bd* *ra2*/*ra2* and

*ra3/ra3 ra2/ra2*combinations were generated the same way as the *ra1/ra1 ra2/ra2* double mutant using *ra2/ra2* as the female parent. Progeny were then self-pollinated the following season. All were expected to segregate 1:3:3:9 (double homozygous: single homozygous: single homozygous: *wild type*).

2.3.3 Agronomy

Plants were grown in summer field nurseries and winter greenhouse nurseries. Husbandry was based on procedures established by Neuffer (1994) and adapted by Allan Hardacre (Maize breeder, Crop and Food Research New Zealand Ltd.).

2.3.3.1 Field Nursery

Six weeks or more prior to planting the field was ploughed in from fresh pasture to remove any Argentine Stem Weevil. Immediately prior to the target planting date of October 20th the field was cultivated with a rotary hoe to a 10cm depth and 300 kg/ha of Nitrophosca 12/10/10 (Ravensdown Ltd.) and 150kg/ha of Urea (Ravensdown Ltd.) per hectare was applied. A springtine cultivator was then used to score lines 75 cm apart in a north-south and east-west pattern. 5m ranges with 1.5m alleys were staked out north south with 90 rows west east per range. Previously packaged labelled manila wage envelopes (Warehouse Stationary Ltd.) containing 20 seeds each were laid out one per row in order. These were then planted 25cm apart at a depth of 2.5cm using jab planters borrowed from Allan Hardacre (Crop and Food Research New Zealand Ltd.). Roundup G2 (Monsanto Ltd.) and Alochlor (Dow Agrosciences) were applied at 7L and 3.5L per hectare respectively immediately post planting to suppress broadleaf weeds. Any remaining weeds were removed by hand. On several occasions it was necessary to irrigate using 20m soaker hoses (Mitre10 Hardware Ltd.) to minimise drought stress.

2.3.3.2 Off-season nursery

An off-season nursery was used to generate material for developmental analysis and generation of new segregating populations for backcrossing and analysis. Kernels were sown in standard greenhouse potting mix; 100L pumice, 100L soil, 0.9kg osmocote (6-9month), 1kg Dolomite, 0.2kg Superphosphate, 40g Calcium Ammonium Nitrate, 40g FTE, 60g 5month coated iron, 100g Potassium Sulphate, 20g

Terrozole in 1.8L P18 plastic bags and grown in the greenhouse. Supplemental light provided 16h days.

2.3.3.3 Pollination

Concurrent with tassel emergence, the best plants expressing the desired phenotype per row were selected and a clear plastic bag 8cm x 18cm (CarterHolt Packaging Supplies) was placed over the ear. The clear plastic bag allows the direct observation of the ear for the determination of silk emergence. Once the silks emerged, pollen from the male parent was collected in a heavy-duty no.1 brown paper bag (CarterHolt Packaging Supplies). The male parent and the pollination date were recorded on the paper bag with a 2B pencil. Removing the plastic ear bag exposed the silks allowing the pollen to be dusted on. The paper bag was then placed over the ear and stapled around the stem (8mm No.1 stapler from Warehouse Stationary Ltd.).

2.3.3.4 Harvesting

Seed was harvested at physiological maturity signified by the appearance of a black layer on the hilum. Paper bags were removed from the plants and the row and female phenotype recorded on the bag. Husk leaves enclosing the ear were then removed and the ear placed in the corresponding paper bag. The paper bag were then stapled twice at the top and placed in an oven at 30°C until the grain at 14% moisture content. The grain was then hand shelled into labelled paper bags and stored at 4°C.

2.3.4 Morphological Analysis

Two sibling populations containing the single allele *bd*, *ra1*, *ra2*, and *ra3* that were segregating 1:3 (homozygous recessive: *wild type*) or a single population double mutant combinations segregating 1:3:3:9 (double homozygous recessive: single homozygous recessive: single homozygous recessive: *wild type*) were planted in blocks of 100 and 200 individuals respectively and grown till anthesis. A third population of *bd* from the co-segregation analysis was also included. As the *bd*/*bd* phenotype in the B73 background produced sterile tassels in the years that it was grown. B73 is an inbred from the United States that matures later than optimal for the New Zealand environment. Work has started to inbreed the alleles into locally adapted

short season inbreds provided by the local maize breeder Allan Hardacre (Crop and Food Research New Zealand Ltd.).

In each population, individuals were scored on the basis of their tassel and ear phenotype at anthesis. A subsample of 10 plants of each phenotype was taken from each population for closer analysis. A number of measurements were made, summarised in tables in appendix III.

Plant height is the distance from the ground to form the uppermost leaf-bearing node.

Peduncle length is the distance form the uppermost leaf-bearing node to the node at which the lowermost branch arises (Nickerson and Dale, 1955).

Tassel length is the distance from the node from which the lowermost branch arises to the tip of the central culm (Nickerson and Dale, 1955).

Primary branches are axes of the secondary order, when the main culm is considered to be an axis of the first order (Nickerson and Dale, 1955).

Secondary and *tertiary branches* are therefore axes of the third and fourth order respectively.

Indeterminate spikelet pairs were determined as indeterminate axes that produced single spikelets in an alternate phyllotaxy (Figure 2-1).

Branch area and *indeterminate spikelet pair area* are the distances along the central culm from the lower to uppermost branch of that type and is included in the tassel length (Nickerson and Dale, 1955). The percentage of the tassel of a particular branch type was calculated from the area of the branch type divided by the respective tassel length.

Three nodes along the tassel axis were analysed in detail. These were 1/4, 1/2 and 3/4 of the tassel length from the lowermost primary branch and are termed *T1*, *T2* and *T3* respectively (Figure 2-2). At each node the type of structure was recorded, it's length, the number of spikelet pairs and spikelets on its axis. The first spikelet pair on the axis was also analysed for the:

Figure 2-1 Primary branch and indeterminate spikelet pair.

(A) is an indeterminate primary branch with spikelet pairs in an alternate phyllotaxy. (B) is an indeterminate spikelet pair with single spikelets in an alternate phyllotaxy.

Spikelet pair axis length was the distance from the central culm to either the pedicel of the sessile spikelet or the tip of indeterminate spikelet pair.

1st spikelet axis length and the *2nd spikelet pair axis length* was the distance from the spikelet pair axis to the lowermost glume of the basal most and second basal-most spikelets respectively.

Glumes were determined to be sterile bract-like structures that enclosed florets in the spikelet.

Florets consisted of a palea and lemma that enclosed a number of floral organs.

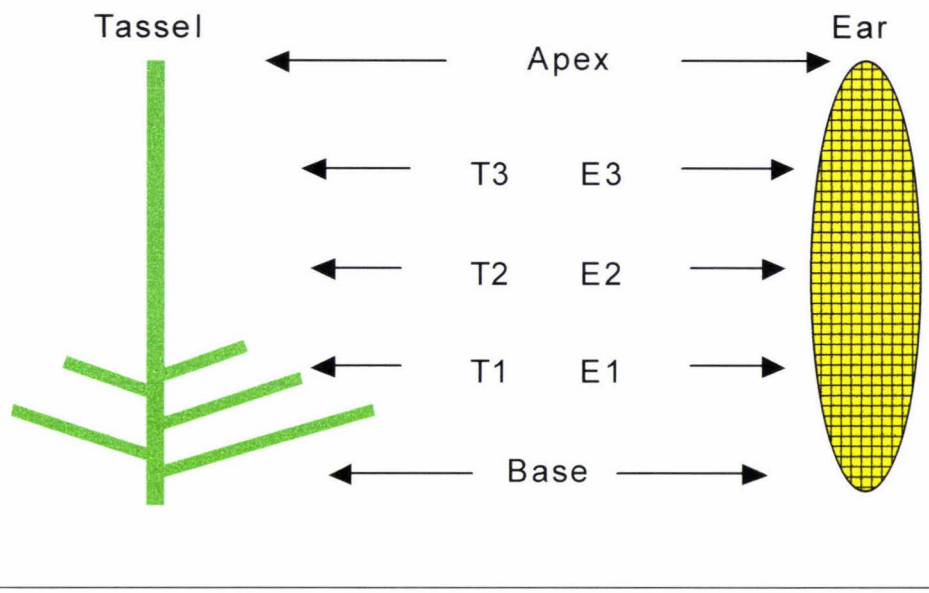


Figure 2-2 Position of sampling points on maize inflorescences

The female ears were analysed in a manner similar to the male tassel. The *length of the ear* was measured from the base of the inflorescence axis to its apex. Similarly, the *mass of the ear* was weighed and recorded. As with the tassel, the *number of primary branches* to which the *number of spikelet pairs around the inflorescence axis* was added. The *type of branch* and the *length of the primary branch* were recorded along with an estimation of the *percentage of functional silks*.

The ear was analysed at three nodes, *E1*, *E2* and *E3* (Figure 2-2), at 1/4, 1/2 and 3/4 the *ear length*, respectively from the base of the inflorescence. The same measurements as at the tassel nodes were carried out under the same criteria with the exception, that the floret contained a functional gynoecium.

The data was entered directly into Microsoft Excel (Microsoft Corporation) before being transferred to SPSS for Windows V9.0 (SPSS Inc.). All charts and data analysis produced using SPSS for Windows V9.0 and then exported to Microsoft Word (Microsoft Corporation).

2.3.5 Developmental Analysis

The samples used in the developmental analysis came from several batches of plants grown in the field and greenhouse at different times of year. Once plants had completed vegetative growth, determined from the periodic sacrifice and dissection of a few representative samples, sample tassels and ears were collected over several weeks from a population of sibling plants. Genotypes of the plants were inferred from the morphology of the tassel in segregating populations. Inflorescences were isolated by peeling away all of the enclosing leaves. Once the innermost leaf was removed, either molds of the inflorescence were made using dental impression material or the plants were placed in fixative for light microscopy and critical point drying.

2.3.5.1 Tissue Preparation

2.3.5.1.1 Replica technique

Molds were made of one side of the freshly dissected inflorescence by placing the inflorescence in dental impression material (Kerr Extrude; polyvinyl siloxane impression material. Type 2: medium consistency-medium bodied, Kerr Manufacturing Co., USA). When the molds had hardened, the inflorescence was removed and filled with glue (Contax modellers glue, Revell) being careful to remove as many bubbles as possible and allowed to set overnight. The casts were then removed from the mold and mounted on stubs with conductive silver paint (Dick Smith Electronics), sputter coated (E5100 SII cool sputter coating unit, Polaron Equipment Ltd.) for 4min with gold/palladium, and viewed with a Cambridge 250 Steroscan MkIII Scanning Electron Microscope (HortResearch New Zealand Ltd.) at 15kV alternating voltage and photographed with Ilford FP4 film. The advantage of using glue replicas was that cell shrinkage was minimal to that observed using standard critical point drying protocols. The disadvantage was the complexity of structures meant that the molds could not always be completely filled and any leaf exudate deposited during dissection, if present, was also preserved as an artifactual surface feature.

2.3.5.1.2 Critical Point Drying

Samples for critical point drying were placed in histological cassettes and left in fixation solution overnight at 4°C. Samples were not treated with Osmium on advice of Doug Hopcroft (Electron Microscope Unit, HortResearch). The cassettes were then removed from the fixative and washed twice for 1hr per wash in 0.05M Sorenson's buffer at pH7.0 before being dehydrated in a graded ethanol series to 100%. The samples were then critical point dried and mounted on stubs with conductive silver paint (Dick Smith Electronics), sputter coated (E5100 SII cool sputter coating unit, Polaron Equipment Ltd.) for 4min with gold/palladium, and viewed with a Cambridge 250 Steroscan MkIII Scanning Electron Microscope (HortResearch.) at 15kV alternating voltage and photographed with Ilford FP4 film. The advantage of critical point drying was that heavily branched inflorescences could be used. However, the disadvantage was that there was considerable cell shrinkage in the critical point dried samples.

2.3.5.1.3 Light Microscopy

Specimens were dissected fresh from the field and placed on a Leica binocular microscope with a camera attachment. The specimens were then photographed with Agfa 200asa Black and White film, which was commercially printed.

2.3.6 Photography

Colour photography of the different structures was done using Kodak Gold 200asa 35mm film. Kodak 200asa T Max and was used for black and white photography. All morphological photography was carried out using a standard 35mm SLR camera (Minolta) with magnifying filter lenses for close-up shots. The photographic prints were scanned into a computer using a Hewlett Packard 4C Scan Jet at 600x600dpi and the resulting images were edited with Abode Photoshop 5.0 (Abode Systems Inc.).

3 Analysis of Maize Inflorescence Morphology

3.1 Wild type inflorescences morphology

The morphology of maize inflorescences has been described numerous times in various degrees of detail (Weatherwax, 1916; Arber, 1934; Evans, 1940; Bonnett, 1948; Nickerson and Dale, 1955; Veit et al., 1993) but for completeness will be reviewed here.

3.1.1 *Wild type* male tassel morphology

The tassel of *wild type* maize at anthesis consists of a thin main rachis (central spike) with several long thin primary branches (rachids) that are restricted to the base of the male inflorescence (Figure 3-1). Spikelet pairs (Figure 3-3) are arranged in four vertical rows on the sides of the tassel and two vertical rows on branches. Each pair of spikelets consists of one pedicelate and one sessile spikelet distinguishable by the length of their pedicels.

Spikelets form the basic unit of the grass inflorescence (Clifford, 1987). A pair of overlapping glumes (sterile bracts) encloses two staminate florets, arranged in an alternate phyllotaxy around a short axis (rachilla). The axis between the glumes and the central rachis is known as the pedicel. Together the pedicel and rachilla combine to form the spikelet axis. Superior to the glumes are two lemmas (fertile bracts). From the axil of each lemma, a short reproductive shoot arises, subtended by an adaxial bikeeled palea (the first lateral appendage produced on an axillary shoot, which is considered homologous to the prophyll; Galinat, 1959). Each lemma and its palea together enclose the floral organs, two lodicules and three stamens, completing the male floret.

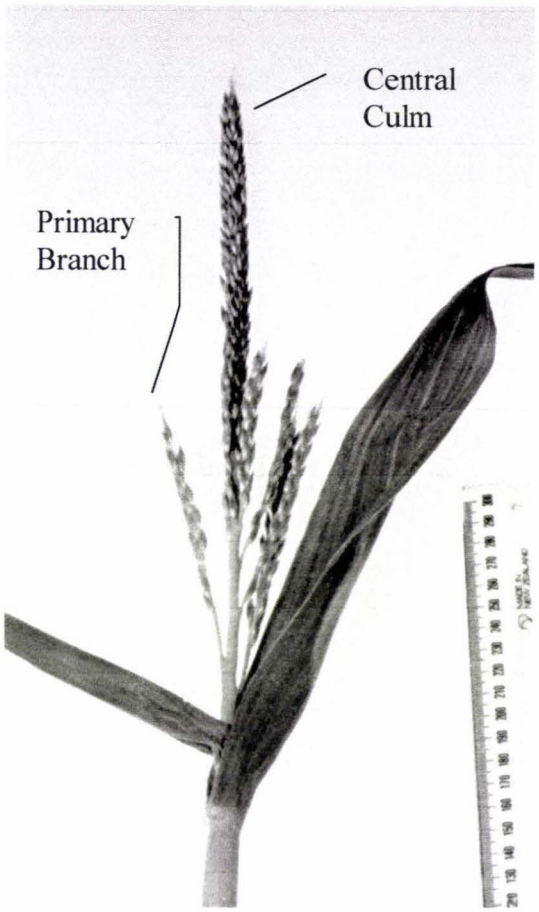


Figure 3-1Maize tassel

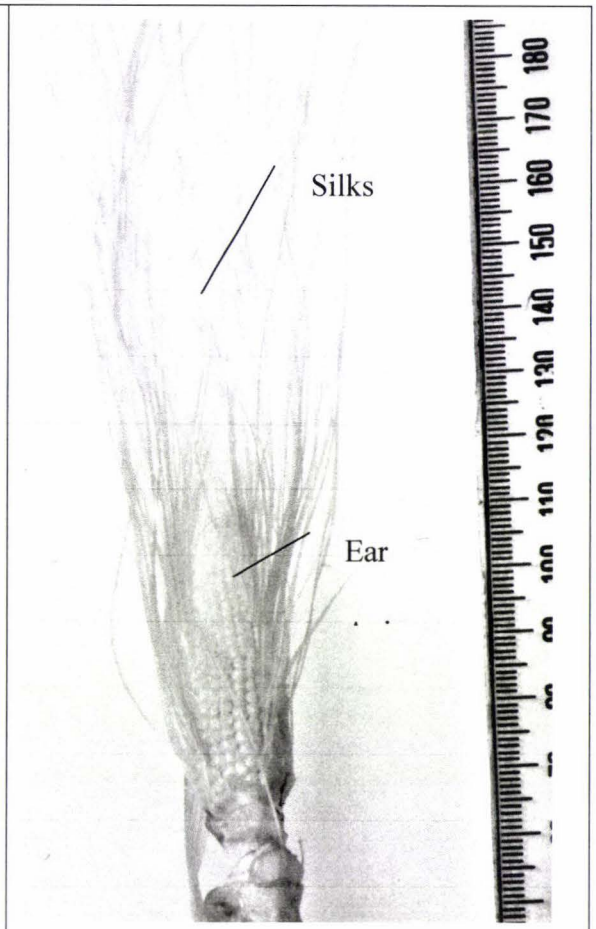


Figure 3-2 Female ear

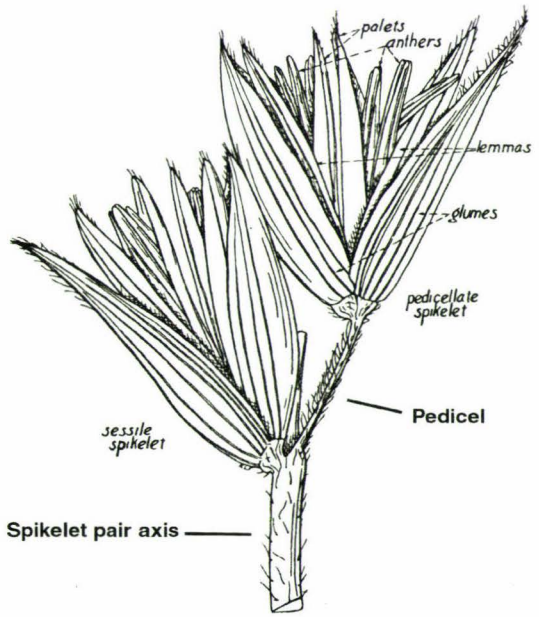


Figure 3-3 Male spikelet pair (Robbins, 1924)

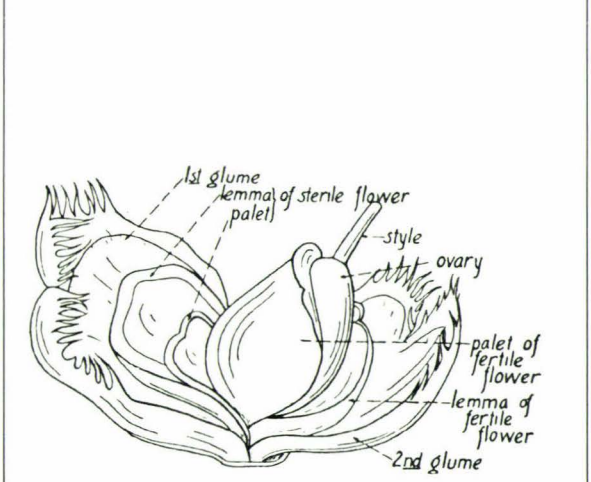


Figure 3-4 Female spikelet (Nees, from Robbins (1924))

3.1.2 *Wild type* female ear morphology

The mature ear of maize (Figure 3-2) differs from the tassel in several respects. Firstly there are no basal branches and the main rachis is thick with little distinction between pedicelate and sessile spikelets. Secondly, though the organisation of the spikelets is the same (Figure 3-4), there are still a number of differences in the morphology. The growth of the bracts (glumes, paleas and lemmas) is normally restricted. However, in some mutant backgrounds, such as *Papyrescent glume* (*Pn*) and *Tunicate* (*Tu)* a tassel like pattern of glume development appears. Although two florets are initiated, only the upper floret develops with the lower aborting early in development. However, some special lines like Country Gentleman have two functional florets (Weatherwax, 1916). The number and arrangement of parts is similar to the staminate spikelet but with the substitution of a functional pistil and abortion of the stamens and pistils (Weatherwax, 1916).

3.2 Ramosa-1 tassel and ear analysis

Gernert (1912) originally described the *ra1* phenotype under the name *Zea ramosa*. The characteristics seen in our, *ra1* stocks (Figure 3-5) were consistent with previous descriptions, with a cone-shaped tassel that was slightly smaller than normal and invariably more branched. The ear (Figure 3-6) was profusely branched and resembled the tassel with numerous pistillate florets (Postlethwait and Nelson, 1964). Kempton (1921) reported upwards of 400 branches in the tassel with the branches decreasing in size acropetally with the transition from branches to spikelet pairs being imperceptible. Moreover, Gernert (1912) found *ra1* ears to be similarly branched with masses of kernels borne on numerous irregular branches. Furthermore, Nickerson and Dale (1955) reported two other features: (1) branches in the *ra1* ear are about as numerous and have the same whorled arrangement as those in the tassel; (2) there are no adaxial cupules at the junctions of ear branches and the central culm. However, cupules were present, adaxial to each spikelet pair of the branches.

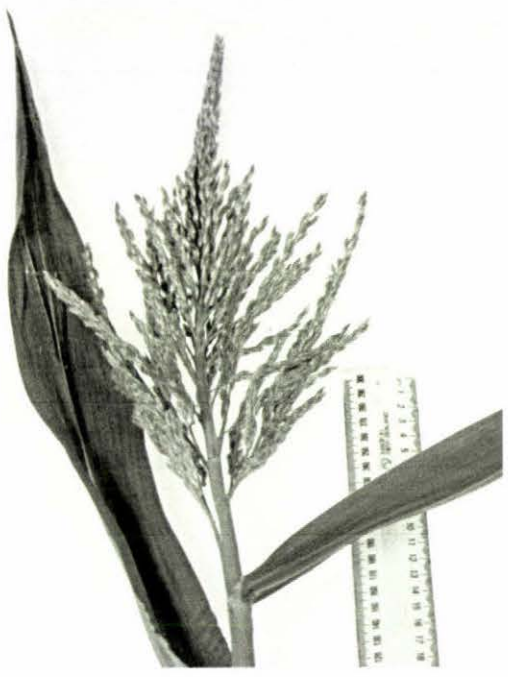


Figure 3-5 *Ramosa*-1 tassel

Figure 3-6 *Ramosa*-1 ear

However, there is some disagreement between Collins (1917) and Nickerson and Dale (1955) about the penetrance of *ra1*. Collin's was unable to distinguish any plants as heterozygous. Though Nickerson and Dale reported plants of a phenotype intermediate between the pyramidal paniculate tassel and a normal one, concluding that these were heterozygous for *ra1*. The penetrance of the *ra1* phenotype is most likely dependent upon the genetic background. Furthermore, no intermediary phenotypes were observed in the two-*ra1* populations examined, where the tassels segregated into two distinct classes based on branch and indeterminate spikelet pair area (Figure 3-7 and Figure 3-8). In contrast, tassels of *ra3* heterozygotes tassel tended to be intermediate between the homozygote and *wild type* classes based on the same criteria (Figure 3-9). Moreover, the presence of heterozygotes effects is likely to be dependent on genetic background.

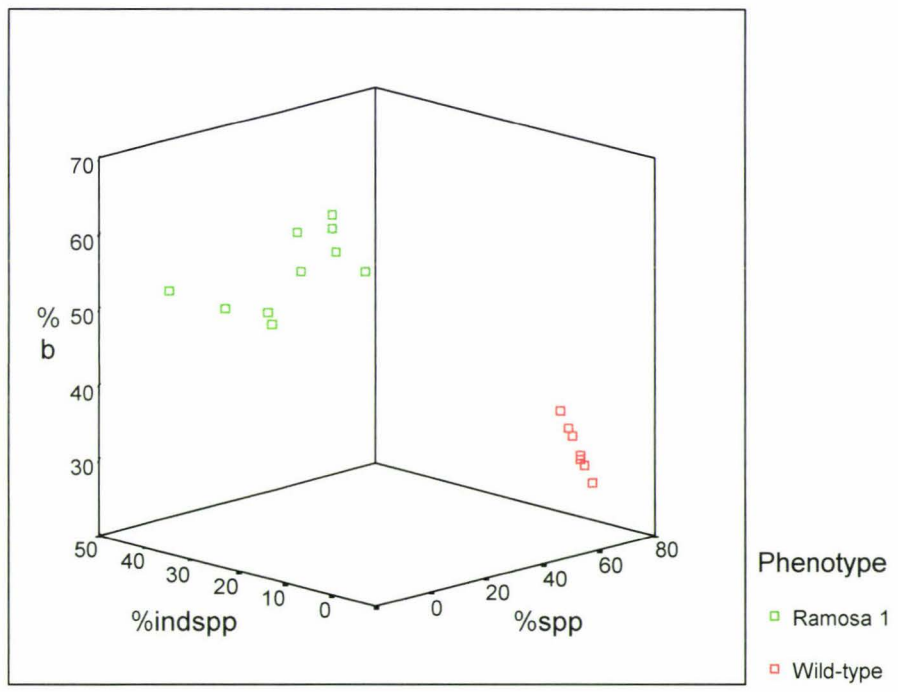


Figure 3-7 3D scatterplot of tassel percentages in *ra1* family 191

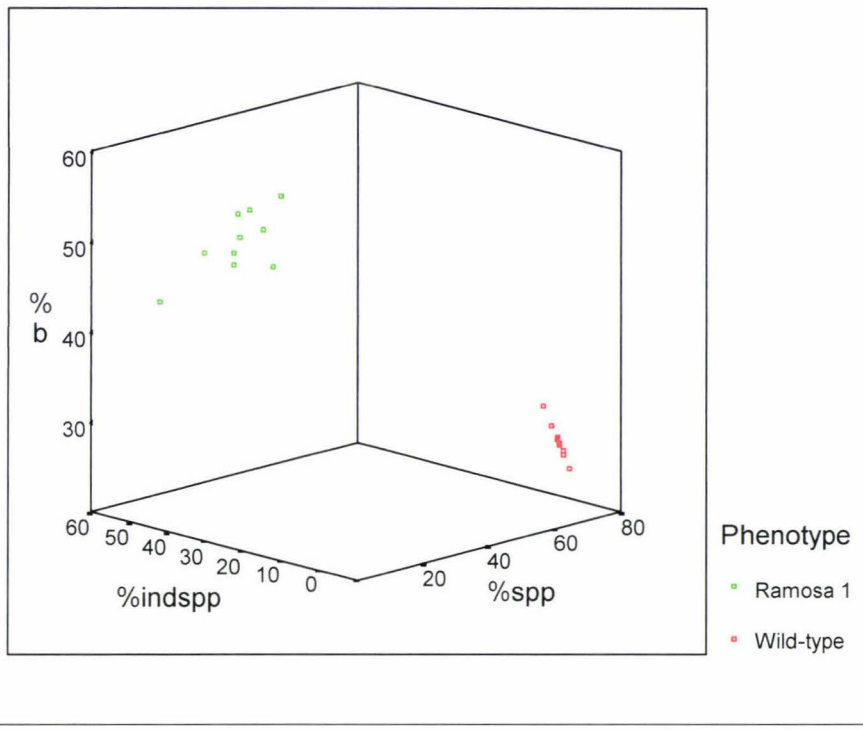


Figure 3-8 3D scatterplot of tassel percentage in *ra1* family 201

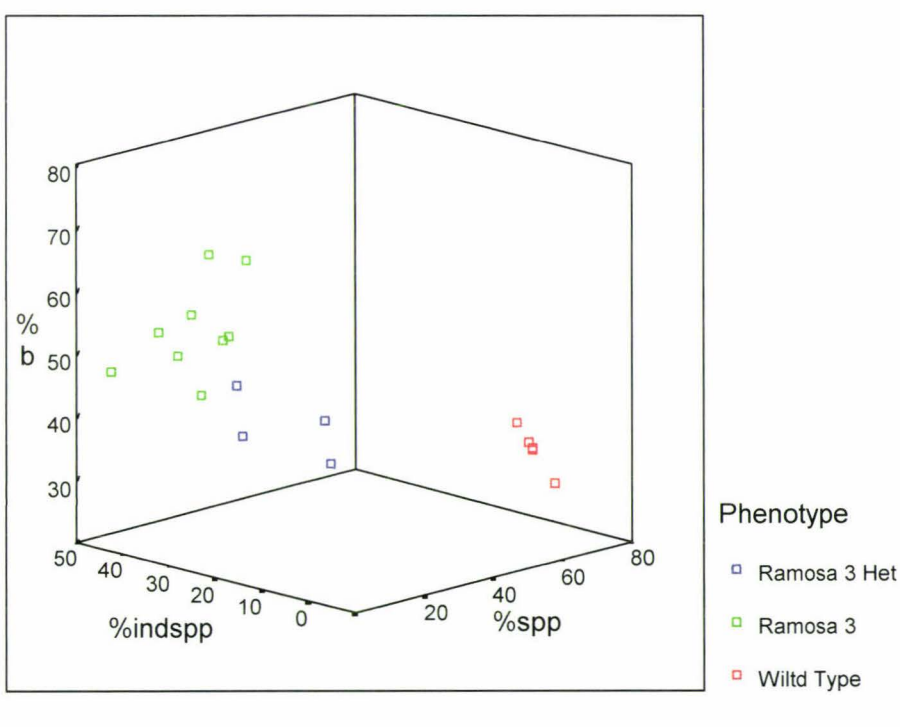


Figure 3-9 3D scatterplot of tassel percentages in *ra3* family 251

3.2.1 Primary axis morphology

The mean length of *ra1* plants, from the ground to either the tip of the tassel or last vegetative node, was greater than that of their normal siblings (Table III-1, Table III-2). Furthermore, in Figure 3-10 (a definition of boxplots is included on page 109) the median of the *ra1* population is very close to the upper quartile of the normal siblings. The distribution of family 201 was skewed towards the higher values, signified by smaller spread of values above the median. However, the distribution of family 191 was more evenly spread indicated by the symmetry of the *ra1* boxplot and therefore was more normally distributed. Though larger sample sizes are needed for more accurate statistical analysis, sufficient data was obtained to conclude that on average *ra1* plants were taller than their *wild type* siblings.

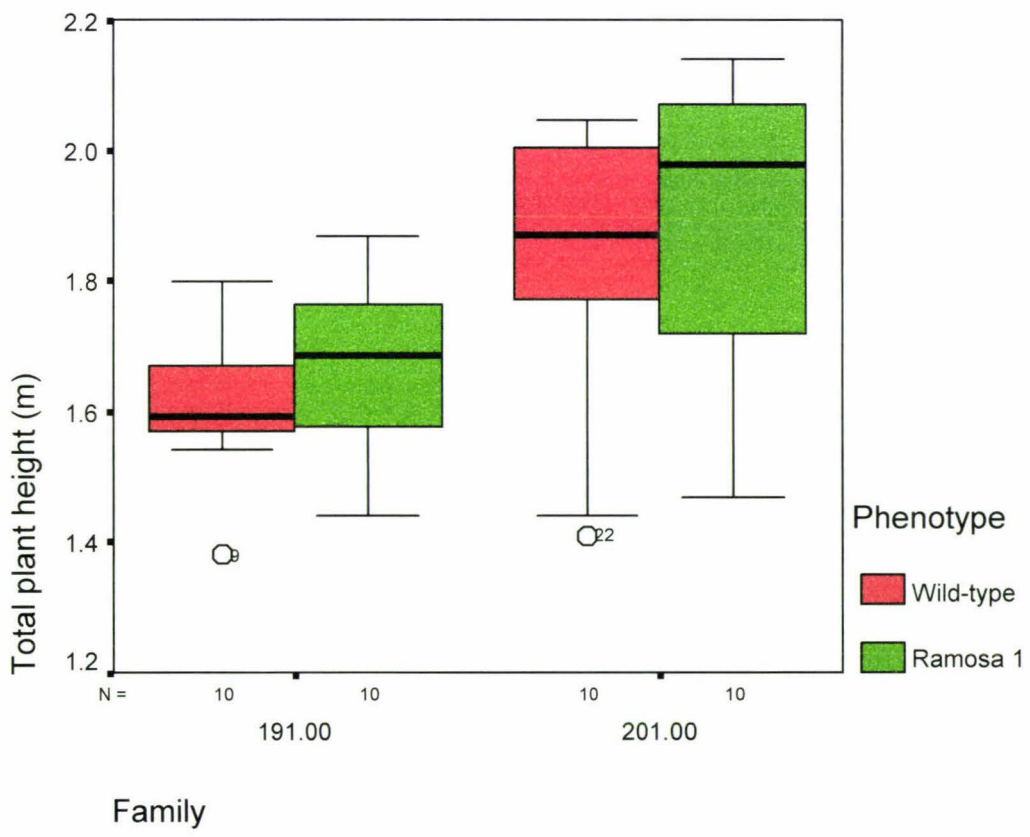


Figure 3-10 Boxplots of the total plant heights for *ra1* and *wild type* siblings

The total plant height is composed of three parts: *plant height*, distance from ground to last vegetative node; *peduncle length*, distance from last vegetative node to the first primary branch; and *tassel length*, distance from first tassel branch to the tip of the tassel. Of these three characters, only plant height was significantly greater in *ra1* plants. In both populations, the median plant heights of *ra1* in both families were above the upper quartile of their siblings as shown in Figure 3-11. In contrast the mean peduncle length of *ra1* plants was less than their normal siblings (Figure 3-12).

Both Kempton (1921) and Nickerson and Dale (1955) reported a short central spike as characteristic of *ra1*. Collins (1917), however, did not mention this. A shortening of the central spike was observed in the *ra1* families used for this study (Figure 3-13). Moreover, the combined peduncle and tassel length was reduced in the *ra1* populations (Figure 3-14). Therefore, the effect of *ra1* on the primary axis is two-fold; the vegetative axis length is increased and the peduncle and tassel length decreased.

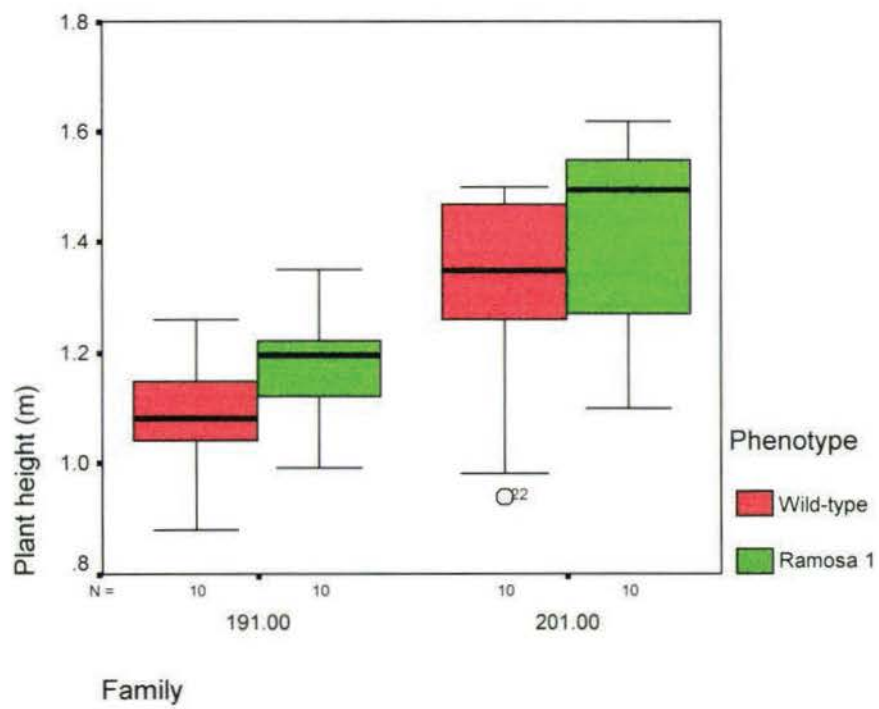


Figure 3-11 Boxplots of vegetative plant height for *ra1* and *wild type* siblings

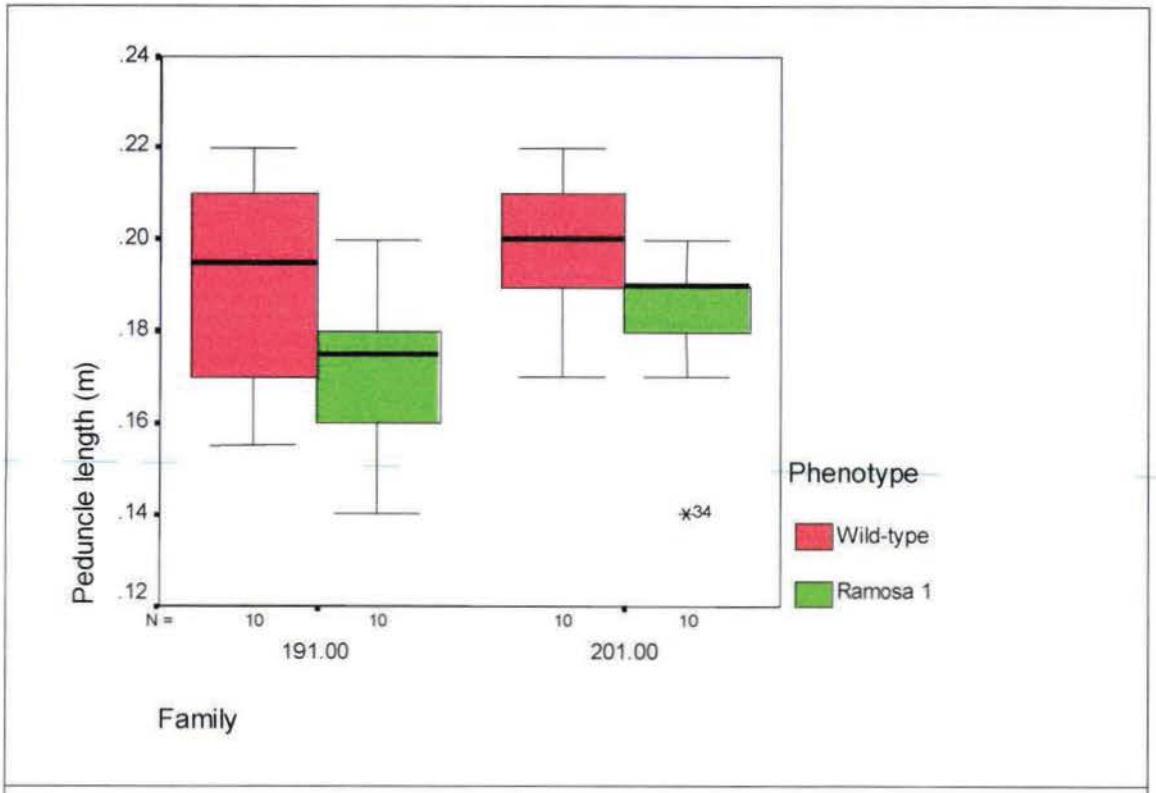


Figure 3-12 Boxplots of peduncle length for *ra1* and *wild type* siblings

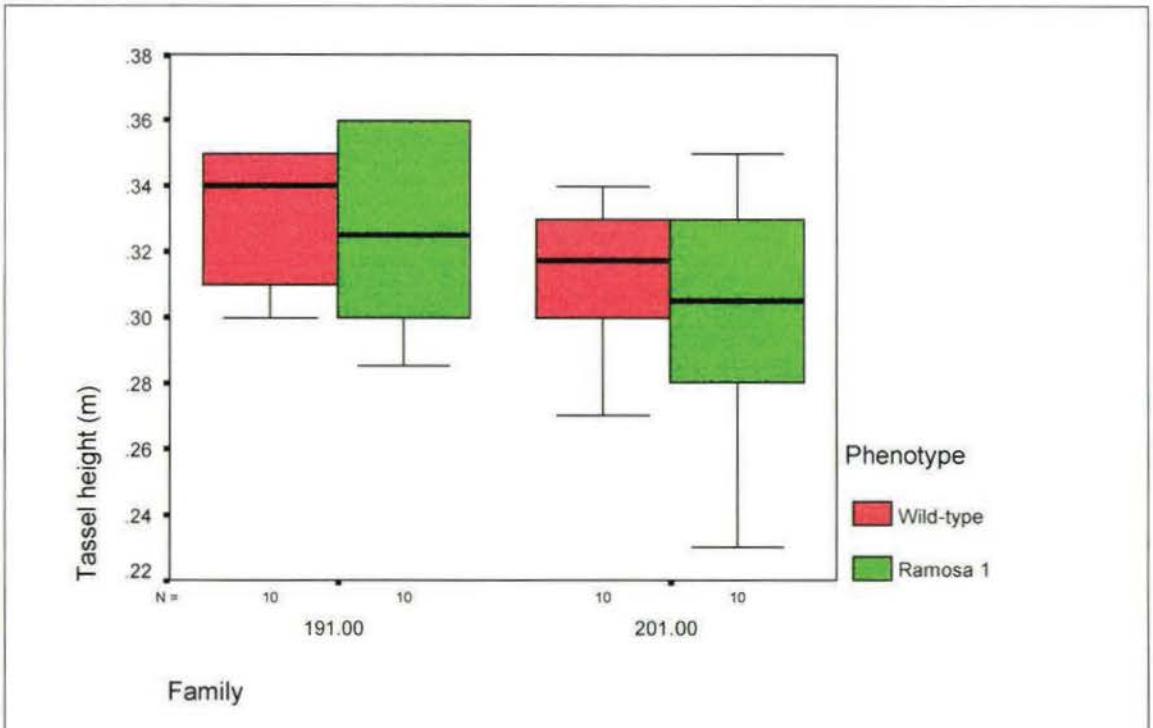


Figure 3-13 Boxplots of tassel height for *ra1* and *wild type* siblings

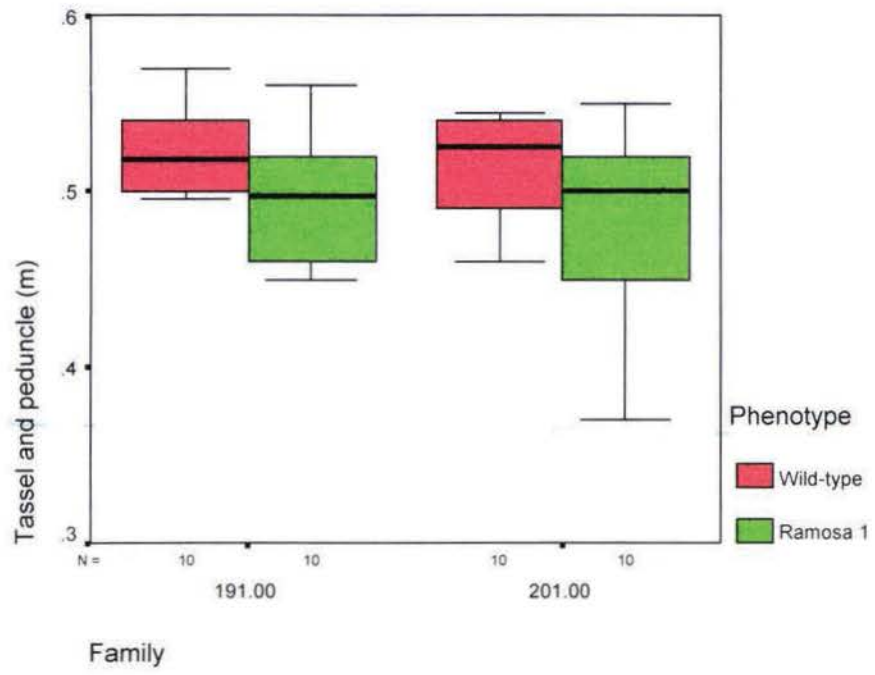


Figure 3-14 Boxplots of tassel and peduncle length combined for *ra1* and *wild type* siblings

3.2.2 Tassel Characters

In the *ra1* populations, a significant increase in primary branch number was observed (Table III-3 and Table III-4). In both families the branch number was greater than twenty, an arbitrary threshold, which was significantly greater than normal. Concurrent with the massive increase in primary branches, a similar increase in branches of higher orders was observed (Figure 3-15 and Figure 3-16). For example the first primary branch is normally unbranched or has only one or two primary branches (Figure 3-16). However, the first primary branch in *ra1* populations will have on average five to seven secondary branches depending upon the vigour of the plant (Figure 3-15).

A distinct pattern in the branching was also observed as shown in Figure 3-17 that shows the basic architectural pattern found in *ra1* tassels. Like Collins (1917) a gradient of branches with decreasing size was observed upwards. However, a number of indeterminate spikelet pairs were observed between the last branch on an axis and a proper spikelet pair. Moreover, the axis of the indeterminate spikelet pairs decreased in length acropetally like the primary branches.

Figure 3-15 *Ramosa*-1 tassel primary branch

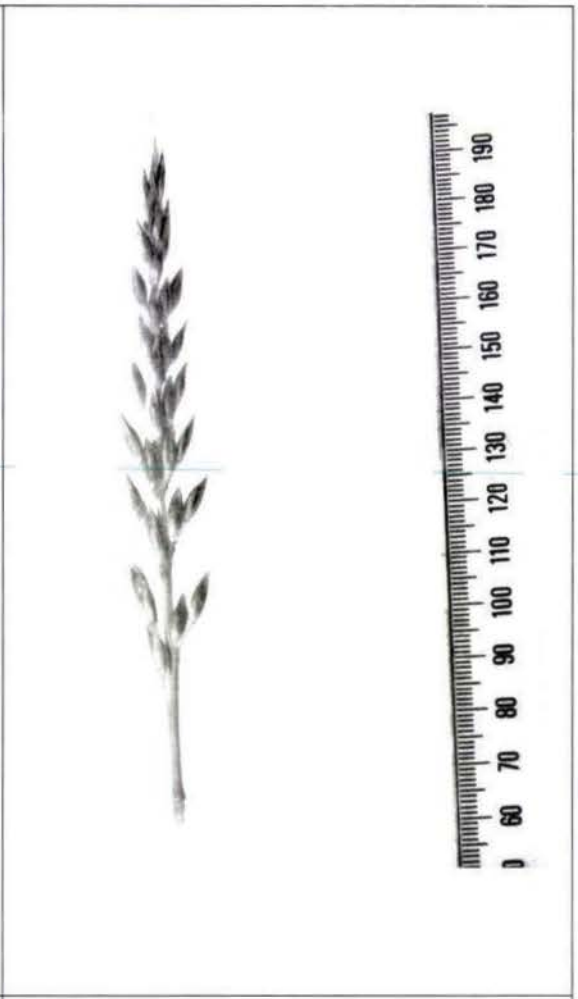


Figure 3-16 Normal tassel primary branch

A simultaneous acropetal shortening of indeterminate spikelet pair axes and decrease in spikelets number per axis was observed, until only two spikelets per axis were observed towards the tassel apex. This decrease in indeterminate axis length combined with the acropetal decrease in branch length results in the conical shape of the *ra1* male tassel.

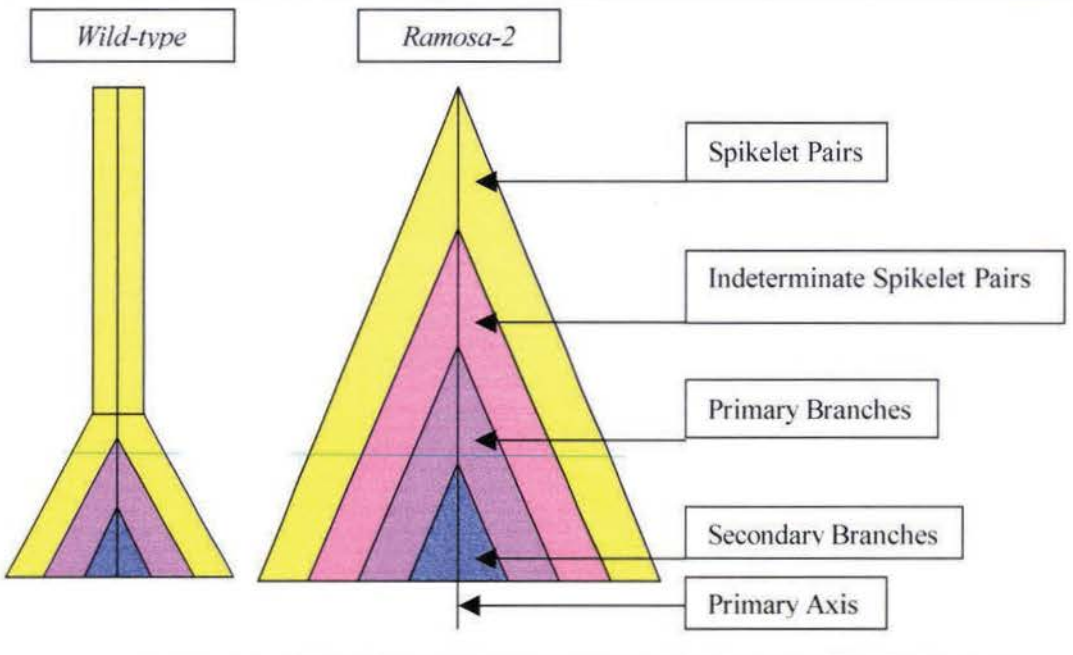


Figure 3-17 Diagrammatic representation of branch types in *wild type* vs. *ra1* inflorescences

The *ra1* tassels had three types of nodes: branches, indeterminate spikelet pairs and spikelets pairs. In contrast *wild type* tassels have only branches and spikelet pairs. Furthermore, the relative ratios of the central culm in the tassel that was occupied changed in the *ra1* phenotype (Figure 3-18). First, the area of primary branches almost doubled relative to the normal siblings to approximately 50% of the total tassel length shown in Figure 3-18. Moreover, indeterminate spikelet pairs that are absent in the wild type tassel comprised a further 30% of the *ra1* tassels distal to the area of primary branches. The net result was a reduction of the total spikelet pair area from around 3/4 of the total area in wild type tassels to approximately 1/8 of the tassel area in *ra1*.

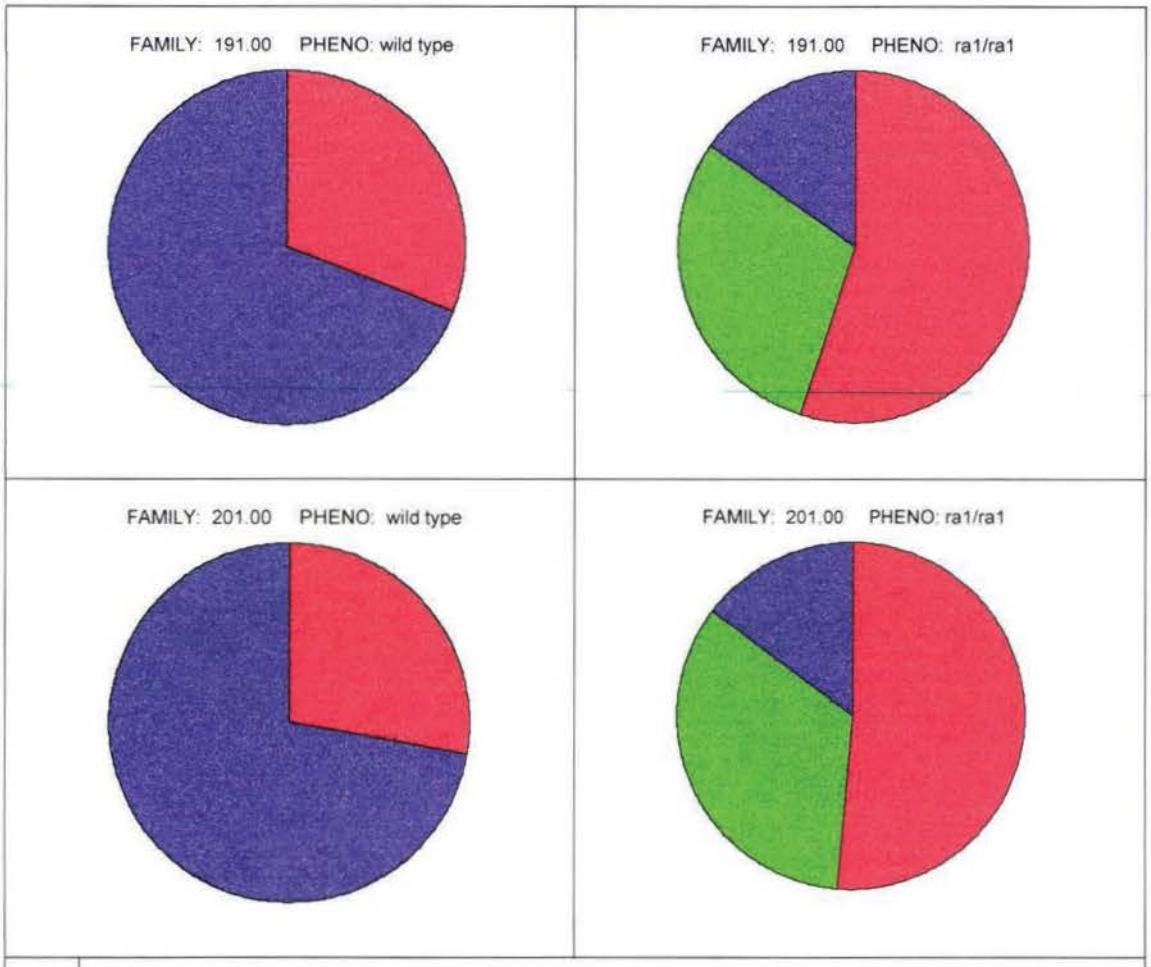


Figure 3-18 Pie charts of Tassel area in *ra1* and *wild type* siblings

The use of relative area in tassels provides a quick and simple method to estimate the effect of the *ra1* and other phenotypes on the branching and their distribution. Admittedly, it is not as accurate as counting the individual branch types but is much quicker for the analysis of large populations. The first draw back of using branch area is that the transition between the different branch types is not clear-cut with often one type of branch area being longer on one side of the tassel relative to the other. Moreover, the spacing between the nodes is not constant and decreases acropetally,

resulting in more spikelet pairs per unit area at the apex compared to lower down on the central axis. However, the estimation of relative branch area is useful not only for the comparison within a family but also between families as it is based on the ratio of branch area divided by the tassel length, thereby removing any bias based on segregation of factors that determine tassel length. A similar division of the branch number divided by the total number of nodes would enable a similar comparison but would be time consuming as there is often more than one hundred and fifty nodes on each inflorescence axis that would need to be individually counted. Therefore, the use of relative branch areas is an excellent tool for documenting the effect of branching mutants on the determination of different meristem types.

3.2.2.1 Analysis of T1 in *ramosa-1* tassels

At T1, branches are produced in both *ra1* and wild type sibling populations with no major differences between the two phenotypes (summarised in Table III-5 and Table III-6). However, several outliers exist where the extra spikelets or floral parts were present. These were not common. The extra spikelets in the first spikelet pair at T1 were due to the presence of a single secondary indeterminate spikelet pair while the increased mean of *ra1* florets and their parts in the second spikelet was also due to a single sample with multiple florets that sporadically occur.

There was one significant difference with *ra1*, the length of the first spikelet pair axis, which was considerably larger than those in the normal siblings. Figure 3-19 shows the consistent difference between the *ra1* and wild type populations spikelet pair axis lengths. In turn this influences the total spikelet pair axis length (Figure 3-20). No other significant differences could be determined at node 1 in *ra1* tassels.

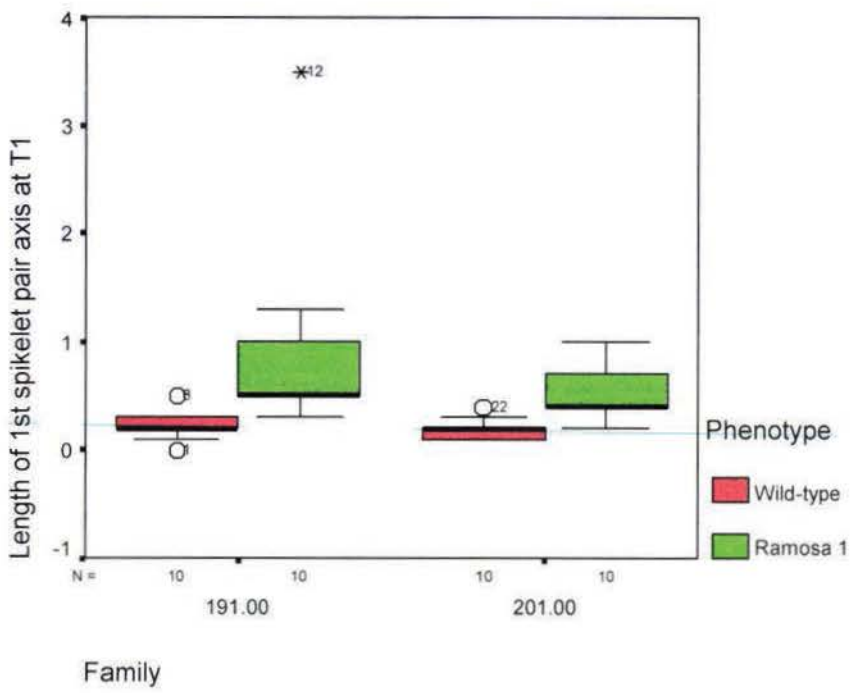


Figure 3-19 Boxplots of spikelet pair axis lengths in *ra1* and *wild type* siblings

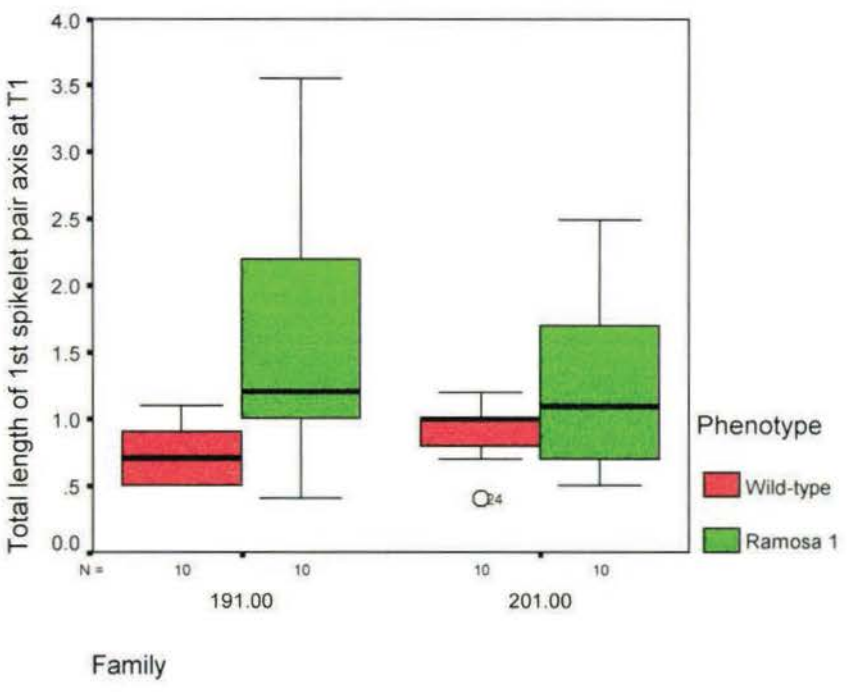


Figure 3-20 Boxplots of the total length of the 1st spikelet pair axis at T1 in *ra1* and *wild type* siblings

3.2.2.2 Analysis of T2 in *ramosa-1* tassels

At T2, both intermediate and full branches occur in *ra1* populations as opposed to the spikelet pair's of *wild type* populations (Table III-7 and Table III-8). These branches are shorter and have fewer spikelet pairs than the branches at T1 (Table III-5 and Table III-6). The presence of indeterminate spikelet pairs in *ra1* populations at T2 accounts for the increase in the number of spikelets (Table III-7 and Table III-8) that was observed. Furthermore, the indeterminate nature of the spikelet pairs also resulted in a dramatic increase in the total size of the spikelet pair axis of the *ra1* population in family 201, while the *ra1* population in family 191 exhibited a much smaller difference. In both families the length of the *wild type* spikelet axes were significantly greater than those of the *ra1* sibling populations. There appears to be a negative correlation between the spikelet pair axis length and the spikelet axis length that probably demonstrates the effect of apical dominance and resource availability.

3.2.2.3 Analysis of T3 in *ramosa-1* tassels

Both populations of *ra1* produce indeterminate spikelet pairs at T3 (Table III-9 and Table III-10). These indeterminate spikelet pairs produce on average twice as many spikelets as the normal siblings on an axis approximately three times the length of the *wild type* siblings. The spikelet pair axis below the first spikelet axis is correspondingly increased. The length of the first spikelet axis in *ra1* plants is also on average three times as great as that observed in the normal siblings, though this reverses in the second spikelet axis. Finally, the variation in the second floret axis was due to a single outlier in each family. Otherwise, the number of glumes and florets produced per spikelet is the same for *ra1* and *wild type* plants in family 191 and 201.

3.2.3 Ear Characters

Compared to the *wild type* siblings, the ears of *ramosa*-1 plants are highly branched and under-developed with their length and mass being significantly smaller (Table III-11 and Table III-12). The base of the ear is highly branched, however, spikelet pairs are present and short silks produced. Immediately above basal branches, there is a transition to indeterminate spikelet pairs, much like the tassel. The spikelets at the

base of the indeterminate spikelet pairs may produce short silks (Figure 3-21). These silks however, fail to elongate past the husk leaves; however, they remain functional, and as careful hand-pollination will produce a few viable kernels.

3.2.3.1 Analysis of E1 in *ramosa-1* ears

Characteristically *ra1* has full branches with many undeveloped spikelets at E1 compared to spikelet pairs of wild type sibling populations (Table III-13 and Table III-14). Moreover, the axis of these spikelet pairs and the spikelets that they contain are much reduced compared to their more developed siblings. Failure of the *ra1* spikelets to develop also resulted in fewer florets being observed in each spikelet.

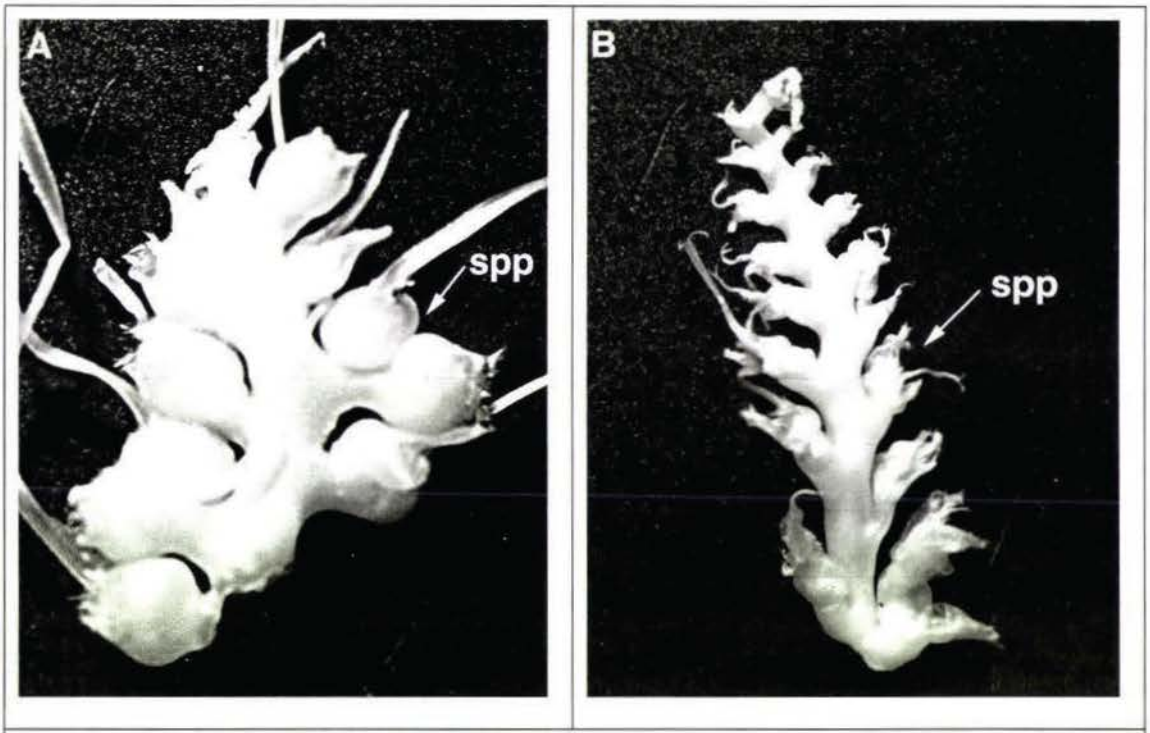


Figure 3-21 Fertile and sterile female *ramosa-1* primary branches in *ra1* and *wild type* siblings

3.2.3.2 Analysis of E2 in *ramosa-1* ears

Indeterminate "spikelet pairs" with more multiple spikelets in an alternate phyllotaxy on an indeterminate axis replace the spikelet pairs in *ra1* ears at E2 (Table III-15 and Table III-16). The spikelets that are produced are determinate but are still much smaller than those of the wild type siblings. The indeterminate nature of the spikelet pair results in the length of the spikelet pair axis being significantly longer compared

to its siblings with a corresponding increase in the number of spikelets. These spikelets, however, are too small to count due to their under-developed nature.

3.2.3.3 Analysis of E3 in *ramosa-1* ears

The *ra1* indeterminate spikelet pairs at T3 are almost identical to those at E2 except smaller, shown by the spikelet pair axis length in Table III-17 and Table III-18. Moreover, these indeterminate spikelet pairs extend up the ear axis form E3 to the tip. This gives the upper ear of *ra1* a matted appearance due to dense small branches.

3.3 Ramosa-2 Tassel and Ear analysis

The first reference to the *ramosa-2* (*ra2*) phenotype was by Emerson et al. (1935) who simply listed *ra2* as being located on chromosome 3 and credited its discovery to Brink. The first description of the *ra2* phenotype was by Nickerson and Dale (1955) who found that the *ra2* tassels (Figure 3-22) were characterized by having stiff upright branches closely appressed to the central culm, similar to the phenotype observed in our material (Figure 3-24). Furthermore, the branches in our material, also acropetally decreased in length and replaced the pedicelate spikelets of the central region of the spike, where there was a well-developed transition from tassel branches bearing many spikelet pairs to smaller branches bearing few spikelets, some of which occurred singly. Acropetal to these multi-spikelet branches were spikelet pairs on stalks with longer pedicels than normal (Figure 3-25). Moreover, these abnormally elongated pedicels could also be found on the lower branches as well.

Figure 3-22 *Ra2* tassel

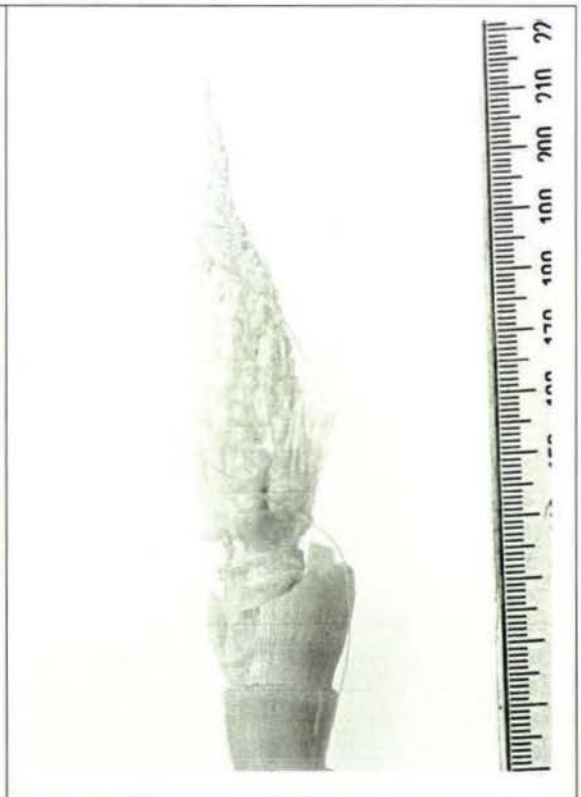


Figure 3-23 *Ra2* ear

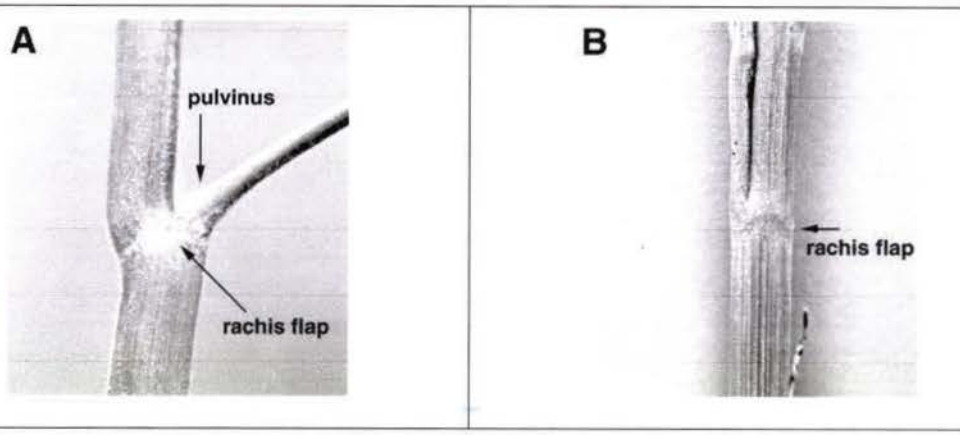


Figure 3-24 Separation of branch axis from inflorescence axis

Separation of the primary branch axis away from the inflorescence axis in *wild type* (A) and *ra2* (B).

Nickerson and Dale (1955) also reported the presence of small cupule-like depressions in the central culm on the adaxial surface of the branches and stalks. These depressions, though not just restricted to *ra2*, were more common and well developed. However, the spikelet pairs in the proximal areas of the lowermost branches had no adaxial depressions.

The *ra2* ears (Figure 3-23) studied by Nickerson and Dale (1955) sometimes ended in staminate structures that looked very similar to the male spike. Furthermore, scattered female branches on the upper ear in *ra2* individuals sporadically occurred. These branches arise from the pedicelate spikelet, while the sessile spikelet never forms anything but a normal caryopsis. Moreover, the spikelet pairs themselves were borne on stalks that were bent nearly at right angles, and were adherent half to the stalk and half to the cob. In addition, Nickerson and Dale (1955) noted that the *ra2* cob with its chaff removed resembled a similarly treated cob of Coroico maize (Cutler, 1946).

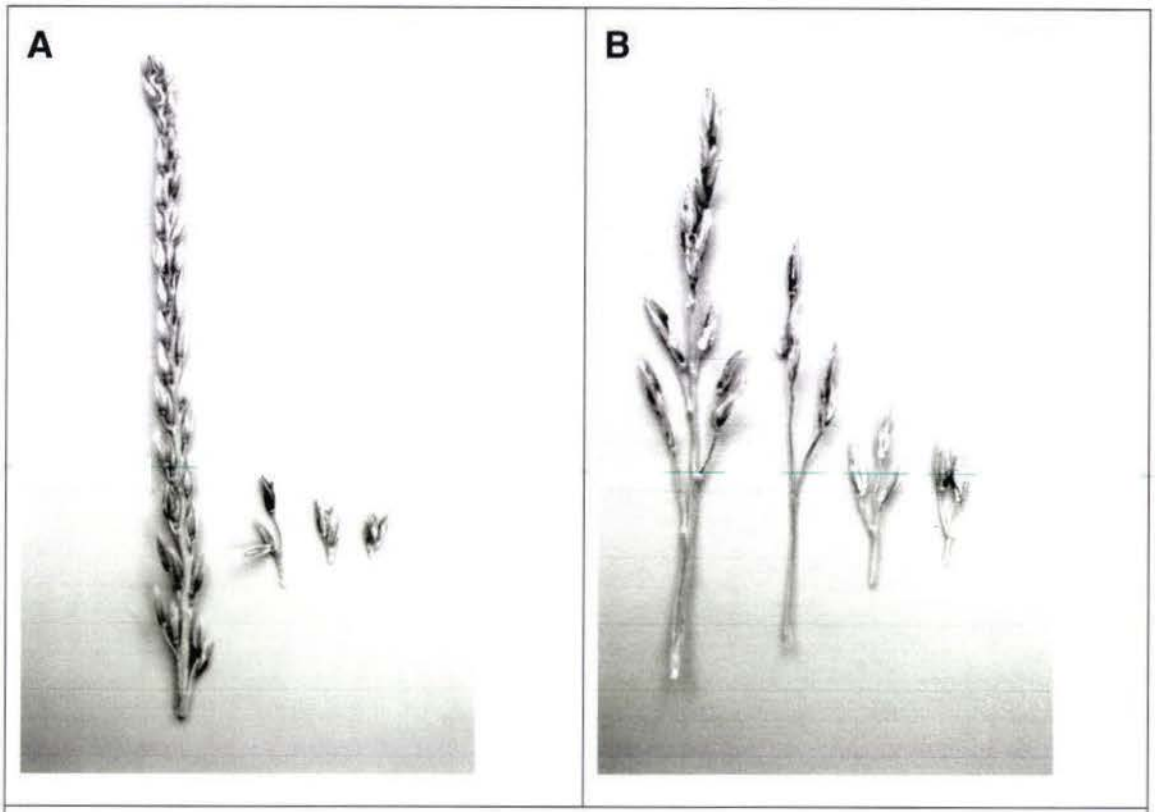


Figure 3-25 *Wild type* vs. *ra2* branches and spikelet pairs

Wild type (A) branch and spikelet pairs vs. *ra2* (B) branch, indeterminate spikelet pair and spikelet pairs showing the acropetal decrease in axis size.

3.3.1 Primary axis morphology

The only significant difference between *ra2* and wild type sibling's primary axis was the length of the peduncles, which were shorter in *ra2* (Table III-19, Table III-20, and Figure 3-26). In both the families, this difference was sufficient to account for the differences in the observed plant heights. Otherwise, there were no significant differences in the vegetative development.

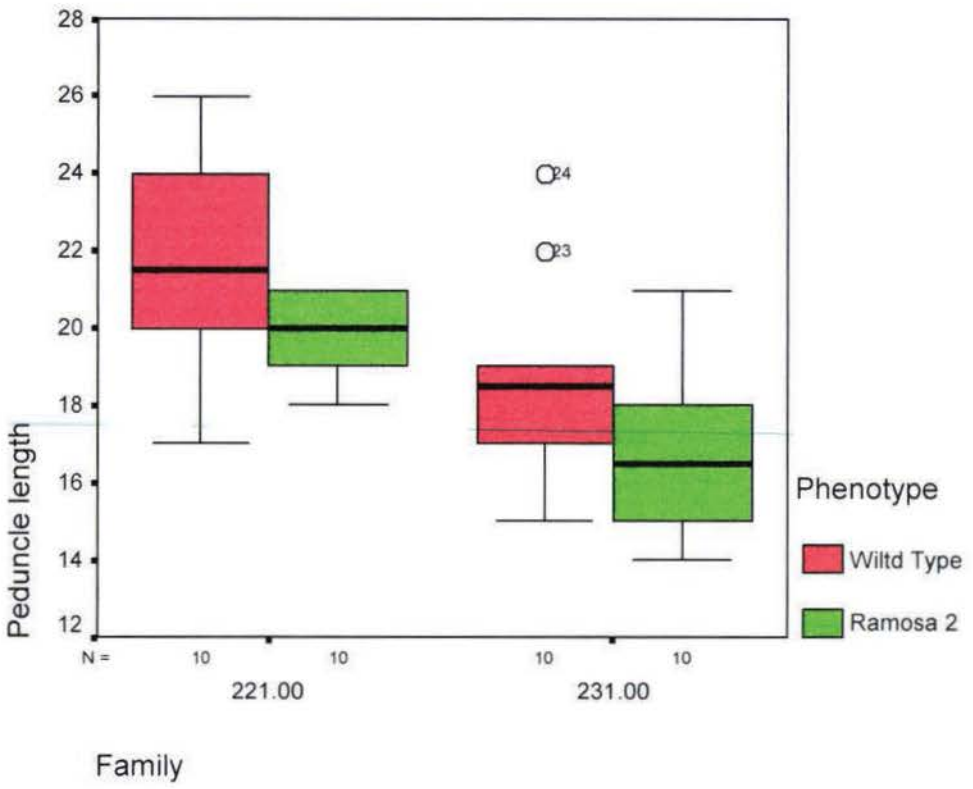


Figure 3-26 Peduncle length of *Ra2* vs. wild type

3.3.2 Tassel Characters

Unlike *ra1*, there is no significant increase in the number of primary branches on the central culm (Table III-21 and Table III-22). However, there is an increase in the number of higher order branches, though indeterminate spikelet pairs like those alluded to by Nickerson and Dale (1955) were present. The indeterminate spikelet pairs occupy about 35% of the tassel axis immediately above the primary branches (Figure 3-27).

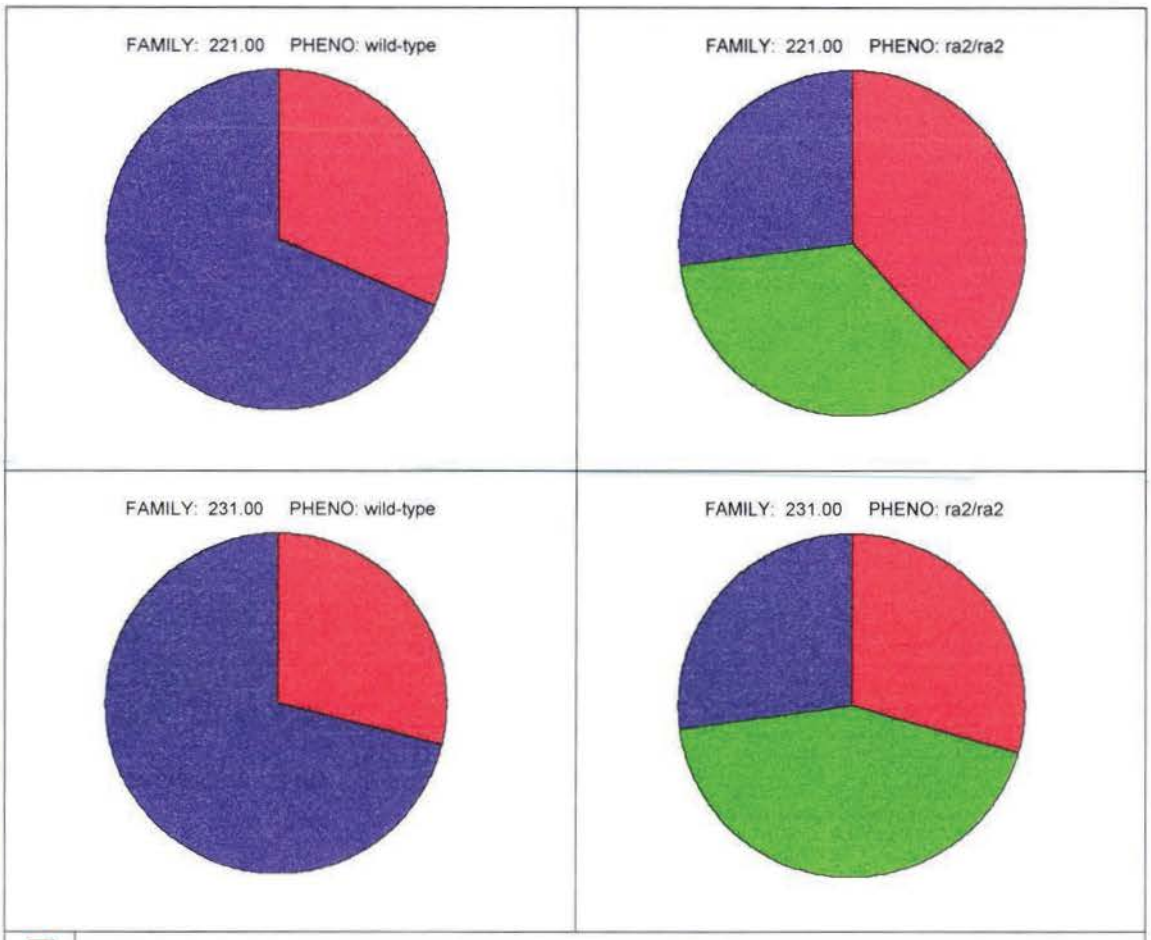


Figure 3-27 Pie Charts of tassel areas in *Ra2* families

3.3.2.1 Analysis of T1 in *ramosa-2* tassels

Branches were formed at T1 in both *ra2* and wild type sibling populations (Table III-23 and Table III-24). However, the branches of *ra2* at T1 had several dramatic differences to those of the normal siblings. These differences primarily related to the elongation of the spikelet pair axis and the pedicels of the otherwise normal spikelet pairs. The lengths of these axes decrease acropetally up the branch (Figure 3-28). Otherwise, the organization and numbers spikelets and their parts were comparable.

wild type branch from T1	*ra2* branch from T1

Figure 3-28 Branch morphology at T1

3.3.2.2 Analysis of T2 in *ramosa-2* tassels

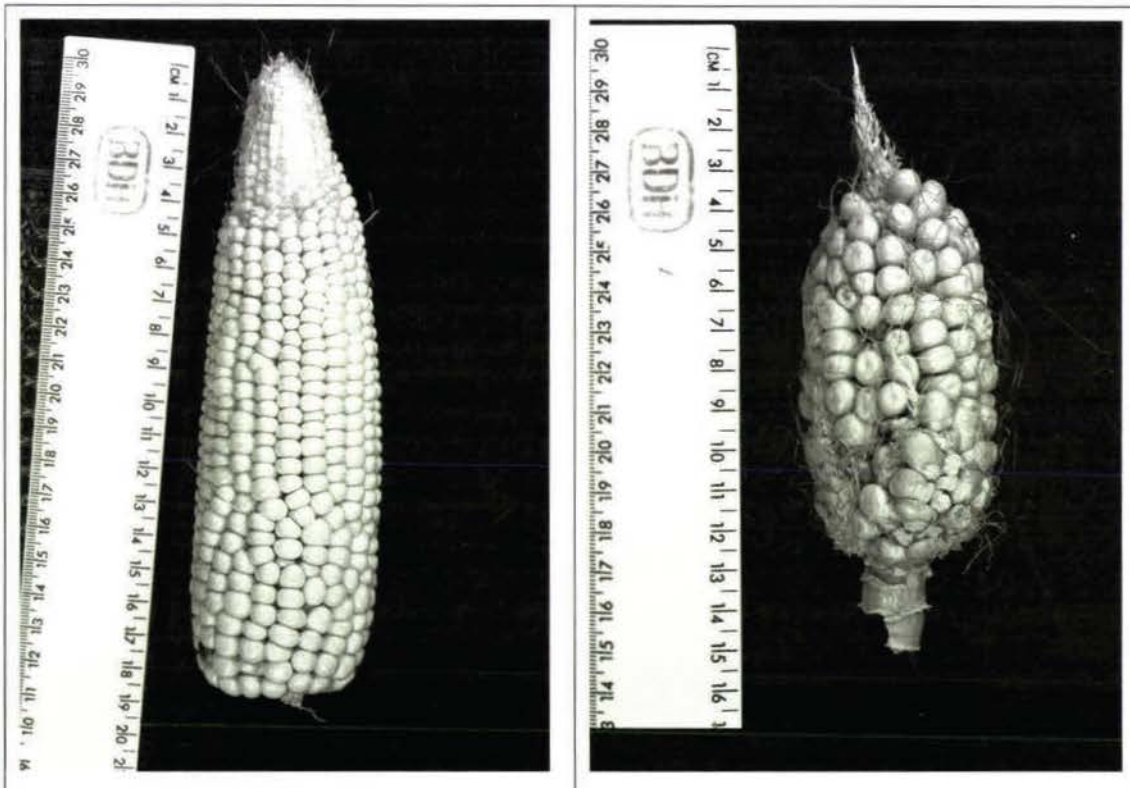
The type of axis produced at T2 in the *ra2* populations in both families was variable and produced a number of different branch types from indeterminate spikelet pairs to full branches depending upon the genetic background. In almost all cases, the number of spikelet pairs and spikelets and the size of spikelets pair axis and pedicel were greater in *ra2* populations than the wild type sibling populations (Table III-25 and Table III-26). Of particular significance is the elongation of all the different types of axes (branch, spikelet pair, spikelet) in this middle region. The variance in the number of glumes and florets is due to a single outlier in each population.

3.3.2.3 Analysis of T3 in *ramosa-2* tassels

Usually, the axis at T3 in *ra2* plants bore spikelet pairs (Table III-27 and Table III-28); however, in family 221, indeterminate spikelets occasionally still occurred. As at T2, the spikelet pairs of T3 had longer spikelet pair axes and pedicels, which were long and wiry. Like in the previous *ra2* spikelet pairs, the variance in the number of glumes and florets is due to a single outlier in each population.

3.3.3 Ear Characters

The fertile ears of *ra2* though similar in length, mass and number of spikelet pairs around the ear axis to wild type, also had branches at the base and spikelet pairs replaced with multiple spikelets (Table III-29 and Table III-30). The replacement of spikelet pairs with multiple spikelets disrupted the even ranks of kernels resulting in the disorganized appearance of mature *ra2* ears (Figure 3-29).



Wild type	*ra2*

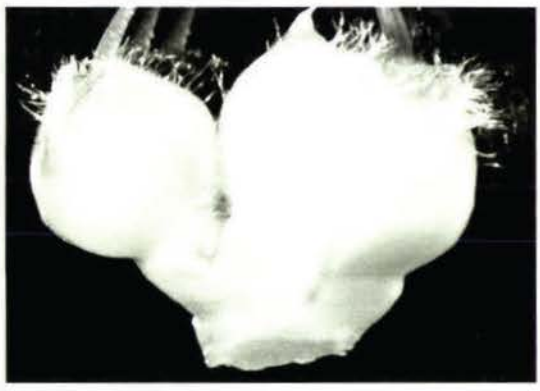
Figure 3-29 mature *ra2* ear compared to mature normal ear

Typically, *ra2* produces a number of branches at the base of the ear that bear spikelet pairs in an alternate phyllotaxy. Occasionally, smaller branches may be produced in the upper portion of the ear. This was rare in the *ra2* populations observed, however, Nickerson and Dale (1955) noted similar branches as a frequent occurrence.

The difference in branching in the distal portion of the ear probably reflects differences in the genetic backgrounds and environments. The branches that Nickerson and Dale (1955) observed originated from what would have been the pedicelate spikelet of a pair of spikelets while the sessile spikelet never produces anything but a caryopsis. Branches found in the upper portion of the ear were similar in structure to those observed by Nickerson and Dale (1955). However, the basal most branches were more like the tassel branches with a single branch producing female spikelets in pairs in an alternate phyllotaxy attached directly to the ear axis.

Occasionally, Nickerson and Dale (1955) noted that the ear of *ra2* sometimes ends in a staminate structure that looks like a normal central spike. In our analysis, a similar structure was observed in some *ra2* ears.

3.3.3.1 Analysis of E1, 2 and 3 in *ramosa-2* ears



wild type

ra2

Figure 3-30 *wild type* and *ra2* spikelet pairs

In *ra2* ears at E1 in *ra2*, indeterminate spikelet pairs have replaced spikelet pairs (Table III-31 and Table III-32), producing on average three to five spikelets before

terminating in a terminal spikelet (Figure 3-30). The organization of spikelets, however, is not altered with only a single caryopsis developing per spikelet. As in the *ra2* tassels, the lengths of the different axes decrease acropetally with a corresponding reduction in the number of spikelets. The differences in axis size, however, are very small and imperceptible to casual analysis.

3.4 Ramosa-3 tassel and Ear analysis

Unlike the other *ramosa* mutants, the *ramosa-3* (*ra3*) phenotype has not been described in the literature. Nueffer et al. (1997) list *ra3* locus on chromosome 4 (H.S. Perry, unpublished). Three distinct phenotypes could be identified in the only *ra3* population used: wild type, *ra3* heterozygote, *ra3* homozygote based on the ear phenotype. The *ra3* phenotype was very similar to *ra1*, however, current maps list *ra1* on chromosome 7 (Nueffer et al., 1997).

3.4.1 Primary axis morphology

The *ra3* homozygote and *ra3* heterozygotes were both significantly smaller compared to wild type siblings (Table III-37). The mean total plant height of the wild type siblings was 2.03 m as opposed to 1.80 and 1.85 for *ra3* homozygote and heterozygote respectively. Broken down into corresponding parts, the major differences occur between the plant height and the tassel length (Figure 3-31 and Figure 3-32).

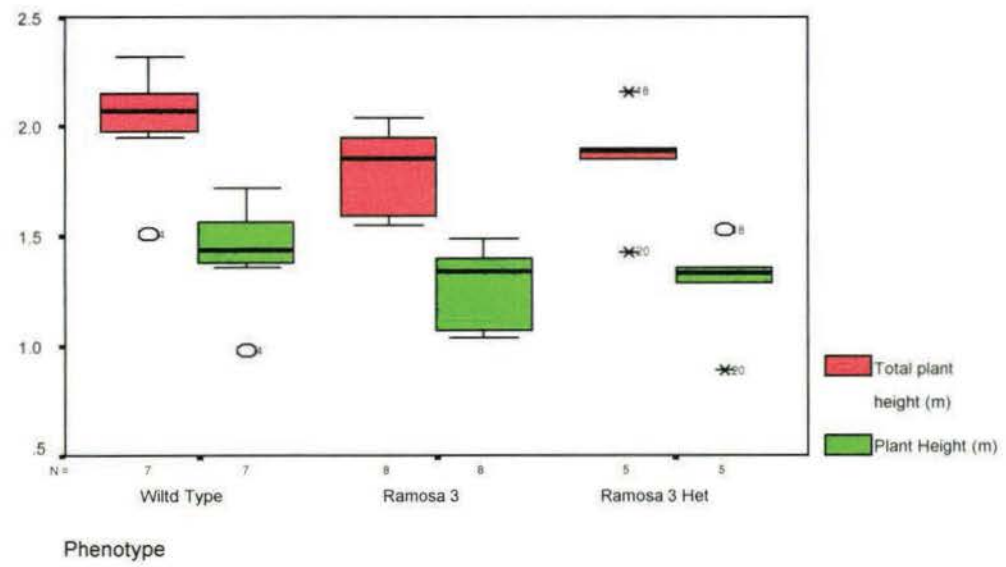


Figure 3-31 A comparison of total plant height and plant height plant height between *wild type* and *ra3*

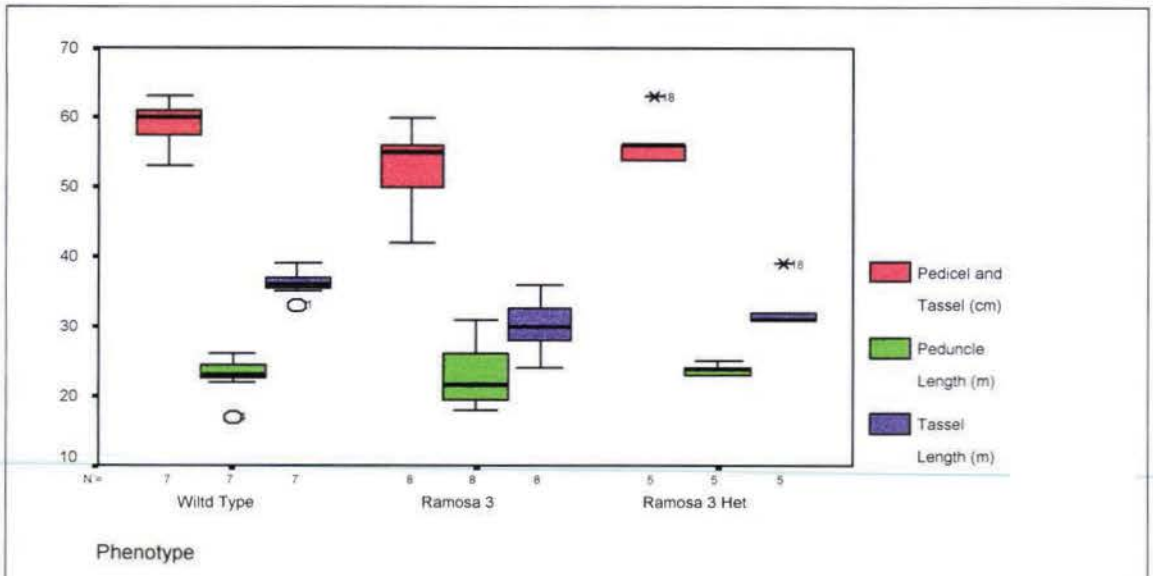


Figure 3-32 A comparison of tassel and peduncle length between *wild type* and *ra3*

3.4.2 Tassel Characters

The branching patterns of *ra3* homozygotes are very similar to *ra1*, being conical in appearance and heavily branched (Figure 3-33), while *ra3* heterozygotes were of an intermediary nature with a more modest increase in the number of primary and secondary branches (Figure 3-34). Both the *ra3* homozygote and heterozygote branches are organized like *ra1* with an acropetal decrease in the branch and indeterminate spikelet pair length.

As such, the model for *ra1* is also valid for *ra3* homozygote and heterozygotes (Figure 3-17). Figure 3-38 shows mean areas of the different branch types in the tassel of the *ra3* homozygotes and heterozygotes compared to the normal siblings. The *ra3* heterozygotes are very similar to *ra2* (Figure 3-27) with an almost equal distribution of primary branches, indeterminate spikelet pairs and spikelet pairs, while the distribution of branches and indeterminate spikelet pairs in the *ra3* homozygotes (Figure 3-38) is very similar to that of *ra1* homozygotes (Figure 3-18). Furthermore, though no data on the number of secondary branches was collected, there was often between three and five secondary branches in *ra3* homozygotes (Figure 3-35), while heterozygotes had only one or two (compressed structure on the left of the branch axis in Figure 3-36) and the wild type siblings had none (Figure 3-37).

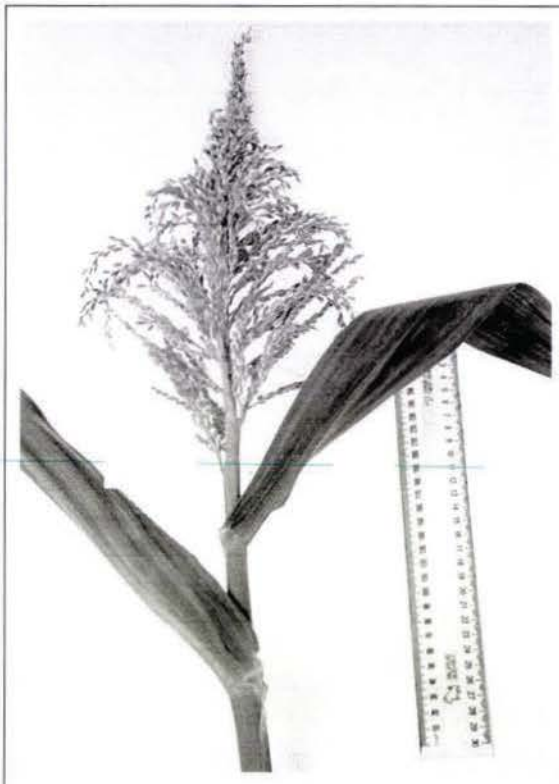


Figure 3-33 *ra3* tassel

Figure 3-34 *Ra3* heterozygote tassel

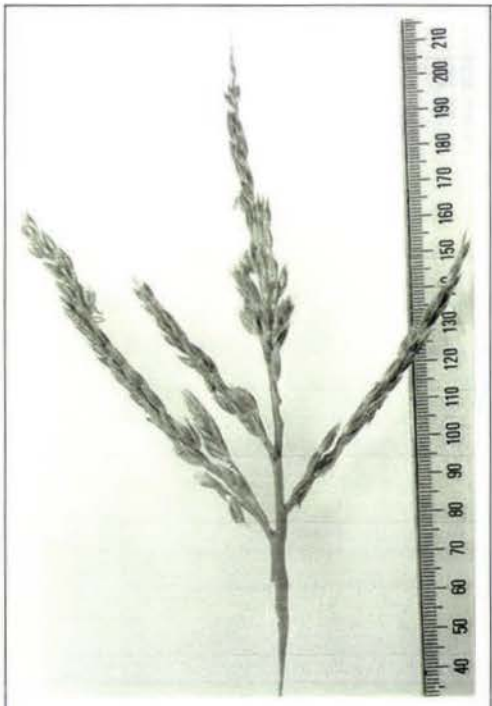
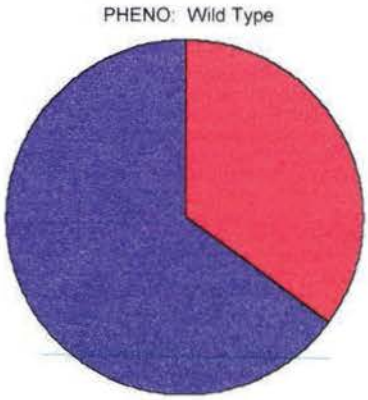
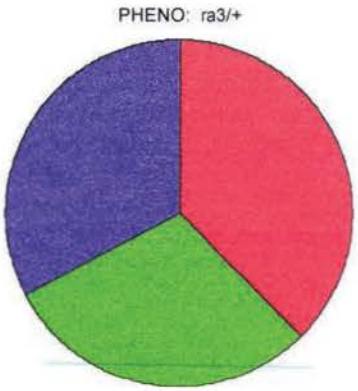


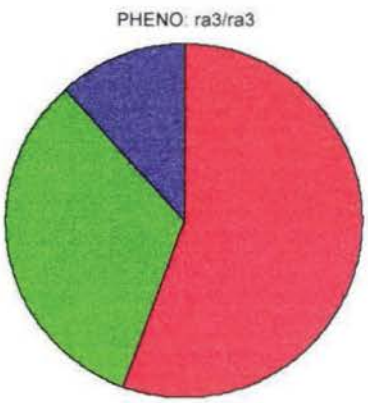
Figure 3-35 *Ra3* primary branch

Figure 3-36 *Ra3* het primary branch

Figure 3-37 *Wild type* tassel branch







	Primary branch area
	Indeterminate spikelet pair area
	Spikelet pair area

Figure 3-38 Pie charts of mean tassel percentages for *wild type* and *ra3*

3.4.2.1 Analysis of T1 in *ramosa-3* tassels

At T1, the *ra3* homozygotes, heterozygotes and *wild type* siblings all have primary branches with more than 20 spikelet pairs each. However, these are significantly longer on average in the *wild type* compared to the *ra3* heterozygotes and *ra3* homozygotes (Table III-39). The spikelet pairs are found along the flanks of the branches of *ra3* heterozygotes and homozygotes. The mean length of the spikelet pair

axes and the pedicels is greatest in the *ra3* heterozygote, though these traits are subject to significant variation in the homozygotes. The *ra3* heterozygotes and homozygotes did not have an altered number of glumes and florets in their spikelets at T1.

3.4.2.2 Analysis of T2 in *ramosa-3* tassels

The *ra3* homozygotes have branches at T2 compared to the indeterminate spikelet pairs in the *ra3* heterozygote and spikelet pairs in the *wild type* siblings (Table III-40). The branch lengths of the *ra3* homozygotes are shorter compared to the *wild type* at T1 with fewer spikelet pairs. Mean values for all the number of spikelet pairs, spikelets and total length of the spikelet pair axis is greatest for *ra3* homozygotes followed by *ra3* heterozygotes. However, the *ra3* heterozygote spikelet pair axis length is greater than the *ra3* homozygote but more variable. Furthermore, pedicel length of the *wild type* pedicelate spikelet is greater compared to either *ra3* homozygotes or *ra3* heterozygotes. The number of florets and glumes per spikelet were not altered in the *ra3* homozygote and *ra3* heterozygote phenotypes a T2.

3.4.2.3 Analysis of T3 in *ramosa-3* tassels

The *ra3* heterozygotes are almost identical to the *wild type* siblings at T3, although the axis length of the *ra3* heterozygote tended to be greater compared to *wild type* siblings. The *ra3* homozygotes, however, have indeterminate spikelet pairs at T3 that increased the axis lengths mean. The number of glumes and florets per spikelet was the same as the *wild type* siblings in both *ra3* homozygotes and *ra3* heterozygotes.

3.4.3 Ear Characters

The *ra3* homozygous ears (Figure 3-39) are highly branched structures, which produced functional caryopsis that can produce viable kernels. The branches in *ra3* heterozygote ears (Figure 3-39) closely resemble the branches of the tassels. The glumes of *ra3* homozygous ears often enclose caryopsis at anthesis. The *ra3* heterozygotes (Figure 3-39), however, are more like *ra2* ears, having several branches at the base with a disorganized appearance of their kernels. The disorganized kernels are primarily due to the production of extra spikelets in the ear (Figure 3-39).

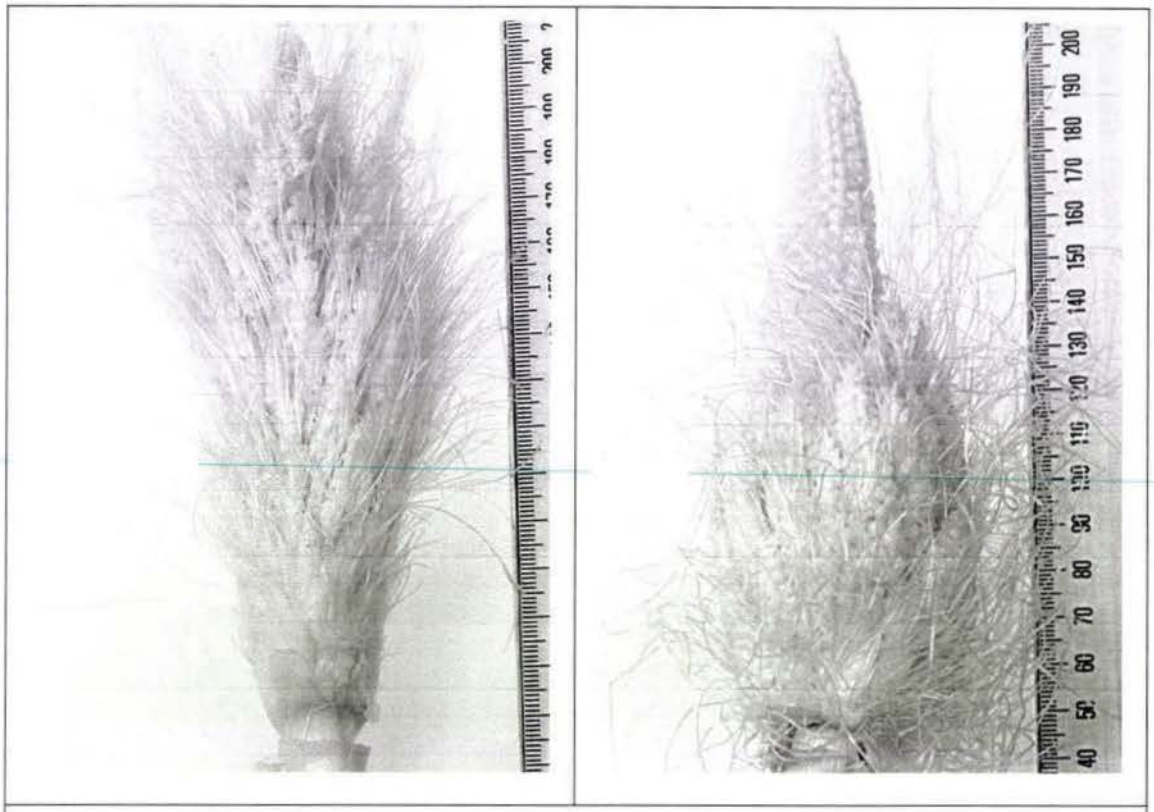


Figure 3-39 *Ra3* homozygous and heterozygous ears showing different degrees of branching along the 1° axis

The quantifiable traits of the ear such as length and mass are very similar between *ra3* homozygotes, *ra3* heterozygotes and *wild type* siblings (Table III-42). The only significant differences were the different branch types on the ear and the percentage of silks.

3.4.3.1 Analysis of E1 in *ramosa-3* ears

The *ra3* homozygotes typically have branches at E1; these have a mean size of 2.59 cm with more than 20 immature spikelet pairs in an alternate phyllotaxy (Table III-43). The spikelet pairs and spikelets of the *ra3* homozygotes are essentially the same as the *wild type* siblings.

The *ra3* heterozygotes (Table III-43), however, primarily have indeterminate spikelet pairs similar to *ra2*. Branches occasionally occur at a low frequency in some *ra3* heterozygous individuals. Apart form the increased spikelet number; the *ra3* heterozygotes are almost the same as *wild type* siblings.

3.4.3.2 Analysis of E2 in *ramosa-3* ears

The organisation of *ra3* homozygous and heterozygous ears at E2 is similar to that of E1. The size and number of spikelets on branch and indeterminate axes have decreased (Table III-44) proportionally, in keeping with the acropetal decrease in branch size observed in the tassel.

Figure 3-40 Branches on *ra3* homozygous ear

3.4.3.3 Analysis of E3 in *ramosa-3* ears

The *ra3* heterozygotes at E3 show primarily spikelet pairs while the homozygotes are indeterminate spikelet pairs (Table III-45) like at T3 in the tassel. The spikelets on the indeterminate spikelet pair in the *ra3* homozygous ear are very small and underdeveloped, failing to reach maturity by anthesis and therefore are often barren at harvest. The *ra3* heterozygotes, like *ra2* ears (Table III-35 and Table III-36), have normal spikelet pairs at E3, which remain fertile. The size and number of the spikelet pair parts are correspondingly very similar between the *wild type*, *ra3* heterozygotes and *ra2*.

3.5 Branched silkless1 tassel and Ear analysis

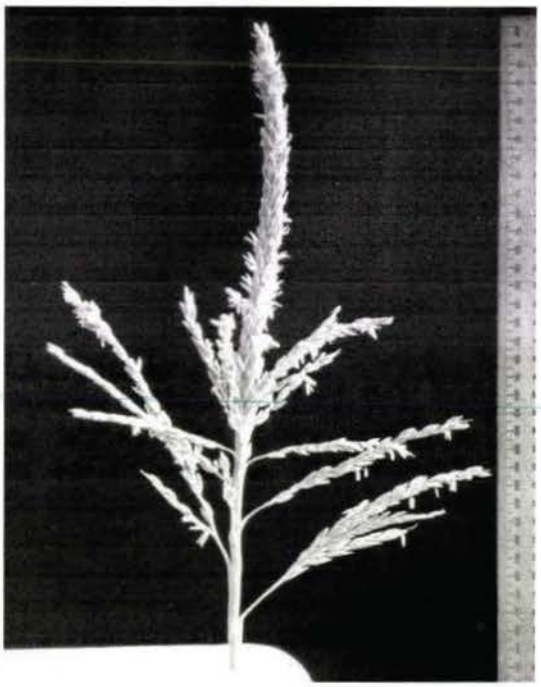
Branch silkless (*bd1*) is unique in this study of branching mutants because the outwards tassel architecture of *bd1* is similar to the *wild type*, where there is a definite division of tassel into branches and central spike, except *bd1* has a slightly thickened appearance (Kempton, 1934). Furthermore, the original description of the *bd1* phenotype was of an ear with excessive branches reminiscent of *ramosa* mutants, but with the additional character of being devoid of silks (Kempton, 1934).

Kempton (1934) attributed the thickened appearance of the tassel to the presence of short branches in the place of spikelet pairs, a condition confirmed by Nickerson and Dale (1955) and seen in our co-segregation population (104; Figure 3-42). Nickerson and Dale (1955) also reported the presence of sterility towards the end of the branches and sometimes the tip of the central spike. Furthermore, in the sterile areas, the spikelets were less developed as the distance from the central culm increased and sporadically occurred for the lowermost branches to be sterile throughout. Kempton (1934) also noted that the plants shed plenty of pollen, but the stamens were often not fully dehisced. Furthermore, Colombo et al. (1998) noted that the *bd1-2* tassels were sometimes less branched than in *wild type*. In two of our *bd1* populations, this sterility was exacerbated and the whole tassel was often sterile (Figure 3-42).

There are two types of *bd1* ears (Figure 3-42), a fact noted by Kempton (1934) and Nickerson and Dale (1955). In one type, a short branch bearing female rudiments that never develop beyond the earliest stages replaces each spikelet. In the other, these branches are confined to the basal quarter of the ear; the remaining three quarters are sterile spikelets with elongated, sometimes thickened glumes. Furthermore, the latter type of *bd1*ear may occasionally develop silks, too short to be exerted (Nickerson and Dale, 1955). Similarly, Emerson et al. (1935) noted that the ear "often occurs without silks" compared to Kempton (1934) who stated that his material "occurred wholly without silks."

More recently, Colombo et al. (1998) reported that *bd1-2* played an important role in regulating the transition from spikelet to floral meristem during the development of the maize female inflorescence, demonstrated by the indeterminate growth of floret-like structures in *bd1-2* spikelets that develop from the axil of glume-like structures.

Family 151



Family 104

Figure 3-41 sterile and fertile *bd1* tassels

Family 151

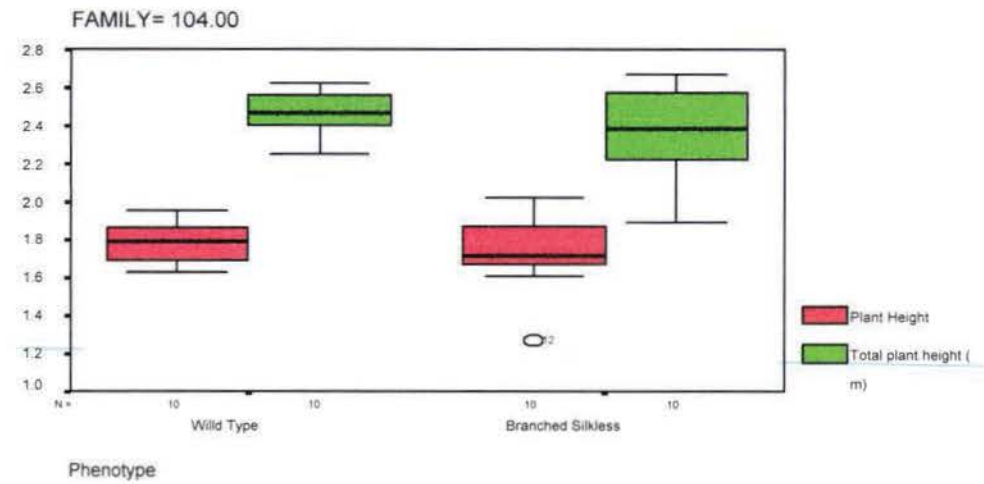
Family 104

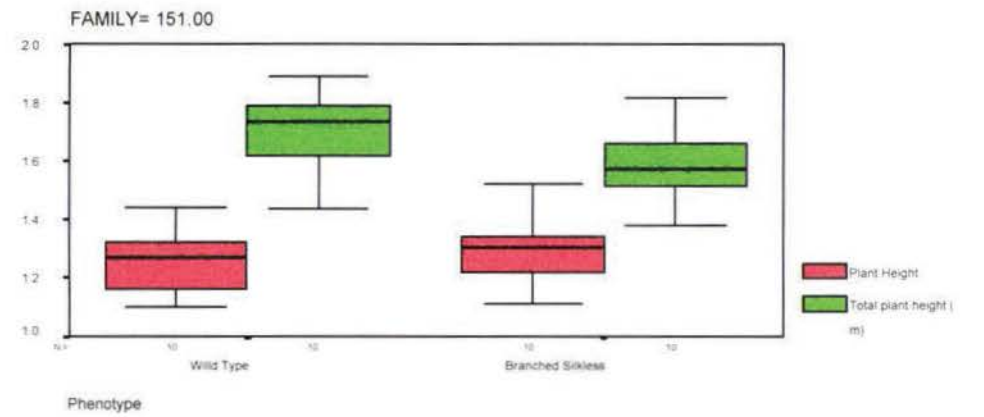
Figure 3-42 un branched and branched *bd1* ears

3.5.1 Primary axis morphology

Three families were used in this study of inflorescence morphology; the two families generated by backcrossing into B73 that had sterile tassels and sterile unbranched ears and family 104 from the cosegregation analysis that closely resembled the tassels and ears described by Kempton (1934) and Nickerson and Dale (1955).

The length of *bd1* plants in families 104, 151 and 161 was not significantly different from that of their *wild type* siblings (Table III-46, Table III-47, Table III-48 and Figure 3-43). The difference in the total plant height in family 151 is due to the poorly developed tassel that can be seen in the reduced peduncle and tassel sizes in Figure 3-44. Otherwise, there was no significant difference between the *bd1* and *wild type* primary axes.





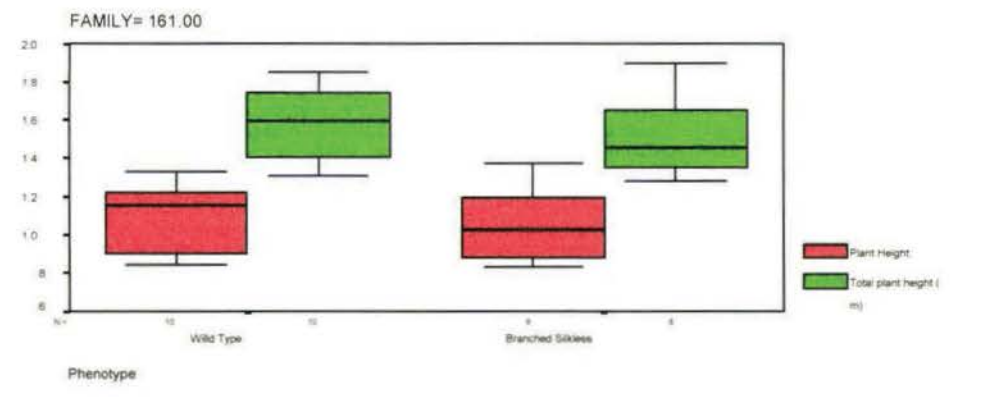


Figure 3-43 Plant height of *bd1* vs. *wild type*

Families 151 and 161 have a B73 background, while 104 is the segregation analysis family

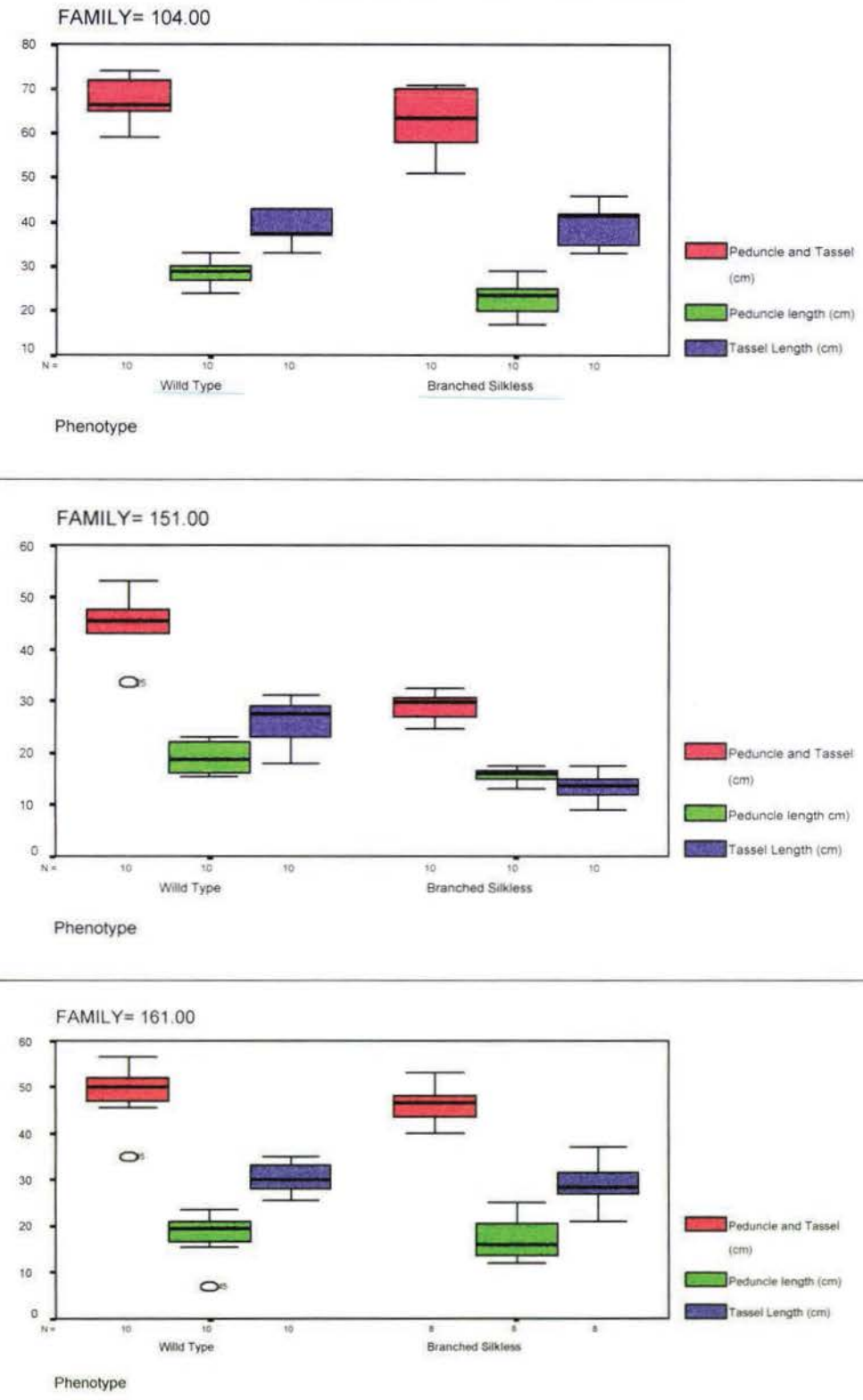
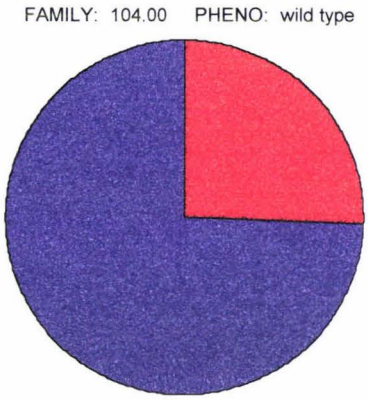


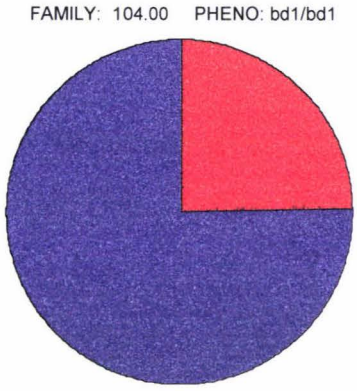
Figure 3-44 Tassel and peduncle heights for *bd1* vs. *wild type*

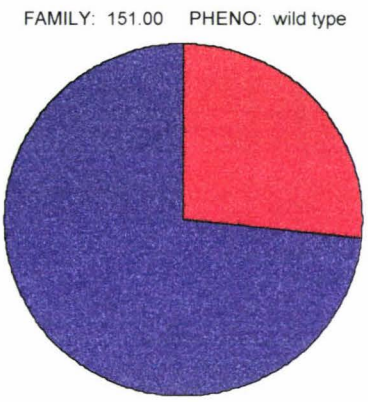
Families 151 and 161 have a B73 background, while 104 is the segregation analysis family

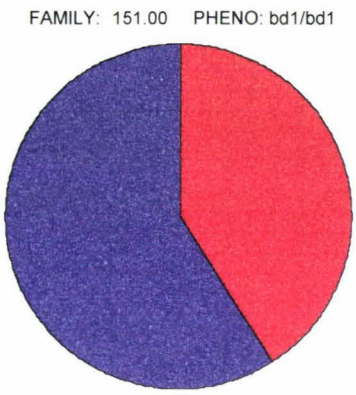
3.5.2 Tassel Characters

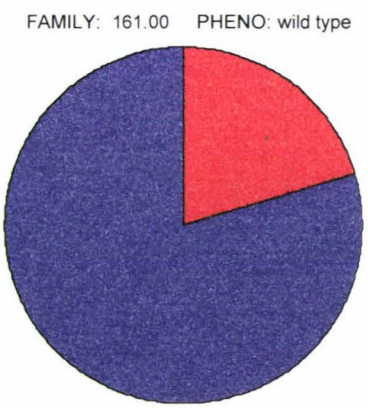
The number of the different types of branches in the *bd1* tassel was not significantly different from those of the wild type siblings (Table III-49, Table III-50, and Table III-51). In addition, there are no indeterminate branches and tertiary branches in the *bd1* and wild type tassels in all the families' analyses. Comparison of the distributions of the branch types in the tassel shows that the basic architecture of *bd1* tassel and wild type siblings is the same (Figure 3-45).











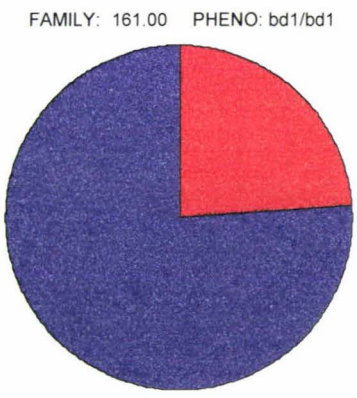


Figure 3-45 Pie charts of mean tassel percentages for *bd1* vs. *wild type*

Families 151 and 161 have a B73 background, while 104 is the segregation analysis family

3.5.2.1 Analysis of T1 in *branched silkless1* tassels

The branch types at T1 in *bd1* and wild type siblings are primary branches (Table III-52, Table III-53, and Table III-54). The lengths of these branches vary between the phenotypes relative to the tassel sizes. Typically, the spikelet pairs produce two spikelets, however, family 161 showed some variation in the branch type and the number of spikelets.

The mean lengths of family 104 spikelet pair axes and pedicels are almost identical (Table III-52). However, family 104 has a reduction in the number of glumes with an increase in the number of florets in each spikelet. Both the number of glumes and florets per spikelet in *bd1* are subject to substantial variation (Figure 3-46).

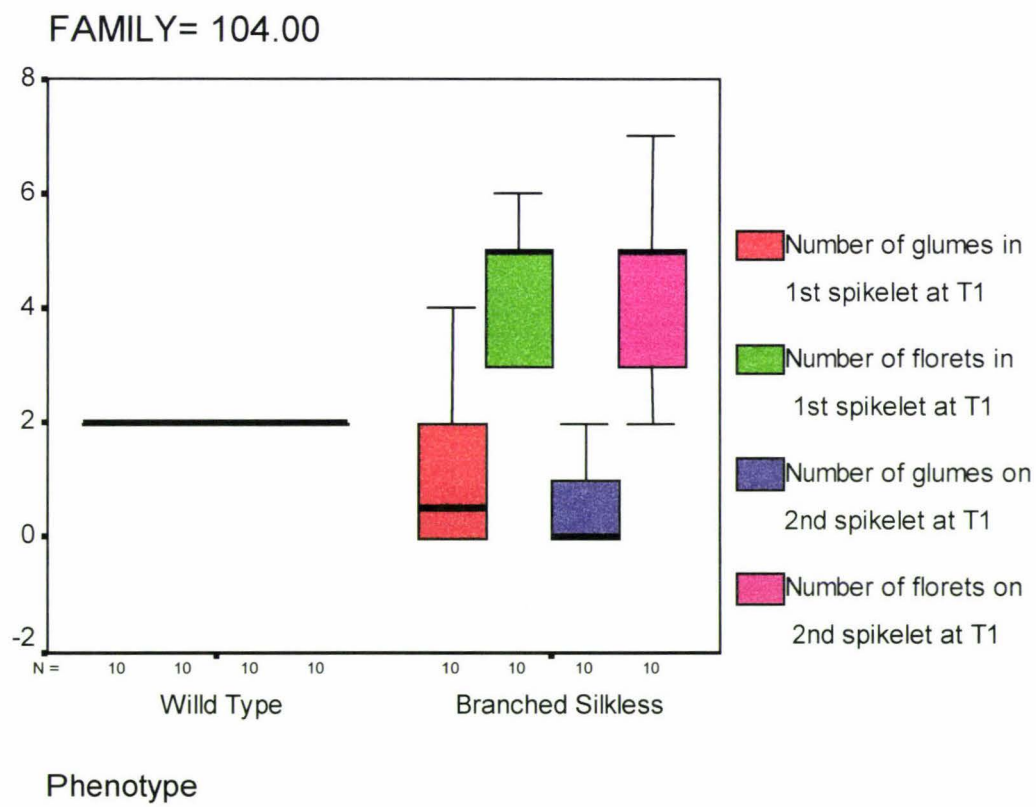


Figure 3-46 Variation in the spikelets at T1 in family 104

Family 151, however, has no florets in the spikelet with a variable number of glumes (Table III-53). Moreover, the *bd1* phenotype in family 161 is intermediary between that of family 104 and 151.

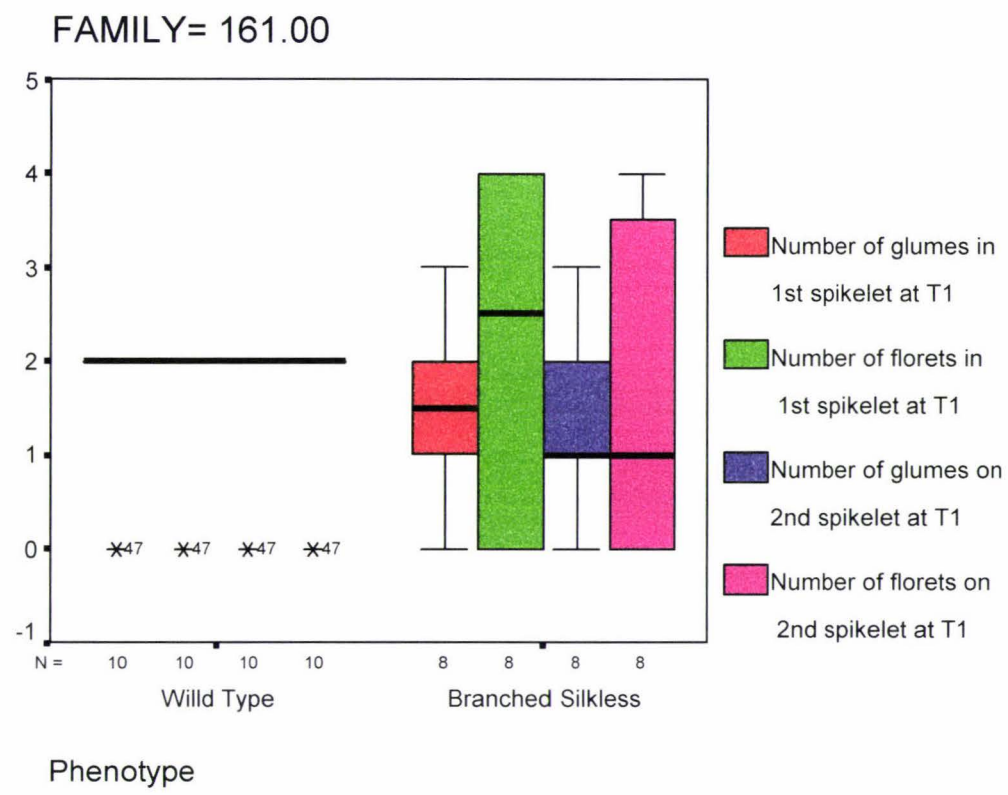


Figure 3-47 Variation in the spikelets at T1 in family 161

3.5.2.2 Analysis of T2 and T3 in *branched silkless1* tassels

Spikelet pairs predominate in both *bd1* and wild type tassels at T2 and T3. The mean length of the spikelet pair axis and the spikelet axis shows considerable variation between families (Table III-55, Table III-56, Table III-57, Table III-58, Table III-59, and Table III-60). *Bd1* spikelets at T2 and T3 are essentially identical to those at T1 of each family.

3.5.2.3 Spikelet Morphology in family 104

Due to the sterility of the tassel in families 151 and 161, the morphology of the spikelet pairs could not be observed. However, family 104 produced developed *bd1*

tassel with fertile male spikelets. The morphology of these spikelets was unique and deserved closer attention.

Indeterminate growth of floret-like structures in the axil of glume like structures in male spikelets of *bd1* was observed in family 104. Furthermore, these were equivalent to the structures noted by Colombo et al (1998) in the female spikelets of *bd1*-2 ears. These floret-like structures generally had a lemma, palea and a variable number of floral parts (Figure 3-48) that were subtended by thick glume-like bracts.

Figure 3-48 indeterminate male *bd1* spikelet

The spikelet axis with the glumes removed had three florets instead of the normal two with between two and three anthers per floret.

The florets were typically spread on an indeterminate spikelet axis (Figure 3-50). The florets in Figure 3-48 were on a secondary indeterminate axis from the axil of one of the glume-like structures at the base of the indeterminate pedicelate spikelet axis. However, similar structures were observed on the pedicelate spikelet axis. Interestingly the plane of these secondary axes was perpendicular to the plane of the florets on the indeterminate spikelet axis as shown in Figure 3-49. Therefore, the *bd1*

spikelets produce florets in an alternate phyllotaxy around an indeterminate spikelet axis. Furthermore, the glumes are no longer sterile and have secondary indeterminate spikelet axes in their axis that produce florets in an alternate phyllotaxy perpendicular to the plane of the primary indeterminate spikelet axis.

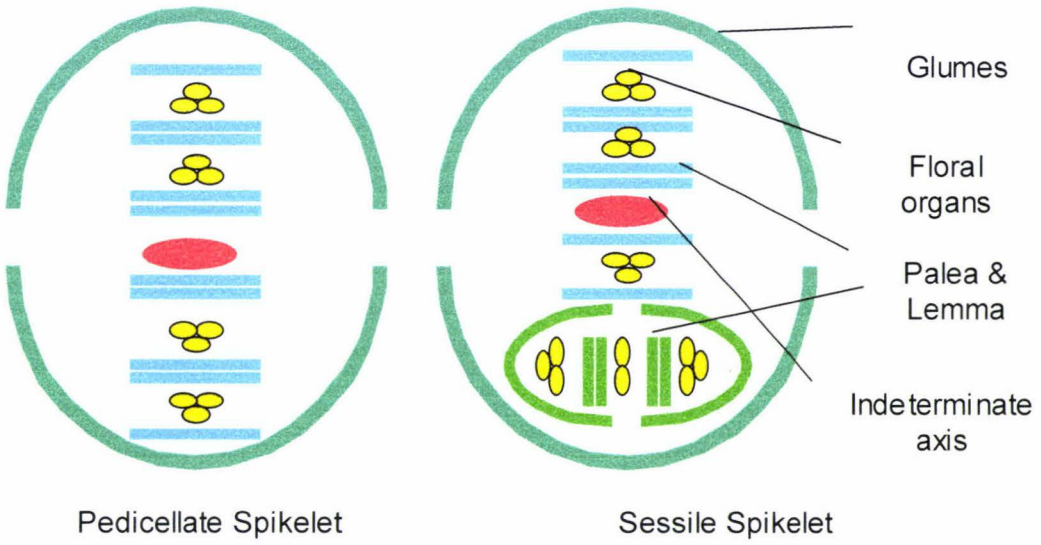


Figure 3-49 Diagram of *bd1* spikelet pair

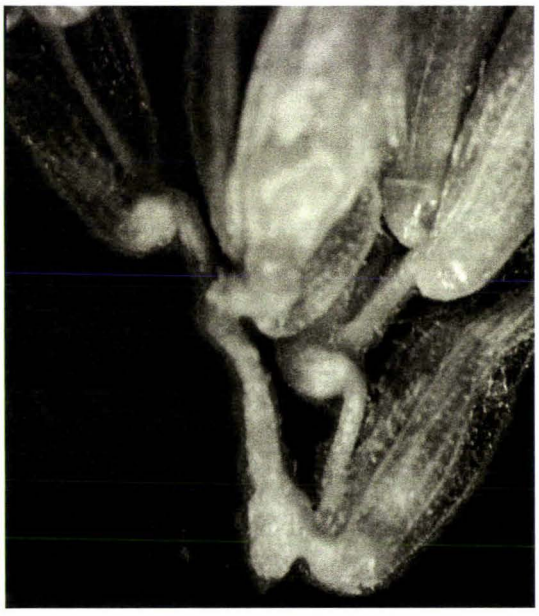


Figure 3-50 *bd1* pedicellate spikelet axis

3.5.3 Ear Characters

As previously noted by Kempton (1934) and Nickerson & Dale (1955), there are two types of *bd1* ear. However, the mean length and mass of *bd1* ears are generally reduced compared to their wild type siblings. This difference can be accounted to their sterile nature. In the more vigorous families 104 and 161, a number of branches (Figure 3-51) were observed at the proximal end of the cob. Distil to these branches were malformed spikelets arranged in pairs (Figure 3-52). Sporadically, pistils would arise from the malformed spikelets; however, they only grew to a few centimeters in length. In family 151, only structures observed on the distal portion of the cob of family 104 and 161 were present. The branches in *bd1* ears develop from the upper floret of the spikelet and subtended at the base by a glume and sterile lower floret (Figure 3-53).

3.5.3.1 Analysis of E1, E2 and E3 in *branched silkless1* ears

At E1, E2 and E3, all three *bd1* families used in this study had spikelet pairs (Table III-64, Table III-65, Table III-66, Table III-67, Table III-68, Table III-69, Table III-70, Table III-71, and Table III-72) compared to the branches in the *ramosa* phenotypes. However, the spikelet pairs of *bd1* had indeterminate branches that produced florets in an alternate fashion. The florets of *bd1* spikelets were much reduced, and showed an acropetal gradient of maturity along the branch. The indeterminate branches were subtended at the base by two or more glumes with a short axis in the axil of the normally sterile glume.

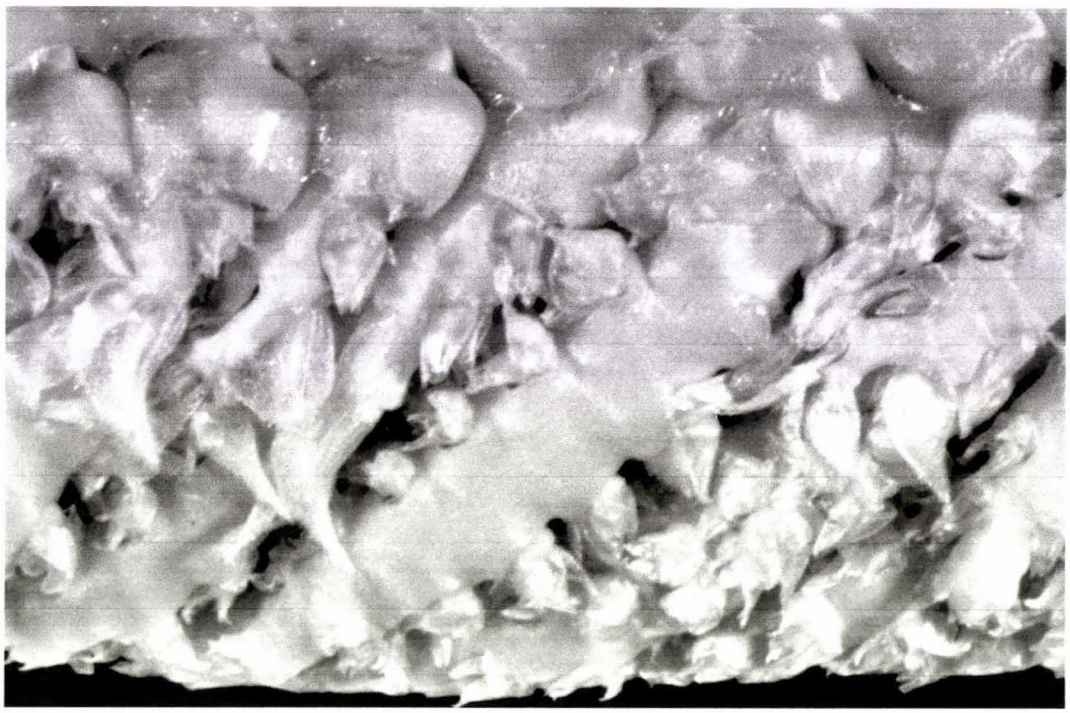


Figure 3-51 *Bd1* female branches

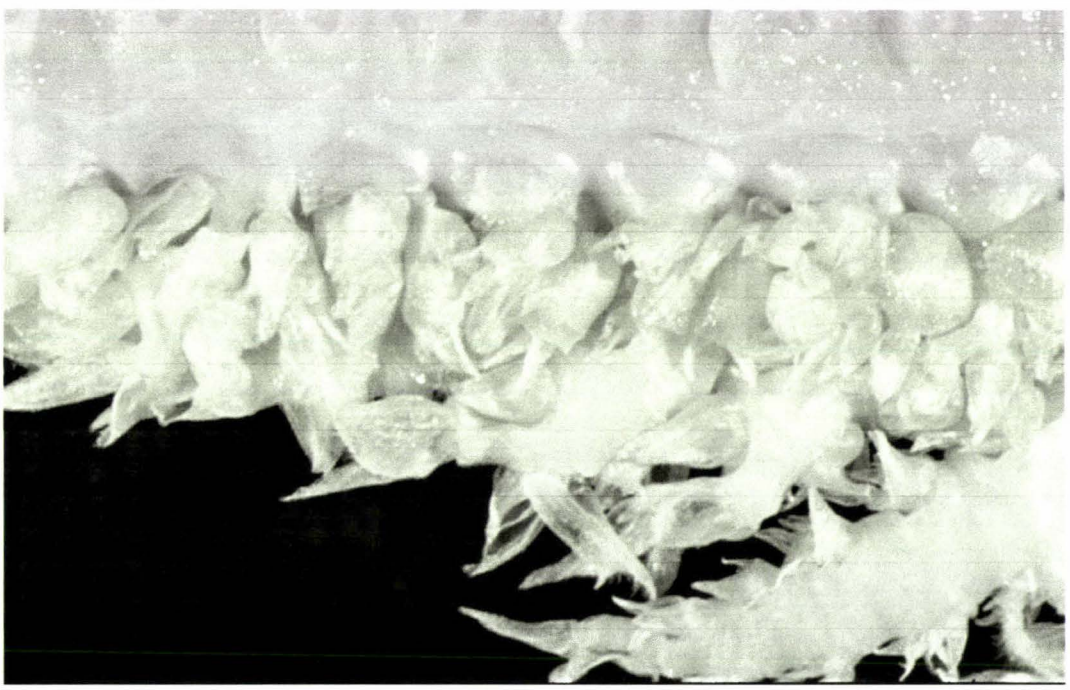


Figure 3-52 *Bd1* female spikelet pairs

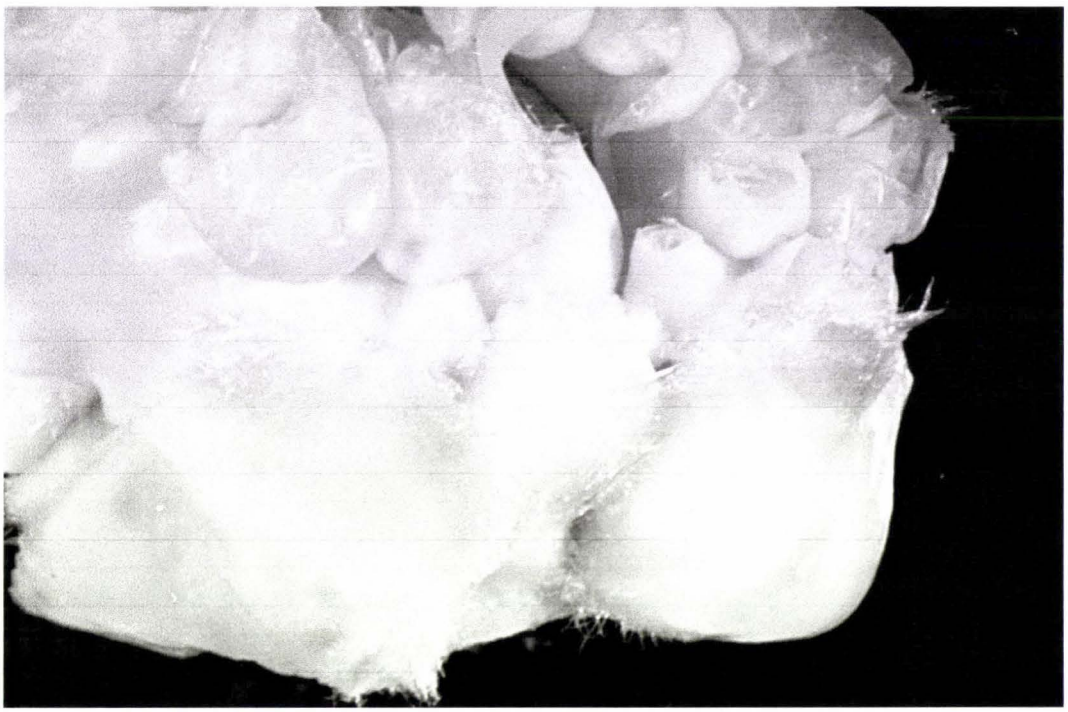


Figure 3-53 Basal end of *bd1* female branch

4 Analysis of Maize Inflorescence Development

In order to determine the developmental stage at which the four mutant phenotypes, *ra1*, *ra2*, *ra3* and *bd1* first become apparent, and thereby learn more of when the corresponding genes are normally required. Several inflorescences of each phenotype were examined at various developmental stages. Although the development of wild type inflorescences has been well describe previously (Weatherwax, 1917; Miller, 1919; Bonnett; 1940, 1948; Cheng et al., 1983; and Stevens et al., 1986) it will be reviewed here to emphasize key steps in normal morphogenesis.

4.1 Normal Development

4.1.1 Vegetative development

In the early stages of vegetative development, an axillary shoot is produced in the axil of each leaf. However, in the later stages of development these axillary shoots are no longer produced. The cessation of axillary shoot development seems to be associated with the elongation of the internodes of the stem and the development of the tassel (Bonnett, 1948) from the shoot apical meristem once a complete set of leaves or vegetative nodes has been produced. Thus a typical plant might then produce 20 or so leaves, the basal-most dozen or so would produce axillary buds with the more distal leaves having no axillary buds. Although the ears are laterally placed in the axil of a leaf in the middle of the stem (Bonnett, 1948) and develop somewhat later than the tassel, having first initiated a number of husk leaves, the ear shares many common developmental features with the tassel as summarized below (Cheng et al, 1983).

4.1.2 Inflorescence development and branch primordia initiation

In both tassel and ear development, the initiation of inflorescence development is associated with the elongation of the vegetative meristem into an inflorescence meristem that initiates outgrowths called branch primordia (Figure 4-1). Branch primordia are initiated in multiple ranks and in an acropetal sequence (Figure 4-2, Figure 4-4, Figure 4-5).

In some genetic backgrounds, narrow, horizontal ridges called rachis flaps may subtend developing branch primordia (Figure 4-2)(Cutler and Cutler, 1948; Bonnett,

1953; Stevens et al., 1986). Rudiments of the rachis flaps can be observed at the base of adult branches (Figure 4-3), while in the ear, these ridges increase in size and form the cup-like depressions (alveoli) in which the spikelets occur (Bonnett, 1948). In studies made on barley, wheat and oats (Bonnett, 1935, 1936, 1937) and grasses (Evans and Grover, 1940), there was always an indication of leaf fundaments on the central axis in the axils of which the lateral shoots of the inflorescence were formed (Bonnett, 1948). However, in most cases these initials do not develop any further.

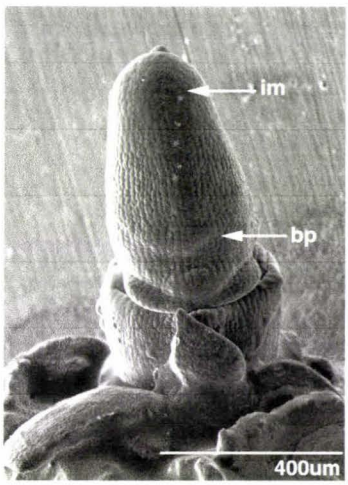


Figure 4-1 Elongated tassel meristem with branch primordia initiating along the flanks

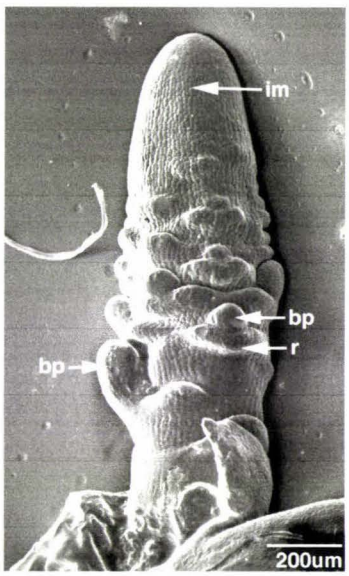


Figure 4-2 Initiation of branch primordia in the tassel with subtending rachis flaps.

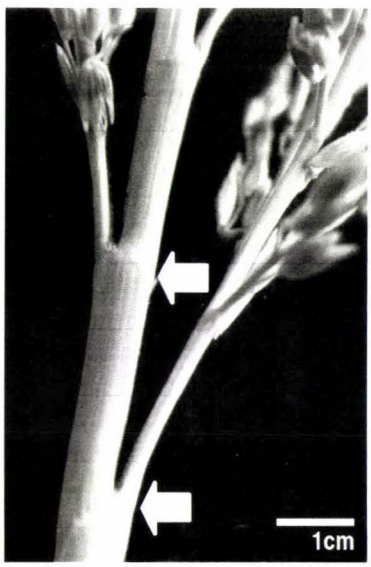


Figure 4-3 Rachis flaps on the inflorescence axis at the base of primary branches at maturity

Key: (im) inflorescence meristem; (bp) branch primordia; (r) rachis flap

4.1.3 Branch development

The branch primordia on the male tassel axis can have two different developmental fates, indeterminate branches or determinate spikelet pairs. Typically, it is the basal-most branch primordia in the tassel that elongate into indeterminate branch meristems (Figure 4-4). These branch meristems initiate secondary branch primordia similar to inflorescence meristems in an alternate phyllotaxy, perpendicular to the plane of the

tassel axis. Each branch primordia must increase considerably in size before secondary branch primordia are produced (Bonnett, 1948). Occasionally, secondary branch meristems may develop in only the basal-most primary branches. Bonnett (1937) reported such secondary branches in oats and Evans and Grover (1940) grasses. Furthermore, at any stage of development, the central spike of the tassel is in advance of the branches. This should be expected as the central axis is formed first with the branches differentiating from it.

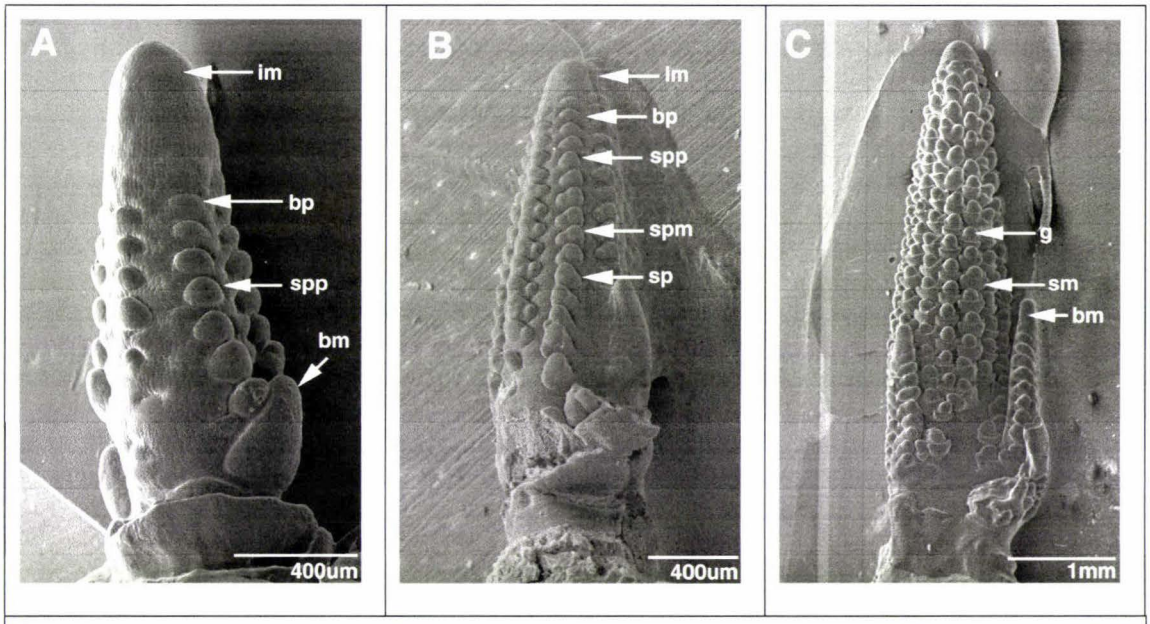


Figure 4-4 Wild type tassel developmental series

Key: (im) inflorescence meristem; (bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (spm) spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume

Figure 4-4 shows a series of developing tassels at different stages of development. Branch meristems arise from the basal-most branch primordia (Figure 4-4a). The remaining branch primordia develop into spikelet pairs.

4.1.4 Spikelet pair development

The remaining branch primordia on the central culm and primary branches of the tassel and the ears enlarge asymmetrically and divide unequally to produce two spikelet primordia (Figure 4-4, Figure 4-5). The orientation of the asymmetry (right vs. left side larger) appears random (Irish, 1997). Moreover, Bonnett (1948) observed that there was no morphological differences between branch primordia destined to

form branches or spikelet pairs. The spikelet primordium that was initially larger develops into the pedicelate spikelet and the smaller into the sessile spikelet (Cheng et al., 1983; Irish, 1997). Although the spikelet primordia are initially unequal in size, they enlarge into spikelet meristems of equivalent size whose subsequent development is nearly equivalent and coincident (Cheng et al., 1983).

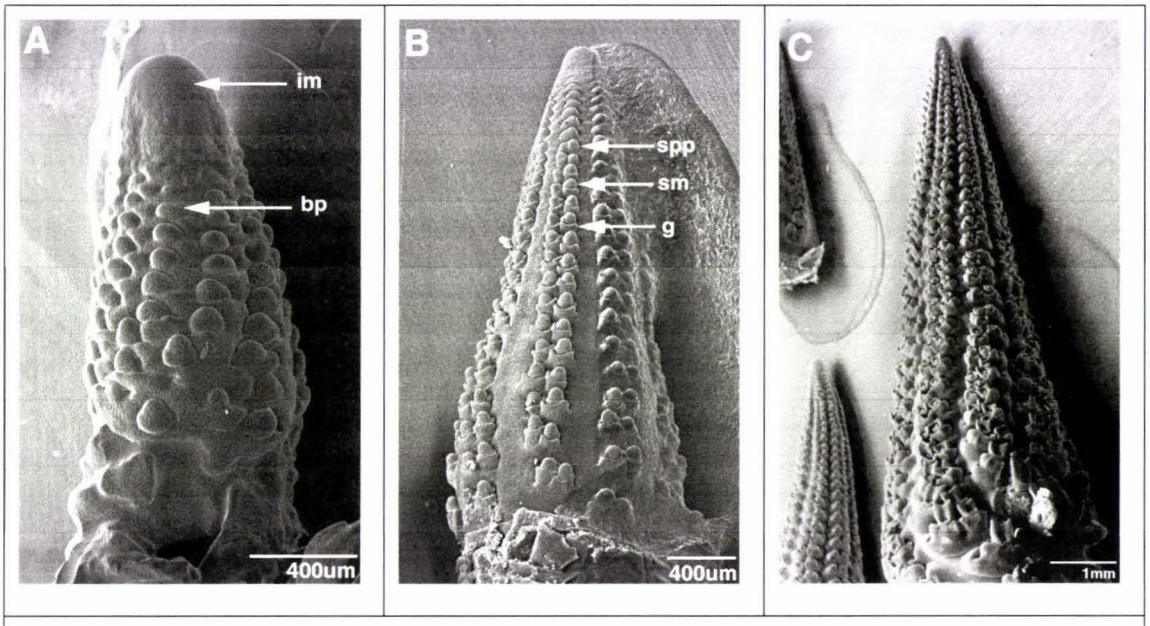


Figure 4-5 Wild type ear developmental series

Key: (im) inflorescence meristem; (bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (spm) spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume

4.1.5 Spikelet and floret development

Each spikelet primordia initiates four bracts in an alternate phyllotaxy in the plane of the inflorescence axis: a pair of sterile glumes then a pair of fertile lemmas. The outer bracts are initiated on the adaxial surface of the spikelet meristem, while the inner bracts initiate on the abaxial surface. In the tassel the glumes quickly grow to enclose the developing spikelet meristem, obscuring later development. In contrast, the glumes in the ear only partially enclose the developing spikelet meristem.

Basal and abaxial to the outer lemma, growth activities generate a flower which will become the lower member of the two flowered spikelet (Cheng et al., 1983) while the remaining spikelet meristem becomes the first or upper flower. The development of the upper flower is somewhat ahead of the lower sessile flower and this difference is maintained through to anthesis (Cheng et al., 1983).

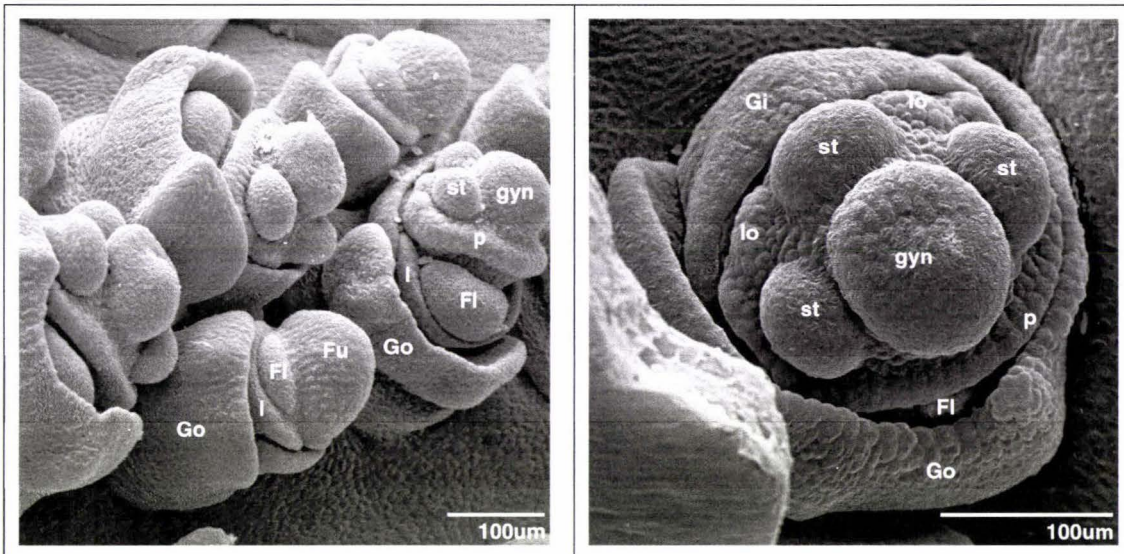


Figure 4-6 Spikelet and floret development in wild type ears.

Key: (Go) outer glume; (Gi) inner glume; (l)=lemma; (Fl) lower floret; (Fu) upper floret; (p) palea; (st) stamen; (lo) lodicule; (gyn) gynoecium.

Two paleas initiate between the two-floret primordia as thin ridges in the tassel spikelets and together with the lemmas these grow to enclose the developing floret. Wild type ears, however, only initiate the single palea of the upper floret that develops into a much smaller structure than in the male flower (Cheng et al., 1983), while the palea of the lower floret in female spikelets either does not initiate or remains rudimentary (Cheng et al., 1983).

Internal to the palea and lemma of each floret are the floret meristems. Each floret meristem initiates two lodicules internal to the lemma before initiating the floral organs. Three anther primordia appear around the remaining spikelet meristem. The remaining spikelet meristem then terminates in gynoecium. All the florets in the tassel develop fully (not shown). In contrast the lower floret of each female spikelet though initiated fails to develop fully. However, in a few types like Country Gentleman sweet corn, both flowers develop fully (Bonnett, 1948). The production of unisexual flowers from bisexual initials is discussed in detail by Cheng et al. (1983) and is outside the scope of this study, except to say that in male flowers the gynoecium aborts early in development, while it is the stamens that are aborted in the female flowers.

4.2 Ramosa-1

Postlethwait and Nelson (1964) proposed that the *ra1* phenotype was due to the failure of the branch primordia to make the transition to spikelet pair primordia. The result of this failure is the massive proliferation of branch meristems in the developing *ra1* inflorescences (Figure 4-7, Figure 4-8). The morphogenesis of the *ra1* tassels and ears are homologous up to the selective abortion of floral organs. Furthermore, the development of *ra1* can be broken into three distinct phases correlated with the structures observed in the mature *ra1* inflorescences: (1) initiation of branch meristems from branch primordia (2) replacement of branch meristems with indeterminate spikelet pair primordia (3) initiation of spikelet development, associated with the termination of indeterminate growth of the branch and spikelet pair meristems.

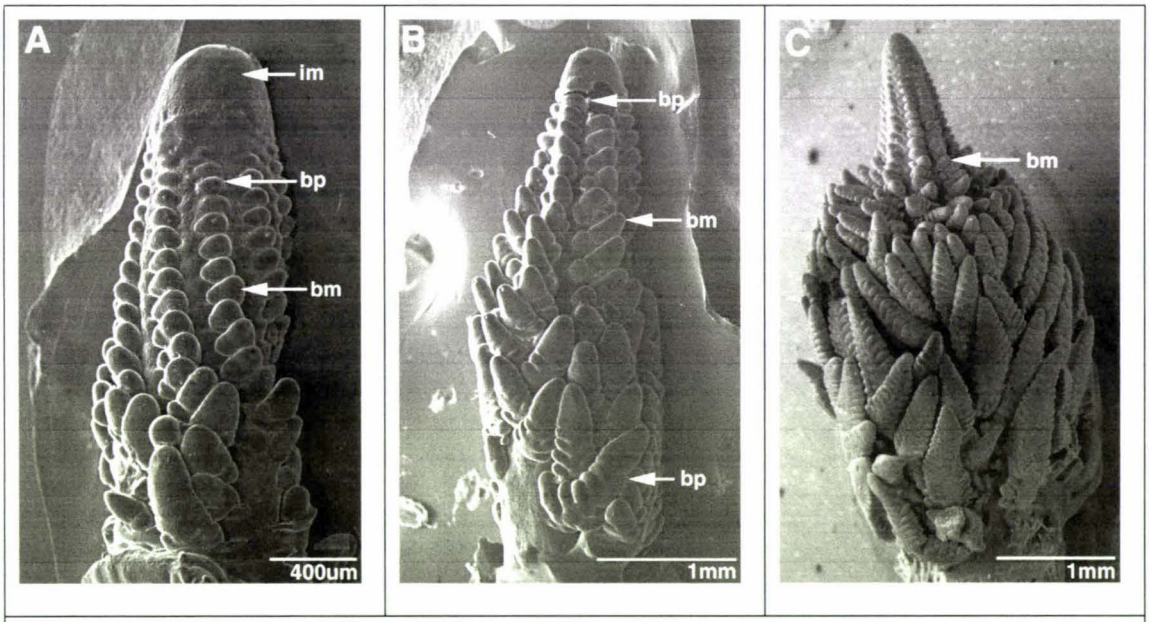


Figure 4-7 *Ra1* tassel developmental series

Key: (im) inflorescence meristem; (bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (spm) spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume

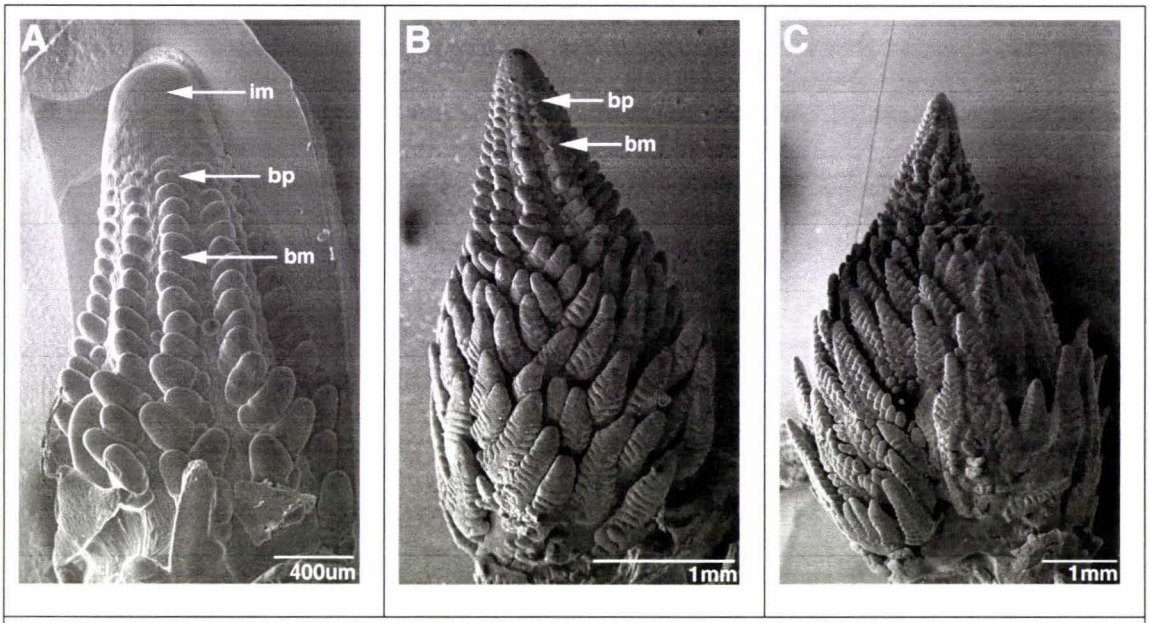


Figure 4-8 *Ra1* ear development

Key: (im) inflorescence meristem; (bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (spm) spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume

4.2.1 Branch development

Correlated with the massive increase in primary and higher order branches in mature *ra1* tassels and ears, is the presence of a large number of branch meristems in both the tassel and ear inflorescences as can be seen in Figure 4-7 and Figure 4-8. The development of these branch meristems is identical to the development of the branch meristems in the wild type tassel, except for increase in secondary branch meristems from the secondary branch primordia (Figure 4-9).

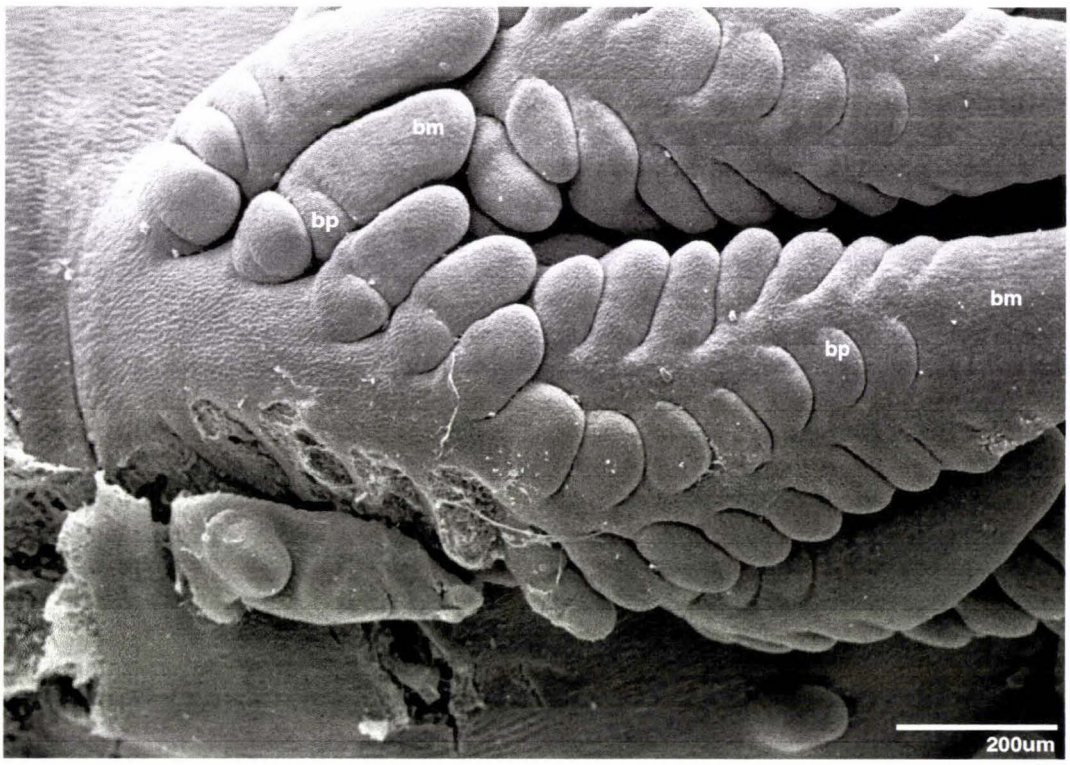


Figure 4-9 Secondary branch development in *ra1* ears

Key: (bp) branch primordia; (bm) branch meristem

4.2.2 Indeterminate spikelet pair development

The early development of indeterminate spikelet pair primordia in the *ra1* inflorescences is similar to the development of determinate spikelet pairs up until glumes are initiated. No distinctions can be made between the primordia that develop into indeterminate spikelet pair primordia and determinate spikelet pair primordia.

The first observable difference between indeterminate spikelet pair development and the development of determinate spikelet pairs is the appearance is the appearance of the glumes on the spikelet pair meristem. In wild type spikelet pairs, these are in the plane of the inflorescence axis. However, in *ra1* indeterminate spikelet pairs, the plane of glume initiation is perpendicular to the plane of the inflorescence axis (Figure 4-10). Coincident with the appearance of the glumes in indeterminate spikelet pairs is the appearance of a third actively growing region on the side of the indeterminate spikelet pair meristem opposite the sessile spikelet primordia (Figure 4-10).

Furthermore, while the indeterminate spikelet pair meristem continues to initiate bracts in an alternate phyllotaxy, the indeterminate spikelet pair axis begins to elongate. Associated with this elongation is the appearance of buds in the axils of the bracts. It is from these buds that the spikelet meristems develop.

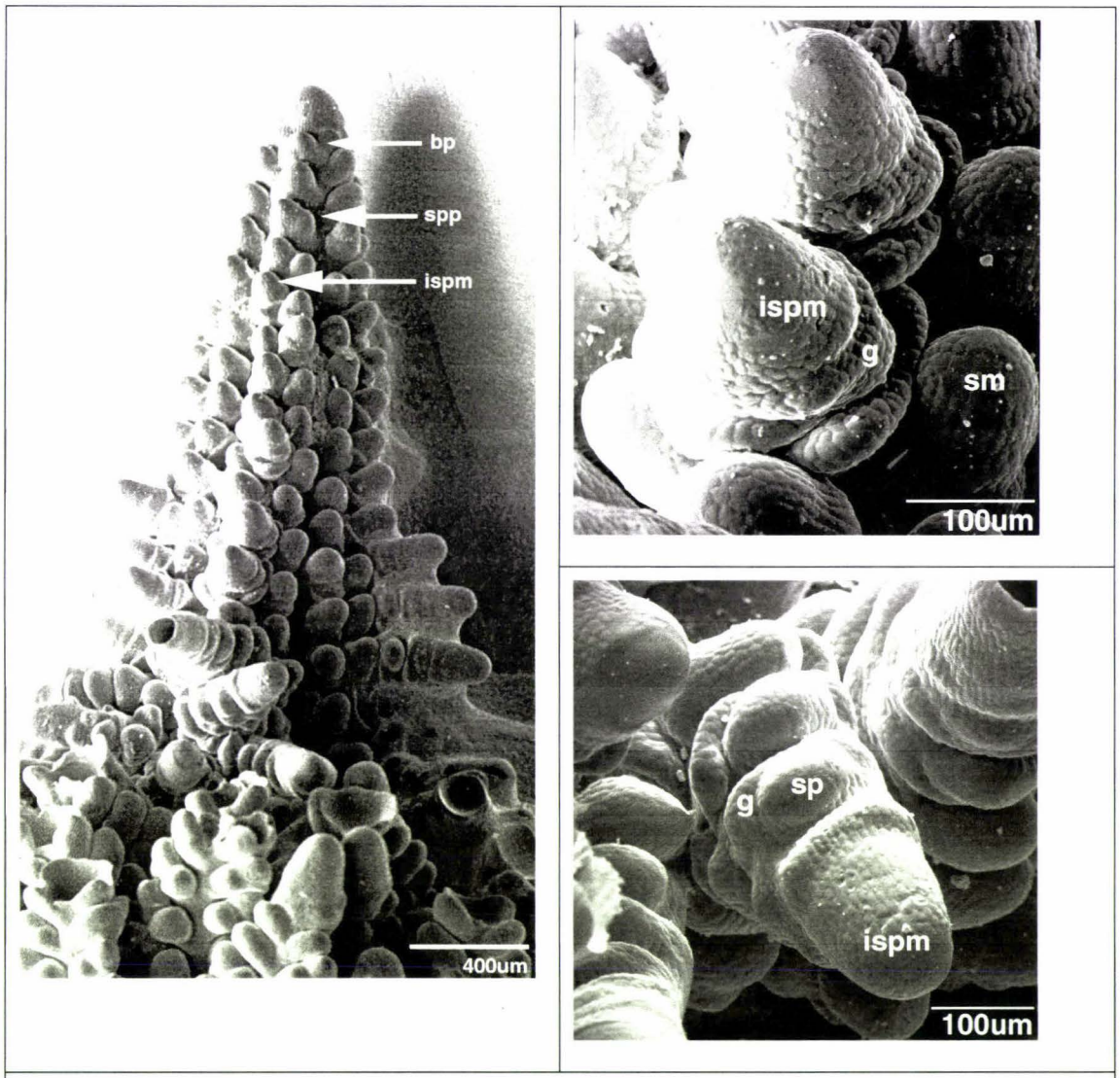


Figure 4-10 indeterminate spikelet development in *ra1* tassels

Key: (bp) branch primordia; (spp) spikelet pair primordia; (ispm) indeterminate spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume.

As noted in the adult *ra1* tassel morphology, a number of determinate spikelet pairs can be found at the tip of the tassel axis. It is highly likely that these determinate spikelet pairs were determined having only initiated the sessile spikelet from the indeterminate spikelet pair meristem, when the growth of all the other indeterminate meristems was determined.

4.2.3 Spikelet development

The development of the spikelet meristems from the spikelet primordia initiated by the indeterminate spikelet pair meristem is identical to the wild type siblings in the tassel (not shown). Though the pattern of pistillate spikelet development in *ra1*ears was identical to that in the wild type siblings, most of the female spikelets remain rudimentary. This is a direct result of a significant delay in the initiation of their development due to the prolonged initiation of branch meristems. Furthermore, only the sessile spikelets from the first initiated indeterminate spikelet pairs are sufficiently developed to have short silks.

4.3 Ramosa-2

Unlike *ra1*, there have been no studies that have documented the early development of the *ra2* phenotype in the literature. Morphological analysis of the *ra2* phenotype shows two distinct architectures in *ra2* inflorescences. Firstly, primary branches may sporadically occur in *ra2* ears. Secondly, indeterminate spikelet pairs replace spikelet pairs in the middle of both the tassel and ear.

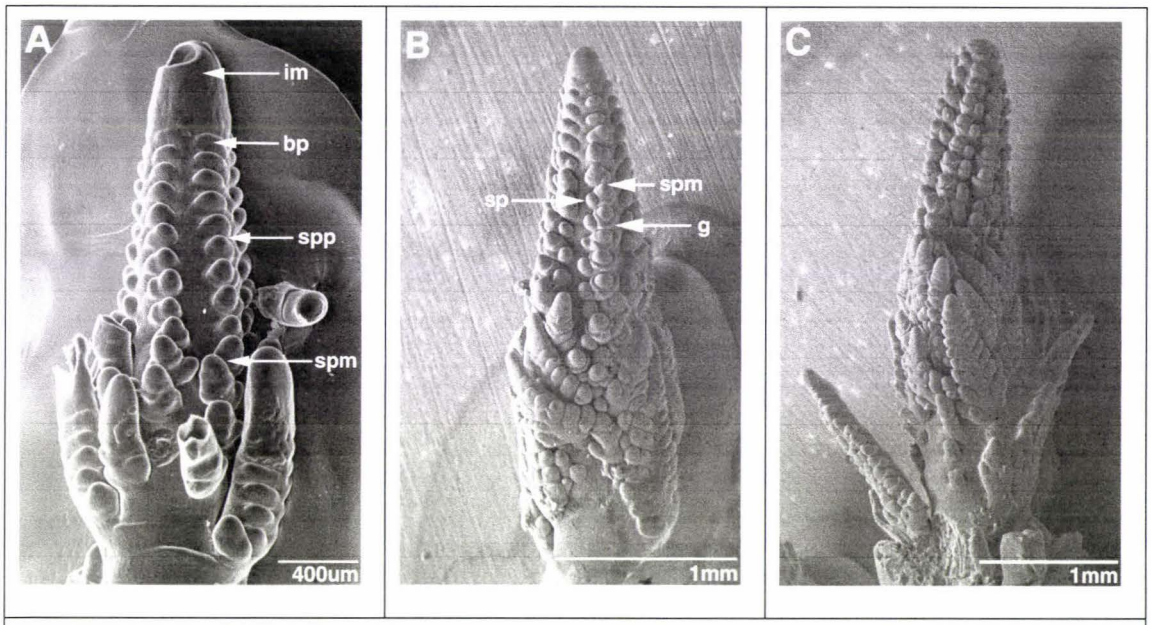


Figure 4-11 Morphogenesis in *ra2* tassels

Key: (im) inflorescence meristem; (bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (spm) spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume

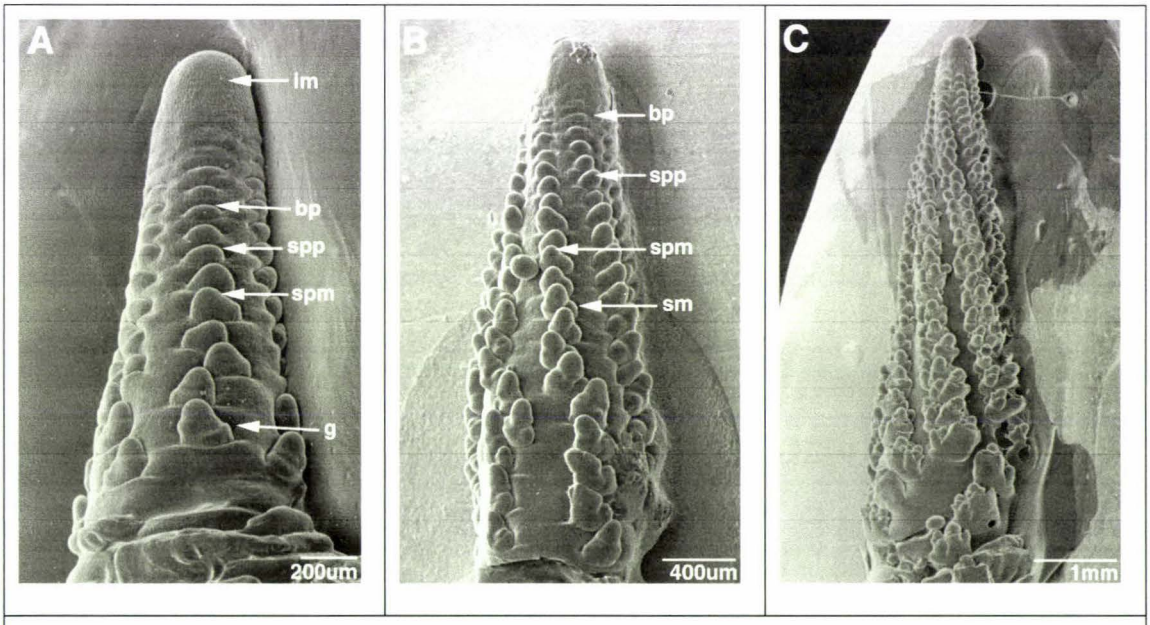


Figure 4-12 Morphogenesis in *ra2* ears

Key: (im) inflorescence meristem; (bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (spm) spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume

4.3.1 Branch development

The development of branch meristems in the *ra2* ears (not shown) is identical to the patterns observed in the *ra2* and wild type tassels. These branches develop directly from a single branch primordia and initiate indeterminate spikelets spikelet pairs in an alternate phyllotaxy (Figure 4-14). It is possible that the sporadic primary branches on the ear inflorescence is due to the determination of spikelet pair meristem from branch primordia becoming unstable and occasionally reverting to a branch meristem.

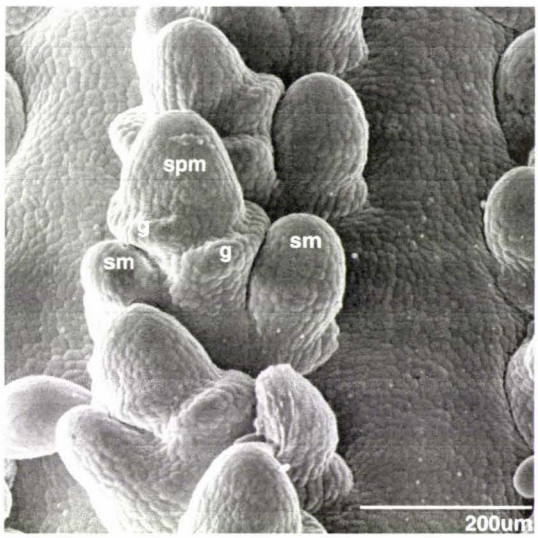


Figure 4-13 Indeterminate spikelet pair development in *ra2* ears

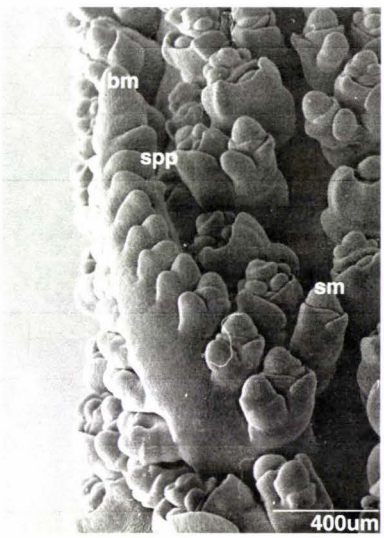


Figure 4-14 Primary branches on *ra2* ears

Key: (im) inflorescence meristem; (bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (spm) spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume

4.3.2 Indeterminate spikelet pair development

The development of the indeterminate spikelet pairs in *ra2* tassels and ears is homologous to the development described for *ra1* indeterminate spikelet pairs. In Figure 4-13, the initiation of second spikelet primordia coincident with the appearance of bracts is clearly visible. Furthermore, it is worth noting that the indeterminate spikelet pairs in the *ra2* ears don't develop elongated axis like the *ra1* and *ra3* siblings. Instead, between seven and two spikelet primordia are formed before the indeterminate spikelet pair meristem becomes determined. Moreover, it is the

presence of these extra spikelets that give *ra2* ears their disorganised appearance. Not the presence of extra florets as in Country Gentleman (Bonnett, 1948). In addition, like *ra1* the development of the *ra2* spikelets is not altered by the *ra2* phenotype and is otherwise homologous to the development of the wild type siblings.

4.4 *Ramosa-3*

There are three distinct phenotypes apparent in *ra3* populations: homozygous wild type, heterozygous *ra3* and homozygous ramsoa-3. Plants heterozygous for *ra3* closely resemble *ra2* in their development, while the *ra3* homozygotes resemble *ra1*. As with *ra1* and *ra2*, both *ra3* homozygotes and heterozygotes have indeterminate spikelet pairs, primary branches in the ears and in the case of *ra3* homozygotes, supplementary primary and secondary branches in the tassel similar to *ra1*. The development of the primary branches and indeterminate spikelet pairs is identical to the development of these structures in *ra1* and *ra2* inflorescences. Furthermore, like the *ra1* and *ra2* inflorescences, spikelet development is unaffected by the *ra3* phenotype.

4.4.1 *Ramosa-3* homozygote tassels and ears

The development of *ra3* homozygous inflorescences (Figure 4-15 and Figure 4-16) and *ra1* inflorescences is basically interchangeable. As in *ra1* tassels, *ra3* homozygous tassels initiate extra primary branch meristems from branch primordia with a transition to indeterminate spikelet pairs soon after half the total number of branch primordia have been initiated on the inflorescence axis.

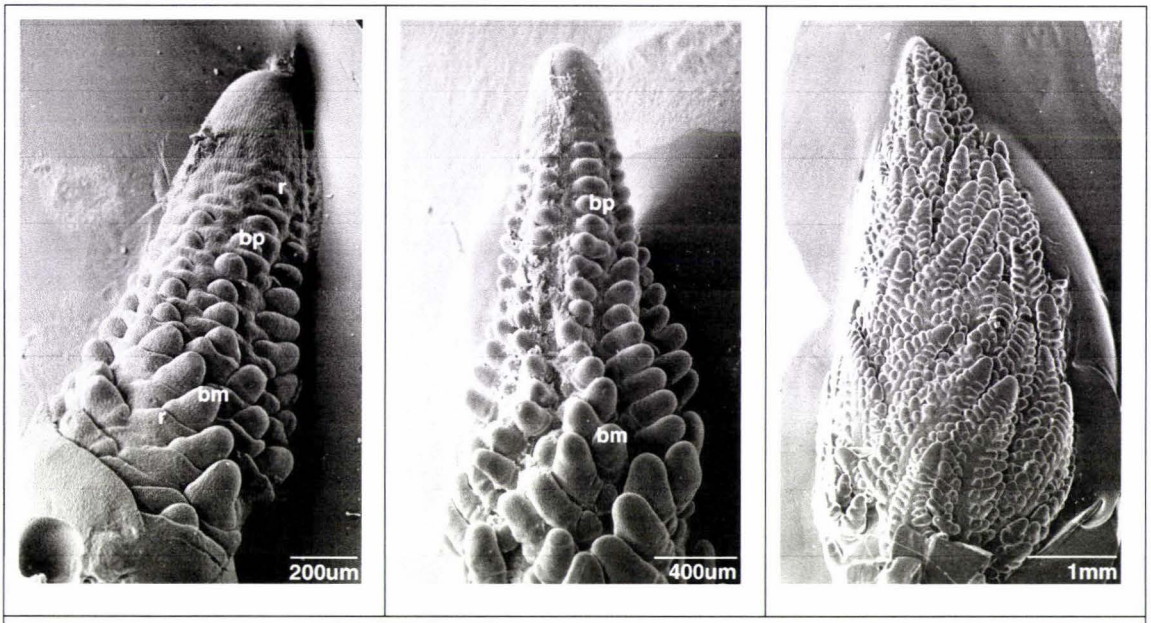


Figure 4-15 Development of *ra3* homozygous tassels

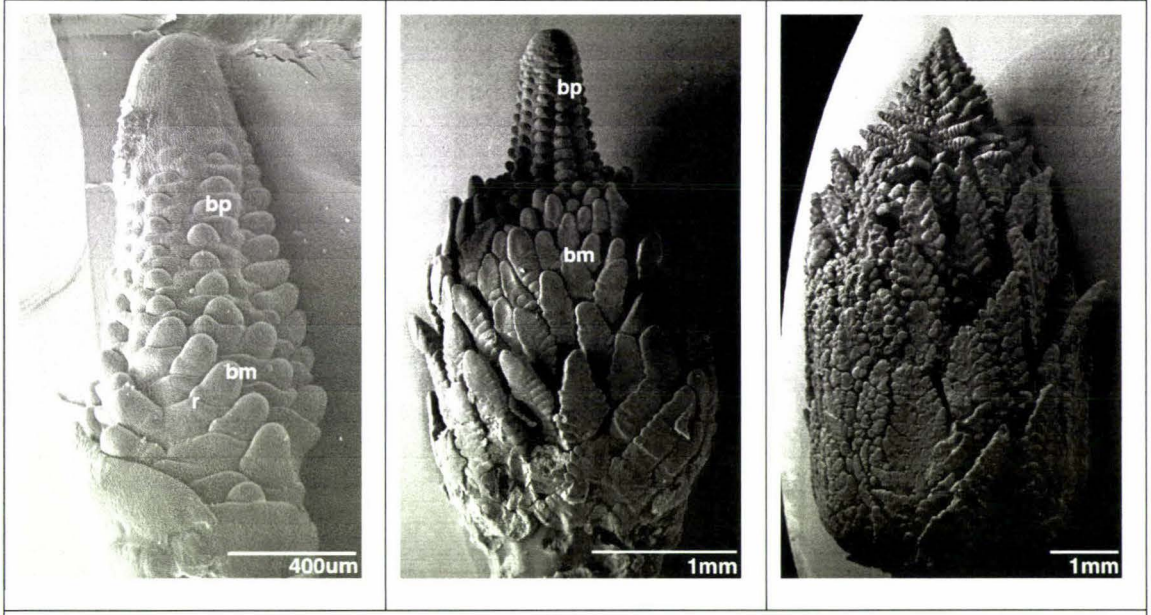


Figure 4-16 Development of *ra3* homozygous ears

Key: (im) inflorescence meristem; (r) rachis flap:(bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (ispm) indeterminate spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume

4.4.2 *Ramosa-3* heterozygote inflorescences

At maturity, the morphology of *ra3* heterozygous inflorescences closely resembles that of *ra2* tassels. As with the *ra2* tassels, both the *ra3* heterozygous inflorescences

initiate a number of branch meristems from the basal most branch primordia. Like *ra2* tassels, *ra3* heterozygous inflorescences initiate a number of indeterminate spikelet pairs and spikelets, of which the development has already been documented for *ra1* and *ra2*.

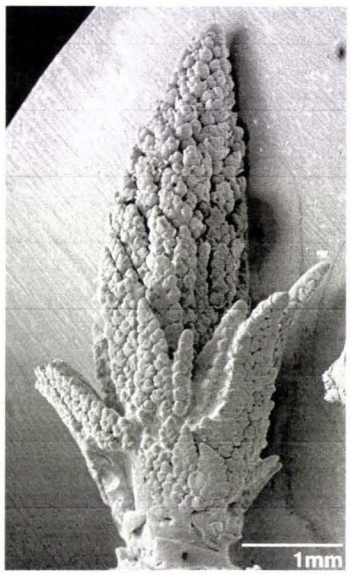
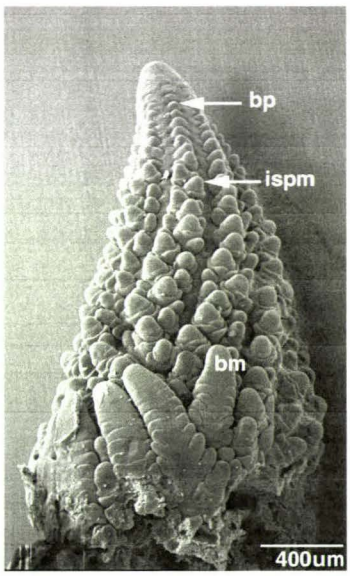
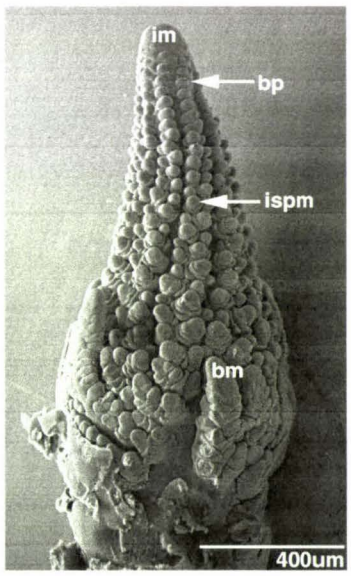


Figure 4-17 Development of *ra3* heterozygous tassels





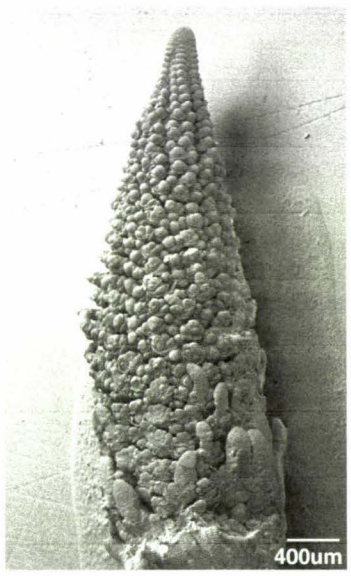


Figure 4-18 Development of *ra3* heterozygous ears

Key: (im) inflorescence meristem; (r) rachis flap:(bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (ispm) indeterminate spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (g) glume.

4.5 Branched silkless1

Veit et al. (1991) first documented *bd1* development and found that "development proceeds normally up to the point when floret primordia are formed. Although each spikelet gives rise to what appears to be the upper and lower floret primordia, an indeterminate number of abnormal divisions ensue to produce a highly branched structure. Thus, in contrast to *ra1*, which interferes with determination of the spikelet, bd appears to block determination of the floret." More recently, Colombo et al. (1998) concluded that "*bd1* plays an important role in regulating the transition from spikelet to floret development in the ear and that this function is duplicated by another unknown gene or set of genes during male inflorescence development." Although both of these observations are essentially correct, they are not explicit about the role of *bd1* in the determination of inflorescence architecture. Furthermore, it is obvious from the morphological analysis of bd inflorescences that *bd1* only affects spikelet development, which is what was proposed by Veit et al. (1991) and Colombo et al. (1998).

4.5.1 Spikelet development

As stated above, morphogenesis in *bd1* inflorescences proceeds normally until the initiation of spikelet primordia (Figure 4-19 and Figure 4-20). Several primary branch meristems are initiated at the base of the *bd1* tassel that develop into the primary tassel branches. Otherwise, the remaining branch primordia in the tassel and ear form determinate spikelet primordia.

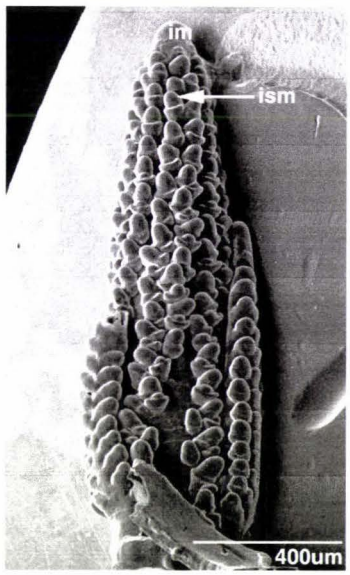


Figure 4-19 Morphogenesis of *bd1* tassels

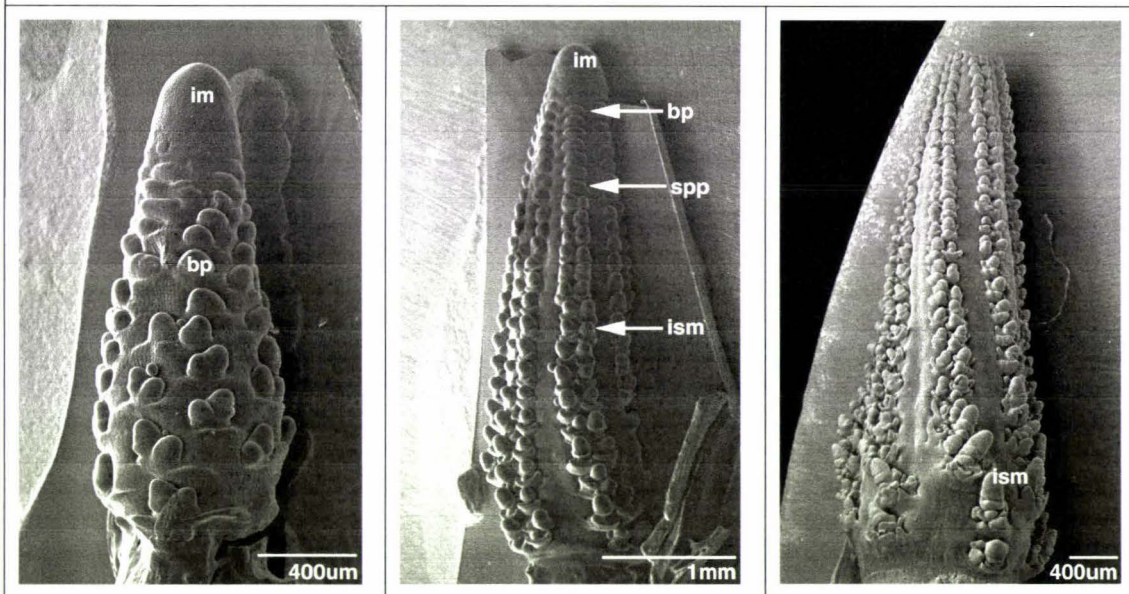


Figure 4-20 Morphogenesis of *bd1* ears

Key: (im) inflorescence meristem; (r) rachis flap:(bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (ispm) indeterminate spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (ism) indeterminate spikelet meristem (g) glume.

However, the initiation of spikelet development results in the indeterminate elongation of the spikelet meristem and the initiation of secondary meristem from the axils of the sterile outer glume (Figure 4-21). These spikelet meristems continue to elongate, initiation floret primordia in the axils of fertile bracts. Moreover, sometimes

the spikelet pair meristem reverts to a branch meristem (Figure 4-21) that initiates spikelet pairs in an indeterminate manner. The conversion of the spikelet meristem into a primary branch can determined because at its base is a glume and a floret. Therefore, *bd1* has a specific effect on the determination of spikelet development as well as the fertility of the bracts produced by spikelet meristems.

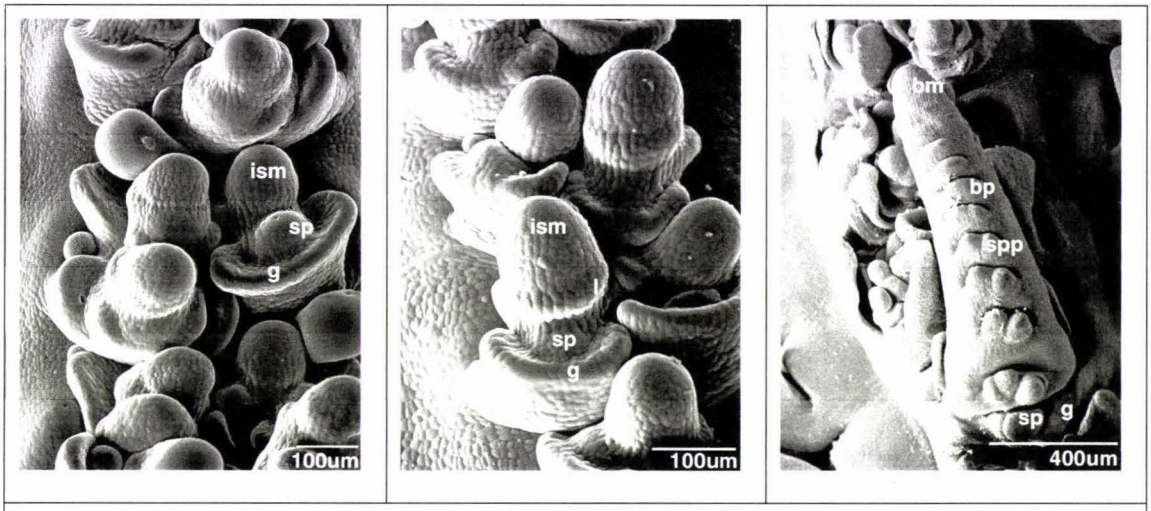


Figure 4-21 Development of spikelets in *bd1* ears

Key: (im) inflorescence meristem; (r) rachis flap:(bp) branch primordia; (bm) branch meristem; (spp) spikelet pair primordia; (ispm) indeterminate spikelet pair meristem; (sp) spikelet primordia; (sm) spikelet meristem; (ism) indeterminate spikelet meristem; (g) glume; (l) lemma.

5 *ramosa-2 ramosa-3* Double Mutant Analysis

The population segregating *ra2 ra3* double mutants had six distinct phenotypes that could be identified based on tassel and ear morphology. Out of a total population of seventy individuals, six distinct phenotypes could be distinguished: 14 +/- +/+, 24 +/- *ra3*/+, 15 +/- *ra3*/*ra3*, 3 *ra2*/*ra2* +/+, 10 *ra2*/*ra2* *ra3*/+ and 4 *ra2*/*ra2* *ra3*/*ra3*. Homozygous *ra2* plants were distinguishable by the upright primary branches, elongated pedicels and spikelet pair axes in the tassels. The *ra3* heterozygotes were primarily distinguished primarily by the ear phenotype where numerous primary branches were at the base of the ear inflorescence axis. Furthermore, the branch area of *ra3*/+ tassels was larger than in the *wild type* and *ra2*/*ra2* siblings. Individuals homozygous for *ra3* were defined based on their ear, which was heavily branched, as the upright branches of the *ra2* phenotype often obscured the increased branching area in the *ra3* homozygous tassels.

The results for the relative tassel areas are summarized in Figure 5-1. The primary branch and indeterminate spikelet pair areas in *ra2*/*ra2* *ra3*/+ were not significantly greater than in the +/- *ra3*/+ individuals. Similarly, the relative proportions of the primary branches and indeterminate spikelet pairs were not significantly different between the *ra2*/*ra2* *ra3*/*ra3* and +/- *ra3*/*ra3* individuals. As stated previously, this evidence is only preliminary. However, the *ra3* phenotype appears to be epistatic to *ra2*, as the *ra2*/*ra2* phenotype does not significantly alter the branching in both *ra3*/+ and *ra2*/*ra2* individuals.

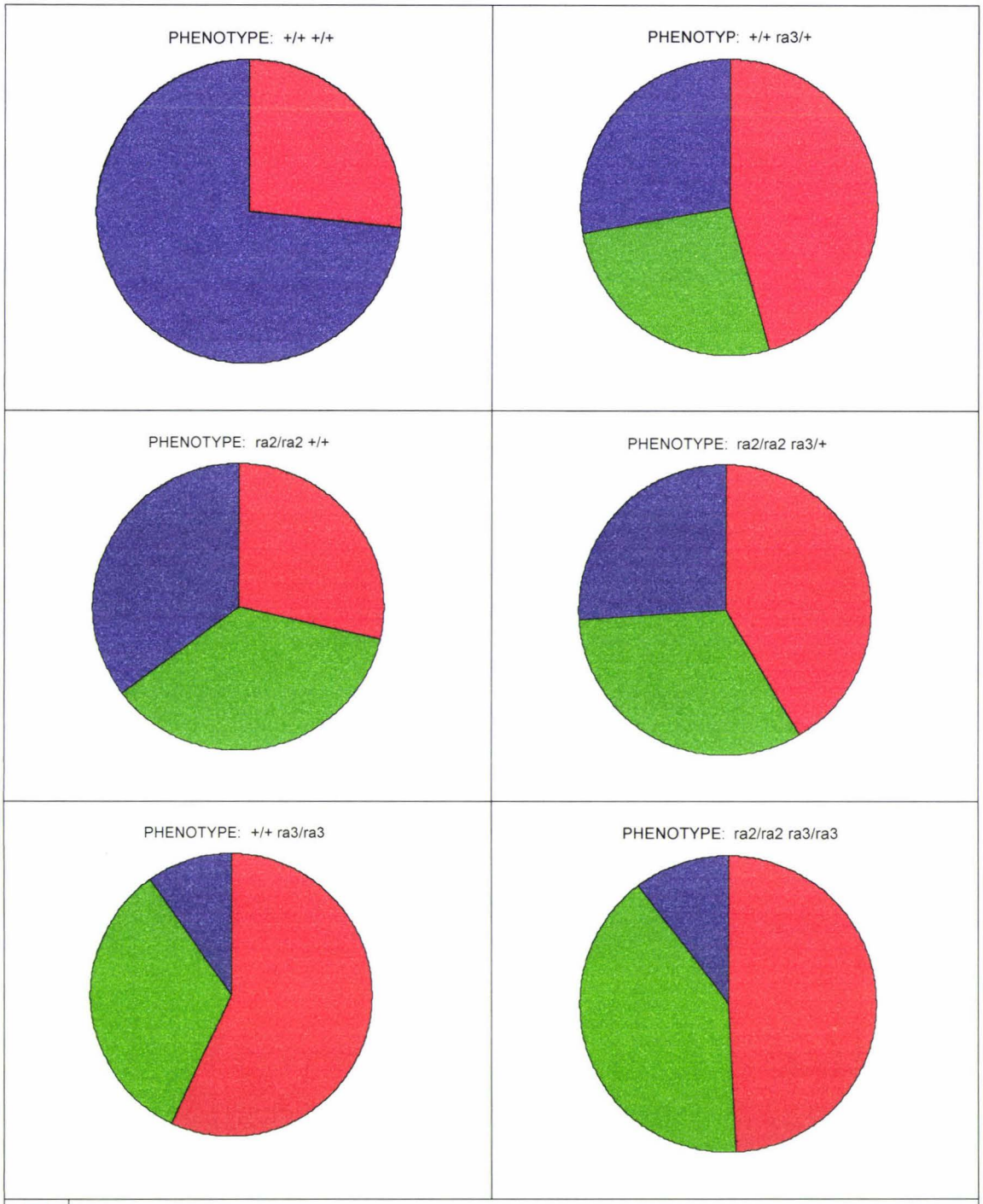


Figure 5-1Pie charts of the relative tassel areas of *ra2ra3* populations

6 Discussion

Branched silkless1 (*bd1*) and the *ramosa* (*ra1*, *ra2*, *ra3*) family of genes each appear to have specific roles in the determination of maize inflorescence architecture. From morphological and developmental analysis, the earliest detectable requirement for gene expression of the *bd1* and *ramosa* family of mutants can be determined and the effects of the absence of functional gene product documented. Therefore, it is possible to establish a role for *bd1* and the *ramosa* family of genes in the regulation of maize inflorescence development and refine the proposed genetic model for morphogenesis.

6.1 Ramosa-1 Morphogenesis

Postlethwait and Nelson (1964) proposed that the *ra1* phenotype was due to an interruption of the inflorescence developmental pathway at the initiation of spikelet primordia, leading to a massive increase in primary branching. The morphological and developmental data from this study confirms the fact that *RA1* is required for the determination of spikelet pair primordia from branch primordia. Furthermore, sufficient evidence also exists, which demonstrates that *RA1* either directly or indirectly determines the determinate growth of spikelet pair meristems.

Typically, both the *ra1* tassels and ears are profusely branched with around half the tassel length being comprised of indeterminate branches. These indeterminate branches are elaborated by branch meristems that initiate from branch primordia in place of spikelet pairs in developing *ra1* inflorescences. Furthermore, the branch primordia initiated by the branch meristems often develop into secondary branches as can be seen in the developing *ra1* inflorescences. Therefore, *RA1* is required for the commitment of meristematic cells in developing branch primordia to form spikelet pair primordia. Absence of *RA1* consequently results in the retardation of the transition between a branch developmental fate and a spikelet pair developmental fate.

RA1 is also required for the determination of indeterminate growth in the spikelet pair meristem that initiates the sessile spikelet before being determined to form the pedicelate spikelet. In *ra1* tassels and ears, indeterminate axes intermediate to branches and spikelet pairs occupied approximately 30% of the tassel by length. These indeterminate axes bear solitary spikelets in an alternate phyllotaxy. Doebley et

al. (1995) observed similar indeterminate branches with solitary spikelets in the *Suppressor of sessile spikelet1* (*Sos1*) phenotype. However, in the *Sos1* phenotype, these structures were due to the absence of sessile spikelets on branch axes. Analysis of the developing indeterminate axes showed that they were formed by the indeterminate growth of spikelet pair primordia. However, this maybe an indirect effect of the *ra1* phenotype due to the absence of expression of other genes triggered by *RA1*. Accordingly, the *ra1* phenotype results in a delay in the transition between indeterminate and determinate growth of the spikelet pair meristem.

Two further important points about can be made about the development of *ra1* inflorescences. Firstly, spikelet development is not affected by the *ra1* phenotype and therefore is part of a separate developmental program to the development of spikelet pairs. Secondly, spikelet pair development is determined at a later stage than usual in the *ra1* tassels, suggesting that another gene or group of genes is involved in the regulation of inflorescence development.

The effect of *ra1* on the length of the inflorescence and vegetative axis compared to *wild type* siblings points to *ra1* being in other developmental programs outside of the determination of inflorescence branching. The decrease in the length of *ra1* inflorescences was significant compared to their *wild type* siblings. However, this is probably a side effect of the prolific branching of the inflorescences. Potentially, the development of these branches increases the pressure on resources within the plant, resulting in less resources being available for the growth of the inflorescence meristem, thereby indirectly decreasing the length of the inflorescence axis. The effect of *ra1* on the length of the vegetative axis could potential be due to *RA1* being involved in the transition from vegetative to inflorescence development. A similar role for the *Arabidopsis* mutant *LEAFY* (*LFY*) has been proposed, where the initiation in inflorescence development was delayed in *LFY* plants (Schultz and Hughn, 1991; Weigel et al., 1992; Hula and Sussex, 1992). Therefore, *RA1* could be required for the transition from vegetative to inflorescence development and its absence results in a delay in the transition causing the vegetative meristem length to be increased.

6.2 Ramosa-2 Morphogenesis

Unlike *ra1*, the number of primary branches and higher order branches in *ra2* tassels is not significantly different from the wild type siblings. However, approximately thirty percent of the tassel length is indeterminate spikelet pairs similar in morphology to those described in *ra1*. However, the equivalent structures in the ear had multiple spikelets on a determinate axis that unlike *ra1* were able to fully develop. No other changes in branching were observed in the developing *ra2* inflorescences. Therefore, *ra2* is required for the determination of the growth of the spikelet pair meristem and its absence retards the determination of the indeterminate growth spikelet pair meristems.

The *ra2* phenotype is also characterised by upright branches and elongated spikelet axes and pedicels in the mature tassels. These characters most likely signify the requirement for *ra2* in other developmental pathways. The upright branch habit was linked to a difference in colouration or absence of pulvini-like structures at the base of primary branches. In wild type tassels, these structures allow the primary branches to spread out, increasing the efficiency of pollen release. In *ra2* tassels, these structures appear to be absent or disorganised resulting in the primary branches being unable to separate from the central culm. Furthermore, in the developing tassels, no such structures could be detected axils of developing branch axes while spikelet pairs were differentiating. Therefore, they are temporally separated in their effects. A similar argument can be applied to the regulation of elongation of the pedicels and spikelet pair axes by *ra2* occurs during a different phase of growth to the determination of branching.

Therefore, *ra2* is involved in three distinct processes in the growth and differentiation of maize tassels. First, it is required for the determination of spikelet pair primordia. Its absence results in indeterminate growth of the spikelet pair meristem coincident with an increase in the number of spikelets per spikelet pair. Second, *ra2* is the involved in the formation of pulvini in the axils of primary branch and it absence results in upright branches. Finally, *ra2* is involved in the determination of the length of axes in spikelet pairs. Furthermore, the second and third effects are limited to *ra2* tassels and were not observed in the developing ears. In addition, like *ra1*, the

morphogenesis of spikelets in *ra2* inflorescences was unaffected by the *ra2* phenotype.

Ears expressing the *ra2* phenotype often had a disorganised appearance with the regular ranks of kernels disrupted. As previously noted, this was due to the presence of extra spikelets in determinate spikelet pairs. Bonnett (1948) observed a similar disorganisation in the Country Gentleman line. However, this was due to the failure of the lower floret to abort, resulting in four kernels per spikelet pair. Furthermore, the delayed determination of the pistillate spikelet pairs reflects the fact that other genes may be involved in the determination of indeterminate growth of spikelet pair meristems.

6.3 Ramosa-3 Morphogenesis

The phenotype *ra3* homozygote is very similar to that of *ra1* inflorescences with a prolific number of branches in the tassel and ear. Furthermore, indeterminate spikelet pairs homologous with those in *ra1* inflorescences were also present and occupied a similar sized area. The similarity of the *ra1* and *ra3* phenotypes means that *ra3* has a similar timing of expression and role in development to *ra1*. However, *ra3* differs from *ra1* in that the heterozygote has a phenotype intermediary between the wild type and *ra2* homozygotes. Furthermore, the tassels of *ra3* heterozygote have a similar branch area to the wild type siblings, but have indeterminate spikelet pairs as well, which is very similar to *ra2* tassels. The ears of *ra3* heterozygote are also subtended by a number of branches with a region of determinate spikelet pairs with multiple spikelets distal to the branches. The region of determinate spikelet pair axes, however, is very small relative to the inflorescence length and has a disorganized appearance. However, the multiple spikelets of the area of indeterminate spikelet pairs quickly make the transition to determinate spikelet pair development. Therefore, *ra3* is essential not only for the determination of spikelet pair primordia from branch meristems, but for the determination of indeterminate growth in the spikelet pair meristem. However, the expression of another gene or group of genes is able to compensate for the loss of *ra3*.

Furthermore, the similarity between *ra3* homozygotes and heterozygotes to *ra1* and *ra2* respectively, is primarily due to fact that they both affect similar stages in

development. The *ra3* locus appears to be not only critical for the determination of spikelet pair primordia from branch primordia, but also for the determination of the number of spikelet primordia produced by spikelet pair primordia, similar to *ra1*. Where the amount of *RA3* present in the developing primordia is critical for the development of the primordia. In the *ra3* homozygotes, no *RA3* is present in the developing branch primordia resulting in the formation of branch meristems in place of the basal-most spikelet pair primordia in the tassel and ear. However, due to the functional redundancy in maize inflorescences, the presence of other factors reaches a level sufficient to determine the branch primordia to become spikelet pair meristems. However, in the continued absence of *RA3* in the homozygote means that the spikelet pair primordia are not determined resulting in its indeterminate growth. Sufficient *RA3* is present in heterozygous branch primordia for the transition from branch primordia to spikelet pair primordia. However, the concentration of *RA3* is not sufficient to determine the development spikelets from spikelet pair primordia. This is due to the presence of only one functional gene. As RNA transcripts are transient molecules with a finite life span, the rate at which the functional *ra3* allele can generate new transcripts is half the amount that can be generated by two functional genes. Therefore, if *RA3* is constitutively expressed by the heterozygote in developing spikelet pair primordia. The equilibrium level of *ra3* transcription vs. catabolism in the heterozygote would be much lower than in the wild type homozygote.

Therefore, *RA3* is required for the determination of spikelet pair meristems from branch primordia. Moreover, in the heterozygous condition, sufficient *RA3* is produced to determine this transition. Thus only in the *ra3* homozygote is the transition between branch and spikelet pair development is delayed. Furthermore, in both the *ra3* homozygote and heterozygote, *RA3* is required for the determination of indeterminate growth of the spikelet pair primordia, resulting in a further delay in the determination of spikelet pair meristems.

6.4 Branched silkless1 Morphogenesis

Morphological analysis of the *bd1* inflorescences was complicated by the male sterility observed in two families. However, the *bd1* tassels from the cosegregation analysis (generated for the cloning of *bd1* by transposon tagging) were added to the analyses because they were not sterile and had a similar morphology to that described

by Kempton (1934) and Nickerson and Dale (1955). Furthermore, between the three different populations, the two types of *bd1* ears could be observed. The tassels of *bd1* plants had a similar number of primary branches to the wild type siblings with the remaining tassel being spikelet pairs. However, analysis of the spikelets in individuals from the co-segregation population showed that the thickened appearance of *bd1* tassels was due to the presence of additional florets. Typically, these florets were on the same axis as the glumes, however, secondary axes could be found in the axils of the glumes that also had extra florets. Developmental analysis of the developing branch primordia in *bd1* tassels and ears was similar to wild type development until the initiation of development in the spikelet primordia in the tassel. Spikelet primordia in *bd1* tassels initiate two glumes like the wild type siblings. However, instead of differentiating into two florets, the spikelet primordium elongates and produces florets in an indeterminate fashion. This loss of determinacy in the spikelet meristem is similar to the loss of meristem determinacy in spikelet pair primordia in *ramosa* inflorescences. This is sufficient to explain the presence of additionally florets on the spikelet axes. Furthermore, a similar elongation of the spikelet pair meristem was associated with indeterminacy in the *ramosa* inflorescences. Chuck et al. (1998) proposed a similar loss of determinacy of the spikelet meristem as being the cause of the production of extra florets in indeterminate spikelet1 (*ids1*) spikelets.

The loss of determinacy of the spikelet meristem was not, however, sufficient to explain the presence of secondary spikelet shoots in the axil of the lower glume in the *bd1* tassels of the co-segregating population. *In situ* hybridisation experiments by Chuck et al. (1998) demonstrated that knotted1 (kn1) gene expression, which is normally associated with meristematic tissue and not in determinate organs such as leaves (Smith et al., 1992; Jackson et al., 1994) had an expanded area of expression in *ids1*-mum pistillate spikelets. Where the expanded domains of kn1 transcripts may reflect the extra areas of floret production (Chuck et al., 1998). Certainly, if genes associated with indeterminate growth in spikelets, like kn1, then it is quite possible that the suppression of development in the axillary buds of glumes is lost, resulting in its development. Furthermore, as the axillary buds of the glumes are not determined to form floret meristems, they instead adopt the default program of spikelet meristem development. Where the determination of floret primordia could occur in a manner similar to the determination of spikelet pair meristems from branch meristems.

Furthermore, Colombo et al. (1998) reported that *in situ* studies with ZAG1, ZAG2 and ZMM2 genes, markers for floral development, the expression in *bd1*-2 male spikelets was similar to the wild type. However, there was no apparent expression of ZAG1, ZAG2 and ZMM2 in the female *bd1*-2 spikelets that lead them to conclude that floret development in the ear was not even initiated, which accounts for the sterility of the *bd1* female inflorescence. Colombo et al. (1998) concluded that *BD1* plays an important role in regulating the transition from spikelet to floret development in the ear and that this function is duplicated by another unknown gene or set of genes during male inflorescences development. Moreover, *BD1* potentially could be required for the determination of genes that promote indeterminate growth in the spikelet meristem, similar to *IDS1*. The combination of the Colombo et al. (1998) hypothesis and the requirement of *BD1* for the determination of spikelet meristems would be sufficient to account for the *bd1* phenotype. Therefore, *BD1* is required for the determination of indeterminate growth of floret primordia, where the absence of *BD1* retards this determination in *bd1* spikelets. However, for a more definitive analysis it will be necessary to look at *bd1* in other backgrounds to separate it from the background effects such as sterility.

6.5 Double mutant combinations

Presently, only sufficient data was available on the *ra2 ra3* double mutant combinations. It appears that the *ra3* allele is epistatic to *ra2* with respect to the regulation of the area of the tassel occupied by primary branches and indeterminate spikelet pairs is relatively unaffected by the *ra2* phenotype. However, the stiff upright branches and elongated spikelet pairs axes of *ra2* were easily identifiable in both *ra3* heterozygous and homozygous individuals. These two traits reflect the fact that *ra2* is involved in two developmental independent of the determination of indeterminate growth in spikelet pair meristems. The *tasselseed4* (*ts4*) and *Tasselseed6* (*Ts6*) are both genes that are involved in the abortion of female gynoeciums in the male tassel and the determination of branching patterns in developing maize inflorescences (Irish, 1997a). Therefore, the *ra2* locus is only epistatic to *ra3* with respect to the determination of the development of branch primordia. This is to be expected as the effect of mutation to the *ra3* locus can be detected at a much earlier stage of

development. Furthermore, how the *ramosa* genes and *bd1* interact is the subject of future study.

6.6 Comparison of ramosa and branched silkless1 phenotypes

The phenotype of *ra1* inflorescences primarily stems form the retardation of the transition between branch primordia and spikelet pair primordia. Furthermore, similar comparisons can be drawn for the transitions affected by other mutants, where each mutant phenotype affects specific transitions in inflorescence development. From the evidence accumulated, both *RA1* and *RA3* appear to be required for the determination of spikelet pair meristems from branch primordia. Even though the appearance of the *ra1* and *ra3* tassels is remarkably similar, *ra3* ears are more tassel-like than the *ra1* ears. Similarly, *ra1*, *ra2* and *ra3* are all required for the determination of indeterminate growth in the spikelet pair meristems and resulting the delay of the determination of with indeterminate spikelet pairs with multiple spikelets in an alternate phyllotaxy as a result. Furthermore, the absence of functional *BD1*, however, specifically retards the determination of indeterminate growth of spikelet pair meristems. Resulting in a proliferation of meristems.

Therefore, while the *ramosa* genes have very specific effects on the development of the spikelet pair meristems, these are independent of the floret meristems as normal functional spikelets are present in the *ra1*, *ra2*, and *ra3* inflorescences. As noted earlier, the *ra1* ears though effectively sterile do produce functional spikelets that abort early in development. In contrast, the *bd1* allele affects only the development of the spikelet meristem. In the *bd1* tassel, an indeterminate number of functional florets are produced. Alternatively, the *bd1* ears are sterile with their spikelet meristems aborted early in development (Colombo et al., 1998) with occasional branch-like meristems arising in place of the upper floret. These indeterminate branches in the *bd1* ears reflect the instability of the meristematic fate of their spikelet meristems, which occasionally revert back to the default branch developmental state. In addition, the presence of these branches seems to be dependent on the genetic background of the lines studied with some lines showing more of this trait than others.

Therefore, the *ramosa* and *bd1* loci regulate specific transitions between different developmental phases in the inflorescence and thereby influence the inflorescence architecture. Moreover, these genes play an important part in other developmental programs. *RA1*, for example, could potentially play a role in the transition from vegetative to inflorescence development, while *RA2* is required for the determination of elongation in spikelet pairs and production of pulvini at the base of primary branches.

6.7 Refinement of the genetic model of inflorescence development

Veit et al. (1993) observed that the very specific effects conditioned by many developmental mutants suggest that inflorescence development can be treated as a modular process, with the mutant phenotypes defining the boundaries of component subprograms. In maize inflorescences there are four distinct levels of organisation: the branch, the spikelet pair, the spikelet and the floret. Each level of organisation represents a different phase in inflorescence morphogenesis and is the result of a specific type of meristem. Given the limitation of the effects *ramosa* and *bd1* to a particular phase of development, suggests that once initiated, each phase proceeds independently. Furthermore, primordia that have not been determined for a particular developmental fate will instead adopt the fate of the meristem from which they were derived. Therefore, it is possible to construct a genetic model for each phase of morphogenesis based on the specific effects of different developmental mutants.

6.7.1 Branch morphogenesis

Branch primordia have two possible developmental fates. They can elongate into branch meristems that produce secondary branch primordia or they can form spikelet pairs. At least five genes are potential required to determine the developmental fate of individual branch primordia. The Few branches1 (*Fbr1*) and unbranched1 (*ub1*) mutants have no or few branches in the tassel and therefore required for the indeterminate growth of branch primordia. In contrast, the *ra1* and *ra3* mutants have a massive increase in branching and are therefore required for the determination of spikelet pair primordia from branch primordia. For simplicity, the initiation of branch

meristems from branch primordia can be considered the default fate of branch primordia. Where *RA1* and *RA3* are required to re-determine the fate of the branch primordia to become spikelet pair primordia. The absence of one of these two genes results in the continued specification of branch meristems.

If the presence of *RA1* and *RA3* were the only gene products required and acted in a linear developmental program, then the absence of either of these genes would result in a complete failure of branch meristems to become determined to form spikelet pairs. However, morphological analysis of the *ra1* and *ra3* inflorescences shows that even though the number and area of the primary branches is increased, indeterminate spikelet pairs and spikelet pairs are also present in the *ra1* and *ra3* inflorescences. Therefore, either they are not the only genes that are able to determine this transition, signified by the presence of along the inflorescence axis.

Therefore, two hypotheses can be proposed to account for this phenomenon. Firstly, that some other gene or group of genes apart from *RA1* and *RA3* has a functional redundancy for the determination of spikelet pair meristems from branch primordia and would be part of different developmental program. Alternatively, *RA1* and *RA3* act in a complimentary fashion to determine branch primordia to become spikelet pair primordia.

Many of the genes known to regulate development in plants are transcription factors (Ma et al, 1991; Mandel et al., 1992; Huijser et al., 1992; Clark et al., 1997). Moreover, transcription factors form complexes that differentially bind RNA polymerase to localised regions of DNA. Therefore, one role for *RA1* and *RA3* would be to form a complex that preferentially binds RNA polymerase to sites required to trigger spikelet pair in branch primordia. The presence of both genes is essential for the optimum binding of RNA polymerase with the affinity of only *RA1* or *RA3* being much less. While the *ra3* heterozygote produces sufficient *RA3* to complex with *RA1* and determine spikelet pair identity in branch primordia, but an insufficient amount is present to initiate *RA2* for the determination of indeterminate growth in spikelet pair primordia.

This hypothesis has the advantage in that it can be tested by double mutant analysis, *in situ* hybridisation and characterisation of *RA1* and *RA3*. In double mutant lines the absence of *RA1* and *RA2* should either result in a sterile massively branched structure

without spikelets or spikelet pairs or at least significantly increase in the number and area of primary branches relative to the single mutant phenotypes. However, if the genes don't act in a combinatorial fashion and instead have a linear relationship, then the number and area of primary branches should remain constant. Furthermore, by cloning *ra1* and *ra3*, the cloned gene could be used to identify the localisation of expression by *in situ* hybridisation, while the function of the gene product could be derived from the cloned sequence.

Additionally, the determination of spikelet pair development characterises the end of one phase of development and the beginning of the next. Signified by the change in the type and organisation of organs in adult and developing inflorescences. However, these gross morphological features only reflect subtle changes of gene expression in the meristematic tissue from which different organs differentiate.

6.7.2 Spikelet pair morphogenesis

Spikelet pair development can be divided into four key switch points based on those determined by Irish and Nelson (1991). These switch points are: (1) indeterminate to determinate growth of the spikelet pair meristem, (2) initiation of buds on the meristem and (3) identity of the initiated bud (4) initiation of bud development (sterility vs. fertility). A gene or a small group of genes regulates each switch point, independent of the other switch points.

The indeterminate growth spikelet pair meristem in the three-*ramosa* phenotypes demonstrates that all three-*ramosa* genes are essential for the determination of indeterminate growth in the spikelet pair meristem. Double mutant analysis of *ra2 ra3* populations has demonstrated that *ra3* is potentially epistatic to *ra2* and therefore could regulate *RA2* expression. Where, the indeterminate spikelet pairs in *ra3* inflorescences are a result of the absence of *RA2*. Therefore, it is *RA2* that is required for the determination of indeterminate growth in spikelet pairs, not *ra3*. Similarly, the presence of independent spikelet pairs in *ra1* inflorescences is most likely due to the absence of *RA2*. As noted for branch development either a complimentary gene or a functional redundant gene product from another process is able to compensate for the absence of *RA2* and determine spikelet pair development in *ra3* and *ra2* inflorescences.

Two other genes, Suppressor of sessile spikelet (*Sos1*) and tasselseed 4 (*ts4*) also appear to be involved in the determination of indeterminate growth in spikelet pairs. Doebley et al. (1995) reported that *Sos1*-Ref was a dominant mutation that suppressed branching of the spikelet pair primordia in the ear and the tassel. In the tassel it also reduced the number of tassel branches, and it reduced the number of ear orthostichies in the ear in a quantitative fashion. However, the observation that *Sos1*-Ref reduced the number of orthostichies (an imaginary vertical line connecting different nodes) in the ear indicates that it acts early on inflorescence development before the spikelet pair primordia are formed. Therefore, *Sos1*-Ref does not directly regulate spikelet pair development and was not used in this model. Irish (1997a) reported that in *ts4* mutant tassels spikelet pair meristems fail to form spikelet meristems, resulting in the reiteration of spikelet pair meristems by spikelet pair meristems. Furthermore, at the base of *ts4* female inflorescences normal spikelet pairs developed, in the centre they developed into a sessile spikelet with two florets and an indeterminate branch, while at the tip they resembled tassels by reiterating the formation of spikelet pair primordia by spikelet pair primordia. Therefore, *TS4* is required for the determination of indeterminate growth in spikelet pair meristems like *RA2* and spikelet identity in the buds initiated. Furthermore, the presence of normal spikelet pairs at the base of the *ts4* ears signifies that other genes are involved in the regulation of spikelet pair development independent to *ts4*, like *ra2*. Where the potential relationship between *ra2* and *ts4* could be easily determined in *ra2 ts4* double mutants. Moreover, it appears that more than one gene is involved in the determination indeterminate growth in spikelet pair meristems.

6.7.3 Spikelet morphogenesis

Once primordia become determined to form spikelet primordia, they enter a new phase of development that is independent from the spikelet pair development. This is signified by the production of fertile and functional spikelets in *ra1*, *ra2* and *ra3* inflorescences. The maize spikelet is a determinate structure that has two sterile bracts (glumes) and two fertile bracts (lemmas) with florets in their axils on a short determinate axis. The initiation of spikelet phase of development results in the elongation of the elongation of the spikelet primordia into a spikelet meristem. The spikelet meristem then initiates bracts in an alternate phyllotaxy much like a vegetative meristem initiates leaves. However, once four bracts have been initiated,

the further development of the spikelet meristem becomes determined. In the axils of the bracts are floret primordia that are competent to form floret meristems. The floret primordia in the axils of the upper two bracts then elongate into determinate floret meristems that differentiate the floral organs. Like spikelet development, once the floret phase of development is initiated, it proceeds independently from the regulation of the spikelet meristem that initiated it. Furthermore, the similarity of the *bd1* and *ids1* phenotypes means they could potentially be different alleles of the same locus, which needs to be investigated.

6.8 Role of morphological genes in evolution

Genetic and molecular studies have shown a high degree of conservation between two dicotyledonous species, *Antirrhinum* and *Arabidopsis* for the control of floral development (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994; Weigel, 1995). Recent studies also show that these genes that can affect branching, may also be conserved between species as diverse tobacco (Kato et al., 1998; Amaya et al. 1999), tomato (Pnueli et al. 1998), petunia (Souer et al. 1998), *Brassica napus* (Mimida et al. 1999), rice (Kyozuka et al. 1998), and Eucalyptus (Southern et al. 1998). Consequently, maize can be expected to contain homologues of these genes.

In *Arabidopsis*, a mutation of either *LFY* (D. Weigel and R.J. Schmidt, unpublished data *from* Veit et al. 1993) or *AP1* (*ZAP1*; Mena et al., 1995) genes inhibits the transition to a floral pattern of development and whose gene functions seem comparable to either *ra1* or *bd1*. However, Veit et al. (1993) reported that neither of the *LFY* and *AP1* classes of genes correspond to either *ra1* or *bd1* based on mapping data. However, Chuck et al. (1998) reported that the maize spikelet meristem fate was regulated by the *APETALA2*-like gene *indeterminate spikelet1* (*ids1*). Consequently, the conservation of these gene functions between monocotyledonous and dicotyledonous species as yet remains unclear.

Conversely, the morphological organisation of the *Arabidopsis* and maize inflorescences show a number of differences. *Arabidopsis* typically initiates a number of flowers on an indeterminate inflorescence or branch axis. Alternatively, maize florets and the florets of other grasses are initiated from a spikelet meristem on determinate spikelet pair axis on an indeterminate branch or inflorescence axis.

Hence, maize and other cereal crops have evolved a number of intermediate phases in the vegetative to floral transition that is reflected in their inflorescence organisation.

In addition, Collins (1919) proposed that the "peculiar characteristics" of the "*Zea ramosa*" and pod corn mutations represent the reappearance of ancestral characters common to the Andropogoneae, since none of the maize-teosinte hybrids that were examined had a suggestion of either of these two mutations. Moreover, while the crossing maize and teosinte brought out a series of intermediate forms, it did not return to the point in the maize ancestry where it became differentiated from the Andropogoneae. Consequently, the *ramosa* and *bd1* loci can be use to determine the early evolution of maize and other monocotyledonous species.

7 Conclusion

The morphogenesis of maize inflorescences can be divided into a series of sequential steps. Each step relates to the elaboration of a specific developmental program, which once initiated it proceeds independently of the program that initiated it. Mutants can used to define each phase of developmental and it is through these mutants that it is possible to understand the genetic programs that underlie morphogenesis. The three-*ramosa* genes and *bd1* each affect specific "key switch points" resulting in the retardation of the next phase of development. Consequently, each of these "switch points" determines the architecture of maize inflorescences and their mutation could produce major evolutionary shifts in morphology.

8 Bibliography

Alberch, P. (1985) Problems with the interpretation of developmental sequences. *Systematic Zoology* 34(1): 46-58

Alverez, J., Guli, C.L., Yu, X-H. and Smyth, D.R. (1992) *terminal flower,* a gene affecting inflorescence development in *Arabidopsis thaliana. Plant Journal.* 2: 103-116

Amaya, I., Ratcliffe, O.J., and Bradley, D.J. (1999) Expression of CENTRORADIALIS (CEN) and CEN-like genes in tobacco reveals a conserved mechanism controlling phase change in diverse species. *Plant Cell.* 11(8): 1405-1417

Auger, D. and Sheridan, W.F. (1994) Using cytogenetics to enhance transposon tagging with Ac throughout the maize genome. *In* The Maize Handbook. Freeling, M. and Walbot, V. (eds.) Springer-Verlag, New York.

Bonnett, O. T. (1935) The development of the barley spike. *Journal of Agricultural Research.* 51: 451-457.

Bonnett, O. T. (1936) The development of the wheat spike. *Journal of Agricultural Research.* 54: 927-931.

Bonnett, O. T. (1940) Development of the staminate and pistillate inflorescence of sweet corn. *Journal of Agricultural Research.* 60 (1): 25-37.

Bonnett, O. T. (1948) Ear and tassel development in maize. *Annals of the Missouri Botanical Garden.* 35 (4): 269-288

Bonnett, O.T. (1953) Developmental morphology of vegetative and floral shoots of maize. *University of Illinois Agricultural Experimental Station Bulletin* 568

Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M. and Smyth, D.R. (1993) Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development* 119: 721-743

Cheng, P. C., Greyson, R. I. and Walden, D. B. (1983) Organ initiation and the development of unisexual flowers in the tassel and ear of Zea Mays. *American Journal of Botany.* 70 (3): 450-462.

Chomet, P.S. (1994) Transposon tagging with Mutator *In* The Maize Handbook. Freeling, M. and Walbot, V. (eds.) Springer-Verlag, New York.

Clark, S.E., Williams, R.W. and Meyerowitz, E.M. (1997) The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. *Cell* 89: 575-585

Clifford, H.T. (1987) Spikelet and floral morphology. *In* Grass systematics and evolution (ed. T.R. Soderstrom, K.W. Hilu, C.S. Cambell, and M.E. Barkworth), pp 21-30. Smithsonian Institution Press, Washington, DC.

Coen, E.S. and Meyerowitz, E.M. (1991) War of the whorls: Genetic interactions controlling flower development. *Nature* 353, 31-37

Coen and Nugent (1994) Evolution of flowers and inflorescences. *Development* (Supplement) 107-116

Collins, G.N. (1917) Hybrids of Zea ramosa and Zea tunicata. Journal of Agricultural Research 9: 383-395

Collins, G.N. (1919) Structure of the maize ear as indicated in Zea-Euchlaena hybrids. *Journal of Agricultural Research* 17(3): 383-395

Colombo, L., Marziani, G., Masiero, S., Wittich, P. E., Schmidt, R. J., Gorla, M. S., Pa, M. E. (1998) BRANCHED SILKLESS mediates the transition from spikelet to floral meristem during Zea mays ear development. *The Plant Journal* (1998) 16 (3): 355-363

Cone, K. (1994) Transposon tagging with *Spm. In* The Maize Handbook. Freeling, M. and Walbot, V. (eds.) Springer-Verlag, New York.

Cutler, H.C. and Cutler, M.C. (1948) Studies on the structure of the maize plant. *Annuals of the Missouri Botanical Garden* 35: 301-316

Dellaporta, S.L. and Moreno, M.A. (1994) Gene tagging with AC/DS elements in maize *In* The Maize Handbook. Freeling, M. and Walbot, V. (eds.) Springer-Verlag, New York.

Doebley, J., Stec, A., Wendel, J. and Edwards, M. (1990) Genetic and morphological analysis of a maize-teosinte F2 population: implications for the origins of maize. *Proceedings of the National Academy of Sciences, USA* 87:9888-9892

Doebley, J. and Stec, A. (1991) Genetic analysis of the morphological differences between maize and teosinte. *Genetics* 129: 285-295

Doebley, J. and Stec, A. (1993) Inheritance of the morphological differences between maize and teosinte: comparison of results for two F2 populations. *Genetics* 134: 559-570

Doebley, J. (1993) Genetics, Development and plant evolution. *Current opinion in Genetics and Development* 3: 865-872

Doebley, J., Stec, A. and Hubbard, L. (1997) The evolution of apical dominance in maize. *Nature* 386(3): 485-488

Doebley, J. (1997) Maize as a model system for investigating the molecular basis of morphological evolution in plants. *In* The Society of Experimental Biology symposium no 51. Control of plant development: Genes and Signals. Greenland, A.J., Meyerowitz, E.M., Steer, M. (eds.) The Company of Biologists Ltd.

Dorweiler, J.E. and Doebley, J. (1997) Developmental analysis of Teosinte glume architecture1: a key locus in the evolution of maize (Poaceae). *American Journal of Botany* 84(10): 1313-1322+C67

Easu, K. (1953) Plant Anatomy. Wiley, New York.

Freeling, M. (1992). A conceptual framework for maize leaf development. *Developmental Biology* 153, 44-58.

Galinat, W.C. (1959) The phytomer in relation to floral homologies in the American Maydeae. *Harvard University Botanical Museum Leaflets* 19(1): 1-32

Galinat, W.C. (1983) The origin of maize as shown by key morphological traits of its ancestor, Teosinte. *Maydica* 28: 121-138

Gernert, B. (1912) A new subspecies of *Zea mays* Linn. *The American Naturalist* 46:616-622.

Gottlieb, L.D. (1984) Genetics and morphological evolution in plants. *The American Naturalist*. 123 (5): 681-709

Green, P. (1991) Morphogenesis. *In* Plant Physiology, a treatise. Volume 10 Growth and Development. Steward, F.C. and Bidwell, R.G.S. (eds.) Academic Press, Inc. London

Hair, J.F., Anderson, R.E., Tatham, R.L. and Black, W.C. (1995) Multivariate data analysis with readings, 4th Edition. Prentice-Hall International, Inc. Sydney

Huijser, P., Klein, J., Lonnig, W.E., Meijer, H., Saedler, H. and Sommer, H. (1992) Bracteomania, an inflorescence anomaly, is caused by the loss of function of the MADS-box gene *squamosa* in *Antirrhinum majus. EMBO Journal* 11, 1239-1250.

Hula, E. and Sussex, I.M. (1992) *LEAFY* interacts with floral homeotic genes to regulate *Arabidopsis* floral development. *Plant Cell* 4: 901-913

Iltis, H.H. (1983) From teosinte to maize the catastrophic sexual transmutation. *Science* 222: 886-894

Irish, E.E. and Nelson, T.M. (1991) Identification of multiple stages in the conversion of maize meristems from vegetative to floral development. *Development* 112: 891-898

Irish, E.E. (1997) Class II tassel seed mutations provide evidence for multiple types of inflorescence meristems in Maize (*Poaceae*) *American Journal of Botany* 84 (11): 1502-1515

Jackson, D., Veit, B. and Hake, S. (1994) Expression of maize KNOTTED1 related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. *Development* 120: 405-413

Kato, H., Honma, T. and Goto, K. (1998) CENTRORADIALIS/TERMINAL FLOWER1 gene homolog is conserved in N. tabacum, a determinate inflorescence plant. *Journal of Plant Research* 111: 289-294

Kempton, J.H. (1921). Inheritance of ramose inflorescence in maize. *U.S. Department of Agriculture Bulletin* 971:1-20

Komeda, Y., Takahasi, T. and Hanzawa, Y. (1998) Development of inflorescences in Arabidopsis thaliana. *Journal of Plant Research* 111: 283-288

Kyozuka, J., Konishi, S., Nemoto, K., Izawa, T. and Shimamoto, K. (1998) Down-regulation of RFL, the FLO/LFY homolog of rice, accompanied with panicle branch initiation. *Proceedings of the National Academy of Science. USA.* 95: 1979-1982

Ma, H., Yanofsky, M.F. and Meyerowitz, E.M. (1991) *AGL1-AGL6*, an *Arabidopsis* gene family with similarity to floral homeotic and transcription factor genes. *Genes and Development* 5: 484-495.

Ma, H. (1998) To be, or not to be, a flower- control of floral meristem identity. ------- 14(1): 26-32

Mandel, M.A., Gustafson-Brown, C., Savidge, B. and Yanofsky, M.F. (1992) Molecular characterisation of the *Arabidopsis* floral homeotic gene *APETALA1*. *Nature* 360: 273-277

McClintock, B. (1947) Cytogenetic studies of maize and Neurospora. *Carnegie Institute, Washington Year Book* 46:146-152

Mena, M., Mandel., M.A., Lerner, D.R., Yanofsky, M.F. and Schmidt, R.J. (1995) A characterisation of MADS box gene family in maize. *Plant Journal* 8: 845-854

Meyerowitz, E.M. (1994) De-evolution and re-evolution of maize. *Current Biology* 4(2): 127-130

Miller, E. C. (1919) Development of the pistillate spikelet and fertilization in Zea Mays L.. *Journal of Agricultural Research.* 18 (5): 255-266.

Mimida, N., Sakamoto, W., Murata, M. and Motoyoshi, F. (1999) TERMINAL FLOWER1 like genes in Brassica species. *Plant Science* 142(2): 155-162

Moore, D.S. and McCabe, G. (1993) Introduction to the practice of statistics, 2nd Edition. W.H. Freeman and Company, New York.

Neuffer, M.G. (1994) Growing Maize for Genetic Studies. *In* The Maize Handbook. Freeling, M. and Walbot, V. (eds.) Springer-Verlag, New York.

Neuffer, M.G., Coe, E.H. and Wessler, S.R. (1997) Mutants of Maize. Cold Spring Harbour Press. New York

Nickerson, N.H. and Dale, E.E. (1955) Tassel modifications in Zea Mays. *Annals of the Missouri Botanical Garden* 42(3): 195-212

Page, T., MacKnight, R., Yang, C.H. and Dean, C. (1999) Genetic interactions of the Arabidopsis flowering time gene *FCA*, with genes regulating floral initiation. *The Plant Journal* 17(3): 231-239

Pidkowich, M.S., Klenz, J.E. and Haughn, G.W. (1999) The making of a flower: control of floral meristem identity in Arabidopsis. *Trends in Plant Science* 4(2) 64-70

Pineiro, M. and Coupland, G. (1998) The control of flowering time and floral identity in Arabidopsis. *Plant Physiology* 117(1): 1-8

Pnueli, L., Carmelgoren, L., Hareven, D., Gutfinger, T., Alverez, J., Ganal, M., Zamir, D. and Lifschitz, E. (1998) The self-pruning gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of *CEN* and *TFL1*. *Development* 125(11) 1979-1989.

Poethig, R.S. (1990) Phase change and the regulation of shoot morphogenesis in plants. *Science* 250: 923-930

Postlethwait, S. N. and Nelson, O. E. (1964) Characterisation of development in maize through the use of mutants. I. The polytypic (Pt) and ramosa-1 (ra1) mutants. *American Journal of Botany* 51 (3): 238-243

Ratcliffe, O.J., Bradley, D.J. and Coen, E.S. (1999) Separation of shoot and floral identity in Arabidopsis. Development 126(6): 1109-1120

Robbins, W. (1924) The botany of crop plants. P. A. Blakiston's son & Co., Philadelphia

Rodgers, J.S. (1950) The inheritance of inflorescence characters in maize-teosinte hybrids. *Genetics* 35: 541-558

Schmitz, G. and Theres, K., (1999) Genetic control of branching in Arabidopsis and tomato. *Current Opinion in Plant Biology* 2(1): 51-55

Schultz, E.A. and Hughn, G.W. (1991) *LEAFY*, a homeotic gene that regulates inflorescence development in *Arabidopsis*. *Plant Cell* 3: 771-781

Sheridan, W.F. (1988) Maize developmental genetics: Genes of morphogenesis. *Annual Review of Genetics* 22: 353-385

Smith, L.G., Greene, B., Veit, B. and Hake, S. (1992) A dominant mutation in the maize homeobox gene, *Knotted1* causes its ectopic expression in leaf cells with altered fates. *Development* 116:21-30

Souer, E., Krol, A., Kloos, D., Spelt, S., Bliek, M., Mol, J. and Koes, R. (1998) Genetic control of branching pattern and floral identity during Petunia inflorescence development. *Development* 125: 733-742

Southern, S.G., Strauss, S.H., Olive, M.R., Harcourt, R.L., Deroocq, V., Zhu, X.M., Llewellyn, D.J., Peacock, W.J. and Dennis, E.S. (1998) Eucalyptus has a functional equivalent of the Arabidopsis floral meristem identity gene leafy. *Plant Molecular Biology* 37(6): 897-910

Veit, B., Schmidt, R.J., Hake, S. and Yanofsky, M.F. (1993) Maize floral development: New genes and old mutants. *The Plant Cell* 5:1205-1215

Veit, B., Greene, B., Lowe, B., Mathern, J., Sinha, N., Vollbrecht, E., Walko, R. and Hake, S. (1991) Genetic approaches to inflorescence and leaf development in maize. *Development* (1991) Supplement 1: 105-111

Weatherwax, P. (1917) The development of the spikelets of Zea Mays. *Bulletin of the Torrey Botanical Club* 44: 483-495.

Weigel, D., Alverez, J., Smyth, D.R., Yanofsky, M.F. and Meyerowitz, E.M. (1992) *LEAFY* controls floral meristem identity in *Arabidopsis*. *Cell* 69: 843-859

Weigel, D. and Meyerowitz, E.M. (1994) The ABCs of floral homeotic genes. *Cell* 78, 203-209

Weigel, D. (1995) The genetics of flower development: From floral induction to ovule morphogenesis. *Annual Review of Genetics* 29, 19-39

Wolpert, L. (1981) Positional information and pattern formation. *Philosophical Transactions of the Royal Society of London*. 295: 441-450

Appendix I Boxplots

Adapted from Moore and McCabe (1993) and Hair et al. (1995)

Boxplots are a method of representing the distribution of a variable. A box represents the major portion of the distribution and the extensions reach to the extremes of the distribution. Furthermore, boxplots can be very useful in making comparisons of a single variable across groups or between several variables. (Hair et al., 1995)

Each boxplot is made up of a number of parts:

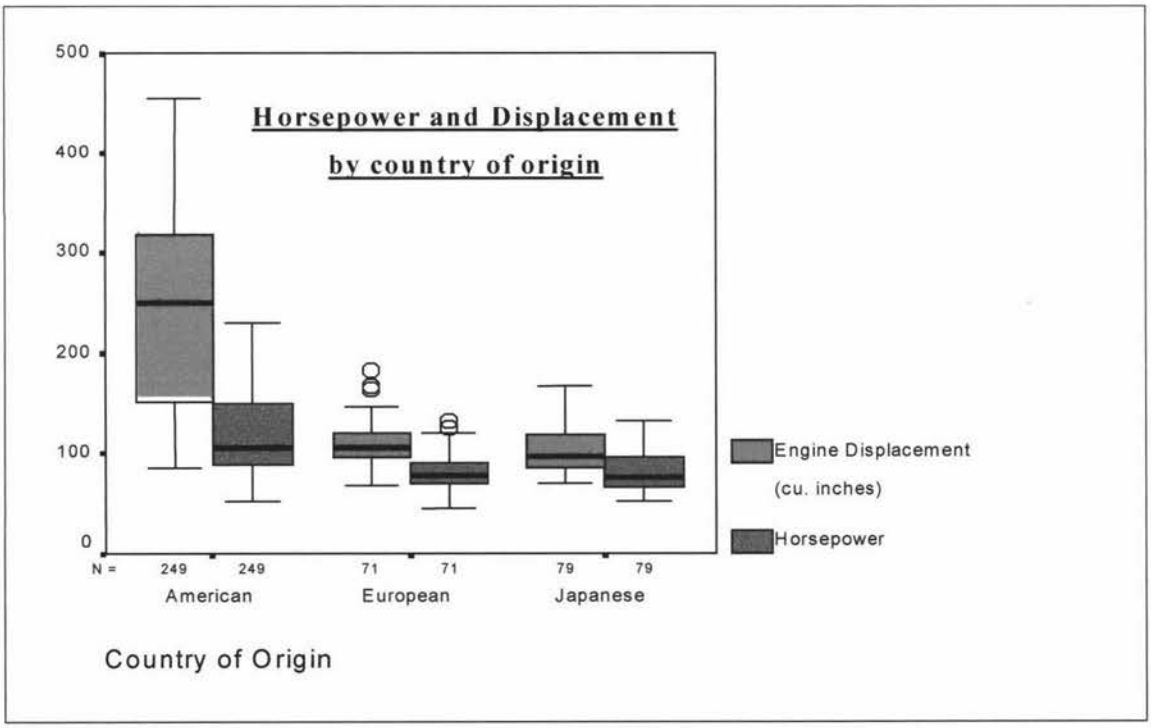
The ends of the box are the quartiles, so that the box length is the interquartile range that gives the range covered by the middle half of the data.

A line within the box marks the median.

Two lines (called whiskers) outside the box extend to the smallest and largest observations.

Suspected outliers are marked separately at the end of each whisker.

The result is that the centre, spread, and overall range of the distribution are immediately apparent from a boxplot (Moore and McCabe, 1993).



Appendix II Genetic Loci that specifically affect inflorescence morphology

Based on descriptions contained in Neuffer, Coe and Wessler (1997)

Barren stalk 1 (ba1)

Hofmeyr 1930

Described by Hofmeyr (1930), *ba1* has a long slender tassel with only a few branches and very few florets, mostly on the main spike. Though the tassel is often completely sterile depending on the severity of alleles. The ear and the concave groove on the culm are completely missing and tillers are usually absent. The phenotype has been mapped to the long arm of chromosome 3.

Barren stalk 2 (ba2)

Hofmeyr 1930

Located on the chromosome 2, near tassel seed 1, is the second barren stalk phenotype. Its phenotype is very similar to ba1. However, the tassel is more normal in its appearance.

Barren stalk fastigate (baf1)

Coe and Beckett 1987

The ear shoots of *baf1* are often absent or few in number. Those few that are present are erect and often fuse with developing internodes, which results in dried stalks having notches in the culm 4-6 cm above each ear node. The stem maybe curved and splits may also be present. The tassel branches are erect, lacking a pulvinus, and often spindly at their base. Located on the short arm of chromosome 9 near the lethal white 11 (*w11*).

Barren inflorescence (Bif1)

Neuffer and Sheridan 1977

A dominant mutation, plants heterozygous for *Bif1* have spikelets missing in large patches along the main axis of the ear and tassel as well as the tassel branches. Moreover, the tassel branching is reduced. The phenotype of plants homozygous for *Bif1* is even more extreme with a completely barren tassel and nearly barren ear. Located on the short arm of chromosome 8.

Barren inflorescence (bif2)

Briggs and Johal 1992; Neuffer and Briggs 1994

This phenotype has a variable expression on the ear resulting in few or no spikelets being produced at each floral node. If no spikelets are produced, the cob is smooth or slightly ridged or hairy where the cupule should have been. The ear spikelets are late to develop with the silks often failing to emerge form the husk. Fertilized silks typically viable seed. The tassel is thin and has few branches. Tassel spikelets are generally unpaired, large, and borne on 1cm-long pedicels. Very little pollen is shed because of the reduced number of spikelets.

Barren sterile (bs1)

Woodworth 1926; Micu 1981; Albertsen et al 1993

First reported by Woodworth (1926) as a weak plant, with little or no tassel and only vestiges of a pistillate inflorescence, the shank and husks. However, this stock was lost but may have been rediscovered again by Micu (1981) and Albertsen et al. (1993), who located an almost identical mutant (*tls1*) on the long arm of chromosome 1.

Branched silkless1 (bd1)

Kempton 1934

Bd1 ears are heavily branched with the basal most branches heavily ramified. Silks are sporadic or lacking, giving a sterile inflorescence. The tassel is branched normally but with

tassel spikelets bearing extra functional staminate florets to give and inflorescence with an overall thickened appearance but normal fertility. Located on the long arm of chromosome 7.

Cob turned out (cto1)

Vahrusheva 1975

The ears of *cto1* are inverted into a sheet or tube with the kernels place internally, though the expression is variable. The ear becomes turned inside out though afasciation with an inward roll following a split (Sarvella and Grogan, 1966).

Compact plant 1 (ct1)

Nelson and Ohlrogge 1957

Located on the long arm of chromosome 8 near *pro1* (proline responsive). This locus in a homozygous condition produces a semi-dwarf plant with all its parts reduced proportionally. The ears of *ct1* plants are also forked.

Compact plant 2 (ct2)

Glover 1968

This is also a semi-dwarf phenotype. However, the tassel is clubbed. It is located on the short are of chromosome 1 near *vp5* (viviparous).

Corngrass (Cg1)

Singleton 1951

The mutant *Cg1* is semidominant with pleiotropic and highly variable phenotype even in inbreed backgrounds. Plants that express the most extreme phenotype are short and highly tillered with narrow waxy leaves and vegetatively transformed ears and tassels. Such plants are usually completely sterile. Intermediate phenotypes, generally lack tillers, have narrow leaves with varying amounts of epicuticular wax, and have partially vegetative tassels and ears. In plants that *Cg1* expression is suppressed, the only obvious phenotype maybe the

presence of epicuticular wax sectors on adult phase leaves. The locus was mapped to the short arm of chromosome 3.

Corngrass (cg2)

Lysikov et al. 1984

Like *Cg1*.

Defective pistil (dep1)

Micu and Mustyatsa 1978

Located on chromosome 6 this locus when mutant produces female florets that have abnormal structure. The ovaries form two or more short defective pistils that do not function.

Fasciated ear (fae1)

Hake and Viet 1988; Sheridan 1988

Early in development, the cob meristem fasciates, resulting in the initiation of many more branch meristems, due to the proliferation of the meristem. The resultant cob, despite the excessive branching does produce seed.

Fascicled ear (Fas1)

Weatherwax 1917; Postlethwait and Nelson 1990

The dominant *Fas1* has repeated dichotomous branching of the inflorescence meristem. The resultant female ear consists of a number of slender ears surrounding a depressed central area. The ovules on the inner surfaces of the ears often abort. The tassel also has a branched central spike.

Few branches (Fbr1)

Neuffer 1989

Fbr1 is a dominant mutation where the tassel consists of a single spike or a few branches (0-3). The leaf bract replaces the second tassel bract (from the base) with a single functional

branch just below it. Irregular silk-like awns form at the tips of the glumes in the more extreme single spike homozygotes.

Indeterminate growth (id1)

Singleton 1946

Mutant plants, require extend growth and short days for flowering, remaining vegetative wile normal segregates flower. When the tassel is present, there is often vegetative proliferation of propagatable plantlets, with roots, arising from inside the glumes of the basal floret of many individual spikelets.

Indeterminate spikelet (ids1)

Chuck et al. 1998

Spikelets of *ids1* mutants are larger than those of the *wild type* due to the presence of a variable number of extra florets. Extra florets are also present in the female spikelets and often contain more than one silk located at ectopic positions. These silks are often unfused and fail to elongate to their proper length. However, manual pollinations will produce a few viable kernels. Approximately 5% of the female spikelets show elongated rachillas that emerge from the spikelet.

Leafy (Lfy1)

Shaver 1983

The dominant *Lfy1* loci produces plants with two extra nodes and leaves produced below the ear placement node. Five or more extra nodes and leaves maybe produced above the ear node. The ears produce extra rows of kernels. Though the expression is variable with background.

Papyrescent glume (Pn1)

Galinat and Manglesdorf 1957

The dominant mutation *Pn1* produces glumes that are long thick and fleshy when immature, becoming thin and papery as they dry with maturation of the ear. Though they are less obvious in the tassel.

Paired rows (pd1)

Langham 1940

The paired spikelet of maize (*Pd1*) is dominant over the single spikelets of teosinte (*pd1*). *pd1* is one of the loci that differentiate maize form teosinte.

Perennialism (pe1)

Shaver 1967

The recessive gene *pe1* is derived form perennial teosinte. It delays tassel formation and depending upon the environment and genetic background may cause failure of ear formation or replacement with a vegetative branch.

Pistillate florets (pi1)

Huelsen and Gillis 1929

Duplicate factor with *pi2*, which in *pi1 pi2* ears cause the secondary florets to develop, such as in the "Country Gentleman" and "Shoe Peg" expression. It is a quantitative character.

Pistillate florets (pi2)

Huelsen and Gillis 1929

Duplicate factor with *pi1*.

Polytypic (Pt1)

Nelson and Postlethwait 1954; Postlethwait and Nelson 1964

Heterozygous ears typically show a massive proliferation of pistillate tissue from the secondary floret. Homozygous ears may have a proliferation of tissue from both florets, along with elongation of the main axis of the ear with all degrees of developmental inhibition of alicole meristems. The development of staminate flowers may or may not be affected.

Ramosa-1 (ra1)

Beadle 1932; Gernert 1912

Ra1 plants have conical inflorescences. The ear is heavily branched with masses of proliferated primordia. Some silks are produced, which though fertile, fail to emerge past the husk leaves resulting in sterile ears. However, treatment with giberellic acid may suppress the mutant phenotype. Located on the long arm of chromosome 7.

Ramosa-2 (ra2)

Brink unpublished; Nickerson 1955

The *ra2* phenotype typically has branched ear with irregular kernel placement, though not conical like *ra1*. The tassels also have an increase in branching. These are arranged in a stiff upright. The *ra2* locus is located on the short arm of chromosome 3.

Ramosa-3 (ra3)

Perry 1954 unpublished

Located on chromosome 4, plants homozygous for *ra3* have branched inflorescences.

Silkless (sk1)

Jones 1925

Located on the short arm of chromosome 2, the plants of the *sk1* phenotype have no silks, due to the abort of the pistils. Otherwise the cobs grow normally.

Silky (si1)

Fraser 1933

Mapped to the long arm of chromosome 6, *si1* plants develop extra silks in the ear. Usually, a few scattered silks develop in the tassel. Pollen is shed sparingly. The arrangement of kernels can be irregular and classification may be difficult in some stocks.

Suppressor of sessile spikelet (Sos1)

Doebley, Stec and Kent 1995

A dominant mutation that blocks the formation of the sessile spikelet in both tassels and ears. Resulting in *Sos1* plants having a single spikelet instead of the usual pair. Also there was a decrease in the number of branches in the tassel and ranks of kernels in the ear. *Sos1* was mapped to the short arm of chromosome 4. Genetic mapping and a comparison of the developmental basis of the single spikelet condition in *Sos1* and teosinte demonstrated that that their similar phenotypes result from distinct genetic-developmental mechanisms.

Tasselless (tls1)

Albertsen et al. 1993

Homozygous mutant plants generally do not develop tassels, although ear shoots are produced. However, these do not develop within the husk tissue. Mutant seedling often appears more pubescent and darker green with a leathery texture. Tassel like structures can be obtained that vary from having only a couple of spikelets to a more complete-looking tassel. The expression of the phenotype is variable, especially among progeny from a self or a heterozygous cross. The locus has been mapped to the long arm of chromosome 1 near *Ts6*.

Tassel seed 1 (ts1)

Emerson 1920

Found on the short arm of chromosome 2, this phenotype has a terminal inflorescence that is usually completely pistillate and pendant and fails to produce pollen. The ear develops if the

tassel is removed soon after emergence. Secondary florets in the ear develop, giving an irregular arrangement of kernels. Often, mutant plants are weaker than their normal siblings.

Tassel seed 2 (ts2)

Emerson 1920

The tassel is almost completely pistillate except for the occasional staminate floret or perfect florets near branch tips. Many kernels are produced in the tassel, which is not compact like *ts4*. Only the branches are pendant rather than the whole tassel. When the tassel is removed the ear develops and has a crowded, irregular kernel arrangement due to abnormal development of both upper and lower florets. The locus has been mapped to the short arm of chromosome 1.

Tassel seed 3 (Ts3)

R.A. Emerson unpublished

Ts3 is a dominant mutation located on the long arm of chromosome 1. The *Ts3* tassels have large sections of either pistillate or staminate flowers in tandem. Some pollen maybe produced. As with the other tassel seed phenotypes, secondary florets develop in the ear. The original mutant was lost, but the current source found by H. Dooner resembles Emerson's description and is located in the same place and considered to be *Ts3*.

Tassel seed 4 (ts4)

Phipps 1928

This recessive mutation, found on the long arm of chromosome 3, produces compact, upright tassels that form a silky mass with both staminate and pistillate florets. The ear develops secondary florets once the tassel is removed and some pollen is usually shed.

Tassel seed 5 (Ts5)

Emerson 1932

The dominant *Ts5* loci, located on the short arm of chromosome 4, as a heterozygote a nearly normal tassel with anthers and scattered short silks. The tassel is not compacted like *ts4* and usually a few kernels develop in the tassel. Secondary florets develop in the ears. The homozygote is the same but more extreme in its phenotype.

Tassel seed 6 (Ts6)

Ts6 is a dominant locus, found on the long arm of chromosome 1, which produces heavy, compact, mostly pistillate tassels in the homozygote. The ear, which develops only when the pistillate tassel is removed, has an irregular kernel placement. Heterozygous plants have well-formed tassels with scattered silks and produce kernels in both the tassel and ear.

Tassel sheath (tsh1)

Briggs 1992

The leaves of *tsh1* plants develop at the base of tassel branches, wrapping the lower portion of the tassel in a husk-like sheath. Leaves also develop at the base of spikelet pairs in both the ear and tassel. (Briggs)

Teopod 1 (Tp1)

Lindstrom 1925

Mapped to the long arm of chromosome seven, *Tp1* is a semi-dominant mutation with pleiotropic effects on both vegetative and reproductive structures. The mature plants are usually highly tillered, with narrow leaves, and produce epicuticular wax and prop roots at higher nodes than normal. Mutant plants may also have one or two small ears and an unbranched tassel. In both the ear and tassel, leaves usually subtend spikelets, and various parts of the spikelet maybe vegetatively transformed. As a result of this transformation, *Tp1* plants are often male sterile and less often female sterile. Mutant plants can usually be

identified after the emergence of leaves 7 and 8 on the basis of leaf width and the presence of epicuticular wax. (Poethig)

Teopod 2 (Tp2)

Peterson 1959

Located on the long arm of chromosome 10, *Tp2* is also a semi dominant mutation with a phenotype similar to *Tp1*. Mutant plants are usually tillered and have narrow leaves with epicuticular wax at their tip and produce prop roots at abnormally high nodes. Leaves usually subtend the spikelets in the tassel and ear. In some cases the tassel maybe absent. *Tp2* differs from *Tp1* in having a more sever effect on vegetative and tassel morphology and a less severe effect on ear morphology. Although the expression of *Tp2* is variable with genetic and environmental modification, mutant plants can be identified at the emergence of leaves 7 and 8 on the basis of leaf width and presence of epicuticular wax and can always be identified from the *wild type* on the basis of tassel morphology. (Poethig)

Teopod 3 (Tp3)

Poethig 1988

Tp3 is a semi-dominant mutation located on the short arm of chromosome three that in most genetic backgrounds increases tillering and decreases the number of lateral tassel branches. In backgrounds that permit more extreme expression, heterozygous plants become highly tillered; have narrow leaves, waxy leaves, and vegetatively transformed ears and tassels. *Tp3* maps closely to *Cg1* and maybe an allele of this locus. J. Beckett originally identified *Tp3*. (Poethig)

Teosinte branched (tb1)

Burnham 1961

Mapped to the long arm of chromosome 1 near *adh1* (*alcohol dehydrogenase1*), *tb1* has been identifies as being critical in the evolution of maize from teosinte. Typically, *tb1* plants resemble teosinte with many tillers and the ears becoming tassel-like bearing a few kernels at the base.

Terminal ear (te1)

Mathews et al. 1974

The terminal meristem produces stalked ear appendages at the tip with silks emerging for the top whorl of leaves. Often the tassel is enclosed in husk leaves and the lower branches become somewhat feminised or may be complete ears with their own set of husks. The first sub-terminal node produces a tassel that pushes past the terminal ear to emerge through the above-mentioned silks to give the appearance of a terminal tassel with silks. The other nodes are also distinctive, often shortened erratically with curved internodes. The leaves emerge at sharp angels, noticeable even at the juvenile stage with the plants being much smaller than the normal siblings. The *te1* gene has been cloned and mapped to chromosome 3.

Tillered (Tlr1)

Neuffer et al. 1987

A dominant mutation located on the long arm of chromosome 1 that produces tillers on non-tillered stocks and extra tillers on others. The heterozygote has smaller ears with staminate tips and a long shank. Homozygotes are more extreme, like *tb1*, with grassy tillers, many small primitive ears, and a single-spike tassel.

Tassels replace upper ears (tru1)

Sheridan 1988

The tassels replace upper-ears with the upper ear branches tassel like and the tillers bear ears (W.F. Sheridan, unpublished)

Tunicate (Tu1)

Collins 1917 a, b

The dominant mutation *Tu1* is located on the long arm of chromosome 4. Typically the kernels are enclosed in long, heavy glumes and the staminate spikelets with large, coarse glumes with some feminisation. Homozygotes are more extreme with grossly vegetative structures that produce no pollen and rarely produce seed in the ear.

Two-ranked (tr1)

Langham 1940

Located on the short arm of chromosome 2, two-ranked spikelets (*tr1*) in the ear and central branch of the tassel of teosinte is recessive to many-ranked spikelets (*Tr1*) in the ear and tassel of maize. Though the mutation to the teosinte form does occur in maize (Langham 1940) with a distichous rather than decussate phyllotaxy in the ear axis. This is another of the family of loci that differentiate maize from teosinte.

Unbranched (ub1)

Neuffer et al. 1968

Located on chromosome 8, this locus in extreme expression produces a single unbranched spike in the tassel but usually one or two short branches develop at the base (Galinat 1991). This simply inherited recessive gene shows incomplete dominance in some genetic backgrounds.

Vestigial glume (Vg1)

Sprague 1939

The dominant *Vg1*, located on the long arm of chromosome 1, produces very small glumes, leaving the cob and anthers exposed. Usually male-sterile due to drying of the exposed anthers before anthesis but some pollen is produced if the tassel is protected. Also the ligule on the leaf blade is vestigial, but unlike liguleless, the leaf blade is oriented at a sharp angle from the culm as in normal plants. This allows good separation of plants at the seedling stage by the absence of ligules (Laughnan 1956).

Appendix III Tables

Ramosa-1 analysis

Table III-1 Comparison of primary axis characters for family 191

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Total plant height (m)	*Wild type*	10	1.61	0.12	0.04
	Ra1	10	1.67	0.13	0.04
Plant height (m)	*Wild type*	10	1.09	0.10	0.03
	Ra1	10	1.17	0.10	0.03
Peduncle length (m)	*Wild type*	10	0.19	0.02	0.01
	Ra1	10	0.17	0.02	0.01
Tassel height (m)	*Wild type*	10	0.33	0.02	0.01
	Ra1	10	0.33	0.03	0.08
Tassel and peduncle (m	*Wild type*	10	0.52	0.03	0.01
	Ra1	10	0.50	0.04	0.01

Table III-2 Comparison of primary axis characters for family 201

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Total plant height (m)	*Wild type*	10	1.82	0.23	0.07
	Ra1	10	1.91	0.22	0.07
Plant height (m)	*Wild type*	10	1.31	0.20	0.06
	Ra1	10	1.42	0.18	0.06
Peduncle length (m)	*Wild type*	10	0.20	0.02	0.00
	Ra1	10	0.18	0.02	0.01
Tassel height (m)	*Wild type*	10	0.31	0.02	0.01
	Ra1	10	0.30	0.04	0.01
Tassel and peduncle (m)	*Wild type*	10	0.51	0.03	0.01
	Ra1	10	0.48	0.05	0.02

Table III-3 Family 191-tassel statistics

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of primary branches	*Wild type*	10	10.00	2.45	0.77
	Ra1	10	>20	0.00	0.00
Number of secondary branches on the 1st primary branch	*Wild type*	10	1.20	0.42	0.13
	Ra1	10	6.60	1.58	0.50
Number of secondary branches	*Wild type*	10	2.00	0.67	0.21
	Ra1	10	18.60	3.47	1.10
Number of tertiary branches	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	0.60	1.07	0.34
Number of intermediate branches	*Wild type*	10	1.20	1.14	0.36
	Ra1	10	>20	0.00	0.00
Tassel Length	*Wild type*	10	33.10	2.09	0.66
	Ra1	10	32.65	2.79	0.88
Branch area (cm)	*Wild type*	10	10.25	1.23	0.39
	Ra1	10	18.00	2.86	0.90
Intermediate branch area (cm)	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	9.60	2.66	0.84
Spikelet pair area (cm)	*Wild type*	10	22.85	1.75	0.55
	Ra1	10	5.05	2.22	0.70
% Branches area/tassel height	*Wild type*	10	30.96	3.24	1.02
	Ra1	10	55.00	6.14	1.94
% Intermediate branch area/tassel Height	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	29.81	9.54	3.02
%Spikelet pair area/tassel height	*Wild type*	10	69.03	3.24	1.02
	Ra1	10	15.18	6.10	1.93

Table III-4 Family 201 tassel statistics

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of primary branches	*Wild type*	10	7.80	1.48	0.47
	Ra1	10	>20	0.00	0.00
Number of secondary branches on the 1st primary branch	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	5.80	1.87	0.59
Number of secondary branches	*Wild type*	10	1.30	0.48	0.15
	Ra1	10	12.00	3.09	0.98
Number of tertiary branches	*Wild type*	10	.00	0.00	0.00
	Ra1	10	.60	0.97	0.31
Number of intermediate branches	*Wild type*	10	2.10	1.52	0.48
	Ra1	10	>20	0.00	0.00
Tassel Length	*Wild type*	10	31.40	2.23	0.71
	Ra1	10	30.10	3.67	1.16
Branch area (cm)	*Wild type*	10	8.75	0.95	0.30
	Ra1	10	15.40	1.88	0.59
Intermediate branch area (cm)	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	10.20	2.35	0.74
Spikelet pair area (cm)	*Wild type*	10	22.65	1.84	0.58
	Ra1	10	4.50	1.43	0.45
% Branches area/tassel height	*Wild type*	10	27.88	2.45	0.78
	Ra1	10	51.29	3.97	1.26
% Intermediate branch area/tassel height	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	33.89	6.42	2.03
%Spikelet pair area/tassel height	*Wild type*	10	72.12	2.45	0.78
	Ra1	10	14.81	3.77	1.1906

Table III-5 Analysis of T1 in Family 191

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T1	Wild type	10	branch		
	Ra1	10	branch		
Length of branch at T1	Wild type	10	9.92	2.15	0.68
	Ra1	10	11.00	2.16	0.68
Number of Spikelet Pairs at T1	Wild type	10	14.00	2.49	0.79
	Ra1	10	16.20	2.25	0.71
Number of Spikelets in the 1st spikelet pair at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.40	1.26	0.40
Total length of 1st spikelet pair axis at T1	Wild type	10	0.74	0.22	0.07
	Ra1	10	1.52	0.92	0.29
Length of 1st spikelet pair axis at T1	Wild type	10	0.24	0.16	0.05
	Ra1	10	0.91	0.96	0.30
Length of 1st spikelet pedicel at T1	Wild type	10	0.16	0.09	0.03
	Ra1	10	0.10	0.11	0.03
Length of 2nd spikelet pedicel at T1	Wild type	10	0.50	0.29	0.10
	Ra1	10	0.55	0.40	0.13
Number of glumes in 1st spikelet at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of florets in 1st spikelet at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of glumes on 2nd spikelet at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.20	0.63	0.20
Number of florets on 2nd spikelet at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.70	0.82	0.26

Table III-6 Characters at T1 in family 201

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T1	Wild type	10	branch		
	Ra1	10	branch		
Length of branch at T1	Wild type	10	9.86	1.35	0.43
	Ra1	10	9.10	3.05	0.96
Number of Spikelet Pairs at T1	Wild type	10	13.40	2.84	0.90
	Ra1	10	12.60	3.44	1.09
Number of Spikelets in the 1st spikelet pair at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.30	0.95	0.30
Total length of 1st spikelet pair axis at T1	Wild type	10	0.92	0.24	0.08
	Ra1	10	1.23	0.63	0.20
Length of 1st spikelet pair axis at T1	Wild type	10	0.19	0.10	0.03
	Ra1	10	0.53	0.24	0.08
Length of 1st spikelet pedicel at T1	Wild type	10	0.19	0.11	0.03
	Ra1	10	0.06	0.05	0.02
Length of 2nd spikelet pedicel at T1	Wild type	10	0.74	0.23	0.07
	Ra1	10	0.49	0.31	0.10
Number of glumes in 1st spikelet at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of florets in 1st spikelet at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of glumes on 2nd spikelet at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of florets on 2nd spikelet at T1	Wild type	10	2.00	0.00	0.00
	Ra1	10	2.30	0.48	0.15

Table III-7 Characters at T2 in family 191

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T2	*Wild type*	10	Spikelet Pair		
	Ra1	10	Int. spikelet pair to Branch		
Length of branch at T2	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	5.82	2.19	0.69
Number of Spikelet pairs at T2	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	7.20	3.29	1.04
Number of spikelets in the 1st spikelet pair at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.60	1.90	0.60
Total length of spikelet pair axis at T2	*Wild type*	10	0.76	0.19	0.06
	Ra1	10	1.05	1.39	0.44
Length of spikelet pair axis at T2	*Wild type*	10	0.13	0.05	0.02
	Ra1	10	0.35	0.26	0.08
Length of 1st spikelet axis at T2	*Wild type*	10	0.18	0.09	0.03
	Ra1	10	0.08	0.05	0.02
Length of 2nd spikelet axis at T2	*Wild type*	10	0.63	0.18	0.06
	Ra1	10	0.33	0.13	0.04
Number of glumes on 1st spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of fertile florets on 1st spikelet at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of glumes on 2nd spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of fertile florets on 2nd floret axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.10	0.32	0.10

Table III-8 Characters at T2 in family 201

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T2	*Wild type*	10	Spikelet Pair		
	Ra1	10	Int. Branch to Branch		
Length of branch at T2	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	3.82	2.76	0.87
Number of Spikelet pairs at T2	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	5.10	3.81	1.21
Number of spikelets in the 1st spikelet pair at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	4.30	3.06	0.97
Total length of spikelet pair axis at T2	*Wild type*	10	0.82	0.20	0.06
	Ra1	10	1.89	1.82	0.58
Length of spikelet pair axis at T2	*Wild type*	10	0.22	0.29	0.09
	Ra1	10	0.49	0.42	0.13
Length of 1st spikelet axis at T2	*Wild type*	10	0.16	0.10	0.03
	Ra1	10	0.07	0.08	0.03
Length of 2nd spikelet axis at T2	*Wild type*	10	0.61	0.20	0.06
	Ra1	10	0.39	0.14	0.05
Number of glumes on 1st spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of fertile florets on 1st spikelet at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of glumes on 2nd spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of fertile florets on 2nd floret axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.20	0.42	0.13

Table III-9 Characters at T3 in family 191

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T3	*Wild type*	10	Spikelet Pair		
	Ra1	10	Indeterminate Spikelet Pair		
Number of spikelets in the 1st spikelet pair at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	3.70	1.16	0.37
Total length of the spikelet pair axis at T3	*Wild type*	10	0.44	0.14	0.05
	Ra1	10	1.55	0.79	0.25
Length of 1st spikelet pair axis at T3	*Wild type*	10	0.09	0.02	0.01
	Ra1	10	0.69	0.50	0.16
Length of 1st spikelet axis at T3	*Wild type*	10	0.06	0.03	0.01
	Ra1	10	0.14	0.10	0.03
Length of 2nd spikelet axis at T3	*Wild type*	10	0.35	0.14	0.04
	Ra1	10	0.17	0.13	0.04
Number of glumes on first spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of fertile florets on 1st spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of glumes on second spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of florets on 2nd spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.20	0.42	0.13

Table III-10 Characters at T3 in family 201

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T3	*Wild type*	10	Spikelet Pair		
	Ra1	10	Indeterminate Spikelet Pair		
Number of spikelets in the 1st spikelet pair at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	4.80	0.79	0.25
Total length of the spikelet pair axis at T3	*Wild type*	10	0.48	0.08	0.02
	Ra1	10	1.66	0.39	0.12
Length of 1st spikelet pair axis at T3	*Wild type*	10	0.10	0.00	0.00
	Ra1	10	0.27	0.18	0.05
Length of 1st spikelet axis at T3	*Wild type*	10	0.09	0.02	0.01
	Ra1	10	0.13	0.15	0.05
Length of 2nd spikelet pair axis at T3	*Wild type*	10	0.38	0.08	0.02
	Ra1	10	0.20	0.19	0.06
Number of glumes on first spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of fertile florets on 1st spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of glumes on second spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Number of florets on 2nd spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.10	0.32	0.10

Table III-11 Family 191 Ear Characters

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Length of the ear (cm)	*Wild type*	10	9.76	2.91	0.92
	Ra1	10	6.53	1.91	0.60
Mass of the ear (g)	*Wild type*	10	32.33	29.01	9.17
	Ra1	10	13.32	7.65	2.42
Number of primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	>20	0.00	0.00
Number of spikelet pairs around the ear axis	*Wild type*	10	9.70	0.82	0.26
	Ra1	10	9.10	0.74	0.23
Presence of silks	*Wild type*	10	100%, mature		
	Ra1	10	10%, undeveloped		
Type of branch on ear	*Wild type*	10	None		
	Ra1	10	Branch		
Length of 1st primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	2.34	0.63	0.20

Table III-12 Family 201 Ear Characters

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Length of the ear (cm)	*Wild type*	10	9.37	3.39	1.07
	Ra1	10	6.37	1.33	0.42
Mass of the ear (g)	*Wild type*	10	27.75	27.66	8.75
	Ra1	10	9.84	7.01	2.22
Number of primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	>20	0.00	0.00
Number of spikelet pairs around the ear axis	*Wild type*	10	8.90	0.32	0.10
	Ra1	10	8.70	0.67	0.21
Presence of silks	*Wild type*	10	100%, mature		
	Ra1	10	10%, undeveloped		
Type of branch on ear	*Wild type*	10	None		
	Ra1	10	Branch		
Length of 1st primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	2.64	.52	0.17

Table III-13 Analysis of E1 in family 191

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E1	*Wild type*	10	Spikelet Pair		
	Ra1	10	Branch		
Length of branch axis at E1	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	2.13	0.63	0.20
Number of spikelet pairs at E1	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	>20	0.00	0.00
Number of spikelets in 1st spikelet pair at E1	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	0.02	0.00	0.00
Length of the 1st spikelet pair axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Length of the 1st spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Length of second spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Type of 1st spikelet at E1	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of florets on 1st spikelet pair axis at E1	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.40	0.52	0.16
Type of 2nd spikelet at E1	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of fertile florets in 2nd spikelet axis at E1	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.40	0.52	0.16

Table III-14 Analysis of E1 in family 201

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E1	*Wild type*	10	Spikelet Pair		
	Ra1	10	Branch		
Length of branch axis at E1	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	2.22	0.49	0.16
Number of spikelet pairs at E1	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	>20	0.00	0.00
Number of spikelets in 1st spikelet pair at E1	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	0.02	0.00	0.00
Length of the 1st spikelet pair axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Length of the 1st spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Length of second spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Type of 1st spikelet at E1	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of florets on 1st spikelet pair axis at E1	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.30	0.48	0.15
Type of 2nd spikelet at E1	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of fertile florets in 2nd spikelet axis at E1	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.30	0.48	0.15

Table III-15 Analysis of E2 in family 191

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E2	*Wild type*	10	Spikelet Pair		
	Ra1	10	Indeterminate Spikelet Pair		
Length of branch axis at E2	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	0.00	0.00	0.00
Number of spikelet pairs at E2	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	1.00	0.00	0.00
Number of spikelets in the 1st spikelet pair at E2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	Tstc	0.00	0.00
Length of spikelet pair axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	1.81	0.73	0.23
Length of 1st spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Length of 2nd spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Type of 1st spikelet at E2	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of florets on 1st spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.60	0.52	0.16
Type of 2nd spikelet at E2	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of fertile florets on 2nd spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.60	0.52	0.16

Tstc= To small to count

Table III-16 Characters at E2 in family 201

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E2	*Wild type*	10	Spikelet Pair		
	Ra1	10	Indeterminate Spikelet Pair		
Length of branch axis at E2	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	0.00	0.00	0.00
Number of spikelet pairs at E2	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	1.00	0.00	0.00
Number of spikelets in the 1st spikelet pair at E2	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	Tstc	0.00	0.00
Length of spikelet pair axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	1.72	0.49	0.16
Length of 1st spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Length of 2nd spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Type of 1st spikelet at E2	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of florets on 1st spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.50	0.53	0.17
Type of 2nd spikelet at E2	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of florets on 2nd spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.50	0.53	0.17

Tstc= To small to count

Table III-17 Analysis of E3 in family 191

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E3	*Wild type*	10	Spikelet Pair		
	Ra1	10	Indeterminate Spikelet Pair		
Length of branch axis at E3	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	0.00	0.00	0.00
Number of spikelet pairs at E3	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	1.00	0.00	0.00
Number of spikelets in 1st spikelet pair at E3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	Tstc	0.00	0.00
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.90	0.24	0.08
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Length of 2nd spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of florets on 1st spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.40	0.52	0.16
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of fertile florets on 2nd spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.40	0.52	0.16

Tstc= To small to count

Table III-18 analysis of E3 in family 201

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E3	*Wild type*	10	Spikelet Pair		
	Ra1	10	Indeterminate Spikelet Pair		
Length of branch axis at E3	*Wild type*	10	0.00	0.00	0.00
	Ra1	10	0.84	0.25	0.08
Number of spikelet pairs at E3	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	Tstc	0.00	0.00
Number of spikelets in 1st spikelet pair at E3	*Wild type*	10	2.00	0.00	0.00
	Ra1	10	2.00	0.00	0.00
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.84	0.25	0.08
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Length of 2nd spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra1	10	0.01	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of fertile florets on 1st spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.10	0.32	0.01
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Ra1	10	Determinate		
Number of fertile florets on 2nd spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Ra1	10	0.10	0.32	0.01

Tstc= To small to count

Ramosa-2 analysis

Table III-19 Primary axis properties in family 221

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Total plant height	*Wild type*	10	1.90	0.26	0.08
	Ra2	10	1.94	0.18	0.05
Plant Height	*Wild type*	10	1.46	0.27	0.06
	Ra2	10	1.51	0.16	0.05
Peduncle length	*Wild type*	10	21.80	2.74	0.87
	Ra2	10	19.90	1.10	0.35
Tassel Length	*Wild type*	10	23.30	4.00	1.27
	Ra2	10	23.00	4.29	1.36
Peduncle and tassel (cm)	*Wild type*	10	45.10	6.14	1.94
	Ra2	10	42.90	4.56	1.44

Table III-20 Primary axis properties in family 231

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Total plant height	*Wild type*	10	1.81	0.14	0.04
	Ra2	10	1.77	0.12	0.04
Plant Height	*Wild type*	10	1.37	0.13	0.04
	Ra2	10	1.37	0.11	0.04
Peduncle length	*Wild type*	10	18.60	2.72	0.86
	Ra2	10	16.60	2.22	0.70
Tassel Length	*Wild type*	10	24.90	2.96	0.94
	Ra2	10	23.80	2.43	0.77
Peduncle and tassel (cm)	*Wild type*	10	43.50	4.40	1.39
	Ra2	10	40.40	3.38	1.07

Table III-21 Analysis of Tassel characters in family 221

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of primary branches	*Wild type*	10	6.70	1.57	0.50
	Ra2	10	7.70	1.70	0.54
Number of secondary branches on the 1st primary branch	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.50	1.51	0.48
Number of secondary branches	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	4.60	2.27	0.72
Number of tertiary branches	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	0.00	0.00	0.00
Number of intermediate branches	*Wild type*	10	0.30	0.48	0.15
	Ra2	10	>20	0.00	0.00
Tassel Length	*Wild type*	10	23.30	4.00	1.27
	Ra2	10	23.00	4.30	1.36
Branch area (cm)	*Wild type*	10	7.20	1.03	0.33
	Ra2	10	8.80	2.46	0.78
Intermediate branch area (cm)	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	8.10	2.22	0.70
Spikelet pair area (cm)	*Wild type*	10	16.10	3.51	1.11
	Ra2	10	6.10	1.71	0.54
% Branches area/tassel height	*Wild type*	10	31.32	4.35	1.38
	Ra2	10	37.96	5.26	1.66
% Intermediate branch area/tassel height	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	35.14	6.78	2.14
%Spikelet pair area/tassel height	*Wild type*	10	68.68	4.35	1.38
	Ra2	10	26.90	6.94	2.19

Table III-22 Analysis of tassel characters in family 231

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of Primary Branches in the tassel	*Wild type*	10	6.80	1.40	0.44
	Ra2	10	5.20	1.62	0.51
Number of Secondary Branches on the Primary Branch of the Tassel	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.10	0.74	0.23
Number of Secondary Branches in the Tassel	*Wild type*	10	1.10	0.32	0.10
	Ra2	10	2.20	0.92	0.29
Number of Tertiary Branches in the Tassel	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	0.00	0.00	0.00
Number of Indeterminate Spikelet Pairs in the Tassel	*Wild type*	10	0.20	0.42	0.13
	Ra2	10	>20	0.00	0.00
Tassel Length	*Wild type*	10	24.90	2.96	0.94
	Ra2	10	23.80	2.43	0.77
Branch Area in Tassel	*Wild type*	10	7.25	1.55	0.49
	Ra2	10	6.95	1.19	0.38
Indeterminate Spikelet Pair Area in the Tassel	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	10.40	2.32	0.73
Spikelet pair area in tassel	*Wild type*	10	17.65	2.14	0.68
	Ra2	10	6.45	1.12	0.35
Percent of the tassel that is branches	*Wild type*	10	29.01	4.57	1.44
	Ra2	10	29.37	4.87	1.54
Percent of tassel that is indeterminate spikelet pairs	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	43.42	6.62	2.09
Percent of the tassel that is spikelet pairs	*Wild type*	10	70.99	4.57	1.44
	Ra2	10	27.21	4.76	1.50

Table III-23 Analysis of T1 in family 221

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T1	*Wild type*	10	Branch		
	Ra2	10	Branch		
Length of branch at T1	*Wild type*	10	8.86	1.33	0.42
	Ra2	10	10.55	2.82	0.89
Number of Spikelet Pairs at T1	*Wild type*	10	13.60	2.72	0.86
	Ra2	10	14.70	3.80	1.20
Number of Spikelets in the 1st spikelet pair at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.00	0.00	0.00
Total length of 1st spikelet pair axis at T1	*Wild type*	10	0.91	0.26	0.08
	Ra2	10	1.66	0.34	0.11
Length of 1st spikelet pair axis at T1	*Wild type*	10	0.19	0.15	0.05
	Ra2	10	0.98	0.23	0.07
Length of 1st spikelet pedicel at T1	*Wild type*	10	0.17	0.15	0.05
	Ra2	10	0.58	0.24	0.08
Length of 2nd spikelet pedicel at T1	*Wild type*	10	0.72	0.20	0.06
	Ra2	10	0.69	0.19	0.06
Number of glumes in 1st spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.90	0.32	0.01
Number of florets in 1st spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.80	0.63	0.20
Number of glumes on 2nd spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.00	0.00	0.00
Number of florets on 2nd spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.00	0.00	0.00

Table III-24 Analysis of T1 in family 231

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T1	*Wild type*	10	Branch		
	Ra2	10	Branch		
Length of branch at T1	*Wild type*	10	8.91	1.70	0.54
	Ra2	10	8.41	2.92	0.92
Number of Spikelet Pairs at T1	*Wild type*	10	12.30	2.36	0.75
	Ra2	10	10.20	3.85	1.22
Number of Spikelets in the 1st spikelet pair at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.00	0.00	0.00
Total length of 1st spikelet pair axis at T1	*Wild type*	10	0.74	0.28	0.09
	Ra2	10	1.65	0.61	0.19
Length of 1st spikelet pair axis at T1	*Wild type*	10	0.17	0.09	0.03
	Ra2	10	0.98	0.26	0.08
Length of 1st spikelet pedicel at T1	*Wild type*	10	0.18	0.10	0.03
	Ra2	10	0.12	0.04	0.01
Length of 2nd spikelet pedicel at T1	*Wild type*	10	0.57	0.26	0.08
	Ra2	10	0.67	0.42	0.13
Number of glumes in 1st spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.80	0.63	0.20
Number of florets in 1st spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.80	0.63	0.20
Number of glumes on 2nd spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.10	0.57	0.18
Number of florets on 2nd spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.50	0.85	0.27

Table III-25 Analysis of T2 in family 221

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T2	*Wild type*	10	Spikelet pair		
	Ra2	10	Indeterminate spikelet pair		
Length of branch at T2	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	1.29	2.17	0.69
Number of Spikelet pairs at T2	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	2.90	3.63	1.15
Number of spikelets in the 1st spikelet pair at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	4.20	1.55	0.49
Total length of spikelet pair axis at T2	*Wild type*	10	0.97	0.21	0.06
	Ra2	10	3.35	1.46	0.46
Length of spikelet pair axis at T2	*Wild type*	10	0.18	0.15	0.05
	Ra2	10	1.25	0.39	0.12
Length of 1st spikelet axis at T2	*Wild type*	10	0.29	0.18	0.06
	Ra2	10	0.77	0.49	0.16
Length of 2nd spikelet axis at T2	*Wild type*	10	0.81	0.20	0.06
	Ra2	10	0.85	0.47	0.15
Number of glumes on 1st spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.90	0.32	0.01
Number of fertile florets on 1st spikelet at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.80	0.63	0.20
Number of glumes on 2nd spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.90	0.32	0.01
Number of fertile florets on 2nd floret axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.80	0.63	0.20

Table III-26 Analysis of T2 in family 231

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T2	*Wild type*	10	Spikelet pair		
	Ra2	10	Indeterminate spikelet pair		
Length of branch at T2	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	0.65	1.42	0.45
Number of Spikelet pairs at T2	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.90	1.91	0.60
Number of spikelets in the 1st spikelet pair at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	3.40	1.65	0.52
Total length of spikelet pair axis at T2	*Wild type*	9	0.91	0.28	0.09
	Ra2	10	3.11	1.46	0.46
Length of spikelet pair axis at T2	*Wild type*	9	0.01	0.00	0.00
	Ra2	10	1.09	0.31	0.10
Length of first spikelet axis at T2	*Wild type*	9	0.29	0.18	0.06
	Ra2	10	0.59	0.37	0.12
Length of 2nd spikelet axis at T2	*Wild type*	10	0.73	0.37	0.12
	Ra2	10	0.74	0.51	0.16
Number of glumes on 1st spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.90	0.32	0.01
Number of fertile florets on 1st spikelet at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.00	1.05	0.33
Number of glumes on 2nd spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.90	0.32	0.10
Number of fertile florets on 2nd floret axis at T2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.20	1.03	0.33

Table III-27 Analysis of T3 in family 221

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T3	*Wild type*	10	Spikelet pair		
	Ra2	10	Spikelet pair		
Number of spikelets in the 1st spikelet pair at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.90	1.73	0.55
Total length of the spikelet pair axis at T3	*Wild type*	10	1.31	2.00	0.63
	Ra2	10	1.51	0.66	0.21
Length of 1st spikelet pair axis at T3	*Wild type*	10	0.01	0.00	0.00
	Ra2	10	0.57	0.18	0.06
Length of 1st spikelet axis at T3	*Wild type*	10	0.13	0.05	0.02
	Ra2	10	0.59	0.32	0.10
Length of 2nd spikelet pair axis at T3	*Wild type*	10	0.58	0.13	0.04
	Ra2	10	0.59	0.245	0.08
Number of glumes on first spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.90	0.32	0.01
Number of fertile florets on 1st spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.80	0.63	0.20
Number of glumes on second spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.80	0.63	0.20
Number of florets on 2nd spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.80	0.63	0.20

Table III-28 Analysis of T3 in family 231

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T3	*Wild type*	10	Spikelet pair		
	Ra2	10	Spikelet pair		
Number of spikelets in the 1st spikelet pair at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.00	0.00	0.00
Total length of the spikelet pair axis at T3	*Wild type*	9	0.52	0.13	0.04
	Ra2	10	0.94	0.31	0.10
Length of 1st spikelet pair axis at T3	*Wild type*	9	0.10	0.00	0.00
	Ra2	10	0.35	0.24	0.07
Length of 1st spikelet axis at T3	*Wild type*	9	0.10	0.00	0.00
	Ra2	10	0.46	0.11	0.03
Length of 2nd spikelet pair axis at T3	*Wild type*	10	0.38	0.18	0.06
	Ra2	10	0.59	0.17	0.05
Number of glumes on first spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.90	0.32	0.10
Number of fertile florets on 1st spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.60	0.84	0.27
Number of glumes on second spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.10	0.57	0.18
Number of florets on 2nd spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	1.20	1.03	0.33

Table III-29 Ear characters of family 221

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Length of the ear (cm)	*Wild type*	10	11.31	3.54	1.12
	Ra2	10	11.62	2.65	0.84
Mass of the ear (g)	*Wild type*	10	46.76	34.60	10.94
	Ra2	10	39.81	32.14	10.16
Number of primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	1.70	2.16	0.68
Number of spikelet pairs around the ear axis	*Wild type*	10	8.00	0.67	0.21
	Ra2	10	7.80	0.63	0.20
Presence of silks	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00
Type of branch on ear	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	3.00	0.00	0.00
Length of 1st primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	3.71	3.55	1.12

Table III-30 Ear characters in family 231

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Length of the ear (cm)	*Wild type*	10	11.50	3.02	0.95
	Ra2	10	11.14	2.10	0.66
Mass of the ear (g)	*Wild type*	10	46.47	33.08	10.46
	Ra2	10	40.29	24.95	7.89
Number of primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	10.50	7.86	2.49
Number of spikelet pairs around the ear axis	*Wild type*	10	8.00	0.67	0.21
	Ra2	10	8.00	0.82	0.26
Presence of silks	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00
Type of branch on ear	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	3.00	0.00	0.00
Length of 1st primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Ra2	10	5.59	1.59	0.50

Table III-31 Analysis of E1 in family 221

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E1	*Wild type*	10	Spikelet pair		
	Ra2	10	Indeterminate spikelet pair		
Number of spikelets in 1st spikelet pair at E1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.60	0.84	0.27
Length of the 1st spikelet pair axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.29	0.09	0.03
Length of the 1st spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.01	0.00	0.00
Length of second spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.13	0.05	0.02
Type of 1st spikelet at E1	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of florets on 1st spikelet pair axis at E1	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00
Type of 2nd spikelet at E1	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of fertile florets in 2nd spikelet axis at E1	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00

Table III-32 Analysis of E1 in family 231

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E1	*Wild type*	10	Spikelet pair		
	Ra2	10	Indeterminate spikelet pair		
Number of spikelets in 1st spikelet pair at E1	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	3.00	0.82	0.26
Length of the 1st spikelet pair axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.26	0.10	0.03
Length of the 1st spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.10	0.02	0.05
Length of second spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.01	0.00	0.00
Type of 1st spikelet at E1	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of florets on 1st spikelet pair axis at E1	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00
Type of 2nd spikelet at E1	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of fertile florets in 2nd spikelet axis at E1	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00

Table III-33 Analysis of E2 in family 221

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E2	*Wild type*	10	Spikelet pair		
	Ra2	10	Indeterminate spikelet pair		
Number of spikelets in the 1st spikelet pair at E2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	3.10	1.29	0.41
Length of spikelet pair axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.29	0.10	0.03
Length of 1st spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.10	0.00	0.00
Length of 2nd spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.11	0.03	0.01
Type of 1st spikelet at E2	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of florets on 1st spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00
Type of 2nd spikelet at E2	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of fertile florets on 2nd spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00

Table III-34 Analysis of E2 in family 231

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E2	*Wild type*	10	Spikelet pair		
	Ra2	10	Indeterminate spikelet pair		
Number of spikelets in the 1st spikelet pair at E2	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	3.30	1.16	0.37
Length of spikelet pair axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.29	0.12	0.04
Length of 1st spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.11	0.05	0.02
Length of 2nd spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.11	0.06	0.02
Type of 1st spikelet at E2	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of florets on 1st spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00
Type of 2nd spikelet at E2	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of fertile florets on 2nd spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00

Table III-35 Analysis of E3 in family 221

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E3	*Wild type*	10	Spikelet pair		
	Ra2	10	Indeterminate spikelet pair		
Number of spikelets in 1st spikelet pair at E3	*Wild type*	10	2.00	0.00	0.00
	Ra2	10	2.60	1.26	0.40
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.26	0.05	0.02
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.01	0.00	0.00
Length of 2nd spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.01	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of fertile florets on 1st spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of fertile florets on 2nd spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Ra2	10	1.00	0.00	0.00

Table III-36 Analysis of E3 in family 231

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E3	*Wild type*	10	Spikelet pair		
	Ra2	10	Indeterminate spikelet pair		
Number of spikelets in 1st spikelet pair at	*Wild type*	10	2.00	0.00	0.00
E3	*Ra2*	10	2.50	0.85	0.27
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.24	0.14	0.04
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.12	0.05	0.02
Length of 2nd spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Ra2	10	0.12	0.07	0.02
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of fertile florets on 1st spikelet	*Wild type*	10	1.00	0.00	0.00
pair axis at E3	*Ra2*	10	0.80	0.42	0.13
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Ra2	10	Determinate		
Number of fertile florets on 2nd spikelet	*Wild type*	10	1.00	0.00	0.00
pair axis at E3	*Ra2*	10	0.60	0.52	0.16

Ramosa-3 analysis

Table III-37 Primary axis morphology of family 251

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Total plant height (m)	*Wild type*	7	2.03	0.26	0.09
	Ra3 Het	5	1.85	0.26	0.12
	Ra3	8	1.80	0.19	0.07
Plant Height (m)	*Wild type*	7	1.43	0.24	0.08
	Ra3 Het	5	1.28	0.24	0.11
	Ra3	8	1.27	0.18	0.06
Peduncle length (cm)	*Wild type*	7	22.86	2.91	1.10
	Ra3 Het	5	23.80	0.84	0.37
	Ra3	8	22.88	4.55	1.61
Tassel Length (cm)	*Wild type*	7	36.14	1.86	0.70
	Ra3 Het	5	32.80	3.49	1.56
	Ra3	8	30.13	3.68	1.30
Pedicel and Tassel Length (cm)	*Wild type*	7	59.00	3.42	1.29
	Ra3 Het	5	53.00	3.71	1.66
	Ra3	8	56.60	5.63	1.99

Table III-38 Tassel characters of family 251

	Phenotype	N	Mean	Std. Deviation	Std. Error of Mean
Number of Primary Branches in the tassel	*Wild type*	7	12.29	1.25	0.47
	Ra3 Het	5	15.80	5.54	2.48
	Ra3	8	21.00	0.00	0.00
Number of Secondary Branches on the Primary Branch of the Tassel	*Wild type*	7	1.29	0.49	0.18
	Ra3 Het	5	2.00	1.58	0.71
	Ra3	8	7.88	1.96	0.69
Number of Secondary Branches in the Tassel	*Wild type*	7	2.29	1.38	0.52
	Ra3 Het	5	10.20	9.96	4.45
	Ra3	8	21.00	0.00	0.00
Number of Tertiary Branches in the Tassel	*Wild type*	7	0.00	0.00	0.00
	Ra3 Het	5	0.00	0.00	0.00
	Ra3	8	0.00	0.00	0.00
Number of Indeterminate Spikelet Pairs in the Tassel	*Wild type*	7	1.14	1.77	0.67
	Ra3 Het	5	21.00	0.00	0.00
	Ra3	8	21.00	0.00	0.00
Tassel Length	*Wild type*	7	36.14	1.86	0.70
	Ra3 Het	5	32.80	3.49	1.56
	Ra3	8	30.13	3.68	1.30
Branch Area in Tassel	*Wild type*	7	12.71	1.63	0.62
	Ra3 Het	5	12.80	1.64	0.73
	Ra3	8	16.88	2.40	0.85
Indeterminate Spikelet Pair Area in the Tassel	*Wild type*	7	0.00	0.00	0.00
	Ra3 Het	5	10.80	2.71	1.21
	Ra3	8	9.56	2.86	1.01
Spikelet pair area in tassel	*Wild type*	7	23.43	1.24	0.47
	Ra3 Het	5	9.20	5.37	2.40
	Ra3	8	3.69	1.65	0.58

	Phenotype	N	Mean	Std. Deviation	Std. Error of Mean
Percent of the tassel that is branches	*Wild type*	7	35.11	3.34	1.26
	Ra3 Het	5	39.40	6.70	2.99
	Ra3	8	56.50	9.03	3.19
Percent of tassel that is indeterminate spikelet pairs	*Wild type*	7	0.00	0.00	0.00
	Ra3 Het	5	33.15	8.85	3.96
	Ra3	8	31.29	6.31	2.23
Percent of the tassel that is spikelet pairs	*Wild type*	7	64.89	3.34	1.26
	Ra3 Het	5	27.45	14.05	6.28
	Ra3	8	12.20	4.86	1.72

Table III-39 Analysis of T1 in family 251

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T1	*Wild type*	7	Branch		
	Ra3 Het	5	Branch		
	Ra3	8	Branch		
Length of branch at T1	*Wild type*	7	14.71	2.16	0.82
	Ra3 Het	5	11.02	3.10	1.39
	Ra3	8	11.03	2.69	0.95
Number of spikelet pairs at T1	*Wild type*	7	>20	0.00	0.00
	Ra3 Het	5	19.20	4.02	1.80
	Ra3	8	>20	0.00	0.00
Number of spikelets in the 1st spikelet pair at T1	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00
Total length of the 1st spikelet pair axis at T1	*Wild type*	7	0.74	0.21	0.08
	Ra3 Het	5	0.82	0.52	0.23
	Ra3	8	0.74	0.35	0.12
Length of 1st spikelet pair axis at T2	*Wild type*	7	0.14	0.14	0.05
	Ra3 Het	5	0.22	0.16	0.07
	Ra3	8	0.31	0.22	0.08
Length of first spikelet pedicel at T1	*Wild type*	7	0.13	0.13	0.05
	Ra3 Het	5	0.16	0.09	0.04
	Ra3	8	0.13	0.05	0.02
Length of 2nd spikelet pedicel at T1	*Wild type*	7	0.63	0.23	0.09
	Ra3 Het	5	0.60	0.36	0.16
	Ra3	8	0.43	0.20	0.07
Number of glumes on 1st spikelet at T1	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of florets in 1st spikelet at T1	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00
Number of glumes on 2nd spikelet at T1	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00
Number of florets in 2nd spikelet at T1	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00

Table III-40 Analysis of T2 in family 251

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T2	*Wild type*	7	Spikelet Pair		
	Ra3 Het	5	Indeterminate spikelet pair		
	Ra3	8	Branch		
Length of branch at T2	*Wild type*	7	0.00	0.00	0.00
	Ra3 Het	5	0.00	0.00	0.00
	Ra3	8	2.89	3.12	1.10
Number of spikelet pairs at T2	*Wild type*	7	1.00	0.00	0.00
	Ra3 Het	5	1.00	0.00	0.00
	Ra3	8	6.38	5.90	2.09
Number of spikelets in the 1st spikelet pair at T2	*Wild type*	7	2.14	0.38	0.14
	Ra3 Het	5	2.60	0.55	0.24
	Ra3	8	7.75	6.25	2.21
Total length of the 1st spikelet pair	*Wild type*	7	1.06	0.32	0.12
	Ra3 Het	5	2.44	1.21	0.54
	Ra3	8	3.36	3.21	1.14
Length of 1st spikelet pair axis at T2	*Wild type*	7	0.24	0.13	0.05
	Ra3 Het	5	0.56	0.34	0.15
	Ra3	8	0.29	0.16	0.05
Length of first spikelet pedicel at T2	*Wild type*	7	0.14	0.08	0.03
	Ra3 Het	5	0.16	0.05	0.02
	Ra3	8	0.18	0.12	0.04
Length of 2nd spikelet pedicel at T2	*Wild type*	7	0.81	0.27	0.10
	Ra3 Het	5	0.10	0.07	0.03
	Ra3	8	0.23	0.10	0.04
Number of glumes on 1st spikelet at T2	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of florets in 1st spikelet at T2	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00
Number of glumes on 2nd spikelet at T2	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00
Number of florets in 2nd spikelet at T2	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00

Table III-41 Analysis of T3 in family 251

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T3	*Wild type*	7	Spikelet Pair		
	Ra3 Het	5	Spikelet Pair		
	Ra3	8	Intermediate Spikelet Pair		
Number of spikelets in the 1st spikelet pair at T3	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	6.25	2.12	0.75
Total length of the 1st spikelet pair axis at T3	*Wild type*	7	0.46	0.13	0.05
	Ra3 Het	5	0.54	0.05	0.02
	Ra3	8	2.53	0.85	0.30
Length of 1st spikelet pair axis at T3	*Wild type*	7	0.09	0.04	0.01
	Ra3 Het	5	0.12	0.04	0.02
	Ra3	8	0.25	0.11	0.04
Length of first spikelet pedicel at T3	*Wild type*	7	0.09	0.04	0.01
	Ra3 Het	5	0.12	0.04	0.02
	Ra3	8	0.13	0.05	0.02
Length of 2nd spikelet pedicel at T3	*Wild type*	7	0.37	0.11	0.04
	Ra3 Het	5	0.42	0.04	0.02
	Ra3	8	0.14	0.05	0.02
Number of glumes on 1st spikelet at T3	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00
Number of florets in 1st spikelet at T3	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00
Number of glumes on 2nd spikelet at T3	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of florets in 2nd spikelet at T3	*Wild type*	7	2.00	0.00	0.00
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00

Table III-42 Ear analysis in family 251

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Length of the Ear (cm)	*Wild type*	7	9.93	4.76	1.80
	Ra3 Het	5	10.04	3.10	1.38
	Ra3	8	7.68	4.09	1.45
Mass of the Ear (g)	*Wild type*	7	27.94	19.22	7.27
	Ra3 Het	5	41.62	29.49	13.19
	Ra3	8	14.09	17.57	6.21
Number of primary branches on the ear axis	*Wild type*	7	0.14	0.38	0.14
	Ra3 Het	5	17.00	8.94	4.00
	Ra3	8	>20	0.00	0.00
Number of spikelet pairs around the axis	*Wild type*	7	7.43	3.46	1.31
	Ra3 Het	5	8.00	0.71	0.32
	Ra3	8	6.88	1.13	0.40
Presence of silks	*Wild type*	7	100%, developed		
	Ra3 Het	5	100%, developed		
	Ra3	8	44%, developed		
Type of branch on the ear	*Wild type*	7	Spikelet pair		
	Ra3 Het	5	Indeterminate spikelet pair		
	Ra3	8	Branch		
Length of 1st primary branch on the ear axis	*Wild type*	7	0.43	1.13	0.43
	Ra3 Het	5	2.84	1.36	0.61
	Ra3	8	3.21	1.48	0.52

Table III-43 Analysis of E1 in family 251

	Phenotype	N	Mean	Std. Deviation	Std. Error of Mean
Type of branch at E1	*Wild type*	7	Spikelet pair		
	Ra3 Het	5	Indeterminate spikelet pair		
	Ra3	8	Branch		
Length of branch axis at E1	*Wild type*	7	0.00	0.00	0.00
	Ra3 Het	5	1.10	1.75	0.78
	Ra3	8	2.59	1.60	0.57
Number of spikelet pairs at E1	*Wild type*	7	0.86	0.38	0.14
	Ra3 Het	5	9.00	10.95	4.90
	Ra3	8	>20	0.00	0.00
Number of spikelets in 1st spikelet pair at E1	*Wild type*	7	1.71	0.76	0.29
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00
Length of the 1st spikelet pair axis at E1	*Wild type*	7	0.04	0.02	0.01
	Ra3 Het	5	0.05	0.00	0.00
	Ra3	8	0.07	0.05	0.02
Length of the 1st spikelet axis at E1	*Wild type*	7	0.04	0.02	0.01
	Ra3 Het	5	0.05	0.00	0.00
	Ra3	8	0.06	0.02	0.01
Length of second spikelet axis at E1	*Wild type*	7	0.04	0.02	0.01
	Ra3 Het	5	0.05	0.00	0.00
	Ra3	8	0.06	0.02	0.01
Type of 1st spikelet at E1	*Wild type*	7	Determinate		
	Ra3 Het	5	Determinate		
	Ra3	8	Determinate		
Number of florets on 1st spikelet pair axis at E1	*Wild type*	7	0.86	0.38	0.14
	Ra3 Het	5	1.00	0.00	0.00
	Ra3	8	0.50	0.53	0.19

	Phenotype	N	Mean	Std. Deviation	Std. Error of Mean
Type of 2nd spikelet at E1	*Wild type*	7	Determinate		
	Ra3 Het	5	Determinate		
	Ra3	8	Determinate		
Number of fertile florets in 2nd spikelet axis at E1	*Wild type*	7	0.86	0.38	0.14
	Ra3 Het	5	1.00	0.00	0.00
	Ra3	8	0.38	0.52	0.18

Table III-44 Analysis of E2 in family 251

	Phenotype	N	Mean	Std. Deviation	Std. Error of Mean
Type of branch at E2	*Wild type*	7	Spikelet pair		
	Ra3 Het	5	Indeterminate spikelet pair		
	Ra3	8	Branch		
Length of branch axis at E2	*Wild type*	7	0.00	0.00	0.00
	Ra3 Het	5	0.00	0.00	0.00
	Ra3	8	1.74	1.18	0.42
Number of spikelet pairs at E2	*Wild type*	7	0.86	0.38	0.14
	Ra3 Het	5	1.00	0.00	0.00
	Ra3	8	>20	0.00	0.00
Number of spikelets in the 1st spikelet pair at E2	*Wild type*	7	1.71	0.76	0.29
	Ra3 Het	5	2.40	0.55	0.24
	Ra3	8	2.00	0.00	0.00
Length of spikelet pair axis at E2	*Wild type*	7	0.04	0.02	0.01
	Ra3 Het	5	0.21	0.22	0.10
	Ra3	8	0.07	0.05	0.02
Length of 1st spikelet axis at E2	*Wild type*	7	0.04	0.02	0.01
	Ra3 Het	5	0.05	0.00	0.00
	Ra3	8	0.06	0.02	0.01
Length of 2nd spikelet axis at E2	*Wild type*	7	0.04	0.02	0.01
	Ra3 Het	5	0.05	0.00	0.00
	Ra3	8	0.06	0.02	0.01
Type of 1st spikelet at E2	*Wild type*	7	Determinate		
	Ra3 Het	5	Determinate		
	Ra3	8	Determinate		
Number of florets on 1st spikelet axis at E2	*Wild type*	7	0.86	0.38	0.14
	Ra3 Het	5	1.00	0.00	0.00
	Ra3	8	0.63	0.52	0.18

	Phenotype	N	Mean	Std. Deviation	Std. Error of Mean
Type of 2nd spikelet at E2	*Wild type*	7	Determinate		
	Ra3 Het	5	Determinate		
	Ra3	8	Determinate		
Number of fertile florets on 2nd spikelet axis at E2	*Wild type*	7	0.86	0.38	0.14
	Ra3 Het	5	1.00	0.00	0.00
	Ra3	8	0.50	0.53	0.19

Table III-45 Analysis of E3 in family 251

	Phenotype	N	Mean	Std. Deviation	Std. Error of Mean
Type of branch at E3	*Wild type*	7	Spikelet pair		
	Ra3 Het	5	Spikelet pair		
	Ra3	8	Indeterminate spikelet pair		
Length of branch axis at E3	*Wild type*	7	0.00	0.00	0.00
	Ra3 Het	5	0.00	0.00	0.00
	Ra3	8	0.80	0.42	0.15
Number of spikelet pairs at E3	*Wild type*	7	0.86	0.38	0.14
	Ra3 Het	5	1.00	0.00	0.00
	Ra3	8	tstc	5.66	2.00
Number of spikelets in 1st spikelet pair at E3	*Wild type*	7	1.71	0.76	0.29
	Ra3 Het	5	2.00	0.00	0.00
	Ra3	8	2.00	0.00	0.00
Length of 1st spikelet pair axis at E3	*Wild type*	7	0.04	0.02	0.01
	Ra3 Het	5	0.05	0.00	0.00
	Ra3	8	0.04	0.02	0.01
Length of 1st spikelet pair axis at E3	*Wild type*	7	0.04	0.02	0.01
	Ra3 Het	5	0.05	0.00	0.00
	Ra3	8	0.04	0.02	0.01
Length of 2nd spikelet pair axis at E3	*Wild type*	7	0.04	0.02	0.01
	Ra3 Het	5	0.05	0.00	0.00
	Ra3	8	0.04	0.02	0.01
Type of 1st spikelet at E3	*Wild type*	7	Determinate		
	Ra3 Het	5	Determinate		
	Ra3	8	Determinate		
Number of fertile florets on 1st spikelet pair axis at E3	*Wild type*	7	0.86	0.38	0.14
	Ra3 Het	5	1.00	0.00	0.00
	Ra3	8	0.13	0.35	0.13

	Phenotype	N	Mean	Std. Deviation	Std. Error of Mean
Type of 1st spikelet at E3	*Wild type*	7	Determinate		
	Ra3 Het	5	Determinate		
	Ra3	8	Determinate		
Number of fertile florets on 2nd spikelet pair axis at E3	*Wild type*	7	0.86	0.38	0.14
	Ra3 Het	5	1.00	0.00	0.00
	Ra3	8	0.00	0.00	0.00

Tstc= To small to count

Branched silkless1 analysis

Table III-46 Primary axis morphology of family 104

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Total plant height (m)	*Wild type*	10	2.46	0.12	0.04
	Bd1	10	2.37	0.24	0.07
Plant Height (m)	*Wild type*	10	1.79	0.11	0.03
	Bd1	10	1.74	0.21	0.07
Peduncle length (cm)	*Wild type*	10	28.60	2.80	0.88
	Bd1	10	23.10	3.96	1.25
Tassel Length (cm)	*Wild type*	10	38.70	3.59	1.14
	Bd1	10	39.90	4.33	1.37
Peduncle and Tassel (cm)	*Wild type*	10	67.30	4.90	1.55
	Bd1	10	63.00	7.13	2.26

Table III-47 Primary axis morphology of family 151

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Total plant height (m)	*Wild type*	10	1.71	0.13	0.04
	Bd1	10	1.58	0.12	0.04
Plant Height (m)	*Wild type*	10	1.26	0.10	0.03
	Bd1	10	1.29	0.11	0.03
Peduncle length (cm)	*Wild type*	10	18.85	2.78	0.88
	Bd1	10	15.60	1.54	0.49
Tassel Length (cm)	*Wild type*	10	26.35	4.08	1.29
	Bd1	10	13.30	2.61	0.82
Peduncle and Tassel (cm)	*Wild type*	10	45.20	5.09	1.61
	Bd1	10	28.90	2.41	0.76

Table III-48 Primary axis morphology of family 161

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Total plant height (m)	*Wild type*	10	1.58	0.18	0.06
	Bd1	8	1.51	0.22	0.08
Plant Height (m)	*Wild type*	10	1.09	0.18	0.06
	Bd1	8	1.05	0.20	0.07
Peduncle length (cm)	*Wild type*	10	18.45	4.73	1.50
	Bd1	8	17.13	4.79	1.69
Tassel Length (cm)	*Wild type*	10	30.45	3.42	1.08
	Bd1	8	29.00	4.69	1.66
Peduncle and Tassel	*Wild type*	10	48.90	5.87	1.86
(cm)	*Bd1*	8	46.13	4.02	1.42

Table III-49 Tassel Characters in family 104

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of Primary Branches in the tassel	*Wild type*	10	9.00	1.25	0.39
	Bd1	10	10.40	3.17	1.00
Number of Secondary Branches on the Primary Branch of the Tassel	*Wild type*	10	0.90	0.32	0.10
	Bd1	10	0.70	0.48	0.15
Number of Secondary Branches in the Tassel	*Wild type*	10	1.60	0.52	0.16
	Bd1	10	1.00	0.67	0.21
Number of Tertiary Branches in the Tassel	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Number of Indeterminate Spikelet Pairs in the Tassel	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Tassel Length	*Wild type*	10	38.70	3.59	1.14
	Bd1	10	39.90	4.33	1.37
Branch Area in Tassel	*Wild type*	10	9.82	1.31	0.41
	Bd1	10	9.89	1.73	0.55
Indeterminate Spikelet Pair Area in the Tassel	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Spikelet pair area in tassel	*Wild type*	10	28.88	3.83	1.21
	Bd1	10	30.01	3.75	1.19
Percent of the tassel that is branches	*Wild type*	10	25.58	4.35	1.37
	Bd1	10	24.84	3.63	1.15
Percent of tassel that is indeterminate spikelet pairs	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Percent of the tassel that is spikelet pairs	*Wild type*	10	74.42	4.35	1.37
	Bd1	10	75.16	3.63	1.15

Table III-50 Tassel Characters in family 151

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of Primary Branches in the tassel	*Wild type*	10	5.70	1.64	0.52
	Bd1	10	4.90	1.10	0.35
Number of Secondary Branches on the	*Wild type*	10	0.90	0.32	0.10
Primary Branch of the Tassel	*Bd1*	10	0.60	0.52	0.16
Number of Secondary Branches in the	*Wild type*	10	1.00	0.47	0.15
Tassel	*Bd1*	10	0.70	0.67	0.21
Number of Tertiary Branches in the	*Wild type*	10	0.00	0.00	0.00
Tassel	*Bd1*	10	0.00	0.00	0.00
Number of Indeterminate Spikelet Pairs in	*Wild type*	10	0.10	0.32	0.10
the Tassel	*Bd1*	10	0.00	0.00	0.00
Tassel Length	*Wild type*	10	26.35	4.08	1.29
	Bd1	10	13.30	2.61	0.82
Branch Area in Tassel	*Wild type*	10	6.95	1.38	0.44
	Bd1	10	5.40	1.22	0.39
Indeterminate Spikelet Pair Area in the	*Wild type*	10	0.00	0.00	0.00
Tassel	*Bd1*	10	0.00	0.00	0.00
Spikelet pair area in tassel	*Wild type*	10	19.40	3.60	1.14
	Bd1	10	7.90	1.71	0.54
Percent of the tassel that is branches	*Wild type*	10	26.68	5.26	1.66
	Bd1	10	40.76	5.16	1.63
Percent of tassel that is indeterminate	*Wild type*	10	0.00	0.00	0.00
spikelet pairs	*Bd1*	10	0.00	0.00	0.00
Percent of the tassel that is spikelet pairs	*Wild type*	10	73.32	5.26	1.66

Table III-51 Tassel Characters in family 161

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Number of Primary Branches in the tassel	*Wild type*	10	5.50	4.25	1.34
	Bd1	8	5.13	3.14	1.11
Number of Secondary Branches on the	*Wild type*	10	0.40	0.52	0.16
Primary Branch of the Tassel	*Bd1*	8	0.50	0.53	0.19
Number of Secondary Branches in the	*Wild type*	10	0.90	1.10	0.35
Tassel	*Bd1*	8	0.50	0.53	0.19
Number of Tertiary Branches in the	*Wild type*	10	0.00	0.00	0.00
Tassel	*Bd1*	8	0.00	0.00	0.00
Number of Indeterminate Spikelet Pairs in	*Wild type*	10	0.00	0.00	0.00
the Tassel	*Bd1*	8	0.00	0.00	0.00
Tassel Length	*Wild type*	10	30.45	3.42	1.08
	Bd1	8	29.00	4.69	1.66
Branch Area in Tassel	*Wild type*	10	6.15	2.72	0.86
	Bd1	8	7.13	2.70	0.95
Indeterminate Spikelet Pair Area in the	*Wild type*	10	0.00	0.00	0.00
Tassel	*Bd1*	8	0.00	0.00	0.00
Spikelet pair area in tassel	*Wild type*	10	24.30	3.89	1.23
	Bd1	8	21.88	2.59	0.91
Percent of the tassel that is branches	*Wild type*	10	20.25	9.02	2.85
	Bd1	8	23.92	6.13	2.17
Percent of tassel that is indeterminate	*Wild type*	10	0.00	0.00	0.00
spikelet pairs	*Bd1*	8	0.00	0.00	0.00
Percent of the tassel that is spikelet pairs	*Wild type*	10	79.75	9.02	2.85
	Bd1	8	76.08	6.13	2.17

Table III-52 Analysis of T1 in family 104

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T1	*Wild type*	10	Branch		
	Bd1	10	Branch		
Length of branch at T1	*Wild type*	10	16.92	1.59	0.50
	Bd1	10	18.10	2.46	0.78
Number of Spikelet Pairs at T1	*Wild type*	10	21.00	0.00	0.00
	Bd1	10	21.00	0.00	0.00
Number of Spikelets in the 1st spikelet pair at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Total length of 1st spikelet pair axis at T1	*Wild type*	10	0.72	0.27	0.09
	Bd1	10	1.12	0.46	0.14
Length of 1st spikelet pair axis at T1	*Wild type*	10	0.13	0.07	0.02
	Bd1	10	0.11	0.03	0.01
Length of 1st spikelet pedicel at T1	*Wild type*	10	0.14	0.09	0.03
	Bd1	10	0.14	0.07	0.02
Length of 2nd spikelet pedicel at T1	*Wild type*	10	0.60	0.24	0.07
	Bd1	10	0.61	0.20	0.06
Number of glumes in 1st spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	1.20	1.62	0.51
Number of florets in 1st spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	4.40	1.07	0.34
Number of glumes on 2nd spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.40	0.70	0.22
Number of florets on 2nd spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	4.50	1.51	0.48

Table III-53 Analysis of T1 in family 151

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T1	*Wild type*	10	Branch		
	Bd1	10	Branch		
Length of branch at T1	*Wild type*	10	9.38	1.86	0.59
	Bd1	10	5.45	1.10	0.35
Number of Spikelet Pairs at T1	*Wild type*	10	14.10	3.45	1.09
	Bd1	10	8.90	2.88	0.91
Number of Spikelets in the 1st spikelet pair at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Total length of 1st spikelet pair axis at T1	*Wild type*	10	0.84	0.18	0.06
	Bd1	10	0.58	0.43	0.14
Length of 1st spikelet pair axis at T1	*Wild type*	10	0.13	0.07	0.02
	Bd1	10	0.06	0.02	0.01
Length of 1st spikelet pedicel at T1	*Wild type*	10	0.15	0.07	0.02
	Bd1	10	0.07	0.02	0.01
Length of 2nd spikelet pedicel at T1	*Wild type*	10	0.71	0.18	0.06
	Bd1	10	0.38	0.23	0.07
Number of glumes in 1st spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	1.50	0.53	0.17
Number of florets in 1st spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Number of glumes on 2nd spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	1.30	0.48	0.15
Number of florets on 2nd spikelet at T1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00

Table III-54 Analysis of T1 in family 161

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of Branch at T1	*Wild type*	10	Branch		
	Bd1	8	Branch		
Length of branch at T1	*Wild type*	10	10.52	6.83	2.16
	Bd1	8	1.38	3.89	1.38
Number of Spikelet Pairs at T1	*Wild type*	10	15.20	8.88	2.81
	Bd1	8	3.13	6.42	2.27
Number of Spikelets in the 1st spikelet pair at T1	*Wild type*	10	1.80	0.63	0.20
	Bd1	8	1.75	0.71	0.25
Total length of 1st spikelet pair axis at T1	*Wild type*	10	0.62	0.29	0.09
	Bd1	8	0.88	0.47	0.17
Length of 1st spikelet pair axis at T1	*Wild type*	10	0.09	0.03	0.01
	Bd1	8	0.10	0.08	0.03
Length of 1st spikelet pedicel at T1	*Wild type*	10	0.12	0.14	0.04
	Bd1	8	0.16	0.20	0.07
Length of 2nd spikelet pedicel at T1	*Wild type*	10	0.54	0.26	0.08
	Bd1	8	0.71	0.38	0.13
Number of glumes in 1st spikelet at T1	*Wild type*	10	1.80	0.63	0.20
	Bd1	8	1.50	0.93	0.33
Number of florets in 1st spikelet at T1	*Wild type*	10	1.80	0.63	0.20
	Bd1	8	2.13	1.89	0.67
Number of glumes on 2nd spikelet at T1	*Wild type*	10	1.80	0.63	0.20
	Bd1	8	1.38	0.92	0.32
Number of florets on 2nd spikelet at T1	*Wild type*	10	1.80	0.63	0.20
	Bd1	8	1.63	1.85	0.65

Table III-55 Analysis of T2 in family 104

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T2	*Wild type*	10	Spikelet pair		
	Bd1	10	Spikelet pair		
Number of spikelets in the 1st spikelet pair at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Total length of spikelet pair axis at T2	*Wild type*	10	0.64	0.14	0.05
	Bd1	10	1.13	0.35	0.11
Length of spikelet pair axis at T2	*Wild type*	10	0.11	0.04	0.01
	Bd1	10	0.12	0.10	0.03
Length of first spikelet axis at T2	*Wild type*	10	0.13	0.07	0.02
	Bd1	10	0.18	0.18	0.06
Length of 2nd spikelet axis at T2	*Wild type*	10	0.54	0.13	0.04
	Bd1	10	0.54	0.17	0.05
Number of glumes on 1st spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.60	0.97	0.31
Number of fertile florets on 1st spikelet at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	5.60	1.96	0.62
Number of glumes on 2nd spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	1.80	2.04	0.65
Number of fertile florets on 2nd floret axis at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	6.70	1.42	0.45

Table III-56 Analysis of T2 in family 151

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T2	*Wild type*	10	Spikelet pair		
	Bd1	10	Spikelet pair		
Number of spikelets in the 1st spikelet pair at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Total length of spikelet pair axis at T2	*Wild type*	10	0.72	0.13	0.04
	Bd1	10	0.38	0.26	0.08
Length of spikelet pair axis at T2	*Wild type*	10	0.11	0.03	0.01
	Bd1	10	0.10	0.14	0.05
Length of first spikelet axis at T2	*Wild type*	10	0.14	0.07	0.02
	Bd1	10	0.10	0.14	0.05
Length of 2nd spikelet axis at T2	*Wild type*	10	0.59	0.15	0.05
	Bd1	10	0.29	0.24	0.08
Number of glumes on 1st spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	1.20	0.42	0.13
Number of fertile florets on 1st spikelet at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Number of glumes on 2nd spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.90	0.57	0.18
Number of fertile florets on 2nd floret axis at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00

Table III-57 Analysis of T2 in family 161

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T2	*Wild type*	10	Spikelet pair		
	Bd1	8	Spikelet pair		
Number of spikelets in the 1st spikelet pair at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	2.00	0.00	0.00
Total length of spikelet pair axis at T2	*Wild type*	10	0.71	0.20	0.06
	Bd1	8	0.95	0.37	0.13
Length of spikelet pair axis at T2	*Wild type*	10	0.08	0.03	0.01
	Bd1	8	0.09	0.02	0.01
Length of first spikelet axis at T2	*Wild type*	10	0.11	0.07	0.02
	Bd1	8	0.09	0.02	0.01
Length of 2nd spikelet axis at T2	*Wild type*	10	0.63	0.19	0.06
	Bd1	8	0.48	0.30	0.10
Number of glumes on 1st spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	2.00	1.31	0.46
Number of fertile florets on 1st spikelet at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	4.00	2.20	0.78
Number of glumes on 2nd spikelet axis at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	2.00	1.85	0.65
Number of fertile florets on 2nd floret axis at T2	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	3.63	2.62	0.92

Table III-58Analysis of T3 in family 104

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T3	*Wild type*	10	Spikelet pair		
	Bd1	10	Spikelet pair		
Number of spikelets in the 1st spikelet pair at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Total length of the spikelet pair axis at T3	*Wild type*	10	0.44	0.17	0.05
	Bd1	10	1.27	0.29	0.09
Length of 1st spikelet pair axis at T3	*Wild type*	10	0.09	0.02	0.01
	Bd1	10	0.09	0.02	0.01
Length of 1st spikelet axis at T3	*Wild type*	10	0.09	0.02	0.01
	Bd1	10	0.09	0.02	0.01
Length of 2nd spikelet pair axis at T3	*Wild type*	10	0.35	0.16	0.05
	Bd1	10	0.34	0.12	0.04
Number of glumes on first spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	1.10	1.20	0.38
Number of fertile florets on 1st spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	4.50	1.35	0.43
Number of glumes on second spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	1.40	1.65	0.52
Number of florets on 2nd spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	6.10	1.79	0.57

Table III-59 Analysis of T3 in family151

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T3	*Wild type*	10	Spikelet pair		
	Bd1	10	Spikelet pair		
Number of spikelets in the 1st spikelet pair at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Total length of the spikelet pair axis at T3	*Wild type*	10	0.48	0.11	0.04
	Bd1	10	0.05	0.06	0.02
Length of 1st spikelet pair axis at T3	*Wild type*	10	0.09	0.02	0.01
	Bd1	10	0.03	0.03	0.01
Length of 1st spikelet axis at T3	*Wild type*	10	0.09	0.02	0.01
	Bd1	10	0.03	0.03	0.01
Length of 2nd spikelet pair axis at T3	*Wild type*	10	0.39	0.10	0.03
	Bd1	10	0.04	0.05	0.01
Number of glumes on first spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.70	0.48	0.15
Number of fertile florets on 1st spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Number of glumes on second spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.50	0.53	0.17
Number of florets on 2nd spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00

Table III-60 Analysis of T3 in family 161

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at T3	*Wild type*	10	Spikelet pair		
	Bd1	8	Spikelet pair		
Number of spikelets in the 1st spikelet pair at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	2.00	0.00	0.00
Total length of the spikelet pair axis at T3	*Wild type*	10	0.46	0.11	0.03
	Bd1	8	0.86	0.36	0.13
Length of 1st spikelet pair axis at T3	*Wild type*	10	0.07	0.02	0.01
	Bd1	8	0.09	0.02	0.01
Length of 1st spikelet axis at T3	*Wild type*	10	0.07	0.02	0.01
	Bd1	8	0.08	0.03	0.01
Length of 2nd spikelet pair axis at T3	*Wild type*	10	0.40	0.11	0.03
	Bd1	8	0.36	0.28	0.10
Number of glumes on first spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	1.75	1.16	0.41
Number of fertile florets on 1st spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	2.88	1.96	0.69
Number of glumes on second spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	2.13	1.81	0.64
Number of florets on 2nd spikelet axis at T3	*Wild type*	10	2.00	0.00	0.00
	Bd1	8	2.88	2.03	0.72

Table III-61 Ear analysis of Family 104

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Length of the ear (cm)	*Wild type*	10	15.90	3.08	0.98
	Bd1	10	10.53	3.96	1.25
Mass of the ear (g)	*Wild type*	10	43.54	20.99	6.64
	Bd1	10	17.97	16.72	5.29
Number of primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	>20	6.01	1.90
Number of spikelet pairs around the ear axis	*Wild type*	10	6.70	0.95	0.30
	Bd1	10	6.80	0.92	0.29
Presence of silks	*Wild type*	10			
	Bd1	10	0.00	0.00	0.00
Type of branch on ear	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	1.00	0.00	0.00
Length of 1st primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	2.62	1.45	0.46

Table III-62 Ear analysis of family 151

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Length of the ear (cm)	*Wild type*	10	8.28	2.37	0.75
	Bd1	10	6.34	0.93	0.29
Mass of the ear (g)	*Wild type*	10	16.56	19.41	6.14
	Bd1	10	4.00	1.60	0.50
Number of primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	12.70	7.09	2.24
Number of spikelet pairs around the ear axis	*Wild type*	10	7.90	0.88	0.28
	Bd1	10	8.00	0.67	0.21
Presence of silks	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Type of branch on ear	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	1.00	0.00	0.00
Length of 1st primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Bd1	10	2.18	0.75	0.24

Table III-63 Ear analysis of family 161

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Length of the ear (cm)	*Wild type*	10	7.79	4.07	1.29
	Bd1	8	6.78	2.64	0.93
Mass of the ear (g)	*Wild type*	10	10.72	15.64	4.95
	Bd1	8	4.48	7.85	2.78
Number of primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Bd1	8	>20	0.00	0.00
Number of spikelet pairs around the ear axis	*Wild type*	10	5.60	2.07	0.65
	Bd1	8	6.00	0.93	0.33
Presence of silks	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	0.00	0.00	0.00
Type of branch on ear	*Wild type*	10	0.00	0.00	0.00
	Bd1	8	1.00	0.00	0.00
Length of 1st primary branch on the ear axis	*Wild type*	10	0.00	0.00	0.00
	Bd1	8	1.16	0.67	0.24

Table III-64 Analysis of E1 in family 104

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E1	*Wild type*	10	Spikelet pair		
	Bd1z	10	Spikelet pair		
Number of spikelets in 1st spikelet pair at E1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Length of the 1st spikelet pair axis at E1	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	1.02	0.99	0.31
Length of the 1st spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Length of second spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Type of 1st spikelet at E1	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of florets on 1st spikelet pair axis at E1	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Type of 2nd spikelet at E1	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of fertile florets in 2nd spikelet axis at E1	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00

Table III-65 Analysis of E1 in family 151

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E1	*Wild type*	10	Spikelet pair		
	Bd1	10	Spikelet pair		
Number of spikelets in 1st spikelet pair at E1	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Length of the 1st spikelet pair axis at E1	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.10	0.00	0.00
Length of the 1st spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Length of second spikelet axis at E1	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Type of 1st spikelet at E1	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of florets on 1st spikelet pair axis at E1	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Type of 2nd spikelet at E1	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of fertile florets in 2nd spikelet axis at E1	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00

Table III-66 Analysis of E1 in family 161

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E1	*Wild type*	10	Spikelet pair		
	Bd1	8	Spikelet pair		
Number of spikelets in 1st spikelet pair at E1	*Wild type*	10	1.80	0.63	0.20
	Bd1	8	2.00	0.00	0.00
Length of the 1st spikelet pair axis at E1	*Wild type*	10	0.05	0.02	0.01
	Bd1	8	0.39	0.49	0.17
Length of the 1st spikelet axis at E1	*Wild type*	10	0.05	0.02	0.01
	Bd1	8	0.10	0.00	0.00
Length of second spikelet axis at E1	*Wild type*	10	0.05	0.02	0.01
	Bd1	8	0.10	0.00	0.00
Type of 1st spikelet at E1	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	2.00	0.00	0.00
Number of florets on 1st spikelet pair axis at E1	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	0.00	0.00	0.00
Type of 2nd spikelet at E1	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	2.00	0.00	0.00
Number of fertile florets in 2nd spikelet axis at E1	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	0.00	0.00	0.00

Table III-67 Analysis of E2 in family 104

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E2	*Wild type*	10	Spikelet pair		
	Bd1	10	Spikelet pair		
Number of spikelets in the 1st spikelet pair at E2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Length of spikelet pair axis at E2	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.43	0.51	0.16
Length of 1st spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Length of 2nd spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Type of 1st spikelet at E2	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of florets on 1st spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Type of 2nd spikelet at E2	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of fertile florets on 2nd spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00

Table III-68 Analysis of E2 in family 151

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E2	*Wild type*	10	Spikelet pair		
	Bd1	10	Spikelet pair		
Number of spikelets in the 1st spikelet pair at E2	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Length of spikelet pair axis at E2	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.10	0.00	0.00
Length of 1st spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Length of 2nd spikelet axis at E2	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Type of 1st spikelet at E2	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of florets on 1st spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Type of 2nd spikelet at E2	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of fertile florets on 2nd spikelet axis at E2	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00

Table III-69 Analysis of E2 in family 161

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E2	*Wild type*	10	Spikelet pair		
	Bd1	8	Spikelet pair		
Number of spikelets in the 1st spikelet pair at E2	*Wild type*	10	1.80	0.63	0.20
	Bd1	8	2.00	0.00	0.00
Length of spikelet pair axis at E2	*Wild type*	10	0.05	0.02	0.01
	Bd1	8	0.11	0.16	0.06
Length of 1st spikelet axis at E2	*Wild type*	10	0.05	0.02	0.01
	Bd1	8	0.05	0.00	0.00
Length of 2nd spikelet axis at E2	*Wild type*	10	0.05	0.02	0.01
	Bd1	8	0.05	0.00	0.00
Type of 1st spikelet at E2	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	2.00	0.00	0.00
Number of florets on 1st spikelet axis at E2	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	0.00	0.00	0.00
Type of 2nd spikelet at E2	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	2.00	0.00	0.00
Number of fertile florets on 2nd spikelet axis at E2	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	0.00	0.00	0.00

Table III-70 Analysis of E3 in family 104

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E3	*Wild type*	10	Spikelet pair		
	Bd1	10	Spikelet pair		
Number of spikelets in 1st spikelet pair at E3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.12	0.06	0.02
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Length of 2nd spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of fertile florets on 1st spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Number of fertile florets on 2^{nd} spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00

Table III-71 Analysis of E3 in family 151

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E3	*Wild type*	10	Spikelet pair		
	Bd1	10	Spikelet pair		
Number of spikelets in 1st spikelet pair at E3	*Wild type*	10	2.00	0.00	0.00
	Bd1	10	2.00	0.00	0.00
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.10	0.00	0.00
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Length of 2nd spikelet pair axis at E3	*Wild type*	10	0.05	0.00	0.00
	Bd1	10	0.05	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Bd1	10	Indeterminate		
Number of fertile florets on 1st spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Bd1	10	Indeterminate		
Number of fertile florets on 2nd spikelet pair axis at E3	*Wild type*	10	1.00	0.00	0.00
	Bd1	10	0.00	0.00	0.00

Table III-72 Analysis of E3 in family 161

	Phenotype	N	Mean	Std. Deviation	Std. Error Mean
Type of branch at E3	*Wild type*	10	Spikelet pair		
	Bd1	8	Spikelet pair		
Number of spikelets in 1st spikelet pair at E3	*Wild type*	10	1.80	0.63	0.20
	Bd1	8	2.00	0.00	0.00
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.02	0.01
	Bd1	8	0.05	0.00	0.00
Length of 1st spikelet pair axis at E3	*Wild type*	10	0.05	0.02	0.01
	Bd1	8	0.05	0.00	0.00
Length of 2nd spikelet pair axis at E3	*Wild type*	10	0.05	0.02	0.01
	Bd1	8	0.05	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Bd1	8	Indeterminate		
Number of fertile florets on 1st spikelet pair axis at E3	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	0.00	0.00	0.00
Type of 1st spikelet at E3	*Wild type*	10	Determinate		
	Bd1	8	Indeterminate		
Number of fertile florets on 2nd spikelet pair axis at E3	*Wild type*	10	0.90	0.32	0.10
	Bd1	8	0.00	0.00	0.00