FLOWERING TIME AND ITS RELATIONSHIP TO VEGETATIVE DEVELOPMENT IN MAIZE (*Zea mays* L.)

Hildrun Jutta Passas

Ph. D.
University of Edinburgh
1994

Abstract

All organisms go through distinct phases of development during ontogenesis. In plants, shoot development has traditionally been divided into two phases of development distinguished by the the ability to flower. The non-flowering, juvenile phase of development is followed by the adult phase of development, during which flowering can occur. Frequently observed modifications in the vegetative morphology of the shoot are thought to reflect the transition to a phase in which the shoot is competent to flower, yet conclusive evidence for this assumption is lacking. An alternative model proposed here may provide a better framework for the genetic analysis of shoot development. In this model, shoot development is partitioned into three phases: the early vegetative and late vegetative phases of development, which are defined by phase-specific vegetative traits, and a reproductive phase of development. These phases are expressed in a spatial sequence as a result of the polar nature of shoot growth. In maize, the early vegetative phase is marked by traits such as the presence of a visible form of epicuticular leaf wax and round epidermal leaf cells in cross section. In the late vegetative phase, the visible leaf wax is absent, the epidermal cells are more rectangular in cross section and trichomes are expressed on the leaf blades. The reproductive phase of development is characterised first by a brief, photoperiod sensitive period and later by the differentiation of inflorescences. The aim of this study is to investigate whether this three-phase model provides an adequate conceptual framework. To this end, the relationship between the transition from early vegetative to late vegetative growth and flowering in maize is studied using a genetic approach.

Results show that the early flowering trait does not alter the rate of leaf initiation. Rather, the overall length of the vegetative development is reduced in early flowering plants, resulting in the production of fewer leaves. This reduction in leaf number affects the vegetative phases of development in different ways; either only the late vegetative phase of development is reduced or the early vegetative and the late vegetative phases are both shortened, as determined by phase-specific traits. This difference is dependent on the inbred background and not on the early flowering trait, as determined by genetic, molecular, and physiological techniques. Therefore, in one species, but in different inbred backgrounds, vegetative phase change and reproductive maturity can be independent or regulated coordinately, indicating that a three-phase model of shoot development is an adequate conceptual framework. Finally, the early flowering trait is placed in a pathway regulating the reproductive phase of development. In the late flowering genotypes examined, a longer vegetative phase is reflected only in a longer late vegetative phase. The expression of the early vegetative phase is unaffected. A working model of shoot development in maize is proposed.

I, the undersigned, hereby declare that this thesis has been composed by myself and that the work is my own.

Hildrun J. Passas

Acknowledgements

First and most of all, I would like to express my heartfelt gratitude to the two persons without whom this work would not have been possible, Prof. R. Scott Poethig and Dr. William Spoor. Scott not only welcomed me into his laboratory and provided me with all the material support necessary to conduct this research, he also created the stimulating and warm environment that I enjoyed thoughout my work in his laboratory and field. Scott introduced me to the world of developmental genetics and to the pleasures of working with maize, and I am thankful for many enlightening discussions. It was Scott who suggested that shoot development may be governed by three phase of development, not two, and he conceived the idea of studying the relationship of vegetative phase change and reproductive maturity in early and late flowering lines of maize. Bill Spoor encouraged me to persue a research career, and stood behind me through all the twists and turns of my PhD. I am grateful to both for their trust and support which allowed me to carry out my research with the greatest possible freedom. I would also like to thank my third supervisor Dr. Robert F. Lyndon for some very thought-provoking and helpful discussions and comments.

A special thank you to all current and former members of the Poethig lab, in particular Kathy Barton, Deverie K. Bongard-Pierce, Laura Conway, Liam Dolan, Mark Dudley, Matt Evans, Emily Lawson, Sam Reck-Peterson, Abby Telfer and Shifra Vega, for many interesting discussions, technical help and guidance, unfailing support and encouragement, and for their friendship.

The technical assistence and advice of greenhouse and technical staff at the University of Pennsylvania and at the University of Edinburgh, Tracy Byford, Mary Lou Oehlert, Laura Philon, Robert Redpath, Edith Cochran, David Smith and Donald Verlenden are gratefully acknowledged. My gratitude goes out to all the undergraduate work-study students, particularly Alison, Pauline, Sue and Robbyn, who worked so hard in helping me to weed, leaf number, pot up plants and with numerous other tasks.

Thanks to Dr. Warren Ewans for helpful discussions of the statistical analysis of some of the data, to Dr. Brenda Casper and Dr. Rich Niesenbaum for the use the areameter and to the librarians both at the University of Pennsylvania and at the University of Edinburgh for their help in locating difficult to find references. The kind gift of seeds and bacterial stocks by Dr. E.H. Coe, Dr. J.M. Gardiner, Dr. D. Glover, Dr. D. Grant, Dr. R. Koester, Dr. M.G.Neuffer, Dr. R.H. Peterson, Dr. D.L. Shaver and the Maize Genetics Stock Center is gratefully acknowledged.

I have been fortunate to be able to count on the loving support from Nikos Passas, my parents, and my family and friends in Philadelphia, Edinburgh and around the world. Finally, I would like to thank my 5th grade biology teacher for opening my mind to the fascinating world of biology.

Table of contents

Table of figures

Table of tables

List of abbreviations

All abbreviations are explained in the text when they are first introduced. This list is provided to serve as a reference for the most frequently used abbreviations in case an explanation cannot be located. Internationally recognised units of measurement and their common abbreviations have been used thoughout.

1. Specific abbreviations

DAN	Days to anthesis
DAP	Days after planting
EAR	Phase-specific trait: number of leaf subtending the primary ear
ED	Early derivative
EFT	Early flowering trait
GF	Gaspé Flint
GL	Phase-specific trait: first partially glossy leaf blade
HR	Phase-specific trait: first leaf blade with hairs
IL	Inbred line
LD	Long day
PHT	Plant height
PR	Phase-specific trait: last node with prop roots
SD	Short day
SHHR	Phase-specific trait: first leaf sheath with hair
SHWX	Phase-specific trait: last partially waxy leaf sheath
SLK	Days to silking
TLN	Total leaf number
WX	Phase-specific trait: last partially waxy (glaucous) leaf blade

2. Frequently used abbreviations in genetics and molecular biology

$(\alpha^{32}P)$dCTP	Deoxycytidine triphosphate, labelled with ^{32}P at the α phosphate group
+	Designates the wild type allele of a gene
BNL	Brookhaven National Laboratory: designator for RFLP markers
Cg1	*Corngrass1*
dlf	*Delayed flowering*
DNA	Deoxyribonucleic acid
EDTA	Disodium ethylenediaminetetraacetic acid
F2	Filial 2: generation produced by self-pollinating or sib-pollinating a heterozygous plant (F1)
id1	*Indeterminate growth1*
Lfy1	*Leafy1*
ltf	*Late flowering*
M2	Generation produced by self-pollinating a heterozygote (M1) following a mutagenesis in the first generation (M0)
M3	Generation produced by self-pollinating a M2 plant
RFLP	Restriction fragment length polymorphism
Rg1	*Ragged1*
SDS	Sodium dodecyl sulfate
SSC	Standard saline citrate
TBE	Tris(hydroxymethyl)aminomethane/ boric acid/ EDTA (buffer)
TE	Tris(hydroxymethyl)aminomethane/ EDTA (buffer)
Tp1	*Teopod1*
Tp2	*Teopod2*
Tp3	*Teopod3*
UMC	University of Missouri, Columbia: designator for RFLP markers

CHAPTER 1

Introduction

1.1 Background and definition of terms

During ontogenesis, organisms pass through a series of distinct phases of development. In plants, the change from one phase to the next affects the meristem, and successively expressed phases of development are permanently laid down in a spatial sequence due to the polar nature of plant growth. The most evident changes in the development of the aerial plant shoot, shifts in the expression of the vegetative and reproductive phases of development, have been studied for hundreds of years (Schaffalitzky de Muckadell, 1959) and concepts and definitions abound. The topic has received much attention, particularly from horticulturists and tree breeders who wish to shorten the time to flowering to obtain fruit and seed faster, or prolong the juvenile phase to be able to use vegetative propagation. Yet, the genetic regulation of these distinct phases of shoot development is little understood. This problem is certainly a complex one, and the long-standing quest to understand the regulation of flowering is but one aspect of it.

Commonly, a juvenile, non-flowering phase has been distinguished from an adult phase, in which the shoot is able to flower (Zimmerman, 1972). The vegetative part of the shoot can also go through changes in a variety of characters in a phase-specific manner (see below). These vegetative changes frequently correlate with the shift from a non-flowering to a flowering shoot and have been assumed to be associated with the changes in the reproductive ability (Doorenbos, 1965; Robbins, 1961; Schaffalitzky de Muckadell, 1954; Zimmerman, 1972; Zimmerman et al., 1985). As a result, the phases of plant shoot development have been defined based only on the reproductive ability of the shoot. This assumption conflicts with observations suggesting that the vegetative and reproductive phases may be regulated independently (see below). It will be argued in the present study that the traditional two-phase model of shoot development is too rigid to accommodate many of the apparent discrepancies reported in the literature and that it provides an inadequate conceptual framework for the study of shoot development. Instead, in a newly proposed model of shoot development, the shoot is thought to go through three, largely independently regulated phases of development; the early vegetative, the late vegetative, and the reproductive phase (Bassiri et al., 1992; Poethig, 1990). The adoption of a three-phase model makes it possible to investigate the relationship between the three phases, in particular to inquire how the expression of the three phases is coordinated to allow proper development of the shoot. In order to assess if this model is likely to provide a more accurate picture of shoot development, it is necessary to establish if the transition from one vegetative phase of development to

the next can indeed proceed independently from changes in the reproductive maturity of the shoot. Using a genetic approach, this question is investigated in the present study by characterising the expression of the vegetative phases of development in early and late flowering maize genotypes. The results indicate that a three-phase model of shoot development provides a useful conceptual framework which is employed in the present study to examine the relationship between the vegetative and reproductive phases of shoot development in maize.

Phase change in plants has been defined as a switch from a juvenile to an adult type of growth (Brink, 1962). The terms "state", "stage", and "phase" are used interchangeably in the literature to describe these alternative growth patterns. To avoid confusion and to stress the persistent, yet dynamic nature of development, the term "phase" will be used preferentially in this work. The juvenile and adult phases of shoot development are traditionally distinguished by the ability of the shoot to flower : a plant cannot flower during the juvenile phase of development, but acquires the ability to do so in the adult phase of development (Schwabe, 1976; Wareing and Frydman, 1976; Zimmerman, 1972, 1973; Zimmerman et al., 1985). Sussex (1976) extended the definition phase change in a more general sense to mean a sudden transition from one persistent developmental state to another. Examples of such phase changes include the transition from production of vegetative organs to production of reproductive organs by the meristem, or the switch from submerged leaf type to emergent leaf type in aquatic plants. Both authors emphasise that these developmental phases are not morphologically and physiologically stable (Sussex, 1976) or permanent (Brink, 1962), but are developmentally discrete phases, or "persistent", in that they can be defined by a characteristic set of traits which persist during somatic growth.

The polar nature of shoot growth causes changes in development to be expressed in a spatial sequence. While it is extremely difficult to establish whether developmental changes are caused by temporal or spatial modifications, or by a gradual shift in the physiology of the shoot, the results can be traced in the spatial arrangement of phase-specific characters. An older structure produced during an early phase of development will have traits pertaining to this early phase, while younger structures that are formed later will display traits of a later phase of development. Older structures are found at the base of the plant or of a branch, while the youngest structures are near the shoot apex. The phases of development are persistent so that new growth originating from axilliary buds will show the same phase-specific characters. For example, the basal parts of plants remain in the juvenile phase of development, creating the juvenile zone (Schaffalitzky de Muckadell, 1959). This is illustrated by the familiar sight of the

basal, juvenile branches of oak or beech trees, which, unlike the adult branches, retain their withered leaves in winter.

Shoot development and phase change have usually been studied in woody species, most prominently English ivy, because of many obvious morphological changes. In woody species, reproductive maturity is reached only after a prolonged period in which the plant does not flower. This observation is reflected in the traditional model of shoot development, where the shoot is partitioned into two phases - the juvenile phase and the adult phase - which are distinguished by the floral competence of the shoot (Schwabe, 1976; Wareing and Frydman, 1976; Zimmerman, 1972, 1973; Zimmerman et al, 1985). In this context, the term phase change relates to the transition from the juvenile phase to the adult phase (Brink, 1962). Since the ability to flower cannot always be assessed prior to the formation of flowers, a transition phase has been postulated in which the shoot is florally competent (adult), but floral differentiation has not yet occurred (Zimmerman, 1972). This concept of a transition phase is of little operational value, and organs produced during the transition phase will often be regarded as juvenile (Zimmerman, 1973). Frequently, changes in the vegetative morphology or physiology are correlated with the time when the plant begins to flower for the first time (Robbins, 1957b; Stein and Fosket, 1969; Stephens, 1944a, b, c). As a result, such vegetative changes have often been thought to be associated with and to reflect the transition to the flowering (adult) phase (Doorenbos, 1965; Robbins, 1961; Schaffalitzky de Muckadell, 1954; Zimmerman, 1972; Zimmerman et al., 1985), in spite of lack of conclusive evidence for this assumption.

A wealth of other terms and definitions, often overlapping, have been used to describe shoot development. They are mentioned here to illustrate the conceptual confusion that surrounds the topic. The term "ageing" has been used by some authors to describe phase change, but its use has been discouraged (Dunberg, 1977) since it does not distinguish chronological ageing from developmental processes, and because it has been applied to maturation and senescence alike. Senescence encompasses the destructive processes that decrease the vitality of an organ or an individual and increase the probability of its death (Comfort, 1964). This phenomenon is not under study here, although it can also be considered part of normal shoot development. Differences in shoot growth rates have been reported in several species to distinguish the juvenile and the adult phases of shoot development (Brink, 1962; Greenwood, 1984; Greenwood et al., 1989; Hackett et al., 1987; Huang et al., 1992; Stein and Fosket, 1969). Yet, some researchers are reluctant to recognise these as phase-specific traits, because shoot growth rates are easily altered and may reflect other changes such as

altered sink-source relationships or the beginning of senescence. Wareing (1959) distinguishes a loss of vigour associated with age from developmental maturation and calls this loss of vigour ageing. Wareing (1959) uses the term mature interchangeably with the term adult. He employs the term maturation to describe the transition from the non-flowering to the flowering phase and associated changes in the vegetative morphology. Fortainer and Jonkers (1976) defined ontogenetic ageing as being similar to Wareing's term maturation, but they also included in it aspects of the shoot growth rate and the statement that a plant need not have flowers to be adult. Physiological ageing was defined as being similar to senescence (Fortainer and Jonkers, 1976). Another definition of ontogenetical and physiological ageing was proposed by Steele (1987). According to his definition, ontogenetical ageing describes the different phases of development, whereas physiological ageing describes changes in the physiological, morphological, cellular, and molecular characteristics that occur during shoot development.

In the alternative model proposed here the vegetative and reproductive phases of development are conceptually separated (Bassiri et al., 1992, Poethig, 1990). Accordingly, the post-embryonic shoot development consists of three phases of development.

The two vegetative phases - the early vegetative and the late vegetative phase - are defined only by a set of vegetative characters such as differences in leaf morphology or physiology and are conceptually dissociated from the reproductive ability of the plant.

The reproductive phase is defined by the expression of traits pertaining to the ability to flower and by the formation of floral structures. According to this definition the reproductive phase includes the three early developmental states of the flowering process (McDaniel et. al., 1992): 1- the competence to sense environmental stimuli and to produce a floral signal, 2- the competence of the meristem to respond to the floral signal and to become florally determined, and 3- the establishment of a florally determined state in the meristem. Floral differentiation then results in the formation of flowers.

Phase change implies the transition from one phase to the next, as represented by changes in the expression of phase-specific traits. A plant with distinct early vegetative and late vegetative phases of development would thus undergo a phase change from early vegetative to late vegetative growth (vegetative phase change), and another phase change from vegetative to reproductive growth.

In contrast to the traditional model, this working model makes no assumptions about the relationship of reproductive maturity to vegetative characteristics, allowing one to formulate and test hypotheses regarding their relationship. Three possibilities must be considered. Firstly, the vegetative and reproductive phases could be regulated completely independently. In this case, vegetative maturity would have no effect on reproductive maturity, and *vice versa*. Secondly, the early vegetative phase and the reproductive phase could be mutually exclusive, so that the transition to the late vegetative phase is required before reproductive maturation can be achieved. This possibility is closest to the traditional concept of shoot development in that the transition from early vegetative to late vegetative growth and flowering would be correlated. In this scenario, either the early vegetative phase may delay the onset of the reproductive phase of development, inhibit its progression, or prevent the differentiation of reproductive structures, or the reproductive phase may repress the expression of the early vegetative phase. Thirdly, the vegetative and reproductive phases could be regulated largely independently, but interact to specify the fate of the shoot in a combinatorial fashion (Bassiri et al., 1992).

If the terms maturity or maturation are used in the context of the present study, they need to be further defined to distinguish vegetative maturation from reproductive maturation. Vegetative maturation of the shoot or vegetative phase change thus refer to the transition from the early vegetative to the late vegetative phase. Reproductive maturation of the shoot describes the change from a phase in which the plant is unable to flower to the flowering phase of development.

1.2 Literature review

This literature review focusses on three aspects of shoot development:

1 - the regulation of flowering,

2- the extent of the differences between the early vegetative and the late vegetative phases and the regulation of the change from the early vegetative phase to the late vegetative phase,

3 - the relationship between the vegetative and reproductive phases of development.

The literature in this field is vast, and there are several excellent reviews (Bernier, 1988; Bernier et al., 1981a, b; Doorenbos, 1965; Evans, 1969; Hackett, 1976, 1983, 1985; Jackson and Sweet, 1972; Kinet et al., 1985; McDaniel, 1984, 1992; Robbins, 1957b, 1961; Wareing, 1987; Zeevaart, 1976; Zimmerman, 1972; Zimmerman et al., 1985). A notable collection of papers has also been published as Proceedings from the Symposium on Juvenility in Woody Plants (Acta Horticulturae **56**, 1976).

1.2.1 The regulation of flowering

The study of shoot development has been concentrated on one of the central problems in plant science, the regulation of flowering. Most plants fall in one of two categories: those that can be induced to flower in a predictable way by environmental stimuli, and those in which flowering cannot be readily induced by external stimuli, but occurs normally in response to endogenous developmental conditions (Bernier et al., 1981a). This difference in reproductive behaviour has shaped research approaches as well as conceptual approaches. Populations of inducible plants can be made to flower in a synchronised, predictable manner. In comparing florally induced plants with uninduced controls, flowering can be studied in a rather direct fashion. The concept of floral induction, which describes flowering in terms of exposure to an environmental stimulus, floral evocation, and differentiation, was developed with such species in mind. In contrast, reproductive maturity in species in the second category tends to be less predictable, and thus much more difficult to study. The presence of a prolonged juvenile phase in many such species has led to the traditional concept of phase change that distinguishes the non-floral from the floral phase of shoot development. The apparent differences between the two groups may not be fundamental, but rather only a matter of degree. The possibility that both types of regulation may be present is exemplified by *Ribes nigrum*, which has a juvenile phase and is also known to be a

short day plant (Nasr and Wareing, 1961, as cited in Schwabe and Al-Doori, 1961). Some factors that determine reproductive maturity in plants will be briefly described to provide an overview of the relevant knowledge.

Flowering is thought to occur when an ontogenetically or environmentally induced floral signal of unknown nature produces the conditions that allow a meristem to differentiate into a flower. The early regulation of flowering is thought to include three major developmental states: 1- the competence to sense environmental stimuli and to produce a floral signal, 2- the competence of the meristem to respond to the floral signal and to become florally determined, and 3- the establishment of a florally determined state in the meristem (McDaniel, 1984, 1992; McDaniel et al., 1992). This is followed by an abrupt change in morphogenesis - floral differentiation. Despite many years of research, a ubiquitous floral signal is as yet unidentified. The switch to reproductive development is thought to be regulated by the production of a floral stimulator and/or by the disappearance of a floral inhibitor (McDaniel et al., 1992; Murfet, 1971, 1977; v. Denffer, 1950).

A large number of environmental and ontogenetic conditions can affect flowering, among them photoperiod, temperature, plant growth substances, light intensity, plant age and size, and nutrient and water availability. Commonly, these conditions interact and one may be replaced by others, indicating that regulation is complex (Bernier et al., 1981a, b; Kinet et al., 1985). A complete review of the available knowledge would be far beyond the scope of this chapter, but a few general points shall be presented here.

The best studied environmental stimulus known to induce flowering is photoperiod (reviewed in Bernier, 1988; Bernier et al., 1981a). Photoperiodic requirements are species-specific and vary from long day conditions to short day conditions, and various combinations of both. Some plants require photoperiodic induction for flowering (qualitative, obligate photoperiodic plants), whereas in others flowering is promoted by photoinduction but is not dependent on it (quantitative, facultative photoperiodic plants). The photoperiodic stimulus is perceived primarily by the leaves, although other plant parts have some photoperiodic sensitivity. Induced leaves are thought to produce a floral signal that is transported out of the leaf to the meristem. The induced state can be transferred by grafting induced plants to uninduced plants (Bernier et al., 1981a).

Vernalisation, or thermoinduction, can also promote flowering in some species (Napp-Zinn, 1962). As a general rule, species with facultative (quantitative)

vernalisation requirements are winter annuals which can be vernalised as imbibed seeds. Plants with obligate (qualitative) vernalisation requirements are usually biennials and perennials that need to reach a certain size before they attain responsiveness to thermoinduction. Thermoinduction is different from photoinduction in that the dividing cells in the apex are thought to perceive the cold signal. The induced state is somatically inherited by the progeny of these cells. Vernalised plants can be devernalised, and *vice versa* (reviewed in Bernier et al., 1981a).

While all plant growth substances can be shown to affect the regulation of flowering in one species or another, gibberellins are thought to play an important role which has been investigated using biosynthetic mutants as well as by applying gibberellins or inhibitors. The effect of gibberellins on flowering time ranges from promoting flowering to delaying flowering, depending on species, time of application, type and amount of gibberellin, and environmental conditions (Bernier et al., 1981b; Zeevaart, 1983). In some species (particularly long day and cold-requiring rosette plants), gibberellins tend to hasten flowering and may substitute for the cold treatment (Bernier et al., 1981b; Zeevaart, 1983). On the other hand, gibberellins can be ineffective in inducing flowering or can delay flowering in other species (Reid et al., 1977; Reid, 1986; Zeevaart, 1983).

Roots also have a large influence on the ability to flower. The presence of roots near the shoot apex prevents or delays floral induction and differentiation in tobacco (McDaniel, 1980; Smith and McDaniel, 1992). Aerial rooting has also been shown to prevent flowering in *Ribes nigrum* (Schwabe and Al-Doori, 1973). Root pruning can hasten flowering in some woody species (Holst, 1961). It has been suggested that hormones (particularly gibberellins) produced in the roots may repress flowering (Bernier et al., 1981b; Schwabe and Al-Doori, 1973). Additionally, in many species the parts of the stem produced during the juvenile phase of development can be distinguished from adult tissues by a greater ability to initiate adventitious roots (Geneve et al., 1988). It is unclear whether the negative correlation between rooting ability and flowering reflects the adverse effect of roots on flowering, or a physiological state that is beneficial to rooting but inhibitory to flowering.

Plant size can also be a factor determining flowering time. Growth conditions favouring rapid attainment of the required plant size favour flowering (Longman and Wareing, 1959; Robinson and Wareing, 1969; Wareing, 1961, 1959). Repeated pruning or hedging can significantly delay flowering in woody plants (Holst, 1961). It is unclear whether plant size itself is the determining factor, or whether this trait is just

correlated with another more critical factor such as distance from the meristem to the roots, complexity of plant structure, or number of cell cycles. This latter possibility has been considered by Robinson and Wareing (1969). *Ribes nigrum* plants usually have to reach a minimum size to flower. When *R. nigrum* cuttings were rooted in repeated cycles, flower formation occurred at a lower position than in normal plants. Robinson and Wareing (1969) suggest that the meristem has to go through a minimum number of cell divisions before flowering can occur.

Growing conditions, such as temperature, light intensity, and the availability of nutrients, water, and carbohydrates also determine flowering time (Holst, 1961; Kinet et al., 1985). Cultural practices, such as pruning, stem ringing, or grafting have been used to manipulate flowering time (Doorenbos, 1965). Hedging can delay or prevent flowering (Doorenbos, 1965; Holst, 1961). Grafting of juvenile scions onto adult stocks can accelerate flowering (Habermas and Sekulow, 1972).

Regardless of whether a species is absolute, facultative, or autonomous in its dependency on specific environmental conditions for flower induction, flowering is under genetic control. Bennett (1972) suggests that the rate of developmental processes can be altered by the amount of nuclear DNA in what he calls a nucleotypic effect. There are indications in several species that early flowering is correlated with small amounts of nuclear DNA (Bullock and Rayburn, 1991; Price, 1988; Tito et al., 1991)

Genes affecting the flowering time are known in many species, and have been identified both in natural populations and in mutagenesis experiments (Koornneef et al., 1991; Murfet, 1989; Nienstaedt, 1961; Poonyarit et al., 1989). Given that a number of different types of environmental stimuli may be substituted for one another it is apparent that flowering is regulated by several pathways. Consistent with this observation, genetic analysis suggests that the time to flowering is a quantitative, polygenic trait. This is most evident in *Pisum sativum* and *Arabidopsis thaliana* , which are preferred models for the genetic analysis of flowering. Several genes regulating flowering and their phenotypic effects under different environmental conditions have been described in both species (Haupt and Nakamura, 1969; Koornneef et al., 1991; Murfet, 1971a, b, c, 1973a, b, 1975a, b, 1977, 1982, 1989; Murfet and Reid, 1973, 1986; Reid, 1977, 1986).

In Arabidopsis, for example, eleven genes conditioning late flowering have been identified (Koornneef et al., 1991). Normally, A. thaliana is a long-day plant that is responsive to vernalisation of the imbibed seed (Laibach, 1951). Mutants carrying the

late flowering genes fall into at least two groups depending on their response to photoperiod and vernalisation. Plants carrying mutations at the loci *LD*, *FCA*, *FLB*, *FMC*, *FPA*, *FVE*, or *FY* are strongly responsive to vernalisation. On the other hand, the late-flowering phenotype of plants carrying mutations at the loci *CO*, *FD*, *FE*, *FHA*, *FT*, *FWA*, or *GI* is only slightly affected by vernalisation, but much enhanced by short-day conditions. These observations and double mutant analyses indicate that these genes are involved in at least two distinct developmental pathways (Araki and Komeda, 1993; Bagnall, 1992; Koornneef et al., 1991; Martínez-Zapter and Somerville, 1990).

A few mutations that cause defects in the biosynthesis of gibberellic acid (*ga1* to *ga5*) or insensitivity to gibberellic acid (*gai*) are known (Koornneef and van der Veen, 1980). Extreme alleles of *GA1* and mutations of *GAI* generate a small delay in flowering time under long-day conditions. Under short-day conditions, flowering is greatly delayed, and the plants are not responsive to vernalisation (Wilson et al., 1992). Additionally, wild type plants as well as plants carrying a mutation in the *FCA* gene flower earlier when treated with gibberellins, particularly when grown under short day conditions (Bagnall, 1992). The gene product of the *spindly* (*SPY*) locus appears to be involved in the gibberellin signal transduction pathway (Jacobsen and Olszewski, 1993). A mutation in this gene causes plants to look like wild type plants that were treated with gibberellin A_3, including an early flowering phenotype. Other late flowering mutants include the ethylene insensitive mutants etr1 and ein2, which have a late flowering phenotype under inductive conditions (Bleeker et al., 1988; Guzman and Ecker, 1990). Finally, plants that carry a mutation at the *deetiolated2* (*DET2*) locus and that show light-independent morphogenesis are also late flowering (Chory et al., 1991).

The few mutations that condition early flowering also fall in at least two phenotypic classes. Mutants of both groups flower earlier than the wild type under long-day and under short-day conditions. Some mutants (*tfl1*, *elf1*, *elf2*) are sensitive to short-day conditions and flowering is delayed under short-day (Alvarez et al., 1992; Shannon and Meeks-Wagner, 1991; Zagotta et al., 1992). Plants with mutations at the loci *ELF3* and *EMF* are insensitive to photoperiod and vernalisation, and fall in the second group (Zagotta et al., 1992; Sung et al, 1992). Three of the long hypocotyl mutants (*hy1*, *hy2*, *hy3*), which are defective in light perception, are also early flowering and have a greatly reduced sensitivity to short-day conditions (Goto et al., 1991; Reed et al., 1993). Another early flowering mutant, *altered meristem program 1* (*amp1*), has been shown to have increased levels of cytokinins and is also thought to be involved in the

signal transduction pathway of light perception (Chaudhury et al., 1993). Based on the analysis of *amp1* plants, Chaudhury et al. (1993) proposed a model of flowering in which flowering is regulated in two independent pathways by cytokinin and gibberellin.

The presence of several pathways that control the transition to flowering in plants is suggested by the fact that different types of floral stimuli can be replaced by one another. Each pathway can respond to a particular stimulus, and in combination they afford the plant the means to detect and respond to favorable conditions for flowering. In the example cited here, *A. thaliana*, these pathways are represented by different classes of mutants. The central pathway regulating the transition from vegetative to reproductive growth of the meristem may be represented by the *EMF* locus. If the *emf* mutation is a loss of function mutation, the wild type gene would normally promote vegetative growth and repress flowering (Sung et al, 1992). Other pathways that are represented by known genes include those that control the responses to photoperiod or vernalisation, and gibberellin biosynthesis and sensitivity. These pathways interact with each other and with the central pathway represented by the *EMF* locus to regulate flower formation. Floral differentiation then occurs as a result of the activity of genes involved in floral morphogenesis.

1.2.2 The vegetative phases of development

1.2.2.1 Characteristics of the vegetative phases of development

Many species, particularly woody ones, show differences in their early and late vegetative growth. A number of different characters may be modified and which trait is altered depends on the species (Brink, 1962; Schaffalitzky de Muckadell, 1954). The early vegetative and late vegetative phases have not been clearly defined as separate developmental phases before, partly because the vegetative differences were thought to occur as a result of reproductive changes, and partly because the traits affected are so diverse. However, these two vegetative phases tend to be persistent in that the sets of traits that define each phase change coordinately. Some of the common characters that are altered in the transition from early to late vegetative growth are listed here to illustrate the distinctive nature of each of the two phases. Since shoot development is discussed in the present study in terms of the three-phase model of shoot development,

phases of vegetative growth will be referred to as early vegetative and late vegetative whenever possible, rather than juvenile and adult.

The presence of adventitious roots or an increased ability to produce adventitious roots is common to the early growth in many species (Doorenbos, 1965; Geneve et al., 1988; Robbins, 1957b; Steele et al., 1990; Stein and Fosket, 1969). In fact, this trait has frequently been used as a characteristic of the juvenile phase of development (Robbins, 1957b, 1961). Rooting ability has attracted attention also because of its importance in plant propagation.

A second obvious difference between early vegetative and late vegetative growth in many species is modification of the leaf morphology (Doorenbos, 1965; Gould, 1993; Robbins, 1957b). In some species, the leaf morphology changes from that typical of a shade leaf to that of a sun leaf or xeromorphic leaf. This ontogenetically regulated transition occurs independently of light exposure or water stress (Bauer and Bauer, 1980; Steele, 1987; Steele et al., 1989). Leaf size and shape also frequently vary between the early and the late vegetative phase (Greenwood et al., 1989).

Photosynthetic efficiency also frequently varies between phases of development. In *Hedera helix*, the net photosynthesis of the early vegetative leaves has been found to be light-saturated at lower light intensities and to attain only two thirds of the light-saturated rate of late vegetative leaves. Major changes in the rate of photosynthesis resulted from differences in leaf volume and in the rate of the Hill reaction and of ribulose bisphosphate carboxylation (Bauer and Bauer, 1980). In maize, the CO_2 exchange rate in newly matured leaves is lower for leaves that are produced early during shoot development than for leaves produced later, and reaches a plateau at leaf position 6 to 8 (Thiagarajah et al., 1981). The total chlorophyll content is higher in late vegetative leaves in larch (Greenwood et al., 1989).

Shoot characteristics, such as phyllotaxis, thorniness, or internode length, have been reported to change during vegetative maturation (Hackett et al., 1987; Schaffalitzky de Muckadell, 1959). Other modifications include changes in the growth habit in ivy (Stoutemyer and Britt, 1965) and in larch (Greenwood et al., 1989). Anatomical differences in the stem tissue of *Hedera helix* have been reported (Goodin, 1965; Stein and Fosket, 1969).

Biochemical differences may also be found between the two phases. Differential distribution of secondary plant compounds between the two vegetative phases has been

reported (Hayman et al., 1986). Increased amounts of acidic components in the epicuticular wax of late vegetative needles have been found in *Pinus radiata* (Franich et al., 1977). Anthocyanin is preferentially produced during the early vegetative phase in ivy (Stoutemyer and Britt, 1965).

There is evidence of developmental differences in pest and pathogen resistance. Rooted cuttings from late vegetative *Pinus radiata* were significantly more resistant to western gall rust than early vegetative cuttings (Zagory and Libby, 1985). Libby and Hood (1976) observed a significant difference in feeding damage caused by hares in *Pinus radiata* . Rooted cuttings from late vegetative growth were more susceptible to browsing, although the leaves of the early vegetative cuttings were greener, less stiff, and more abundant. The authors suggest that under normal circumstances, the early vegetative needles are more likely to be within the reach of hares, and that a feeding repellent in those needles would be adaptive. Kearsley and Whitham (1989) reported that the gall-forming aphid *Pemphigus betae* was much more common on late vegetative *Populus angustifolia* trees than on early vegetative ones, whereas the leaf-feeding beetle *Chrysomela confluens* predominantly inhabited early vegetative trees. In both cases the distribution was adaptive in that growth and survival of the insects were lower in the less preferred environment. The authors suggest that developmental changes in resistance can occur rapidly within one plant and are important components in determining the distribution of plant pests.

Evidently, a large number of traits can distinguish the early vegetative and the late vegetative growth in many species. Differences between the two phases are most informative if the changes occur more or less abruptly and coordinately. Such coordinated changes are frequently observed, yet they have rarely been evaluated for their use as quantitative indices of shoot maturation. In Sitka spruce, a number of needle characteristics and rooting ability have been quantified and mathematically assessed. Morphological and physiological changes are closely correlated and occur in a predictable sequence. In combination, they have been found to be reasonable quantitative indicators of physiological age (Steele, 1987; Steele et al., 1989, 1990).

Although the exact nature of the characters varies with species, it may be concluded that a species-specific set of traits can be used to describe the differences in organs produced during the early vegetative and the late vegetative phases of development. The conceptual distinction of an early vegetative and a late vegetative phase of development appears likely to accurately reflect ontogenetic changes in many, if not all, plant species. Although little is known about the physiological or genetic basis of

regulation of the two vegetative phases of development, the examples cited above suggest that these phases may have a functional significance in presenting different vegetative phenotypes at ontogenetically determined times. The presence of distinct vegetative phases of development and developmental plasticity in the length of these phases may well hold evolutionary advantages for a species.

1.2.2.2 Regulation of the vegetative phases of development

The change from an early to late phase of vegetative growth can be modified by a number of factors, many of which are also effective in the regulation of flowering.

Plant size and closeness to roots may play a role in the regulation of vegetative development. Hedging in trees and shrubs is known to maintain the early vegetative character of the plants (Schaffalitzky de Muckadell, 1954; Zagory and Libby, 1985). On the other hand, small plant size and the presence of roots is not necessarily sufficient to generate early vegetative growth. Rooted cuttings from late vegetative ivy do not revert to an early vegetative growth habit, but maintain the late vegetative characteristics and grow into a plant form known as *Hedera helix arborescens* (Doorenbos, 1954).

Hormones also affect the expression of vegetative phases. Ethylene can promote the change to adult development in daylily (Smith et al., 1989). Reversion to early vegetative growth (juvenile) from late vegetative (adult) growth can be induced in *Hedera helix* by treatment with gibberellins (Frydman and Wareing, 1974; Robbins, 1957a; Rogler and Hackett, 1975a), whereas the late vegetative (adult) plant form is stabilised with abscisic acid and growth retardants (Rogler and Hackett, 1975b). Photoperiodic stimuli can alter vegetative development just as they can affect flowering time (Ashby, 1950). Extreme growing temperatures can promote early vegetative growth (Stoutemyer and Britt, 1961 and references therein).

Once the late vegetative (adult) phase is attained, it is rather stable under normal conditions and reversion to an early vegetative (juvenile) phase occurs only after sexual or apomictic reproduction. The phase of development is thought to be somatically inherited and callus cultures derived from the two phases have been found to possess intrinsic physiological differences (Banks, 1979; Polito and Alliata, 1981; Hackett, 1985; Wareing, 1987). Yet a reversion to early vegetative growth in somatic tissues (rejuvenation) can be brought about under certain conditions (Hackett, 1985; Wareing,

1987). There is some evidence that shoots derived from adventitious buds are rejuvenated (Hackett, 1985). Whereas rooting of late vegetative cuttings or grafting with early vegetative stocks or scions in most cases does not lead to rejuvenation, such rejuvenation has been observed in some species. Examples of this can be found in *H. helix* (Doorenbos, 1954), *H. canariensis* (Stoutemyer and Britt, 1961) and *Sequoia sempervirens* (Huang et al., 1992). Vegetative maturation can also be reversed through *in vitro* propagation (Hackett, 1985; Mullins et al., 1979; Stoutemyer and Britt, 1965), and by hormone treatment (Frydman and Wareing, 1974; Robbins, 1957a; Rogler and Hackett, 1975a, b).

The molecular basis of these changes in vegetative morphology is unknown. Several authors have reported differences in the DNA or RNA content between juvenile and adult tissues, but reports are conflicting and the biological relevance of the observation is unclear (Domoney and Timmis, 1980; Kessler and Reches, 1977; Polito and Alliata, 1981; Schäffner and Nagl, 1979; Stoutemyer and Britt, 1965; Wareing and Frydman, 1976). DNA methylation has been considered as a possible mechanism of gene regulation by several authors, but no differences in DNA methylation have been found between early and late vegetative growth in larch (Greenwood et al., 1989; Hackett and Murray, 1992; Wareing, 1987).

Despite the lack of information about the genetic basis of phase change, it is clear that this process is under genetic control. This is apparent from the highly predictable nature of phase change and from the existence of mutations that dramatically alter the expression of phase-specific vegetative traits. The best characterised of these are the *Teopod* mutations in maize (Bassiri et al., 1992; Dudley and Poethig, 1991; Galinat, 1954a, b, 1966; Lindstrom, 1925; Poethig, 1988a, b, 1989; Ritchings and Tracy, 1989; Singleton, 1951; Weatherwax, 1929; Whaley and Leetch, 1950). These single gene, dominant mutations condition the prolonged expression of the early vegetative phase of development, thus causing phytomers that are produced late during shoot development to assume characteristics of earlier formed phytomers. As a result, the morphology of the shoot is greatly modified, although the onset of the late vegetative phase and the reproductive phase of development is not substantially delayed in these mutants.

1.2.3 Relationship between vegetative phase change and reproductive maturity

Vegetative traits of the shoot usually change in a coordinated manner, and it is frequently suggested that this reflects a correlation between vegetative maturation and flowering (Doorenbos, 1965; Robbins, 1961; Schaffalitzky de Muckadell, 1954; Zimmerman, 1972; Zimmerman et al., 1985). The antagonistic relationship between some characters of the early vegetative phase (roots in particular) and flowering, the observation that vegetative changes often occur at or just prior to flowering, and the similarity between the factors that regulate vegetative maturation and flowering lend some support to this notion. Some of the vegetative changes outlined above may indeed reflect changes in reproductive maturity, but this relationship has been explicitly studied in only a few cases (Stephens, 1944a, c, d). On the other hand, a coordinated regulation between vegetative maturation and reproductive maturation has long been questioned (see Stoutemyer, 1964). For example, there is ample evidence from grafting and rejuvenation experiments suggesting that different aspects of shoot ontogenesis can be regulated independently of each other (Hackett and Murray, 1992). It is clearly desirable, therefore, to gain an understanding of the relationship between vegetative and reproductive maturation. This will not only serve to improve our knowledge of shoot development, but may also aid in breeding efforts. Breeders and horticulturists have long tried to reduce the time to flowering (Nienstaedt, 1961). At the same time, there is an interest in maintaining some characters of the early vegetative phase such as rooting ability for ease of vegetative propagation.

A link between vegetative and reproductive maturation is suggested by genetic analysis in cotton and *Pisum*. In cotton, Stephens (1944a) has shown that leaf shape changes progressively from node to node along the main stem, and that this change follows a 'developmental track' characteristic of each leaf shape allele. The modification of leaf shape depends on flowering time. At flowering or shortly thereafter, leaf shape development is arrested and a climax leaf is produced. The shape of the climax leaf depends on the developmental rates as well as the length of the non-flowering period (Stephens, 1944a, c, d). Thus, the leaf shape alleles Okra and Superokra can be clearly distinguished when no differences in flowering time are present (Stephens, 1944b). By controlling flowering time, Stephens (1944a) showed that a Superokra leaf can be made to phenocopy an Okra leaf, and *vice versa*. Additionally, while the course of the developmental tracks is not altered by flowering

time, the rate of change is accelerated in early flowering lines, thus partially compensating for the earlier attainment of a climax leaf (Stephens, 1944c).

The complex interactions between vegetative and reproductive development have also been observed in *Pisum sativum*. (Reid, 1986). Several genes regulating flowering have been identified in this species (Murfet, 1989). The dominant alleles of two of the genes, *Sn* and *Dne*, are thought to control the production of a graft-transmissible floral inhibitor in the leaves and cotyledons in a photoperiod-dependent manner. A common basis of regulation of the vegetative and reproductive development is suggested by the fact that both genes also have a pleiotropic effect on vegetative development. In the late flowering genotypes, senescence of the shoot and flowers is delayed, the flower peduncle is elongated, and vegetative traits such as the branching habit are modified (Murfet, 1989).

While the change from the early vegetative phase to the late vegetative phase and the onset of reproductive maturity are frequently correlated, there are obvious deviations from this general observation. Because of these exceptions, the linkage between vegetative and reproductive maturation has been doubted for a long time (see Stoutemyer, 1964). Several of these inconsistencies are reviewed here.

One of the most obvious problems is posed by precocious flowering. Precocious flowers are those produced at times or positions where the plant is normally expected to be juvenile. Precocious flowering occurs naturally (v. Denffer, 1950), in mutants (Sung et al., 1992) or can be forced (Longman and Wareing, 1959). In several tree species, precocious flowering is known to be under simple genetic control (see Nienstaedt, 1961). If vegetative development is linked to reproductive maturity, as has been assumed traditionally, then the late vegetative phase of development must also be initiated prematurely. While this aspect has not been studied explicitly, there is no mention of premature late vegetative growth in precociously flowering plants. Indeed, precocious flowering may indicate that vegetative and reproductive development can proceed independently. The latter possibility is supported by the observation that a plant can flower precociously in one season, and then return to vegetative growth for a number of years (Stoutemyer, 1964).

This observation has led some investigators to propose that reproductive maturity is reflected not merely by the ability to flower, but by the ability to do so continuously (Zimmerman et al., 1985). This distinction introduces great difficulties into the assessment of reproductive maturity. How are studies to be evaluated in which

flowering potential is measured after manipulation of the plant, such as in cuttings or tissues *in vitro*? How should monocarpic plants be assessed where a single flowering event cannot be distinguished from continuous flowering? When is full reproductive maturity reached in a species where flowers of one sex are produced at a different time from the other sex? One of the issues here is that the assessment of reproductive maturity strongly depends on the ways in which the ability to flower is measured. The requirement of continuous flowering represents one extreme; the other extreme is represented by the view that the production of any flower indicates reproductive maturity. Logically, the ability to flower must precede the differentiation of floral structures. Thus, the production of any flower shows that the plant is able to flower under a given set of circumstances. The conceptual distinction between flowering once and continuous flowering may be useful in some cases, but is at best problematic, and at worst unacceptable in studies where the ability to flower has to be determined.

It is clear that at least parts of the reproductive phase of development are regulated independently of vegetative development. For example, late vegetative *H. helix* plants can be induced to flower or prevented from flowering by different photoperiodic treatments (Wallerstein and Hackett, 1989), suggesting that the late vegetative phase itself does not condition the ability to flower. Observations in rejuvenation studies also suggest that reproductive maturation and vegetative maturation can be reversed independently of each other. Frequently, different degrees of partial rejuvenation can be induced depending on the length or severity of treatment. In such cases, the ability to flower is lost in the least severe treatments, whereas early vegetative traits are acquired only in more severe treatments. Doorenbos (1954) reported that adult scions of *H. helix* lose their ability to flower or stop flowering when grafted onto early vegetative (juvenile) stocks (or *vice versa*), but do not, or only partially revert to early vegetative growth unless all late vegetative leaves are removed. Based on such defoliation studies, the author suggests that rejuvenation is promoted by the early vegetative leaves and inhibited by the late vegetative leaves. Rogler and Hackett (1975a) used gibberellic acid to induce rejuvenation in *H. helix*. They found that low doses repress flowering ability, whereas increasingly higher doses induce increased expression of early vegetative traits. Independence of vegetative maturation and flowering ability is also suggested by the observations that in some eucalyptus species flowering occurs before a change in foliar characteristics and that in *H. helix* some early vegetative traits can be expressed in plants that have the ability to flower (Hackett and Murray, 1992).

Independent regulation can also be demonstrated in the independent responses of vegetative growth and reproductive development to environmental stimuli. Ashby (1948) and Ashby and Wangermann (1950) have shown that changes in leaf morphology in *Ipomoea* depend on the position of the leaf on the shoot. Short day conditions induce flowering and alter the changes in leaf shape. Ashby (1950) was able to show that both flowering time and leaf shape are regulated by short day conditions, but independently of each other. When a florally induced plant is transferred to long day conditions, leaves that differentiate subsequently have normal long day type of lobing. Finally, the notion that early vegetative growth *per se* is adverse to reproductive maturation has been challenged in maize. Bassiri et al. (1992) demonstrated that the onset of the reproductive phase of development is not affected or only slightly delayed in plants carrying the *Teopod2* mutation. In these plants the expression of the early vegetative phase is greatly prolonged.

In summary, the existing information about the regulation of vegetative and reproductive development suggests that a single switch model such as the traditional model of shoot development and phase change is too rigid and simplistic. Hackett and Murray (1992) instead propose a variety of alternative models in which ontogenetic changes are regulated independently and proceed in parallel or in sequence. Whereas Hackett and Murray (1992) suggest that each phase-specific trait may be regulated independently, it is assumed here based on the phenotype of the Teopod mutants that all the characters of each phase can be regulated together. The model of shoot development used in the present thesis postulates independently regulated, yet interacting phases of development which condition the coordinate regulation of phase-specific traits (Bassiri et al., 1992; Poethig, 1990). The phases of development are thought to interact to guarantee proper shoot development. This model, simpler that those of Hackett and Murray (1992), but more flexible than the traditional model, is believed to be a better conceptual framework for understanding phase change and the relationship between the phases of plant shoot development.

1.3 Maize and its usefulness for the study of plant shoot development

The species used in the present research project is maize (*Zea mays* L.), a herbaceous cereal that was domesticated in tropical and subtropical America. This plant is well suited for a study of shoot development for a number of reasons. Firstly, the growth and development of the plant have been described in detail (Kiesselbach, 1949; Sharman, 1942; Poethig, 1984). During vegetative growth, the shoot produces a series of segmental units called phytomers (Galinat, 1959). Each phytomer is composed of a bud, an internode, a node, and a leaf. The leaves are composed of a sheath and a blade, separated by an auricle with a ligule, and are arranged in a distichous phyllotaxis. In the terminal inflorescence, the tassel, phyllotaxis is spiral. The tassel bears only staminate flowers, and pistillate inflorescences, the ears, arise from axillary buds a few nodes below the tassel. Basal buds can grow into lateral branches, the tillers, and no buds develop between the primary, top ear and the tassel. Two types of root are produced, the seminal root and adventitious roots (also called nodal or prop roots). The adventitious roots develop from basal internodes and make up most of the root system of the mature plant (Martin and Harris, 1976; Sharman, 1942).

Secondly, traits characteristic of the early vegetative and the late vegetative phase have been well characterized and are superimposed on the phytomer structures (Bongard-Pierce and Poethig, in preparation; Poethig, 1988a, 1990). In maize, traits specific of the early vegetative phase include such characters as the presence of adventitious roots and of a form of epicuticular leaf wax that causes a blue-gray bloom on the leaf surface (glaucous leaves), short internodes, the production of vegetative lateral shoots (tillers) in appropriate backgrounds, round epidermal leaf cells (in cross section), and a purple staining of the epidermis with toluidine blue O. The late vegetative phase is characterised by such traits as the absence of adventitious roots, the presence of a different form of leaf wax (glossy, green leaves) and of epidermal hairs on the leaf blade, elongated internodes, rectangular epidermal leaf cells (in cross section), and a purple-and-aqua staining of the epidermis with toluidine blue O. Axillary budsgrow into ears or do not develop at all. The termination of the early vegetative growth takes place over several phytomers, resulting in an overlapping expression of the early vegetative and the late vegetative phases of development during the transition between the two phases. The expression of phase-specific traits usually takes place in spatially distinct regions of the transition phytomers. These phase-specific traits have been identified initially by virtue of their being coordinately expressed in the basal or apical leaves, respectively. Additionally, their expression is

modified coordinately in Teopod plants (Bongard-Pierce and Poethig, in preparation; Poethig, 1988a, 1990).

Thirdly, maize is a monocarpic species with a terminal inflorescence, which allows one to study the developmental changes that occur during shoot development within one meristem and the organs it produces.

Finally, maize is one of the genetically best characterised plants. Many genes that affect the habit of maize have been described (Coe et al., 1988). Among these are four dominant mutations (*Teopod1*, *Teopod2*, *Teopod3*, *Corngrass1*) that greatly extend the expression of the early vegetative phase of development, while the onset of the late vegetative phase and of the reproductive phase is not substantially altered (Bassiri et al., 1992; Galinat, 1954a, b, 1966; Lindstrom, 1925; Poethig, 1988a, b, 1989; Ritchings and Tracy, 1989; Singleton, 1951; Weatherwax, 1929; Whaley and Leetch, 1950). Since these mutations affect all the known phase-specific traits, it is likely that they play a role in the regulation of the early vegetative phase of development. More recently, a gene regulating the expression of phase-specific traits in the leaf epidermis has been described (Evans et al., 1994). This gene called *glossy15* is epistatic to the *Teopod* genes and is thought to act downstream of the *Teopod* genes in the regulation of the vegetative phases of development.

In spite of the extensive knowledge about maize genetics in general, relatively little is known about the genetic regulation of flowering in this species (Hanway and Ritchie, 1985). Flowering time is believed to be a quantitative trait controlled by an estimated 2 to 20 loci (Giesbrecht, 1960a, b; Hallauer, 1965; Mohamed, 1959). Most estimates are for 4 to 6 genes. Mutations at two loci are known to condition late flowering, *indeterminate growth1* (*id1*) and *Leafy1* (*Lfy1*) (Shaver, 1983; Singleton, 1946). *id1* and two other mutations (*grassy tillers1* , *Gt1*, and *perennialism1, Pe1*) in combination cause a perennial growth habit in maize (Shaver, 1967). There is evidence that early flowering correlates with a smaller amounts of nuclear DNA in different lines (Bullock and Rayburn, 1991; Rayburn and Auger, 1990; Rayburn et al., 1985; Tito et al., 1991), but reports are conflicting (McMurphy and Rayburn, 1991). No single genes conditioning early flowering have been isolated. However, several early flowering, near-isogenic lines have been derived from a naturally occurring early flowering race, Gaspé Flint, by recurrent selection (Shaver, 1976; Sisco et al., 1989). Additionally, flowering can be induced by short-day conditions, and the flowering time is modified by temperature and light intensity (Coligado and Brown, 1975; Duncan and Hesket, 1968; Hesket et al., 1969; Hunter et al., 1974, 1977; Russel and Stuber, 1983; Stevenson and Goodman, 1972; Tollernaar and Hunter, 1983; Vig and Limberg, 1986).

1.4 Rationale and outline

As indicated earlier, the relationship between the vegetative and reproductive phases of development is far from clear. In the traditional model of phase change, the development of the shoot is determined solely by the reproductive ability of the plant. This traditional model does not provide the conceptual tools to explain or analyse occurrence of the phase-specific vegetative changes independently of reproductive maturity. The alternative model employed in the present study postulates the presence of three phases of shoot development: an early vegetative phase, a late vegetative phase, and a reproductive phase of development. This conceptual framework assumes no causal relationship between the three phases, and allows for the formation and examination of hypotheses regarding the relationship between the phases of development.

Proper timing of expression of each developmental event relative to other developmental events is crucial for the normal development of an individual. When studying the timing of developmental events it is useful to invoke the concept of heterochrony, which refers to changes in the timing or the rate of one developmental event relative to one another (Gould, 1977; Raff and Wray, 1989; Takhtajan, 1972). In plants, heterochronic changes (changes in timing) can usually not be distinguished from homeotic changes (changes in space) because of the polar nature of shoot growth. When the concept of heterochrony is used here it is not implied that this distinction has been made. Heterochrony has often been considered when changes in timing of vegetative (somatic) development relative to reproductive maturity are observed. In plants with a terminal inflorescence, such as maize, shoot development can be observed in a spatial sequence of phase-specific phytomers laid down by one meristem. The timing of flowering in such species determines the length of the vegetative phase. When flowering time in such a species is modified, three scenarios must be considered, as illustrated in figure 1.1 for the case of early flowering:

1-Only the length of the late vegetative phase of development is changed. The transition from early vegetative growth to late vegetative growth would in this case take place at the same chronological time, but be shifted relative to the onset of flowering. This would indicate that the two events are independently regulated.

2-Both the early vegetative and the late vegetative phases of development are altered in length. Whereas the the vegetative phase change and flowering time are both altered in absolute (chronological) time, this would indicate no change in the relative timing of

developmental events. This would indicate that the vegetative phase change and the reproductive maturity are coordinately regulated.

3-Only the length of the early vegetative phase is altered. In this case, the length of the late vegetative phase is constant and the change in flowering time matches the change in the length of the early vegetative phase. This scenario would also indicate that the vegetative phase change and reproductive maturity are coordinately regulated. It suggests that a minimum number of late vegetative leaves has to be formed before flowering can occur and that expression of the early vegetative phase and of the reproductive phase of development may be incompatible.

To study which of these possibilities occur in maize, vegetative maturation was examined in a variety of early flowering and late flowering plants. Early flowering near-isogenic lines of maize, late flowering mutants, and photoinduction together provide several distinct ways to modify flowering time experimentally and to investigate the relationship between changes in the vegetative maturity of the shoot and the reproductive maturity of the shoot. All three approaches were used in the present study.

In order to study the relationship between the vegetative and the reproductive phases of development in detail, the research is focussed on a characterisation of early flowering, near-isogenic lines of maize. Initially, growth characteristics are investigated to establish that leaf initiation rates are not altered in these lines and if the early flowering trait acts specifically to induce an earlier production of reproductive structures by the shoot meristem. The latter aspect is studied by comparing root growth and the progression of leaf form along the stem in inbred lines and their near-isogenic early derivatives.

In order to investigate if the time of expression of the vegetative phases of development is altered in the early derivatives, vegetative development is characterised in detail in inbred lines and their early derivatives using a number of phase-specific traits. The early flowering lines are found to be particularly useful for investigating the relationship between the vegetative and reproductive phases of development because within one species, but in different inbred lines, the shift from the early vegetative to the late vegetative phase of development and flowering time can be coordinately regulated or not (cases 1 and 2 in figure 1.1).

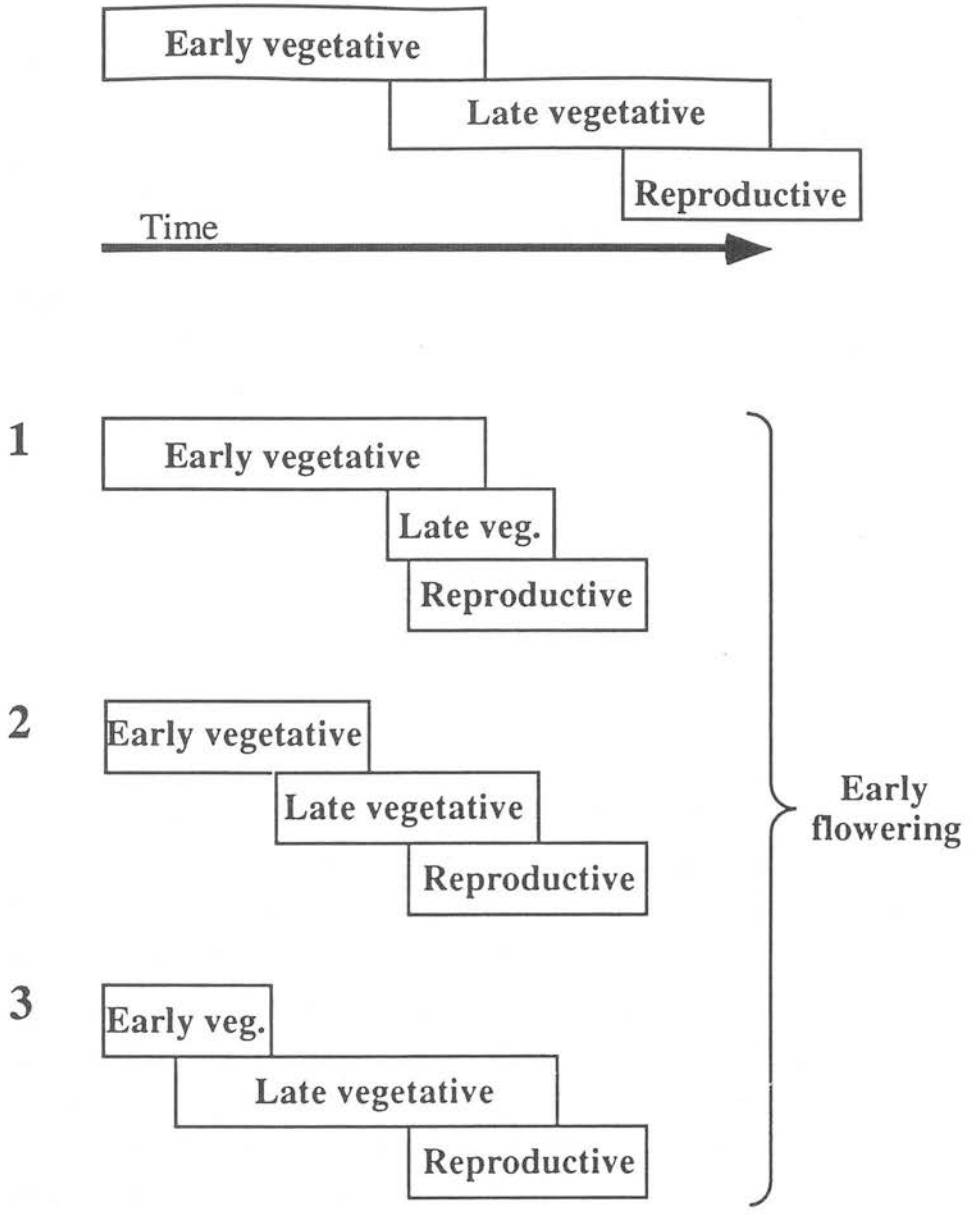

Figure 1.1: Model illustrating the expression of the three phases of shoot development in normal and early flowering plants. The three phases of development are represented by boxes arranged along a time line. The early vegetative and late vegetative phases are thought to overlap because transition phytomers express characters specific to both phases. The placement of the ear suggests an overlap between the late vegetative phase and the reproductive phase. The three possible ways in which the expression of the early vegetative and late vegetative phases can be adapted to fit a shorter vegetative period overall are indicated in the lower three models, compared to a model of shoot development in a normal plant at the top. For further explanation see main text.

Since the early derivatives were developed independently by converging the early flowering trait from Gaspé Flint into the inbreds, the cause for the different patterns of vegetative development could be the presence of different early flowering genes in each line, some of which may also alter vegetative development. Alternatively, the difference between these lines may result from genetic differences in the inbred background. To distinguish between these possibilities, the attempt is made to resolve genetic difference in the early flowering trait between the early derivatives using genetic and RFLP analyses. Moreover, the transition from the early vegetative to the late vegetative phase of development is characterised in additional lines to examine if the pattern of vegetative development is similar in inbreds with related genetic backgrounds or early derivatives developed from the same donor. Finally, photoinduced plants are used to study vegetative development in early flowering plants in the absence of the early flowering trait. Results indicate that the differences in the relationship between vegetative maturation and reproductive maturation are conditioned by the genetic background.

In order to place the early flowering trait in a reproductive pathway, the responsiveness of the early derivative to short day conditions is tested and double mutant analyses with the *Tp1* and *Tp2* mutations are performed. The analysis of leaf form suggested that the early flowering trait is expressed early during shoot development. The time of expression of the early flowering trait is further investigated by measuring the response to short day conditions supplied at a different time of shoot development. Together these results indicate that the early flowering trait acts prior to the photoperiodic requirement in the regulations of the reproductive pathway.

In the final part of the present study, vegetative development is characterised in four different mutations conditioning a late flowering phenotype.

The results are discussed in terms of heterochrony and in terms of their implications for the conceptual framework of plant shoot development. It is suggested that the vegetative phase of development may have a function in adapting the plant phenotype to predictable changes in the environment of the plant and that ontogenetic plasticity in shoot development may hold adaptive advantages for the species. Finally, a three-phase model of shoot development in maize consistent with the findings is proposed.

CHAPTER 2

MATERIALS AND METHODS

2.1 General

2.1.1 Plant material

The plants used in this study are from standard inbred lines and their near-isogenic derivatives unless indicated otherwise. All the early flowering lines were derived from the very early primitive race Gaspé Flint. Tables 2.1 and 2.2 summarise the pedigrees of these early flowering, near-isogenic lines (early derivatives (EDs)) and of the inbred lines (ILs) used as recurrent parents. Dr. D.L. Shaver kindly provided the seeds from the inbred lines A619, A632, Oh43, Mo17, and N28 (yellow cobbed version), the early flowering, near-isogenic lines A619E, A632E, A635E, C123E, H100E, Oh43E, Mo17E, and N28E, and the race Gaspé Flint , as well as with material carrying the *Leafy1* (*Lfy1*) mutation. The lines B73 and Sc76 and the early derivatives B73G and NC76 were a kind gift from Dr. R. Koester. Further plant material was obtained from Dr. R.H. Peterson (A635, C123), Dr. D. Glover (H100), Dr. M.G.Neuffer (*delayed flowering* (*dlf*), Dr. E.H. Coe (ethyl methane sulfonate mutagenised M2 seed in A632), and the Maize Genetics Stock Center (*id1, Rg1*). The phenotypes of each of these lines and mutants is described in the appropriate sections of chapter 3.

2.1.2 Cultivation conditions

All plant material was either grown in the summer nursery at the field sites near Swedesboro, NJ, or in a greenhouse. Field-grown plants were planted at 25 plants per 8.5 metre (m) row, with a distance of 1m between rows. The ground was treated with a pre-planting herbicide to keep weeds at a minimum. Later in the season, weeds were controlled by manual cultivation. Granular inorganic fertiliser was applied between the rows when the plants were about 50 centimetres (cm) tall.

Unless otherwise noted, the greenhouse plants were grown in a sandy soil mixture (24 volumes potting soil, 1.3 volumes fine sand (number zero grain), 1 volume coarse sand (number three grain), 1 volume vermiculite) in 15cm plastic pots in the greenhouses of the University of Pennsylvania with supplemental lighting to ensure long day conditions (90millimols m^{-2} $second^{-1}$of photons in the photosynthetically active range, 400-700nanometres) (18 hours (h) light : 6h dark). The temperature was kept between 20 degrees centigrade (°C) and 30°C. Fertiliser and pesticides were provided as necessary.

Table 2.1 Origin and pedigree of the early derivatives.

Near-isogenic line	Source of early flowering trait	Pedigree	Reference
A619E	NC230E	$BC_8\ S_{11}$[1]	Shaver (pers. comm.)
A632E	Wf9E	$BC_{10}\ S_{10}$	Shaver (pers. comm.)
A635E	A632E	$BC_6\ S_6$	Shaver (pers. comm.)
B73G	Gaspé Flint (GF)	$(B73 \times GF_2)BC_6^2\ S_3$	Koester et al (1993)
C123E	Gaspé Flint	$BC_{11}\ S_{20}$	Shaver (pers. comm.)
H100E	A632E	$BC_6\ S_6$	Shaver (pers. comm.)
Mo17E	Gaspé Flint	$BC_8\ S_{16}$	Shaver (pers. comm.)
N28E	Gaspé Flint	$BC_{20}\ S_{12}$	Shaver (pers. comm.)
Oh43E	Gaspé Flint	$BC_{10}\ S_{12}$	Shaver (pers. comm.)
Nc264	Gaspé Flint	$(Sc76 \times GF_2)BC_4^2\ S_3$	Koester et al. (1993), Sisco et al. (1989)

[1] Backcrosses (number of crosses to recurrent parent) followed by sib or self pollinations (number of crosses).

[2]Each backcross followed by one generation of self pollination

Table 2.2 Origin of the inbred lines (Henderson, 1984; MBS, Inc., 1988; Phillips et al., 1992)

Standard inbred line	Developed from	Time to maturity[1]
A619	(A161 x Oh43) x Oh43	110 (1390)
A632	(Mt42 x B14) x $B14_3$	110 (1440)
A635	(ND203 x B14) x $B14_2$	105 (1450)
B73	Iowa Stiff Stalk Syn. (rec. sel. pop. C5)	116 (1450)
C123	C102 x C103 sel.	115 (1360)
H100	N28 x H91	- (1400)
Mo17	187-2 x C103	118 (1450)
N28[2]	Nebraska Stiff Stalk Synthetic	130 (1475)
Oh43	Oh40B x W8	112 (1350)
Sc76	Hastings Prol x Yel. Tuxpan	- (-)

[1]given in Days to Relative Maturity (and U.S. Heat Units to flowering) as reported in MBS, Inc. (1988).

[2]The original, red cobbed N28 was converted into a yellow cobbed version by means of six back crosses prior to the early flowering convergence (Shaver (pers. comm.)).

2.1.3 Morphological analysis

Whenever possible, the plants used in this study were described in terms of several characters that can be scored under field conditions (Table 2.3). These traits have previously been described as been expressed in a phase-specific manner (Poethig, 1988a; 1990). The early vegetative phase of development is marked by the presence of adventitious roots and of a blue-gray (glaucous) bloom on the leaves, which is caused by a early vegetative form of epicuticular wax (Figure 2.1). Leaves produced during the late vegetative phase of development are brightly green (glossy) and have macrohairs on the leaf surface. These features were commonly scored on the leaf blade, and their expression on the leaf sheath (sheath wax and sheath hair) was used only occasionally. The total leaf number (TLN) describes the overall length of the vegetative phase and the onset of reproductive differentiation in the apical meristem. All leaf numbering was carried out from the base to the apex, so that the oldest, most basal leaves have the lowest numbers and the youngest leaf, the flag leaf, has the highest number. The leaves were numbered several times throughout the season. This was necessary because the basal leaves senesce early in shoot development.

Teopod plants usually produce more leaves than wild type plants, presumably by converting tassel structures into leaves. The apical vegetative phytomers in these plants frequently produce more than one leaf per node. These nodes were considered vegetative as long as no spikelets were produced in the leaf axils. Given that the number of leaves per node varied in these plants, the TLN was recorded as the number of vegetative nodes, not as the total number of leaves.

The tassel morphology in Teopod plants was assessed according to the following scale. Branched Teopod tassels received a score of 1, unbranched tassels with wild type spikelets a score of 2. If elongated glumes were observed, the tassel was given a score of 3. Tassels with leafy structures had scores of 4 (1/4 of the tassel leafy), 5 (1/2 of the tassel leafy), 6 (3/4 of the tassel leafy), or 7 (tassel completely leafy). A score of 8 was given when no floral structures were produced and the rachis was barren. Wild type tassels received a score of 0.

Figure 2.1 Expression of epicuticular leaf wax in a transition leaf. A transition leaf shows both the waxy bloom typical of the early vegetative growth (glaucous leaf surface) and the glossy green leaf surface typical of late vegetative growth.

Table 2.3 Morphological traits specific to the vegetative and reproductive phase of development. Most of these traits were scored on all plants. Sheath wax and sheath hair were not commonly recorded.

Phase	Description of trait
Early vegetative	Last node with adventitious roots, scored according to the number of the leaf arising from the node above them
Early vegetative	Last leaf blade with any early vegetative epicuticular wax, i.e. last partially glaucous leaf blade
Early vegetative	Last leaf sheath with any early vegetative epicuticular wax, i.e. last partially glaucous leaf sheath
Late vegetative	First leaf blade with any visible trichomes (macrohairs) on the adaxial surface of the leaf blade. Marginal hairs were disregarded.
Late vegetative	First leaf blade not covered entirely with early vegetative epicuticular wax, i.e. first partially glossy leaf blade
Late vegetative	First leaf sheath with any visible trichomes (macrohairs)
Reproductive	Ear placement node, scored according to the number of the leaf subtending the ear
Reproductive	Total number of leaves (TLN)

2.1.4 Statistical analysis

Analyses were carried out in software programmes designed for Apple Macintosh computers: Microsoft Excel (Microsoft) , Cricket Graph III (Computer Associates), and Statsworks/Systat 5.2.1 (Systat).

When a test was required to establish whether means of two samples were identical or different, Student's *t* test was the preferred statistical test. However, frequently the sample size was smaller than 25, making it impossible to ascertain that the scores were from a normal distribution. One required assumption in parametric tests is that the data are normally distributed. In cases where such assumptions cannot be made, a non-parametric test, in this case the Mann-Whitney *U* test, must be considered. This test can be applied when differences between two independent samples are assessed, and the data are at least ordinal. However, Student's *t* test does not necessarily need to be rejected because of the problems encountered here. Firstly, parametric tests are robust with regard to violations of some of their assumptions. Secondly, parametric tests generally have a greater power-efficiency than non-parametric tests. Thus, when assessing the difference between the mean of two samples, the *t* test is more likely to detect a difference correctly than is an appropriate non-parametric test for the given sample size. Finally, these two methods do not ask exactly the same question. A parametric test such as the *t* test makes a rather direct assessment of whether the mean of one group of data differs from the mean of another, whereas a non-parametric test asks whether one distribution differs from another in any way (McCall, 1990). In the present study, most samples were distinguished by differences in genotypes. There is no reason to assume severe departures from normality in such data. Thus, Student's *t* test was applied throughout. In a few examples, the *U* test was used in addition to the *t* test to confirm the validity of the assumptions and of the results. When both tests were applied, the significance is indicated in the tables by a letter for the *t* test (a, b, c) and by a number for the *U* test (1, 2, 3). When a hypothesis could be formulated about the direction in which one sample was expected to differ from another, one-tailed tests were employed.

The Pearson Chi-Square test was used to assess segregation of the early flowering trait (EFT) (Crow, 1976). Yates's correction was employed to correct for small expected values where appropriate, otherwise a Poisson distribution approximation or the exact binomial were calculated (Zeller and Carmines, 1978).

All confidence intervals were always calculated as two times the standard error estimate of the means (s.e.m.).

2.2 Experimental procedures

2.2.1 Habit and flowering time (section 3.1.1.1)

Plants grown in the field at Swedesboro in the summer of 1992 were scored for morphological characters and photographed at maturity. Plants used to determine flowering time were grown in 20cm clay pots in the greenhouses of the University of Pennsylvania in the autumn of 1990. The following genotypes (number of plants) were scored for the time to flowering as well as phase-specific characters: Oh43 (10), Oh43/Oh43E (8), Oh43E (10), Oh43/Oh43E x self (F2 family) (110). The time to flowering was measured in days from planting to the beginning of pollen shedding (anthesis).

2.2.2 Leaf initiation and emergence (section 3.1.1.2)

For measurement of leaf initiation and emergence rates in A632 and A632E, plants were grown in potting soil in 5cm peat pots in the Plant Growth Facilities of the East of Scotland College of Agriculture in the spring of 1991. Supplemental lighting was provided to ensure long day conditions (18h light : 6h dark). Temperatures were kept constant at 22°C. 3 to 7 plants were scored for the number of visible leaves and then dissected at each time that measurements were taken in six day intervals until 35 days after planting. For the first experiment in Oh43 and Oh43E (figure 3.4A), plants were grown and the leaf numbers at each time point measured as described in section 2.2.3. For the second Oh43 experiment (figure 3.4B), plants were grown in a sandy soil mix in 5cm peat pots in the greenhouse of the University of Pennsylvania in the winter of 1992/93. 3 to 8 plants were scored for the number of externally visible leaves and for the number of visible ligules every two days until tassel initiation occurred. The number of leaves initiated by the plant was determined by removing all leaves under a dissecting microscope.

2.2.3 Root growth (section 3.1.1.3)

Seeds from the inbred line Oh43 and the near-isogenic early line Oh43E were germinated on moist filter paper in 9cm Petri dishes at 25°C for three days. Depending on the expected date of harvest, the seeds were then planted in the greenhouse in 10cm, 15cm, 20cm, and 25cm pots filled with fine sand (0.5 -1.5 millimetre (mm) aggregate size). This range of pot sizes was used to reduce possible variation in root size due to constriction by the pot. Measurements were taken every three to five days, starting at planting. The pots were flushed with 1/16 dilute Hoagland's solution (Hoagland et al., 1950) at least four times a day to ensure constant nutrient concentrations in the substrate. The plants were grown under long day conditions with supplemental lighting (90millimols m^{-2} $second^{-1}$of photons in the photosynthetically active range, 400-700nanometres, 16h light : 8h dark) at 30°C (light) : 25°C (dark).

For each measurement, five plants of each genotype were removed from the pots and the sand washed off. The shoot, adventitious roots, and seminal roots were separated, blotted dry and weighed. The roots were then spread and the root lengths determined digitally with a Delta-T Areameter (Delta-T Devices) connected to a 8mm video camera (Minolta CR-8000S AF). The exposed leaf blades were photocopied and the leaf areas determined using a ADB MacTablet digitising drawing pad (Summagraphics, Modell MM 1201) and computed with the MacMeasure 1.9 (Hook and Rasband, 1987) and Microsoft Excel 4.0 (Microsoft) software programmes. Leaves that had not fully emerged from the whorl were measured from the last exposed ligule to the tip of the emerging leaf. A dissection of the shoot under a dissecting microscope yielded the number of leaves initiated. The shoot, adventitious roots, and main roots were then oven-dried over night and weighed.

2.2.4 Leaf form (section 3.1.1.4)

Ten to eleven plants each of the lines A632, A632E, Oh43, and Oh43E were grown in the spring of 1993 in the greenhouse of the University of Pennsylvania under standard conditions. All plants were scored for the vegetative and reproductive traits described in table 2.3. The length of each leaf blade along the midrib from ligule to tip and the maximum width of the blade were measured with a ruler accurate to 1mm after the leaf blade had fully emerged.

2.2.5 Vegetative morphology (section 3.1.2.1)

F2 families (IL/ED x self or sib) segregating the EFT and plants from the inbred lines, early derivatives and hybrids were scored for the vegetative and reproductive traits given in table 2.3. Three F2 families segregating the early flowering trait in the A632 background and their controls were scored in the field in summer 1992. Five F2 families segregating the early flowering trait in the Oh43 background and their controls were scored in the greenhouse in the autumn of 1991. The F2 plants were sorted according to the total number of leaves and the data analysed separately for each group.

2.2.6 Epidermal cell shape (section 3.1.2.2)

This protocol follows a method provided by D.K. Bongard-Pierce. Five plants of each genotype (A632, A632E, Oh43, Oh43E) were grown in the autumn of 1991 in the greenhouses of the University of Pennsylvania. All plants were scored for the vegetative and reproductive traits defined in section 2.3. Leaf material was harvested from consecutive leaves of three plants per genotype. A 1cm by 1cm piece of leaf tissue was removed from a position 50% along the length of the leaf blade and midway between the midrib and the leaf margin, and was cut transversely into small strips of about 1mm by 10 mm. The material was fixed for 1 to 2 hours at room temperature, followed by over night fixation at 4°C in a fixative containing 0.05 Molar (M) sodium phosphate buffer, pH 6.8, 3% glutaraldehyde, 1.5% acrolein and 1.6% paraformaldehyde. The tissue was post-fixed in 0.05M sodium phosphate buffer, pH 6.8, 1% osmium tetroxide for at least 2 hours, followed by three rinses in 4°C water. The material was dehydrated in an ethanol/acetone series, using 12.5%, 25%, 35%, 50%, 70%, 80%, 95%, and two 100% ethanol steps, and two 100% acetone steps for 10 minutes each. The imbedding medium was a modified Spurr's resin (Spurr, 1969, as cited in O'Brien and McCully, 1981), made of 10g vinyl-4-cyclohexene dioxide (ERL 4206), 7.17g DER 736 resin, 0.4g dibutyl phthalate, 21g nonenyl succinic anhydride, and 0.65 millilitres (ml) S-1 accelerator (Electron Microscopy Sciences). The leaf strips were infiltrated at room temperature with 1:1, acetone:resin for 30-60 minutes, 1:2, acetone:resin for 30 minutes, 100% resin for 60 minutes, and again with 100% resin over night on a shaker. Polymerisation took place at 60°C over night. A Sorvall MT2-B ultra-microtome with a glass knife was used to cut the specimen into 1µm thick sections. The sections were attached to slides at 60°C, stained with 0.2%

toluidine blue O (C.I.#52040, Basic blue 17, Sigma), 2.5% sodium carbonate, pH 11.1, for 30 to 60 seconds at 60°C, rinsed with water, dried, and mounted in immersion oil under a cover slip (after Trump et al, 1961, as cited in O'Brien and McCully, 1981). The samples were viewed under a Olympus BH2 light microscope at 200 x magnification. A blue (LBD-2N) and green (IF 550) filter were used for photography at 83 x magnification. The outline of the cells of the abaxial epidermis was traced from prints using an ADB MacTablet digitising drawing pad (Summagraphics, Modell MM 1201) and computed with the MacMeasure 1.9 (Hook and Rasband, 1987) and Microsoft Excel 4.0 (Microsoft) software programmes. The shape factor is calculated by the MacMeasure programme to equal 4π (area)/(circumference)^2. The shape factor of a circle calculated with this formula would be one, that of a line zero. Thus, cells that appear round in cross section will have a higher shape factor than elongated cells. Guard cells and their neighbouring cells were excluded to reduce variability. Similarly, the adaxial epidermal cells were not measured to avoid increasing the variability by including bulliform cells into the analysis.

2.2.7 Tissue staining (section 3.1.2.3)

Plants were grown in the autumn of 1992 in the greenhouses of the University of Pennsylvania. Halfway along the length of the leaf blade, the abaxial surface of the leaf was carefully abraded with diatomite and a piece of tissue was removed from half way between the midrib and the leaf margin with a cork borer. The samples were fixed in Farmer's fluid (3:1, ethanol:glacial acetic acid) (Johansen, 1940). After complete clearing in 95% ethanol, the leaf discs were put into fresh fixative over night and then stained in a 1/16 diluted toluidine blue O stain. The stain stock solution consisted of 0.05% toluidine blue O (C.I.#52040, Basic blue 17, Sigma) in 0.01M sodium acetate buffer, pH 4.4 (adapted from Sakai, 1973). The stained discs were mounted in water under a cover slip and viewed and photographed under a dissecting microscope in darkfield illumination at 25x magnification (32x magnification at the camera). The light quality was adjusted with a 80A filter for the daylight film (Ektachrome 100, Kodak).

2.2.8 Expression of *Ragged1* (section 3.1.2.4)

Plants heterozygous for the *Ragged1* (*Rg1*) mutation were crossed to the inbred lines and early derivatives (A632, A632E, Oh43, Oh43E) in the summer of 1991. The progeny was scored directly in A632 (A632 x *Rg1/rg1* ; A632E x *Rg1/rg1*), whereas a second backcross was performed in Oh43 (Oh43 x *Rg1/rg1*, Oh43(1); Oh43E x *Rg1/rg1*, Oh43E(1)). Families segregating the *Rg1* mutation were grown in the summer nursery of 1992 and scored for the expression of the mutation and the phase-specific traits.

2.2.9 Estimate of the number of genes (section 3.1.3.1)

Plants in F2 families segregating the early trait in A632 or Oh43 (hybrid x self or sib), backcross families (hybrid x parental), hybrids (F1), and parentals were scored for TLN. The frequencies of each class of TLN were used to estimate the number of segregating genes. The experiment in the A632 background consisted of three F2 populations with a total of 206 plants and was grown in the summer nursery in 1992. The first experiment in Oh43 was grown in the summer nursery of 1992. The second experiment in the Oh43 background consisted of three F2 populations in the Oh43 background with a total of 104 plants and was grown in the summer nursery in 1990. The third experiment in Oh43 consisted of four F2 families with a total of 148 plants and was grown in the greenhouses of the University of Pennsylvania in the autumn of 1992.

2.2.10 Restriction fragment length polymorphism analysis (section 3.1.3.2)

Methods are modified from those described by Sambrook et al. (1989) and Ausubel et al. (1989) except where stated otherwise. Stock solutions and media are described at the end of the section. All materials were purchased from Sigma Chemical Company, unless otherwise stated.

2.2.10.1. Plant methods

Plant material

Three F2 families segregating the early trait in the A632 background, two backcross families to Oh43 (Oh43/Oh43E x Oh43), and three backcross families to Oh43E (Oh43/Oh43E x Oh43E) were grown in the field in the summer of 1992. The phenotypically most extreme segregants were selected in the F2 families. Similarly, the latest plants in the backcross families to Oh43 and the earliest plants in the backcross families to Oh43E were chosen. Four to six mature leaves and occasionally the immature ear of each selected individual were harvested in the field. In the laboratory, tough material (midribs, sheaths, outer husk leaves) was removed. The rest was cut into 2x2cm pieces, sealed into freezer bags, quickly frozen in liquid nitrogen, and stored at -80°C.

Plant DNA extraction

The deoxyribonucleic acid (DNA) extraction protocol is based on a procedure reported in Dellaporta et al. (1983) as adapted by D.K. Bongard-Pierce. The tissue was ground to a fine powder in a tissue grinder (Regal, No. V505) with dry ice. A volume of approximately 20ml of powder was added to 10ml 1 x lysis buffer in a 50ml tube and the mixture was allowed to thaw at room temperature. 10ml of phenol-chloroform in 1:1 volume ratio was blended into the mixture by vigorous shaking. The samples were left a room temperature for at least 20 minutes and shaken intermittently. Centrifuging at 4000revolutions per minute (2700 x earth's gravity) for 15 minutes at room temperature separated the phases. The aqueous phase was pulled off with a Pasteur pipette and filtered through Miracloth (Calbiochem, No. 475855) into a fresh tube. Nucleic acids were precipitated from it by adding 0.9ml ammonium acetate solution (table 2.5) and 10ml isopropanol. The precipitate was spooled on a Pasteur pipette and resuspended in 1ml TE buffer (table 2.5). Another precipitation was performed with 0.1ml ammonium acetate and 2.5ml cold 95% ethanol. The precipitate was rinsed in 5ml 70% ethanol, dried briefly by inverting the tubes on filter paper, and resuspended in 1ml TE buffer. Ribonucleic acid was removed by incubating the sample with 2 microlitres (µl) 10 milligrammes/ml (mg/ml) ribonuclease at 37°C for 30 minutes. A second phenol-chloroform extraction was followed by a precipitation with 200µl 5 M sodium chloride (NaCl) and 2.5ml ethanol to remove excess carbohydrates. After three rinses in 5ml 70% ethanol each, the DNA was dried briefly by inverting the tubes on filter paper, resuspended in 200-600µl TE and stored at 4°C.

2.2.10.2. Southern blotting

Restriction endonuclease digests

20µl DNA of each sample was incubated at 37°C over night with at least 80 units restriction enzyme in 1 x concentration of the appropriate buffer furnished by the supplier. The following enzymes were used: BamHI, EcoRI, HindIII, KpnI, PstI, PvuII, SalI, SstI, XbaI. The total volume of the reaction varied from 30 to 200µl, depending on the original concentration of the enzyme used, because the final enzyme concentration in the reaction was kept below 10%. Following the digestion, the DNA was precipitated with ammonium acetate and ethanol, rinsed in 70% ethanol, dried briefly by inverting the tube on filter paper, and resuspended in 25µl TE buffer. 5µl loading buffer (50% glycerol, 5 milliMolar (mM) ethylene-diamine-tetra-acetic-acid (EDTA), 0.04% bromophenol blue, 0.04% xylene cyanol FF, 10mM NaCl) was added to each sample.

Agarose gel electrophoresis

DNA fragments were separated on the basis of their size by agarose gel electrophoresis. Gels with 24 wells containing 0.7-0.8% agarose in 0.5 x TBE buffer were prepared in large horizontal gel systems (20x20cm) (Fisher) and completely covered with 0.5 x TBE running buffer. A voltage of 25 to 35 Volts (V) (1 to 1.5 V/cm gel) was applied over night until the bromophenol blue marker dye had travelled 3/4 to 4/5 of the gel. The gel was then removed from the gel box, stained for 20 minutes in 0.4µg/ml ethidium bromide with gentle agitation, and subsequently photographed on a UV transilluminator using a polaroid camera and polaroid 665 instant film.

Preparation of gel for DNA transfer

The gel was incubated in 0.25M hydrochloric acid at room temperature with gentle agitation for 10 to 20 minutes until the marker dyes had changed colour. Following two rinses in water, the gel was incubated in denaturation buffer (1.5M NaCl, 0.5MNaOH) for 30 minutes under the same conditions as before, and then twice rinsed in water again. The gel was then placed in neutralisation buffer (1.5M NaCl, 0.5M Tris-hydrochloric acid (Tris-HCl) pH8, 1 mM EDTA for 15 minutes at room temperature with agitation. The neutralisation buffer was replaced with fresh buffer and left for another 15 minutes. After two final rinses in water, the gel was ready for DNA transfer.

DNA transfer to nylon filters

DNA was transferred onto a nylon filter (Hybond N^+) using capillary transfer methods is based on a method of Southern (1975). The gel was transferred to a blotting apparatus which consisted of a tray containing 20 x Standard saline citrate (SSC), covered with a glass plate that rested on 5 cm high supports. The ends of three sheets of Whatmann 3MM paper covering the glass plated were immersed in the 20 x SSC. The gels were placed on the moist Whatman paper. Nylon filter, wetted in 20 x SSC, was cut to the same size as the gel and was placed on the gel, followed by 3 to 6 sheets of 3MM paper cut to size. Care was taken to remove all air bubbles between the layers of the transfer set-up. Contact between the gel and the top 3MM paper was avoided by covering the margins of the filter with strips of parafilm. 10 to 15 cm of paper towels were put on top of the 3MM paper and weighted down evenly with a glass pane and a weight of approximately 500 grammes (g). The transfer was allowed to proceed for at least 15 and up to 36 hours. The blotting apparatus was dismantled, and the gel placed, DNA side up, on fresh 3MM paper. The DNA cross-linked to the filter first by irradiating it with ultra violet light at 0.75 Joules cm^{-2} (Stratalinker, Stratagene) while placed on a piece of 3MM paper moistened with 50mM sodium phosphate buffer, pH 7.0. The cross-linking was completed by drying the filter for 2-3 hours at 80°C. The nylon filter was stored at room temperature until use.

Synthesis of radioactively labelled probe

Probes were synthesised from isolated DNA fragments in a random priming reaction (Feinberg and Vogelstein, 1983). All reagents used were supplied by the manufacturer or made up according to specifications. 10µl of random oligonuclotide primers were added to 1 to 15µl of linear double stranded probe DNA and water in a final volume of 33µl . After the tubes were heated to 95 to 100°C for 5 minutes, 10µl of 5 x primer buffer, 5µl of (α^{32}P) dCTP (Amersham), and 1-2 units of polymerase (Klenow fragment of DNA polymerase 1) (Stratagene) were added. The reaction was incubated at 40°C for 10 minutes, and then stopped with 5µl 0.5M EDTA, pH 8.0. Following an ammonium acetate/ethanol precipitation (10µl ammonium acetate, 150µl ethanol), the probe was resuspeded in 200µl TE. 2µl of the solution was used to measure incorporation of the radionucleotides in a scintillation counter. The volume was then brought up to 1ml . The tube heated to 95 to 100°C for 5 minutes and then chilled on ice.

Hybridisation

The nylon filter was pre-hybridised with prehybridisation solution (0.5M sodium phophate buffer, pH7.2; 7% sodium dodecyl sulfate (SDS); 1% bovine serum albumin)

for 2 hours at 65°C. The prehybridisation solution was replaced with hybridisation solution (prehybridisation solution, 5% dextran sulfate) and the denatured radioactively labelled probe was added. Hybridisation took place over night at 65°C. The following day, the filter was washed three times at high stringency in 0.2 x SSC, 0.1% SDS at 65°C for 10 minutes each. The nylon filters were monitored to establish whether sufficient removal of labelled probe was achieved. The filter was then sealed into plastic bags, and an autoradiographic image obtained by exposing Kodak XAR5 film to the filters for 12 hours to one week at -80°C.

Filter stripping

Between hybridisations, probe was removed by placing the filter in freshly boiled 0.1 x SSC, 0.1% SDS. The liquid was allowed to cool with gentle agitation at room temperature. Filters were then stored in a sealed plastic bag at 4°C until the next use.

2.2.10.3. Bacterial methods

RFLP markers used

The chromosomal location of the RFLP markers and the origin of the clones used in this study is given in table 2.4. These markers were selected because they have been linked to aspects of the early flowering phenotype, particularly the TLN, in other studies (Koester et al., 1993; Phillips et al., 1992). Bacterial strains containing plasmids that carry the restriction fragment length polymorphism (RFLP) marker fragment were kindly provided by Dr. J.M. Gardiner, University of Missouri, and Dr. D. Grant, Pioneer Hi-Breed International.

Table 2.4 RFLP markers used in the present study

RFPL marker	Chromosome region	Source
BNL5.59	1L	Dr. J. Gardiner, University of Missouri
UMC119	1L	Dr. J. Gardiner, University of Missouri
UMC32	3S	Dr. J. Gardiner, University of Missouri
UMC39	3L	Dr. J. Gardiner, University of Missouri
NPI419	6L	Dr. D. Grant, Pioneer Hi-Breed International
UMC12	8L	Dr. J. Gardiner, University of Missouri
NPI445	10L	Dr. D. Grant, Pioneer Hi-Breed International

Small scale isolation of plasmid DNA ('Miniprep')

Single bacterial colonies were picked into 5 ml of Luria broth containing 200µg/ml ampicillin and grown overnight at 37°C in a shaking incubation. 1.5ml of the bacterial broth was centrifuged in a Eppendorf microfuge tube and the pellet resuspended in 100µl of GTE (0.9% glucose, 10mM EDTA, pH 8.0, 2.5mM Tris-HCl pH 8.0). After a 5 minute incubation, 200µl of 0.2M sodium hydroxide (NaOH), 1% SDS was added and the solutions mixed by inversion. The preparation was the incubated on ice for 5 minutes before the addition of 150µl of 3M potassium acetate, pH 4.8. After mixing by inversion the sample was centrifuged in a microfuge at top speed for 5 minutes . The supernatant was removed to a new tube and 800µl cold ethanol added. Nucleic acid was spun down in a microfuge for 15 minutes at room temperature, washed with 70% ethanol, and resuspended 50µl TE.

Digestion of DNA by restriction enzymes

The isolation of DNA fragments for miniprep analysis and for use as radioactive probes was performed with Pst1 and the reaction buffer supplied by the manufacturer (Stratagene). A reaction mixture, made up from the plasmid DNA isolated in the miniprep, 50 units of restriction enzyme and reaction buffer in the appropriate concentrations, was incubated at 37°C for at least two hours.

Gel electrophoresis

Gel loading buffer was added to samples to a final concentration of 10% and the DNA fragments separated on the basis of their size through agarose gel electrophoresis. Gels containing 1% agarose in 0.5 x TBE buffer, 0.4µg/ml ethidium bromide measuring 6 x 8cm were used. A size marker (1kb ladder, Gibco BRL) was run alongside to aid analysis. Gels were run at voltages between 5 and 10 V/cm. After electrophoresis, gels were examined in a ultra violet transilluminator and photographed using a polaroid camera and instant polaroid 665 film.

Isolation of DNA fragments from agarose gels

Larger gels were used for fragment isolation (13 x 20cm). The gels and samples were prepared and run as before. The gels were examined with a hand-held UV illuminator. The band containing the required fragment was identified and excised with a razor blade. The resulting well in the gel was enlarged by about 1cm in the running direction (anode side) and then lined with dialysis tubing. The piece of gel containing the fragment was put back into its original position, inside the lined well. The well was filled with fresh running buffer and current was run through the gel again for 30 minutes. Progress was monitored with a hand-held ultra violet illuminator. When

the fragment was seen to have accumulated along the dialysis tubing on the far end of the well, the current was reversed for 10 seconds. The fragment was the removed with a pipette in 200µl, precipitated with 0.1 volumes of ammonium acetate and 2 volumes cold ethanol, and spun down at top speed in a microfuge. After a wash in 70% ethanol, the DNA was allowed to air dry before being resuspended in 20-50µl TE buffer. DNA concentration was estimated by running aliquots on a gel.

Table 2.5 Stock solutions and media

Stock solutions	
0.5M EDTA	186.1g disodium ethylenediaminetetraacetic acid, dihydrate (EDTA), about 20g of sodium hydroxide, pH 8.0, in 1l deionised water (diH_2O), autoclaved.
4M NaCl	233.8g sodium chloride in 1l diH_2O, autoclaved.
1M Tris	121.1g Tris(hydroxymethyl)aminomethane (Trizma base) in 1l diH_2O, pH adjusted, autoclaved. about 60 ml hydrochloric acid for pH 7.6 about 42 ml hydrochloric acid for pH 8.0
20% SDS	200g sodium dodecyl sulfate (SDS) in 1l diH_2O, pH7.2.
4.4M ammonium acetate	105ml diH_2O, 50ml glacial acetic acid, 45ml ammonium hydroxide, pH 5.2.
1M phosphate buffer	423ml 1M sodium phosphate, monobasic (137.99g $NaH_2PO_4 \cdot H_2O$ per 1l diH_2O), 577ml 1M sodium phosphate, dibasic (268.07g $Na_2HPO_4 \cdot 7H_2O$ per 1l diH_2O), pH7.0. For pH7.2 volumes adjusted accordingly.
10% Sarcosyl	100g N-lauryl sarcosine, sodium salt in 1l diH_2O.
TE buffer	10mM (5ml 1M) Tris, pH 8.0, 1mM (1ml 0.5M) EDTA, pH 8.0.
10 x lysis buffer	87.5ml 4M NaCl, 1ml 1M Tris, pH 7.6, 2ml 0.5M EDTA, pH 8.0.
1 x lysis buffer	7M (42g) urea, 2% (20ml 10%) Sarcosyl, 50mM (10ml 0.5M) EDTA, pH 8.0, 1 x (10ml 10 x) lysis buffer, made up to 100ml.
5 x TBE buffer	54g Trizma base, 27.5g boric acid, 20ml 0.5M EDTA, pH 8.0, in 1l diH_2O.
Luria broth	10g bacto-tryptone, 5g yeast extract, 5g NaCl pH 7, in 1l diH_2O (add 15g/l agar for plates)

2.2.11 Additional inbred lines and early derivatives (section 3.1.4.1)

B73, B73G, Mo17, Mo17E, Sc76, and NC264 were planted in the summer nursery in 1992. A number of additional inbreds A619, A635, B73, C123, H100, and N28 and their respective early early derivatives A619E, A635E, B73E, C123E, H100E, and N28E were planted in the summer nursery in 1993. The plants were scored for phase-specific traits as described in section 2.1.3.

2.2.12 Short day experiments (sections 3.1.4.2 and 3.1.5.2)

During winter 1992/1993, plants of the lines A632, A632E, Oh43, and Oh43E were grown in the greenhouses of the University of Pennsylvania. Long day (LD) conditions (18h light : 6h dark, constant at 20°C) were provided with supplemental lighting at 90millimols m^{-2} $second^{-1}$of photons in the photosynthetically active range (400-700nanometres). For short day (SD) conditions (11h light : 13h dark), plants were moved to a dark chamber constructed with 98% shade cloth in the same room. Light intensity was reduced 300 fold to 0.3millimols m^{-2} $second^{-1}$ of photons in the photosynthetically active range. The dark chamber was kept at 15-20°C with an air conditioner during dark hours. The developmental stage of the plants were recorded at the beginning and the end of the SD treatment as the number of ligules and the number of leaves showing. SD treatments were administered in 5 day intervals between 8 and 38 days after planting (DAP). Care was taken to keep pairs of a wild type and an early flowering plant together during the SD treatment to ensure equal conditions. Control plants were grown under continuous SD and continuous LD until the last day of treatment (38 DAP). The plants were then grown to maturity under LD conditions at 25 to 30°C and scored for the presence of epicuticular wax and the position of the ear and TLN. Six to ten plants were scored in each genotype/treatment combination.

2.2.13 *Teopod* and the early flowering trait (section 3.1.5.1)

F2 families segregating the *Teopod* (*Tp*) mutation and the EFT were generated by the cross *Tp*/+, IL/ED x +/+, IL/ED or the reciprocal cross. In each experiment F2

families from several ears were grown and analysed. In A632, *Tp1* and *Tp2-E2* were used. The *Tp2-E2* allele is an ethyl methane sulfonate induced partial reversion allele that causes a less severe phenotype than the standard *Tp2* allele. This facilitated the generation of sufficient seed for this experiment. The *Tp1*, A632 experiment was grown in the greenhouse in the autumn of 1991 and the *Tp2-E2,* A632 experiment in the greenhouse in the autumn of 1992. Families segregating Tp and either lacking the EFT, heterozygous for the EFT, or backcrossed twice to A632E served as controls.

In Oh43, the standard *Tp1* and *Tp2* alleles were used because these where the only alleles that were converged into Oh43. Theses alleles condition a less severe Teopod phenotype in Oh43 than in A632. The *Tp1*, Oh43 experiment was grown in the greenhouse in the autumn of 1990 and the *Tp2* , Oh43 experiment in the summer nursery of 1993. The controls were families segregating *Tp* in the Oh43 (*Tp*/+; Oh43 x Oh43) or in hybrids (*Tp*/+; Oh43 x Oh43E) and families backcrossed to Oh43E (*Tp*/+; Oh43E(2 to 3 times) x Oh43E). Early flowering control families segregating *Tp1* were crossed to Oh43E three times and those segregating *Tp2* four times. Inbred Oh43E plants were used as early flowering, wild type controls in the Tp2 experiment. All plant were scored for the phase-specific traits (table 2.3).

2.2.14 Late flowering (section 3.2)

A families segregating plants carrying the *Leafy 1* (*Lfy1*/+) mutation in A632 were grown in the summer nursery in 1992 and scored for the characters listed in table 2.3. Additionally, the number of leaves between the ear and the tassel and the number of husk leaves were scored. The families were either wild type with regard to the EFT (*Lfy1*/+, A632 x +/+, A632), heterozygous for the EFT (*Lfy1*/+, A632 x +/+, A632E), or backcrossed 4 times to A632E (*Lfy1*/+, A632E(3) x +/+, A632E).

Families segregating for the *indeterminate growth habit1* (*id1*) were scored in the summer nursery in 1993. A final score of the TLN in the plants homozygous for the *id1* mutation was taken at harvest time. At that time, the shoot was dead and no tassel was visible. The last visible leaf was scored as the TLN.

Seed carrying another new late flowering mutation from an ethyl methane sulfonate induced mutagenesis in hybrid stock was supplied by Dr. G.M. Neuffer and grown in the summer nursery in 1993. This mutation is called *delayed flowering* (*dlf*) pending

the results of allelism tests. Families segregating for *dlf* or homozygous for *dlf* were scored for the traits given in table 2.3.

Another new late flowering mutant was found in an ethyl methane sulfonate induced mutagenesis in A632 that was performed by Dr. E.H. Coe who kindly provided the M2 seed. The M2 seed was screened in the summer plantation 1992 and one family segregating late flowering plants was identified. One mutant and several wild type plants of this family were selfed and planted in the summer nursery 1993. M3 seed homozygous for the mutation, M3 families segregating the mutation and A632 families were scored for the phase-specific traits (table 2.3). While the results from allelism tests are pending, this mutation has been named *late flowering* (*ltf*).

Suppliers

Amersham Corporation, 2636 South Clearbrook Drive, Arlington Heights, IL 60005

Boehringer Mannheim Biochemicals, P.O. Box 50414, Indianapolis, IN 46250

Calbiochem-Novabiochem Corporation, La Jolla, CA 92039, USA.

Computer Associates International, One Computer Associates Plaza, Islandia NY 11788-7000

Delta-T Devices Ltd., 128 Low Road, Burwell, Cambridge, UK

Eastman Kodak Company, Rochester, NY 14650

Electron Microscopy Sciences, 321 Morris Road, Box 251,Fort Washington, Pa 19034, USA, Telephone:1-800-523-5874, 1-215-646-1566, Fax: 1-215-646-8931

Fisher Scientific, 711 Forbes Avenue, Pittsburgh PA 15219-4785, Telephone:1-800-766-7000

Gibco BRL, Life Technologies, P.O.Box 68, Grand Island, NY 14072-0068

Microsoft, 1Microsoft Way, Redwood, WA 98052-6399, USA

Regal Ware, Inc., 1675 Reigle Drive, Kewaskum, WI 53040, USA.

Sigma Chemical Company, P.O. Box 14508, St. Louis, Mo 63178-9916, USA, Telephone: 1-800-325-3010, 1-314-771-5750, Fax: 1-800-325-5052, 1-314-771-5757.

Stratagene, 11099 North Torrey Pine Road, La Jolla, CA 92037

Summagraphics, 777 Commerce Drive, Fairfield, CT 06430 USA, Telephone: 1-203-384-1344, Fax: 1-203-333-4131

Systat, Inc., 1800 Sherman Avenue, Evanstown IL 60201-3793, Telephone: 1-708-864-5670, Fax: 1-708-492-3567

CHAPTER 3

RESULTS

AND

DISCUSSION OF INDIVIDUAL EXPERIMENTS

Introduction

As indicated in Chapter 1, the aim of this thesis is to investigate if a three-phase model of shoot development provides a more accurate picture of plant shoot development than the traditional two-phase model. To this end, it is important to establish if the vegetative phase change and flowering time are coordinately regulated or not. This can be done by experimentally altering flowering time and monitoring the expression of vegetative, phase-specific traits: early flowering plants will have a shorter period to produce vegetative growth, late flowering plants will have a longer period. As discussed earlier (section 1.4), there are three ways in which the expression of the two vegetative phases can be adapted to fit an altered vegetative period overall:

1-Only the length of the late vegetative phase of development is changed. The vegetative phase change would in this case be shifted relative to the reproductive maturity. This would indicate that the two events are independently regulated.

2-Both the early vegetative and the late vegetative phases of development are altered in length. Whereas the vegetative phase change and the onset of reproductive differentiation are both altered in absolute (chronological) time, this would indicate no change in the relative timing of developmental events, suggesting that the vegetative phase change and the reproductive maturity are coordinately regulated.

3-Only the length of the early vegetative phase is altered. In this case, the length of the late vegetative phase is constant and the change in flowering time matches the change in the length of the early vegetative phase. This scenario would also indicate that the vegetative phase change and the reproductive maturity are coordinately regulated. It would suggest that a minimum number of late vegetative leaves has to be formed before flowering can occur and that expression of the early vegetative phase and of the reproductive phase of development may be incompatible.

The results obtained from studying early flowering plants are presented in the section 3.1, whereas section 3.2 deals with late flowering mutants.

3.1 Early flowering

Genes conditioning early flowering have been described in a number of species. In maize, flowering time is thought to be a quantitative trait regulated by several genes. No individual genes causing early flowering have been isolated. However, the early flowering trait (EFT) has been introgressed into a variety of inbed lines from the very

early flowering, primitive race Gaspé Flint by Dr. D. Shaver (see table 2.1 for pedigrees). Two of the inbred lines (ILs) that were used as recurrent parents, A632 and Oh43, and the near-isogenic, early flowering derivatives (EDs) A632E and Oh43E were used in the present research. As will be shown in this chapter, these two sets of lines represent two different types of relationships between the reproductive and the vegetative phases of development.

Before testing which of the three possibilities outlined earlier (section 1.4) applies, the following needs to be considered: Firstly, the correlation between flowering time and the overall length of the vegetative period have to be confirmed for the early flowering lines (section 3.1.1.1). Secondly, the way in which the length (time of onset or end) of each phase of development is measured has to be determined. As pointed out earlier, temporal and spatial changes occur together in plants due the polar nature of plant growth. If growth rates are constant, the length of the phases of development can be seen, and easily measured, in the spatial arrangement of phase-specific structures. For the study of shoot development it is most imporant to know if the leaf initiation and emergence rates are comparable in normal and early flowering lines, so that the number of leaves can be used to measure the length of the vegetative phase of development and the onset of the reproductive maturity. The leaf initiation and emergence rates will be examined in section 3.1.1.2. Thirdly, it has to be shown that the early flowering trait regulates the expression of the reproductive phase of development, and does not cause a general deficiency with a pleiotropic effect on plant growth and development. Genes that regulate flowering time directly are likely to act specifically during shoot development and not to have a primary effect on root development. To test if the early flowering trait act specifically in shoot development, root growth will be examined in Oh43 and Oh43E (section 3.1.1.3). Additionally, modifications of a complex character that is not thought to be phase-specific, leaf form, have been associated with changes in flowering time. By examining leaf form it is possible to show if and when the early flowering trait affects shoot development (section 3.1.1.4). Finally, it has to be established which traits are expressed phase-specifically. This research has been carried out previously, and will not be dealt with here (see section 1.3, table 2.3, Bongard-Pierce, in preparation; Evans, personal communication; Poethig, 1988a).

The expression of the two vegetative phases of development in the inbred lines and the early derivatives is examined in section 3.1.2 by examining a variety of phase-specific traits. The results indicate that the vegetative phase change and reproductive maturity are independently regulated in A632E (figure 1.1: case 1), whereas they are coordinately regulated in Oh43E (figure 1.1: case 2).

The different patterns of shoot development may be conditioned either by a different set of components of the early flowering trait or by the genetic background of the inbred lines. The former possibility will be investigated in genetic and restriction fragment length polymorphism analyses (section 3.1.3). If a different set of genes conditioning early flowering have been converged into A632E and Oh43E, these differences may be detectable in a RFLP analysis. The latter possibility will be examined in a number of other inbred lines and their early derivatives as well as in photoinduced plants (section 3.1.4). If factors present in the genetic background determine whether vegetative phase change and reproductive maturity are coordinately regulated or not, related lines should have the same patterns of shoot development. Additionally, photoinduced A632 and Oh43 plants should show the same pattern of shoot development as A632E and Oh43E, respectively. These experiments demonstrate that the pattern of shoot development is conditioned at least in part by the inbred background.

Finally, the three-phase model will be employed to place the early flowering trait in the developmental pathway regulating the reproductive phase of development. In crosses with Teopod1 and Teopod2 plants, the early flowering trait will be found not to be epistatic to these genes regulating vegetative development (3.1.5.1). This suggests that the early flowering trait is part of a distinct developmental pathway that regulates reproductive maturity. In order to determine when the early flowering trait acts in the regulation of the reproductive phase, it will be placed relative to the photoperiodic requirement in the regulation of flowering. If the early flowering trait acts early during shoot development and before the photoperiodic requirement, the photosensitive period should occur at an earlier time in the early derivatives. This hypothesis will be investigated in section 3.1.5.2 by measuring the effectiveness of short day conditions applied at various times during shoot development in the inbred lines and their early derivatives.

3.1.1 Phenotype of the early flowering lines

3.1.1.1. Habit and flowering time

Due to the polar nature of shoot growth, developmental changes that occur during shoot growth are manifested in the spatial succession of phase-specific organs that are produced during that time. In species with a terminal inflorescence, the progression of developmental phases that takes place in one meristem can thus be monitored by examining the phytomers produced by that meristem. For the total number of leaves (TLN) to be used as a measure of flowering time, a positive correlation between flowering time and TLN needs to be confirmed in the lines examined in this study.

In maize, as in many other species, flowering time has been found to be significantly and positively correlated with the total number of leaves produced by the plant before flowering (Chase and Nanda, 1967; Hesket et al., 1969). Plant height and ear placement nodeare also correlated with flowering time, and have occasionally been used as a measure of early flowering (Koester et al., 1993; Phillips et al., 1992; Troyer, 1990). Shaver (1976) confirmed these correlations for some of the early derivatives generated in his conversions from Gaspé Flint. A similar study has not been carried out as yet in A632, A632E, Oh43, and Oh43E.

In order to determine how the early flowering trait affected habit and total leaf number (TLN) in the different lines, plants were grown as described in chapter 2. The TLN and the ear placement node were recorded, and selected plants of each genotype photographed. The habit of these plants is shown in figure 3.1. Plants carrying the early flowering trait were less tall at anthesis than their respective inbred lines or hybrids (IL/ED) (Figure 3.1). This decrease in plant height did not result from a stunted or dwarf habit, but from the reduced leaf number of these early lines. The ear was also located at a lower position (Table 3.1).

The early derivatives A632E and Oh42E flowered about a week earlier than the inbred lines. Figure 3.2 shows that flowering time is positively correlated with TLN, here illustrated by Oh43 families. Oh43 flowered after 61.8 ± 0.8 days with 14.4 ± 0.3 leaves, hybrid plants flowered after 59.1 ± 1.1 days with 13.1 ± 0.5 leaves, and Oh43E plants flowered after 54.5 ± 1.1 days with 9.9 ± 0.5 leaves. In a family segregating the early trait, flowering time and TLN were significantly correlated ($r=0.47$, $df=107$, $p<0.001$)). The ear placement node was also positively and

significantly correlated with flowering time (r=0.49), while the number of leaves between the ear and the tassel remained constant (Table 3.1). These results are consistent with previously published reports. A positive correlation between flowering time, TLN, and plant height has been established for a variety of cultivars and species, and for plants with environmentally induced changes in flowering time (Arnold, 1969; Chase and Nanda, 1967; Hesket et al., 1969; Hunter et al., 1977; Russel and Stuber, 1983; Troyer, 1990).

Table 3.1 Total leaf number and ear placement node in inbreds, hybrids, and early flowering lines in A632 and Oh43 (N is the number of plants measured, all errors given as ±2 s.e.m.).

	A632	A632 / A632E	A632E	Oh43	Oh43 / Oh43E	Oh43E
Total no. of leaves	19.3±0.3	18.5±0.3^{c}	16.3±0.3^{c}	14.4±0.3	13.1±0.5^{c}	9.9±0.5^{c}
Ear node	13.8±0.2	12.8±0.2^{c}	10.7±0.3^{c}	9.3±0.3	8.3±0.5^{b}	6.4±0.3^{c}
N	18	15	17	10	8	10

b: significantly different from next later flowering genotype at $p<0.01$ (2-tailed *t* test)
c: significantly different from next later flowering genotype at $p<0.001$ (2-tailed*t* test)

Hybrids between a inbred and its early derivative have an intermediate phenotype with regard to flowering time, plant height, and TLN (figure 3.1, table 3.1). This observation is consistent with previous reports on similar early derivatives (Shaver, 1976; Phillips et al., 1992), and demonstrates that the early flowering trait is an incompletely dominant trait. As will be described later in this chapter, the frequency distribution of the TLN in segregating families suggest that the early flowering trait is a quantitative, probably polygenic trait. Given that the conversions were carried out by selecting for early flowering plants in a backcross progeny, selection for incompletely dominant or dominant genetic factors was to be expected. Both dominant and incompletely dominant (additive) gene action have been demonstrated for the early flowering trait in other studies using inbred lines that differ in flowering time (Giesbrecht, 1960a, b; Hallauer, 1965; Mohamed, 1959).

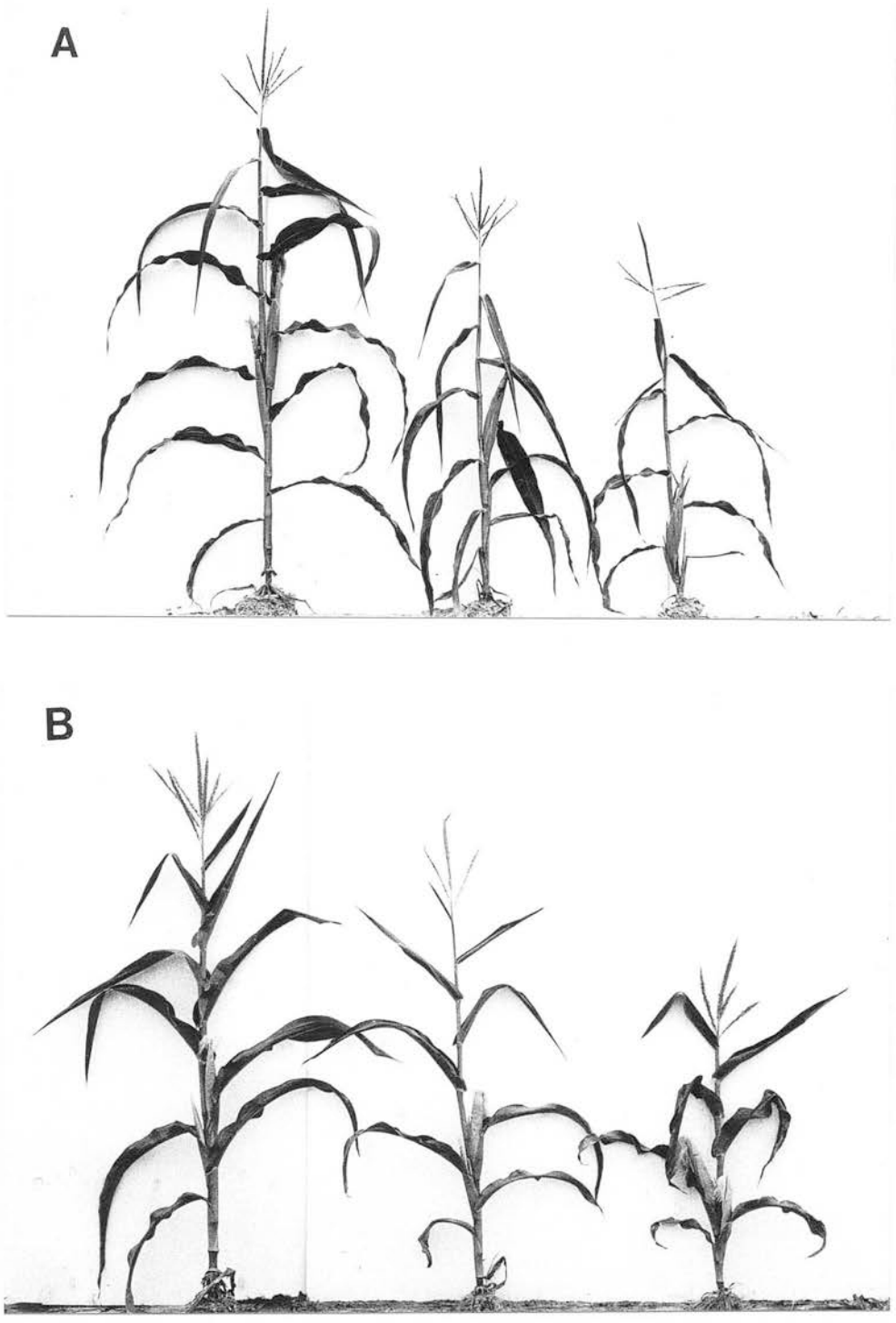

Figure 3.1 Photographs showing the habit of A632, A632/A632E, and A632E plants (A, left to right) and Oh43, Oh43/Oh43E, and Oh43E plants (B, left to right).

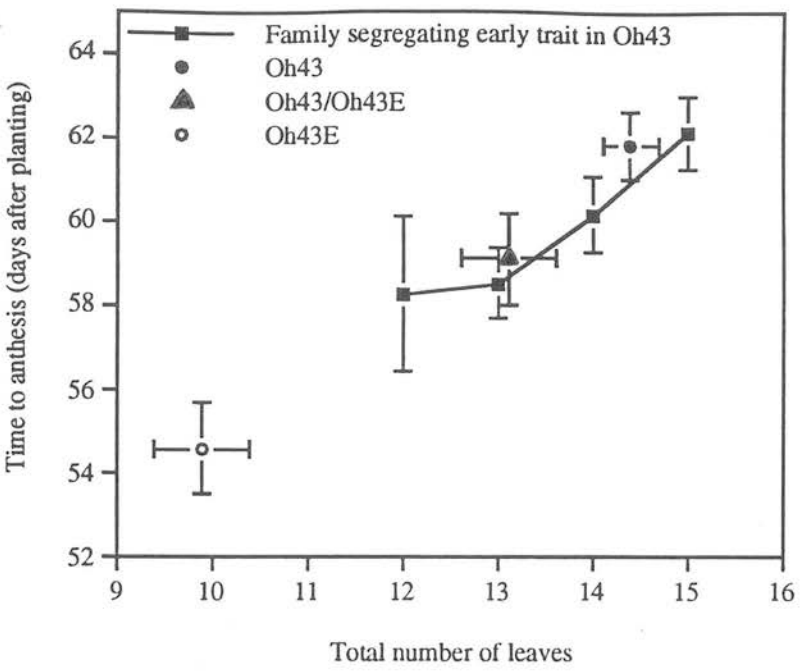

Figure 3.2 Time to flowering as a function of total leaf number in Oh43. All errors given as ±2 s.e.m.

3.1.1.2. Leaf initiation and emergence

Leaf initiation and emergence rates have commonly been used to measure the growth rate of the shoot in maize and other cereals (Abbé and Phinney, 1951; Gallagher, 1979; Greyson et al., 1982; Cao and Moss, 1989a, b). Genotypic differences as well as differences in growing conditions such as temperature, day length, and nutrient availability are known to affect these growth rates (Eik and Hanway, 1965; Tollenaar et al., 1984; Hay and Delécolle, 1989). In the majority of such studies, leaf initiation and emergence rates in maize were found to be constant for a given genotype and environment. However, Thiagarajah and Hunt (1982) reported an increased rate of leaf initiation in maize just prior to the initiation of the tassel, a phenomenon also demonstrated in other species. Additionally, leaf emergence rates in maize may also be affected by the rate of internode elongation (Warrington and Kanemasu, 1983). Results with genotypes of maize that differ in flowering time are inconsistent. Eik and Hanway (1965) found that early maturing hybrids tended to have higher rates of leaf emergence than later hybrids, whereas Shaver (1976) noted a tendency towards longer plastochrons in the early derivatives than in the inbred lines.

Early flowering may be associated with a change in the duration of leaf production, as demonstrated in section 3.1.1.1, or by a changes in the rate of leaf initiation. The length of developmental phases during shoot development can be measured from the spatial arrangement of phase-specific traits only if the rate of leaf initiation is known. In order to confirm that leaf number can be used as an adequate measure of the lenght (or beginning and end) of developmental phases throughout shoot development, plants were grown as described in chapter 2, and the leaf initiation and emergence rates were measured. These data are presented in figures 3.3 and 3.4.

There was no significant difference in the leaf initiation or leaf emergence rate of either inbred line and its corresponding early derivative. The rates of leaf initiation and of leaf emergence in both A632 and A632E were constant and similar until the 13th plastochron (figure 3.3). Unfortunately, these plants grew much slower than expected during the 35 days of recording, and the final part of shoot growth could not be monitored. During this period, the plastochron length was approximately 5.1 days for A632 and 5.8 days for A632E.

In Oh43 and Oh43E, the leaf initiation rate was constant until 13 DAP (figure 3.4B). During this period, the length of the plastochron was approximately 2.3 days for Oh43 and 2.6 days for Oh43E. At 10 to 13 DAP, tassel initiation began in Oh43E and the leaf initiation rate was accelerated (figure 3.4A). Leaf initiation was constant in Oh43 until 23 DAP in the same experiment. An increase in the leaf initiation rate was also observed in Oh43 just prior to tassel initiation at 26 DAP (figure 3.4A). While the increase in leaf initiation rates prior to floral initiation has been reported before in maize (Warrington and Kanemasu, 1983), it is possible that this result is an artifact resulting from the difficulty of distinguishing the leaf primordia from the branch primordia of an early inflorescence meristem, which produces primordia at a much greater rate. The beginning of leaf emergence was delayed in Oh43 in one experiment, but proceded at a similar rate as in Oh43E (figure 3.4A).

These results show that the rates of leaf initiation and emergence are constant and similar in the inbred lines and their early derivatives; the early derivatives produce leaves for a shorter period of time, not at a faster rate. Therefore, it is possible to use leaf number as an adequate measure of the timing of developmental events during shoot growth.

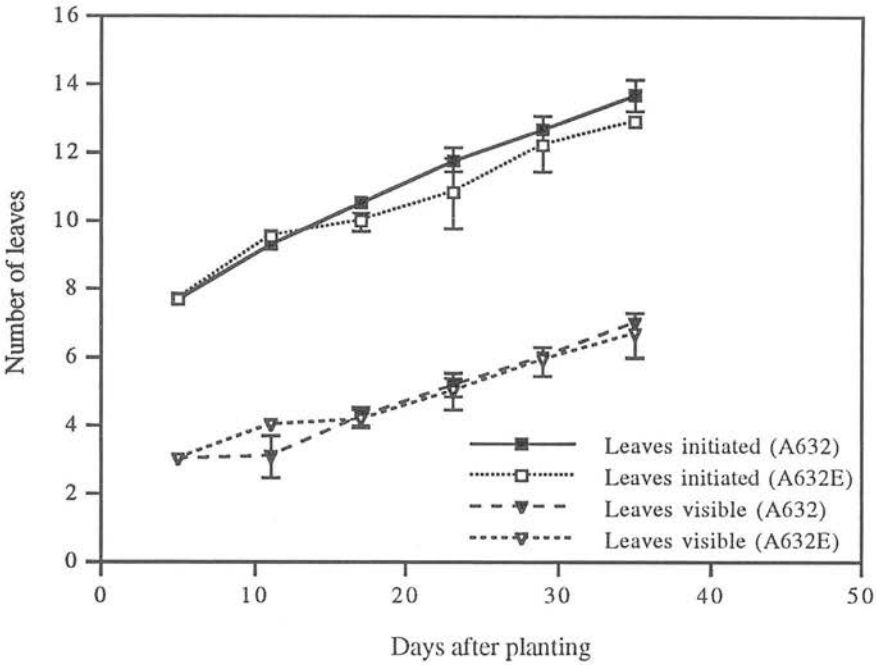

Figure 3.3 Leaf initiation and emergence in A632 and A632E plants. At 35 days after planting the tassels had not been intitated. All errors given as ±2 s.e.m.

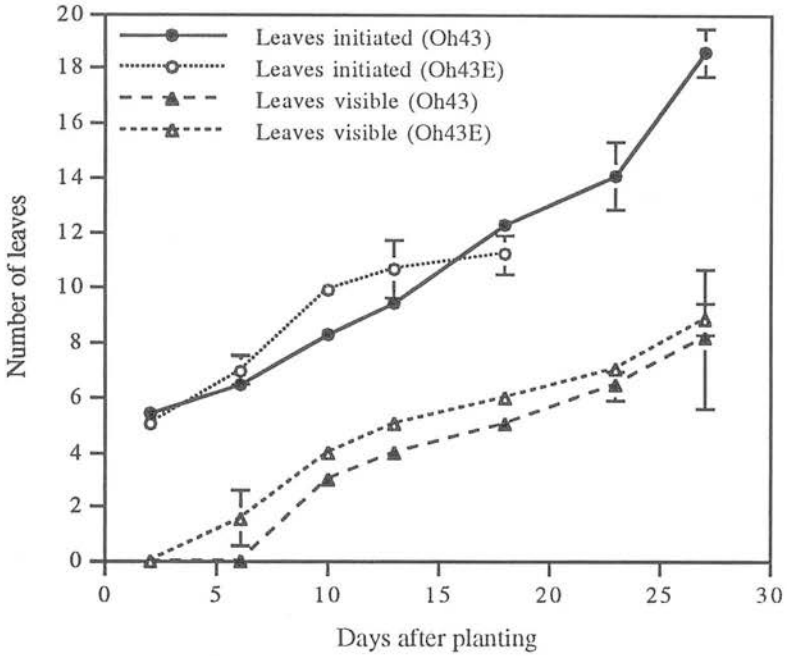

Figure 3.4A Leaf initiation and emergence in Oh43 and Oh43E plants. Tassel intitiation began at 13 days after planting in Oh43E and at 27 days after planting in Oh43. All errors given as ±2 s.e.m.

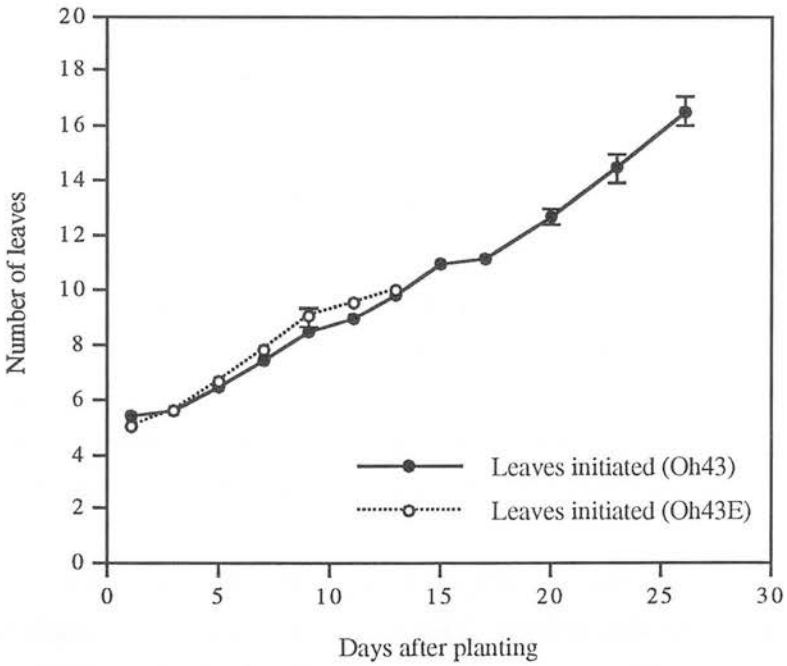

Figure 3.4B Leaf initiation in Oh43 and Oh43E plants. Tassel intitiation began at 13 days after planting in Oh43E and at 26 days after planting in Oh43. All errors given as ±2 s.e.m.

3.1.1.3. Root growth

Roots can affect flowering in at least two distinct ways. Roots have been shown to inhibit the transition to flowering in several species (Schwabe and Al-Doori, 1973; McDaniel, 1980; Smith and McDaniel, 1992), yet little is known about the relationship between the development of the root system and reproductive maturation of the shoot. Additionally, roots take up nutrients and water, and nutrient and water supply can affect flowering time, although results are inconsistent across species (Jackson and Sweet, 1972; Kinet et al., 1985).

If substances produced by the root system, or good nutrient and water supply, directly or indirectly inhibit floral development in maize, early flowering may result from a decrease in the size of the root system. Thus, early flowering would be one aspect of a pleiotropic phenotype including a modified root size. A significant difference in root size prior to tassel initiation would be expected in this case. Alternatively, if the early flowering trait plays a role in the regulation of flowering time, its primary effect would be on the development of the shoot and any change in root size would likely be a secondary effect. To establish which possibility applies, the correlation between root length and weight and several shoot traits (weight, leaf area, leaf number) were examined in Oh43 and Oh43E from germination to tassel formation.

In maize, the root system is composed of the seminal root and a number of adventitious roots, also called nodal roots or prop roots, which originate from the basal nodes of the shoot and make up a large part of the root mass of a mature maize plant. The root size is known to vary with a large number of factors, including genotype, temperature, aggregate size, and water and nutrient availability (Alexander and Miller, 1991; Demotes-Mainard and Pellerin, 1992; Donald et al., 1987; Veen et al., 1992; Teyker, 1992). In order to minimise such environmentally conditioned effects and to provide constant nutrient levels, the plants were grown in sand that was regularly flushed with dilute Hoagland's solution.

Results show that there was no difference in root growth or shoot growth between Oh43 and Oh43E before tassel intiation. As discussed previously (figure 3.4A), tassel intitation occurred in Oh43E at about 13 days after planting when 4 leaves were partially visible and 2 leaves had fully emerged from the whorl (ligule visible). In Oh43, tassel initiation occurred when 7 leaves were visible and 5 leaves had fully emerged. Until that time, the total root length (adventitious roots and seminal root) and the shoot and total root dry weight increased in the same way in both genotypes

(figures 3.5 and 3.6). Over time, the relative contribution of the adventitious and seminal roots to the total root change. To ensure that the adventitious and main roots had contributed to the root in the same way, the lengths and weights were examined separately for each part of the root (appendix). Again, there was no difference between the genotypes. Some aspects of root morphology such as the branching pattern may affect shoot development by way of changing the number of root meristems, but visual assessment of the roots did not indicate any difference in branching pattern here.

The amount of photosynthate can significantly affect the growth and development of a plant. To ensure that plant growth was not altered by differences in energy availability, the light exposed leaf areas were measured. No differences in the light exposed leaf areas were found between the genotypes. In both Oh43 and Oh43E, the area increased in the same manner when plotted against the number of leaves emerged (figure 3.7). Similarly, the shoot dry weight increased in the same fashion in the two genotypes (figure 3.5).

In summary, no differences in root length, root weight, shoot weight, or light exposed leaf area were found between Oh43 and Oh43E during the time measured here. These results indicate that changes in the flowering time conditioned by the early flowering trait are not correlated with changes in the growth rate of the root system or the shoot system in the time between germination and tassel initiation. This suggests that the early flowering phenotype is not caused by changes in the size of the root or by a reduction in the photosynthetically active leaf area. It is possible that other features of the root system, such as the meristem mass (as sites of hormone/ messenger production), transport efficiency or solute concentrations differ in the inbred lines and the early derivatives, but an analysis of these parameters was beyond the scope of this study.

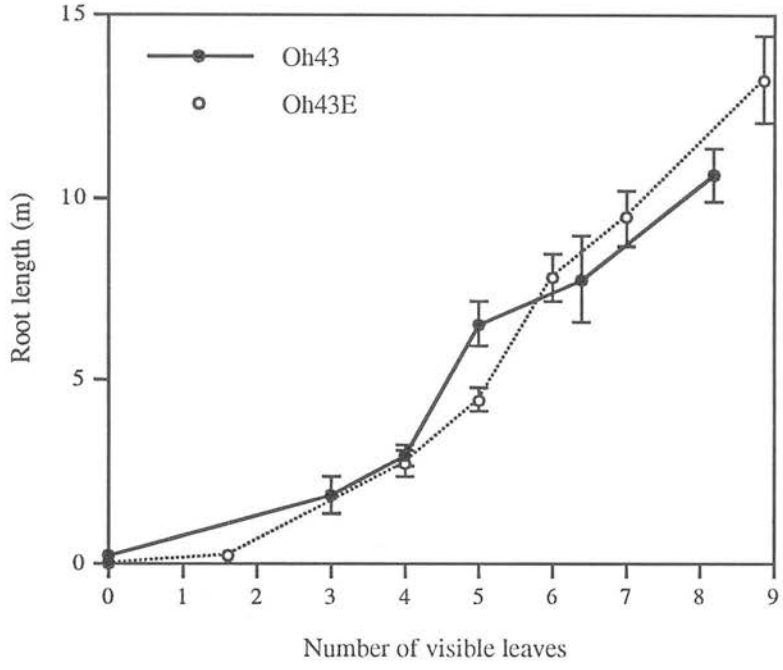

Figure 3.5 Root length as a function of developmental state (number of visible leaves) in Oh43 and Oh43E. All errors given as ±2 s.e.m.

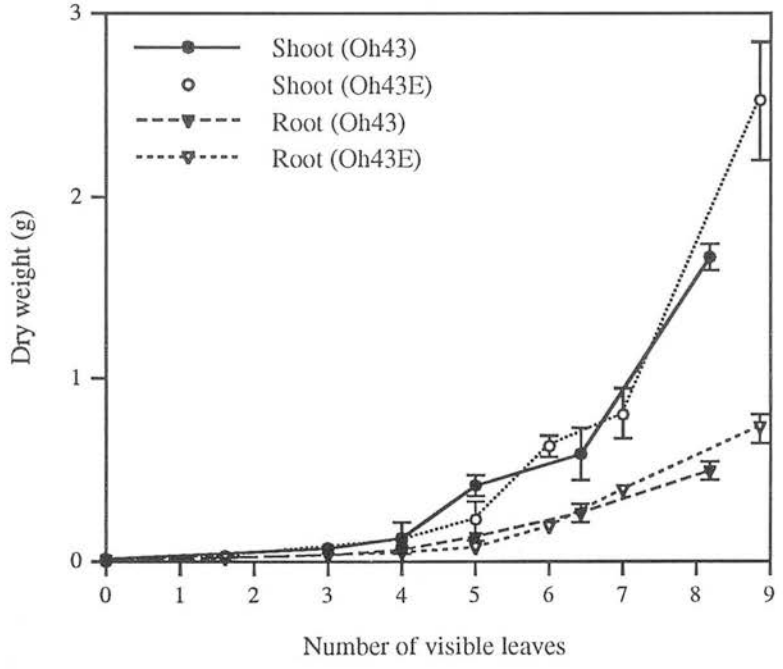

Figure 3.6 Shoot and root dry weights as a function of developmental state (number of visible leaves) in Oh43 and Oh43E. All errors given as ±2 s.e.m.

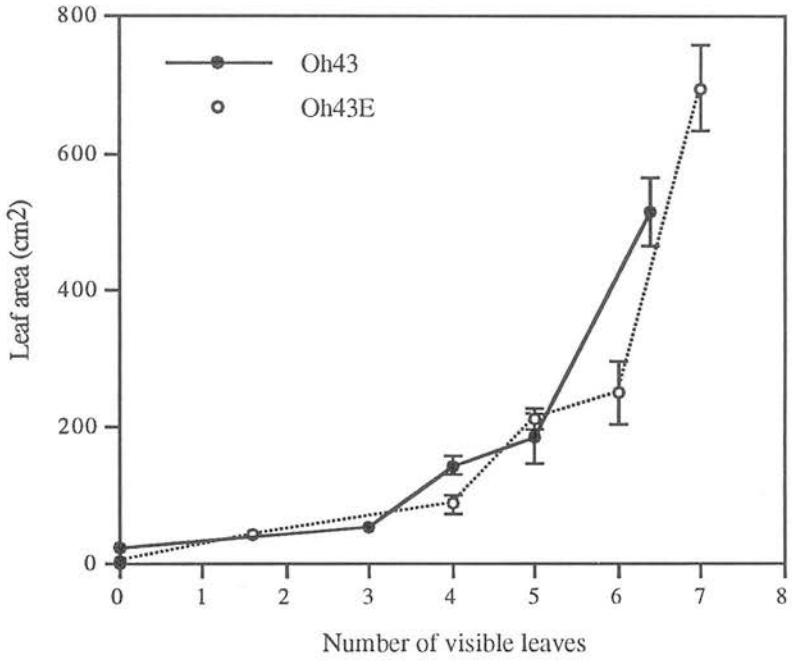

Figure 3.7 Leaf area as a function of developmental state (number of visible leaves) in Oh43 and Oh43E. All errors given as ±2 s.e.m.

3.1.1.4. Leaf form

Because of the polar nature of shoot growth, many of the complex morphological changes that occur during ontogenesis result in morphological variation of the structures produced along the shoot. This phenomenon, called heteroblasty, is often most evident in the morphology of the leaves. Leaf shape is thought to be regulated in a complex manner by several factors (Ashby, 1948, 1950; Allsop, 1967; Jones, 1993). The polar nature of shoot growth makes it difficult to distinguish genetically regulated causes of heteroblasty from heteroblasty caused by quantitative changes in size or in the physiology of the plant, particularly if the morphology of the shoot changes gradually.

There is some indication that changes in leaf shape are related to the reproductive maturity of the plant. In cotton, the leaf shape changes from an entire, cordate leaf to a lobed leaf in the vegetative, non-flowering portion of the shoot. At flowering or shortly there after the final leaf shape, the climax leaf, is produced. Studies have shown that the rate at which the leaf shape changes and the ultimately attained leaf shape of the climax leaf can be greatly modified by the time to flowering (Stephens, 1944a, b,c). A gradual change in leaf size and shape has also been described in maize (Abbe et al., 1941; Greyson et al., 1982; Poethig, 1988a). These changes reflect cultivar specific differences as well as developmental changes during shoot growth. For example, Greyson et al. (1982) found a relationship between leaf form and ear position, while Poethig (1988a) reported a change in leaf form in Teopod mutants, which have an altered expression of the early vegetative phase of development. Thus, these reports suggest that leaf form in maize is not thought to be a phase-specific trait, but rather a complex character that can be altered by a number of factors including the vegetative phases of development and the reproductive maturity of the shoot.

To determine when the early flowering trait acts during shoot development, its affect on leaf shape was assessed. Activity of the early flowering trait early during shoot development would be expected to appear as a precocious change in leaf form to a form usually found at a later leaf position. Alternatively, if the early flowering trait acts very late, no such change in leaf form would be observed. To test these hypotheses, the dimensions of successive leaves of A632, A632E, Oh43, and Oh43E plants were measured. The length to width ratio was calculated as an indicator of leaf shape.

The leaf form in all four lines changed gradually and characteristically with leaf position, and the change in the early derivatives was distinctly different from that in the

inbred lines beginning very early in shoot development. In all genotypes, the length to width ratio, rose steeply from leaf 1 to an early maximum at leaf 3, reached a second maximum, and then decreased in the upper half of the shoot (Figures 3.8; 3.9). The shapes of the seedling leaves was identical in the inbred lines and the early derivatives, but leaf shape between the inbred line and the early derivative was significantly different in the A632 background by leaf 7 and by leaf 5 in the Oh43 background. Thus, the early flowering trait has an effect on leaf shape very early in shoot development. The length to width ratios remained lower and decreased earlier in the early derivatives. The leaf shapes were alike again by leaf 12 in A632 and by leaf 11 in Oh43.

In both the inbred lines and early derivatives, leaf length and leaf width increased steadily from the first seedling leaf to higher leaf positions, reached a maximum value near the ear and then decreased again (figures 3.10; 3.11; 3.12; 3.13). The rate of increase was greater in the early derivatives than in the inbred lines. However, the early derivatives produced the longest leaf earlier, that is, at a lower leaf position, than the inbred lines, and as a result, their maximum leaf length was shorter. A632 plants reached a maximum leaf length of 78.4cm ± 1.9 at leaf position 9, A632E have a maximum length of 68.6cm ± 4.1 at leaf position 8. Similarly, the longest leaf in Oh43 measured 82.2cm ± 2.3 at leaf position 8 , and 73.0cm ± 3.7 at leaf position 7 in Oh43E. Leaf width also changed in a similar manner in the inbred lines and the early derivatives, only it did so faster, that is, in fewer leaves in the early derivatives than in the inbred lines. The maximum leaf width in A632 was 5.3cm ± 0.2 at leaf position 12, 5.1cm ± 0.6 at leaf 10 in A632E, 7.4 cm ± 0.2 at leaf 11 in Oh43, and 7.7 cm ± 0.3 at leaf 8 in Oh43E. While the leaf length in the early derivatives never increased as much as in the inbred lines, the maximum leaf width was not statistically different between the inbred lines and their early derivatives. This explains the difference in the length to width ratio between the lines. The range in total leaf number in each line was 17 to 18 leaves in A632, 11 to 16 leaves in A632E, 14 to 15 leaves in Oh43, and 10 to 12 leaves in Oh43E; this accounts for the greater variability of the data at the higher leaf positions.

The results indicate that the changes in leaf shape and leaf size are affected by the time to flowering in maize. In the early derivatives, the leaf shape is altered by leaf 7 in A632 and leaf 5 in Oh43. The present results suggest that leaf length and leaf width are modified at a much earlier position, but it is not clear whether these changes are just a result of increased vigour in the early derivatives in this particular experiment, or an effect of the early flowering trait. Further experiments would be required to clarify this point. For now, it is concluded that the early flowering trait changes the leaf form no

later than by leaf position 7 in A632 and position 5 in Oh43. Thus activity of the early flowering trait can be demonstrated before the vegetative phase change is completed, as indicated by the expression of phase-specific traits (table 3.2).

The two components of shape, length and width, are modified in a slightly different fashion. The increase in length proceeds at a faster rate in the early flowering lines, and comes to a stop earlier. Leaf width also increases at a slightly faster rate in the early derivatives, but reaches the same maximum width as the inbred line at a lower position. A similar effect has been reported in cotton (Stephens, 1944b, c), where early flowering on one hand limits the duration of the changes in leaf shape thus arresting the series earlier, and on the other hand accelerates the rate of changes in leaf shape.

Overall, the results in the inbred lines compare well with findings of other studies (Sharman, 1942 ; Greyson et al., 1982; Poethig, 1988a). Several different factors have been suggested to be involved in the changes of leaf shape (Abbe et al., 1941; Greyson et al., 1982). Abbe et al. (1941) found the increase in leaf width in leaves 6 to 12 to be associated with an increase in the circumference of the shoot apex, which is correlated with an increase in the number of cells in the apex (Abbe et al., 1941, 1951; Abbe and Phinney, 1951; Ledin, 1954). Greyson et al. (1982) point to the possibility that leaf shape changes as the reproductive maturity of the shoot changes. They also suggested that the developing ear may alter leaf shape, since the widest leaf was found to be located near or just below the ear. Greyson et al. (1982) suggested that the proximity of the developing inflorescence may cause leaves to grow larger, possibly through hormonal stimulation. In the present study, the ear was located near or above the longest leaf and near or below the widest leaf in all genotypes. The ear formed above the leaf with the greatest length to width ratio (arrows in figures 3.8 to 3.13). In the A632 background the length to width ratio had already decreased considerably before the ear was produced, whereas in Oh43, the ear was very close to the leaf with highest length to width ratio. Thus, the relationship between the changes in leaf shape and size and ear position are maintained in the early flowering lines. This is consistent with the hypothesis that the heteroblastic series is conditioned by a gradual shift from a vegetative to a reproductive physiology, with possible regulative interaction between the leaves and the inflorescence. However, the leaf shape is affected by the early flowering trait no later than by leaf 7 in A632E and leaf 5 in Oh43E. These leaves are initiated long before any reproductive structures are produced. If leaf form is determined by the developing inflorescence, the possibility that the shape of these leaves is determined late in the organogenesis of the leaves rather than at the time of their initiation may explain this effect.

A different cause for the changes in leaf shape is suggested by the results of Poethig (1988a). In that study, leaf shape was found to be altered by the *Teopod* mutations, which are thought to cause constitutive expression of the early vegetative phase of development. Teopod plants have narrower leaves (higher length to width ratio) than wild type plants (Dudley and Poethig, 1991; Poethig, 1988a). If this is due to the prolonged expression of the early vegetative phase of development, then the production of fewer leaves with lower length to width ratios by the early derivatives might suggest that the early vegetative phase of development terminated earlier in these lines. Yet, it is not clear if leaf width is a phase-specific trait and if so, precisely which length to width ratio is correlated with early vegetative or late vegetative development. In order to determine whether changes in leaf shape correlate with the vegetative phases of shoot development, several traits specific to the early vegetative phase and the late vegetative phase were scored (see table 2.3 for a description of the traits) (table 3.2). All the traits were located at a significantly lower position in Oh43E than in Oh43; in A632E only the location of the reproductive organs and prop roots was affected. Thus, the early flowering trait affects leaf shape in A632E, but does not affect any of the known morphological, phase-specific traits of the leaves. This is inconsistent with a link between the expression of the vegetative phases of development and leaf shape as suggested by the Teopod results. These results indicate that reproductive maturation does affect leaf shape, whereas the expression of one or the other vegetative phase does not necessarily do so.

Results also indicate that the vegetative phase change occurs at the same leaf position in A632 and A632E, whereas early vegetative growth ceases earlier and late vegetative growth begins earlier in Oh43 in the presence of the early flowering trait. This phenomenon is studied further in the following sections.

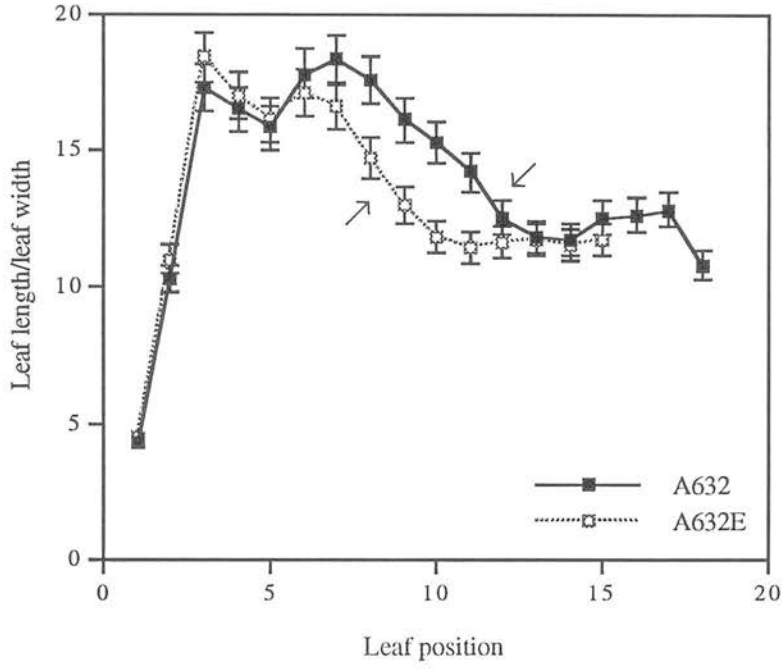

Figure 3.8 Leaf shape, as described by length/width ratio, as a function of leaf position in the lines A632 and A632E. The position of the ears is indicated by arrows. All errors given as ±2 s.e.m.

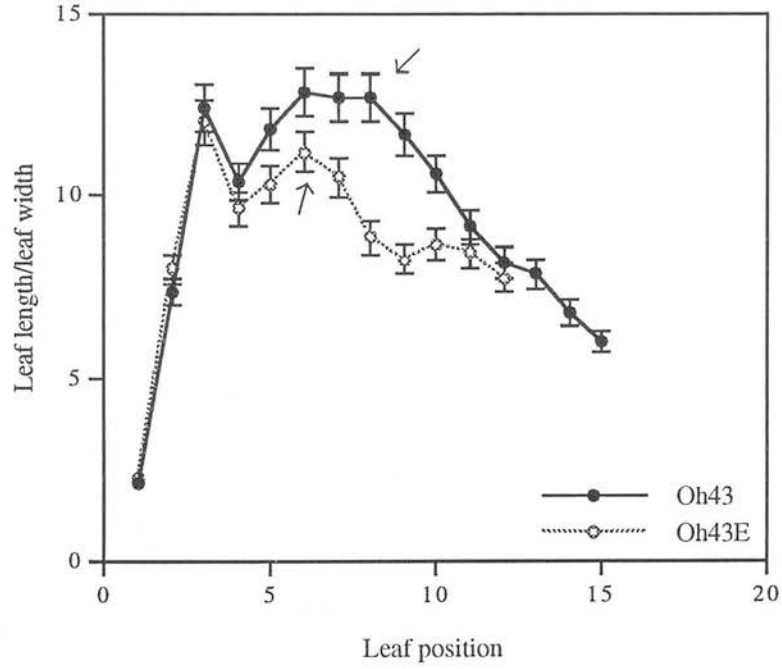

Figure 3.9 Leaf shape, as described by the length/width ratio, as a function of leaf position in the lines Oh43 and Oh43E. The position of the ears is indicated by arrows. All errors given as ±2 s.e.m.

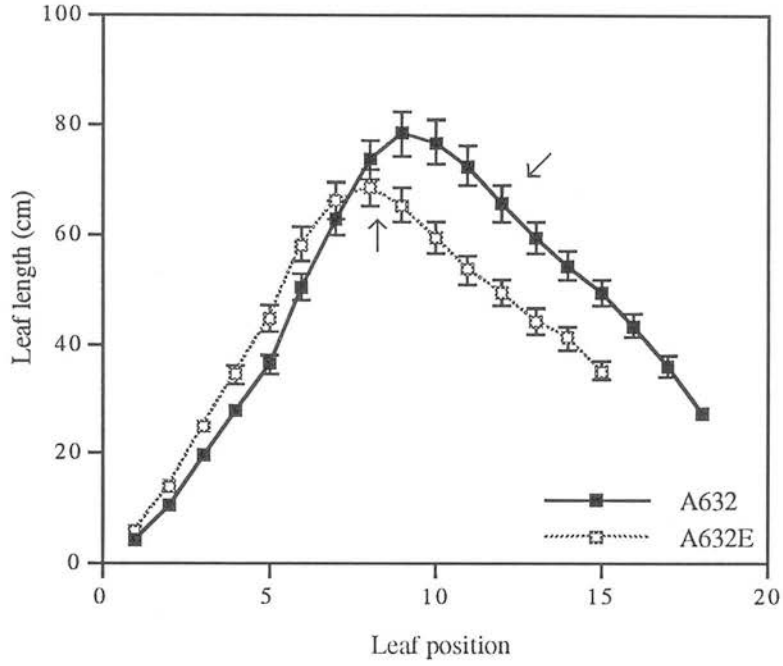

Figure 3.10 Leaf length as a function of leaf position in the lines A632 and A632E. The position of the ears is indicated by arrows. All errors given as ±2 s.e.m.

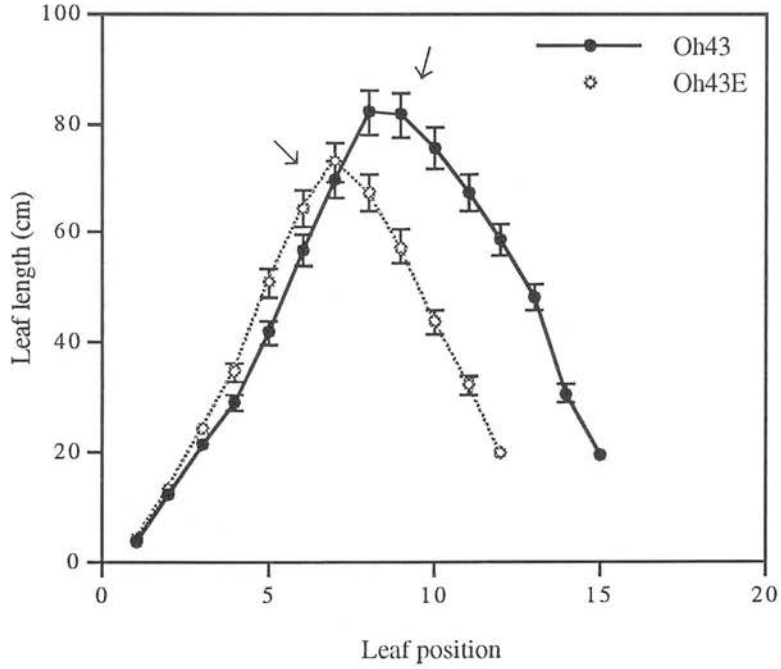

Figure 3.11 Leaf length as a function of leaf position in the lines Oh43 and Oh43E. The position of the ear is indicated by arrows. All errors given as ±2 s.e.m.

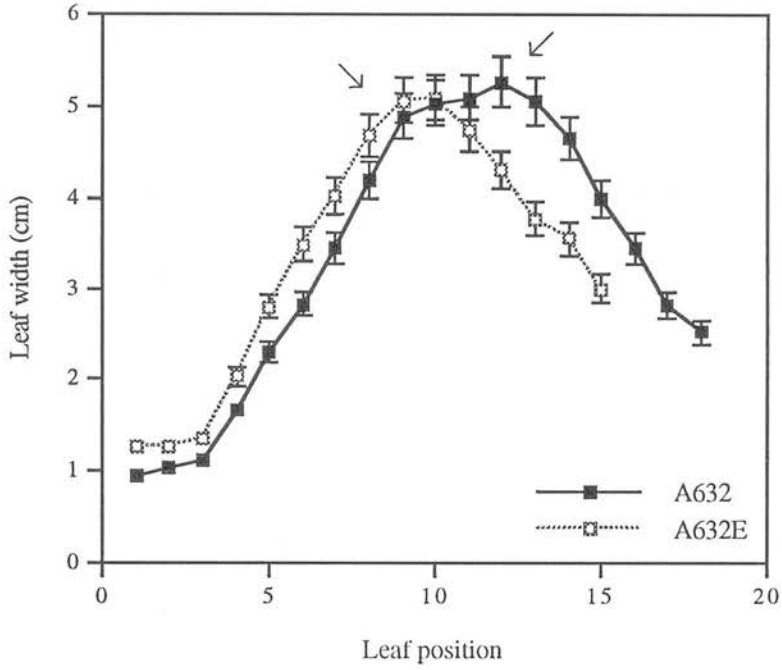

Figure 3.12 Leaf width as a function of leaf position in the lines A632 and A632E. The position of the ears is indicated by arrows. All errors given as ±2 s.e.m.

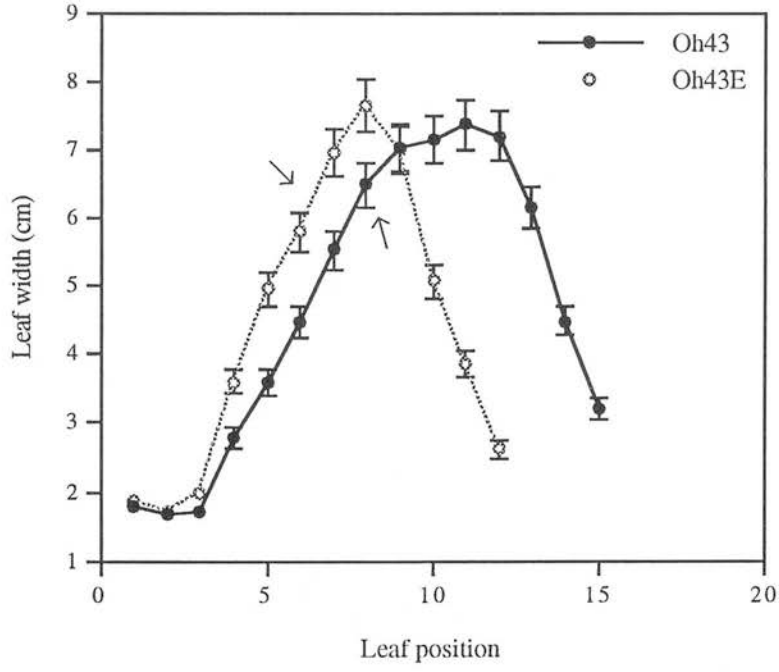

Figure 3.13 Leaf width as a function of leaf position in the lines Oh43 and Oh43E. The position of the ear is indicated by arrows. All errors given as ±2 s.e.m.

Table 3.2 Phase-specific, morphological traits of the plants used for leaf form measurements (N is the number of plants scored. All errors given as ±2 s.e.m. Significant differences between the inbred and its early derivative are indicated by bold print).

	Last node with prop roots	First partially glossy leaf	First leaf with trichomes	Last partially glaucous leaf	Ear placement node	Total number of leaves	N
A632	6.5±0.3	6.5±0.3	4.5±0.3	10.6±0.3	12.0±0.0	17.5±0.3	11
A632E	**5.7±0.3**[b]	6.1±0.2	4.6±0.3	10.2±0.4	**8.5±0.4**[c]	**13.7±0.9**[c]	11
Oh43	6.1±0.3	6.6±0.3	4.5±0.3	9.6±0.3	9.6±0.3	14.6±0.3	11
Oh43E	**4.7±0.3**[c]	**5.1±0.2**[c]	**3.5±0.3**[c]	**8.1±0.3**[c]	**6.4±0.3**[c]	**11.1±0.5**[c]	10

b: significantly different from inbred at $p<0.01$ (2-tailed *t* test)

c: significantly different from inbred at $p<0.001$ (2-tailed *t* test)

3.1.2 Early flowering and the vegetative phases of development

Having established that leaf number is an adequate way to measure the beginning or end of the phases of development, and that the early flowering trait acts early in shoot development, a study of the relationship between vegetative and reproductive development was undertaken. A preliminary examination of vegetative development of inbred lines and early derivatives (table 3.2) indicated that the vegetative phase change is independent of reproductive development in A632;A632E, whereas these two events appear to be coordinately regulated in Oh43;Oh43E. This would suggest that of the possibilities presented in figure 1.1 (section 1.4) both case 1 and case 2 can occur. To confirm this observation, a detailed examination of the effect of the early flowering trait on a variety of phase-spcific traits, including shoot morphology, leaf anatomy, leaf staining pattern, and the expression of genetic markers was conducted.

3.1.2.1. Vegetative morphology

Several characters specific to the early vegetative or the late vegetative phases in maize are known, some of which can easily be scored in the field by visual examination (Bongard-Pierce, in preparation; Poethig, 1988a, 1990). These traits are considered phase-specific for two reasons. Firstly, they occur consistently either at basal (early) or at more apical (late) positions, and always in the same combinations of traits. Secondly, the expression of the phase-specific traits is modified coordinately by the Teopod mutations, which prolong the expression of the early vegetative phase (Poethig, 1988a). The early vegetative phase is thus characterised by the presence of adventitious roots, the absence of macrohairs on the leaf blades and the presence of a visible form of epicuticular wax. This leaf wax produces a blue-gray bloom on the leaf surfaces, giving the leaf a glaucous appearance. In the late vegetative phase, the adventitious roots are absent, and macrohairs are produced on the adaxial leaf blades. The lack of visible epicuticular wax gives the leaves a green, glossy appearance.

Most plant surfaces are coated with a mixture of very hydrophobic lipids which form a protective cover called the cuticle. The waxy components of the cuticle are thought to be synthesised in the epidermal cells (reviewed in Kolattukudy, 1970).The inner layers of the cuticle are made up of a network of cutin, waxes, and carbohydrate polymers (Kolattukudy, 1980). The outermost layer of the cuticle, the epicuticular

wax, is deposited on the plant surface in the shape of plates, rods, tubes, or other forms. The chemical composition and morphology of the deposits is often characteristic of a species and a particular organ (see Kolattukudy, 1980; Bianchi and Avato, 1984; Thompson, 1980; Tulloch, 1973). The amount and nature of the wax may vary with environmental factors, such as humidity, light intensity, photoperiod, and temperature (Baker, 1974; Sutter and Langhans, 1982), or with factors such as leaf age (Faboya et al., 1980; Reicosky and Hanover, 1976; Sahai and Chinall, 1932; Wilkinson and Kasperbauer, 1972). Changes in the expression of epicuticular wax have been associated with the transition from the early vegetative to the late vegetative phase in several species, including maize (Blaker and Greyson, 1988; Franich et al., 1977; Kurtz, 1950; Poethig, 1988a; Tulloch, 1973).

In order to investigate the relationship between the vegetative phase change and reproductive maturity in A632; A632E and Oh43; Oh43E, several traits specific of each phase of development were scored in families segregating the early flowering trait. By scoring F2 families derived from self-pollinated plants that were heterozygous for the early flowering trait (IL/ED x self) it is also possible to distinguish whether any vegetative changes are associated with the early flowering trait or result from another, unlinked factor accidentally introgressed into an early derivative from Gaspé Flint (GF). The plants were categorised according to their TLN and the expression of the phase-specific traits averaged in each group. The data are presented in three different ways. Firstly, the absolute position of each trait in leaf number is presented as a function of the TLN. This type of presentation was chosen because it can best show which traits are expressed at the same time in all maturity classes (i.e. at constant leaf position, and which traits are expressed at an earlier position in the earlier maturity classes (i.e. increasing leaf position with increasing TLN). Secondly, the position of each traits relative to TLN is presented as the percent of TLN for each maturity group. This kind of presentation will allow to distinguish if the vegetative phases of development are shortened proportionately in the early flowering lines (case 2 in figure 1.1) or if only the early vegetative phase is shortened (case 3 in figure 1.1). If case 2 applies, the phase-specific traits will be expressed at the same relative position (i.e. constant percent of TLN). If case 3 applies, the relative position would increase with increasing TLN. Finally, table 3.3 lists the regression coefficients for the absolute and relative position of each trait and TLN.

Several observations can be made from these experiments. Firstly, the ear placement node in leaf number and TLN are highly correlated in both A632 and Oh43 (figures 3.14 and 3.16, table 3.3), whereas the relative position of the ear placement

node is constant and either not correlated with TLN (A632) or poorly correlated with TLN (Oh43) (figures 3.15 and 3.17, table 3.3). Thus, the ear is placed at a proportionately lower position as the TLN is reduced. This demonstrates that the positions of the ear and the tassel are equally affected by the early flowering trait, confirming the results of section 3.1.1.1.

Secondly, whenever vegetative traits are modified in early flowering plants, they are changed in a consistent pattern. The low variability of each trait indicates that the early flowering plants did not segregate into groups where in one group a trait was unaffected by the early flowering trait, whereas the same trait was found at a proportionately lower position in the other group. This indicates that the vegetative changes are associated with the early flowering trait and not with an unlinked factor that may have been introduced into an early derivative from Gaspé Flint.

Thirdly, the position of the last partially glaucous leaf blade describes two different patterns in A632 and Oh43, confirming earlier results. In A632, the position of this trait in leaf number remains the same in all maturity groups and correlates poorly with TLN. On the other hand, the relative position of this trait is negatively correlated with TLN; it increases as the TLN is reduced in the earlier plants (figures 3.14 and 3.15, table 3.3). In early flowering plants, a larger proportion of the shoot has glaucous leaves, which is indicative of the early vegetative phase of development. This can be seen best when the position of the shoot trait is graphed as percent of the TLN (figure 3.15). This suggests that in A632 families segregating the early flowering trait, the early vegetative phase of development is independent of changes in flowering time. In contrast, the position of the last partially glaucous leaf blade in leaf number is highly correlated with TLN in Oh43 (figures 3.16 and 3.17, table 3.3). In all maturity groups, the same proportion of the shoot has early vegetative leaves. This suggests that in the Oh43 families segregating the early flowering trait, the termination of the early vegetative phase of development and flowering time are coordinately regulated. Moreover, the expression of this character suggests that both phases are proportionately shortened in the earlier maturity groups (figure 3.17).

The expression of macrohairs and the loss of epicuticular wax in Oh43, and of adventitious roots in A632 do not conform with the above result. The appearance of macrohairs and of glossy patches on the blade is unaffected by changes in the flowering time in Oh43 families (figures 3.16 and 3.17, table 3.3). The position in leaf number of these traits is poorly correlated with TLN, whereas the relative position of these traits correlates well with TLN. The number of nodes with adventitious roots does not show

the pattern of any other vegetative trait, but is highly correlated with TLN in A632 and Oh43 (figures 3.14 to 3.17, table 3.3). This is inconsistent with the results from the other vegetative traits and raises doubts about the validity of considering nodal roots as indicators of early vegetative development. These observation will also be discussed below.

The position of the last leaf with wax suggestes that in A632, the vegetative phase change is independent of reproductive maturity, and early flowering only reduces the number of late vegetative leaves. The leaf shape of A632E demonstrates that the effects of the early flowering trait can be detected no later than by leaf 7 (section 3.1.1.4). Since the visible epicuticular wax disappears by leaf 9 to 10 in all maturity groups, it is clearly not affected by the early flowering trait. In contrast, in Oh43 the early vegetative phase of development, as measured by the expression of epicuticular wax, terminates earlier in early flowering plants, indicating that the early vegetative phase and reproductive development are coordinately regulated. In Oh43, this result is supported by another observation. The expression of epicuticular wax and hairs on the leaf sheath of a F2 family was scored in order to establish whether these two additional traits show a similar pattern. As is the case for the leaf blade, sheaths are characterised by a glaucous bloom and the absence of hairs in the early vegetative phase of development, whereas sheaths produced during the late vegetative are glossy and have visible hairs. Figures 3.18 and 3.19 and table 3.3 show that both of these traits change in the same fashion as leaf wax. The bloom disappears and hairs appear at a lower leaf position in the plants with fewer leaves, whereas the proportionate position of change (as percent of TLN) remains constant. This indicates that changes in epicuticular wax on the leaf blade are paralleled by changes in other phase-specific traits, including the production of trichomes, which is considered an indicator of the late vegetative phase. The expression of additional characters will be examined in the following sections.

The phase-specific, visible differences in wax expression are likely to result for differences in the wax composition. Differences in the composition of leaf wax between early vegetative and late vegetative leaves of maize have been demonstrated (Bianchi et al., 1984, Blaker and Greyson, 1988). Studies with *glossy* mutants suggest that the glaucous bloom on the early vegetative leaves is a result of wax projections that are present in at least the first five leaves of wild type plants (Bianchi and Marchesi, 1960; Lorenzoni and Salamini, 1975). Blaker and Greyson (1988) studied the composition and amount of leaf wax in Oh43 at different leaf positions and plant ages. They reported that overall, a higher amount of total wax was present in seedlings than in mature plants. They found that leaves could be grouped in to a

seedling group (leaves 1 to 6) and a mature group (leaves 7 to 16) based on analysis of six compounds. In the analysis of leaf wax using gas chromatography, five peaks were highly correlated with seedling leaves (10 to 20 DAP), whereas another four peaks were highly correlated with mature plants (40 to 80 DAP). Two of the peaks representative of each group were analysed by leaf position. Peak 21 (n-C_{32} alcohol) constitutes a high fraction of the wax in early vegetative leaves. Its relative amount decreases slowly between leaves 5 and 9, reaching a plateau by leaf 10. In contrast, peak 13 (n-C_{31} alkane) is present in a low proportion in leaves 1 to 4. Its relative amount increases quickly in leaves 5 and 6, reaching a plateau by leaf 7. These changes are in good agreement with the changes observed in the waxy bloom on Oh43 leaves. The transition from early vegetative to late vegetative wax begins by leaf 5 to 6, when glossy patches first appear, and the area that is covered with bloom decreases slowly with leaf position. In Oh43, the last partially glaucous leaves are observed around leaf position 10. This suggests that the observed change in epicuticular wax bloom is not a single, isolated modification. Rather, it is part of a complex change in the composition and morphology of cuticular waxes conditioned by the transition from one vegetative phase of development to the next.

The position of leaves with macrohairs or glossy patches is not affected by the early flowering trait in either A632 or Oh43. This observation is not consistent with the results for the last glaucous leaf, and with the findings in section 3.1.1.4 (table 3.2). This result means either that the expression of these traits is not modified by the early flowering trait, suggesting that the beginning of the late vegetative phase of development is unaffected, or that the early flowering trait does not consitently affect leaf traits that are expressed so early in shoot development, i.e. in basal leaves. The former possibility would imply that early vegetative and late vegetative development are not coordinately regulated. This is consistent with results from the Teopod mutants, in which independent regulation of the early vegetative and the late vegetative phases of development has been suggested (Poethig, 1988a). However, the results presented in figures 3.18 and 3.19 suggest that the production of hairs on the leaf sheath, considered to be a trait characteristic of the late vegetative phase of development, can be affected by the early flowering trait in Oh43. The possibility that the early flowering trait does not consistently affect the expression of macrohairs and of glossiness, possibly because these leaves are formed at or before the time the early flowering trait is expressed, is supported by the following considerations. The effect of the early flowering trait on leaf shape is not detectable until leaf 5 in Oh43, suggesting that in earlier leaves leaf development is complete, or at least determined, before the effects of the early flowering trait are apparent. In Oh43, macrohairs appear on the leaves at leaf

position 5 and glossy patches at leaf position 6. It is possible that these traits cannot be expressed at lower leaf positions because the character of the lower leaves is already specified by the time early flowering trait activity begins. Maize embryos initiate 4 to 5 leaves before dessication (Hubbard, 1951). It is possible that leaves which are present in the embryo are affected differently by the early flowering trait. Also, the early flowering trait may have a smaller effect early in shoot development and a small proportionate change in the expression of phase-specific traits at a lower leaf position may be undetectable. Thus, an analysis of these two traits neither rejects nor supports the result obtained by assessing partially glaucous leaves. In order to understand why the position in leaf number of these two traits is not affected by the early flowering trait an assessment of further phase-specific characters, especially late vegetative traits and of leaf traits expressed in leaves 4 to 6, would be needed. For this purpose it would also be interesting to know when early flowering trait activity begins, and when the character of a leaf is specified.

Adventitious roots are produced at a proportionately lower position in early flowering plants in A632 and Oh43 (figures 3.14, 3.15, 3.16, and 3.17, table 3.3). This result suggests that the formation of adventitious roots and flowering time are coordinately regulated in both genetic backgrounds. In Oh43, this is consistent with the position of partially glaucous leaves. The number of nodes with adventitious roots is reduced in early flowering Oh43 plants even though adventitious roots occur only on the first 4 to 6 nodes, possibly because the adventitious roots are produced so late in shoot development. Adventitious roots develop at successively higher nodes well after the leaves at those nodes are fully mature (Sharman, 1942). In contrast to the leaf characters, where there may be a lower limit at which a trait can be affected by the early flowering trait, the position of adventitious roots is less likely to be limited by early specification. On the other hand, the number of nodes with adventitious roots is the only vegetative trait that is affected by the early flowering trait in A632. This observation casts doubt on whether this character is truly characteristic of the early vegetative phase, or instead is associated with the reproductive phase of development. Consistent deviation from the expression of other vegetative, phase-specific shoot traits would at least suggest that the number of nodes with adventitious roots is not a trait specific to the early vegetative phase of development. Given the inhibiting effect of roots on flower initiation and differentiation in other species (Schwabe and Al-Doori, 1973; McDaniel, 1980; Smith and McDaniel, 1992), it is conceivable that the adventitious roots in maize are indicative of a non-reproductive phase of development in maize. Roots may affect flowering, or vice versa, or they may be regulated in an opposite manner by a common factor. Support for the suggestion that the presence of

adventitious roots is not a character specific to the vegetative phase of development, but associated with flowering time comes from observations in several mutant genotypes, including *glossy15* mutants (Evans et al., submitted) and the late flowering mutants that will be described in section 3.2.

Overall, the analysis of the experiments presented in this section indicates that vegetative development is affected by the early flowering trait differently in A632 and Oh43. In A632, vegetative phase change is independent of reproductive maturity, whereas in Oh43, the vegetative phase change and reproductive maturity are coordinately regulated. Because not all vegetative traits showed the same pattern, an examination of further traits to support this result is clearly desirable and will be presented in the following sections.

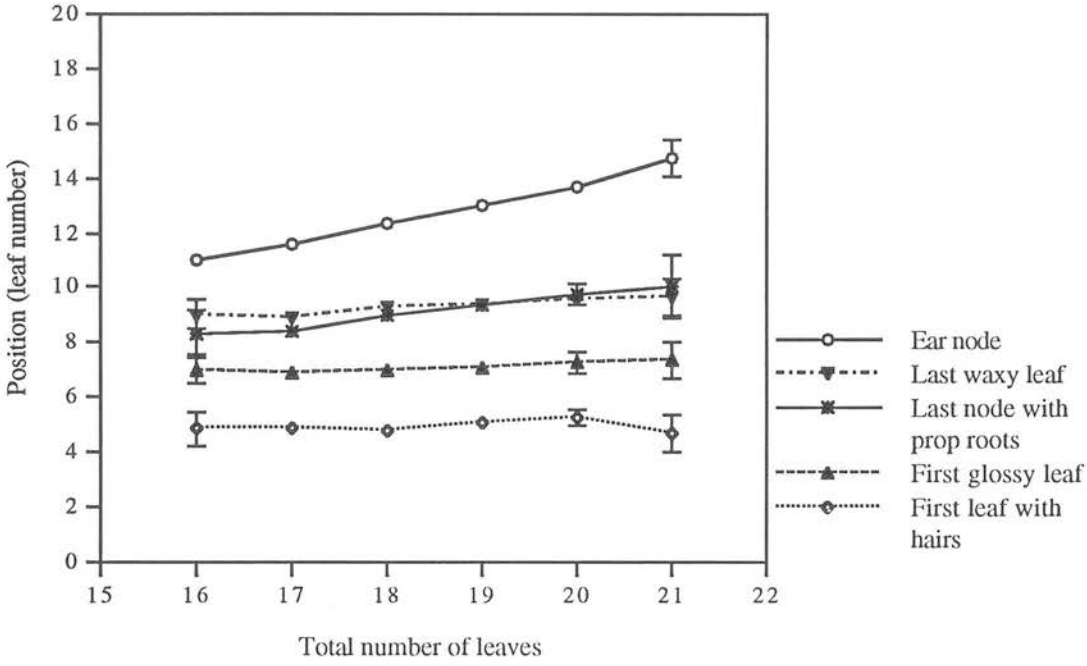

Figure 3.14 Position of morphological traits in plants from three families segregating the early flowering trait in A632 (in leaf number).

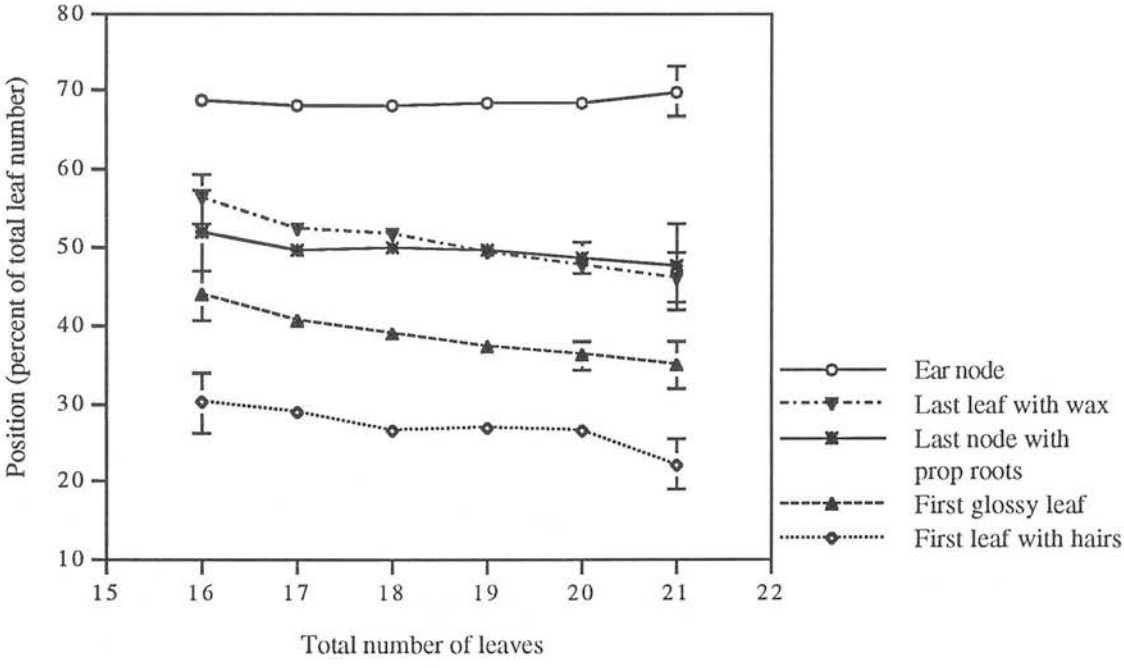

Figure 3.15 Relative position of morphological traits in plants from three families segregating the early flowering trait in A632 (in percent of total leaf number). All errors given as ±2 s.e.m.

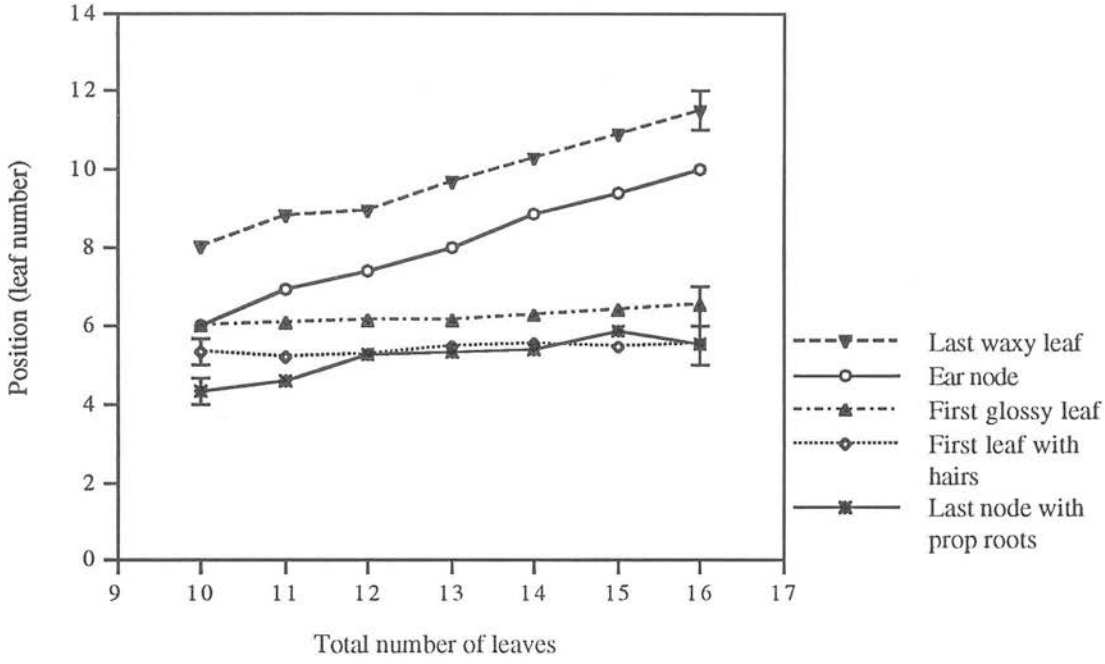

Figure 3.16 Position of morphological traits in plants from five families segregating the early flowering trait in Oh43 (in leaf number).

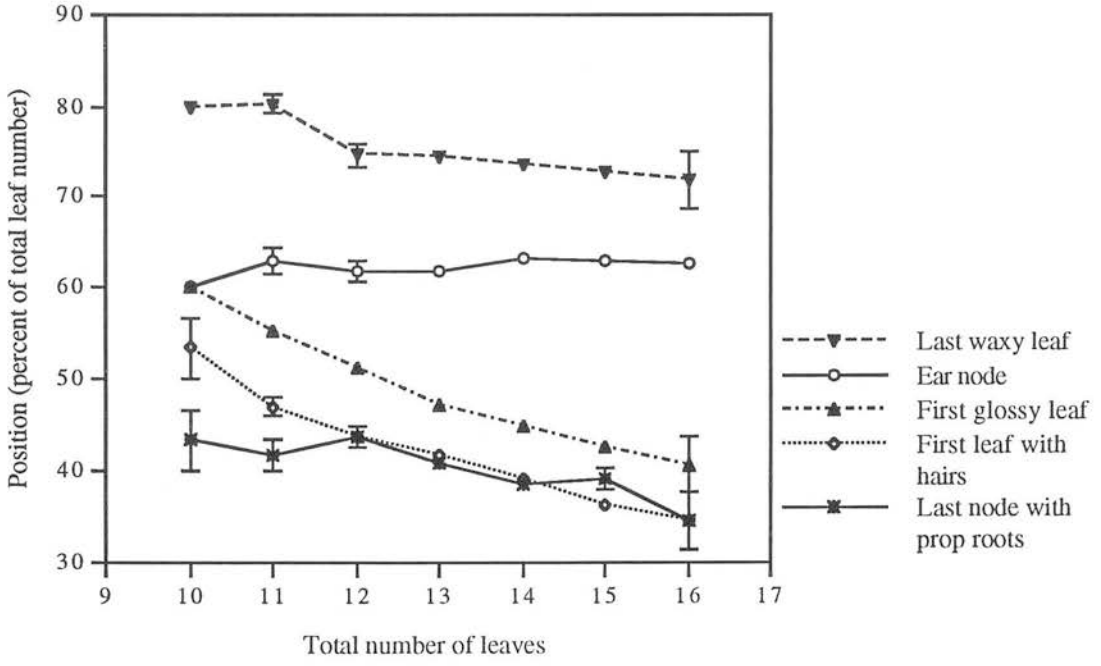

Figure 3.17 Relative position of morphological traits in plants from five families segregating the early flowering trait in Oh43 (in percent of total leaf number). All errors given as ±2 s.e.m.

Table 3.3 The correlation coefficient r for the correlation of each phase-specific trait with TLN (in absolute leaf number and in relative position) in A632 and Oh43.

Trait	A632		Oh43	
	Absolute position	Relative position	Absolute position	Relative position
Last node with prop roots	**0.54**	0.05^{ns}	**0.46**	0.28
First leaf with hairs	0.16^{a}	**0.29**	0.20^{a}	**0.64**
First partially glossy blade	0.17^{a}	**0.58**	0.27	**0.77**
Last partially glaucous blade	0.29	**0.52**	**0.83**	0.40
Ear node	**0.83**	0.07^{ns}	**0.89**	0.17^{a}
Last partially glaucous sheath			**0.74**	0.31
First leaf sheath with hairs			**0.78**	0.18^{a}

All traits were significantly correlated with TLN at least at $p<0.01$, unless otherwise noted. The best correlations are emphasised in bold print.

ns: not significant. a: trait is significantly correlated with TLN at $p<0.05$.

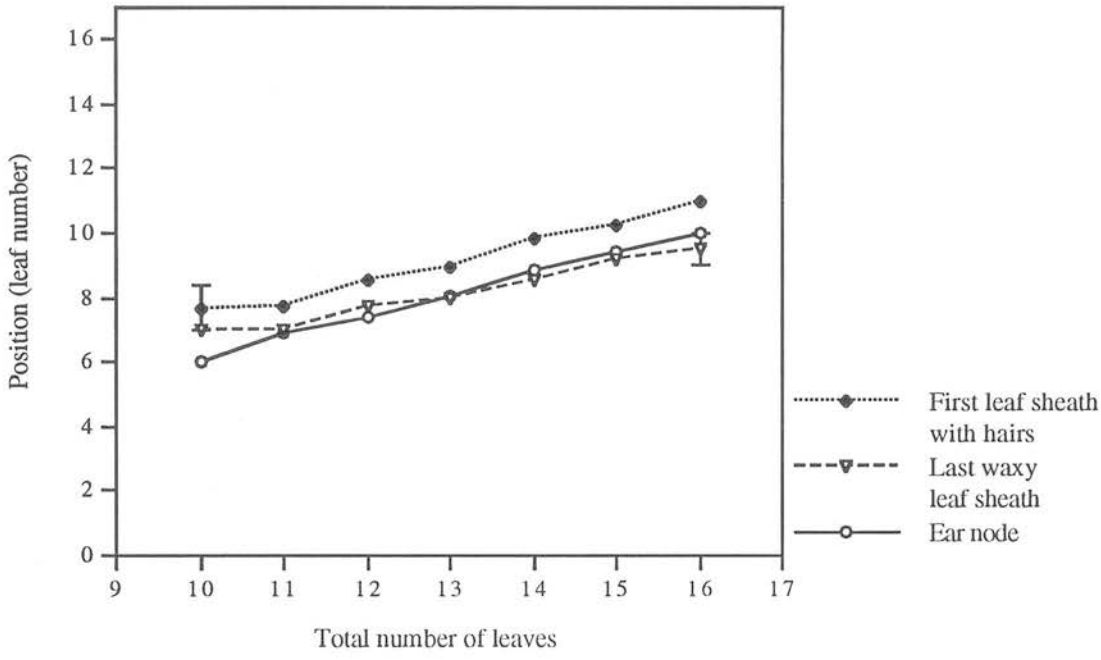

Figure 3.18 Position of morphological traits in plants from five families segregating the early flowering trait in Oh43 (in leaf number). Errors given as ±2s.e.m.

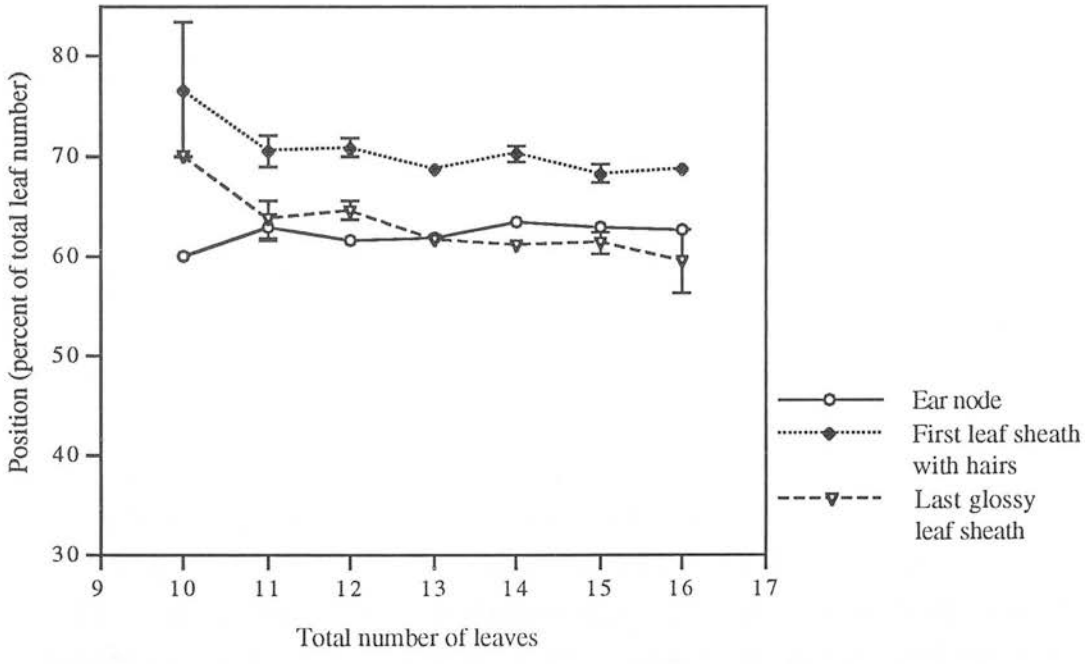

Figure 3.19 Relative position of morphological traits in plants from five families segregating the early flowering trait in Oh43 (in percent of total leaf number). All errors given as ±2 s.e.m.

3.1.2.2. Epidermal cell shape

The aim of the experiments presented in this section is to further investigate the effect of the early flowering trait on the vegetative phases of development by assessing additional phase-specific markers. A histological examination has revealed phase-specificity in the anatomy of leaves. In early vegetative leaves, the cells of the abaxial epidermis have few interdigitations and appear round in cross section, whereas in late vegetative leaves they are highly interdigitated and elongated in cross section (Bongard-Pierce and Poethig, in preparation). Since changes in the vegetative development were found to be associated with the early flowering trait (section 3.1.2.1), and not another unlinked, introgressed factor, it is sufficient to compare vegetative development in the inbred line and the early derivative. Leaf material from each line was sectioned and the changes in cell shape of the abaxial epidermal cells measured.

As illustrated in figures 3.20 and 3.21, the shape of the abaxial epidermal cells in cross section is round in early vegetative leaves and elongate in late vegetative leaves. This change can be quantitated by a shape factors calculated from the digitally traced sections. A perfectly round shape would be described by a shape factor of 1, whereas a shape approaching a line would have a shape factor approaching zero (for formula see section 2.2.6). Thus, the round epidermal cells in early vegetative leaves have a high shape factor, whereas this value is low for elongated, late vegetative epidermal cells.

When the line A632Ht and its early derivative A632E are compared, the shape of the epidermal cells is the same at each leaf position (figures 3.20 and 3.21). The cells in the early vegetative leaves have a shape factor of approximately 0.90 to 0.93, which decreases to less than 0.84 in the late vegetative leaves. In both genotypes, the transition takes place between leaf positions five and eight, with the greatest drop between six and seven. This result demonstrates that the early onset of reproductive maturity in A632E does not alter the timing of the vegetative phase change as measured by epidermal cell shape. Late vegetative traits, such as the first glossy patches on the leaf blade usually appear first in the middle of the leaf blade. This is also the leaf region that was sampled in this experiment. A comparison with the scores for epicuticular wax and epidermal hairs in the same plants reveals that the position of transition leaves as described by the phase-specific morphological traits and by the epidermal cell shape coincide (table 3.4). However, for unknown reasons, in this experiment, the first partially glossy leaf is at a significantly lower position in A632E than in A632 and the number of nodes with adventitious roots was unchanged, while the other traits are expressed as expected.

In contrast, the timing of the transition from early vegetative to late vegetative cell shapes is significantly different between the inbred Oh43 and Oh43E. In Oh43, the shape factor of the epidermal cells decreases from an initial early vegetative value of 0.90 to 0.85 to a value of 0.70 to 0.75 in the late vegetative leaves (figures 3.21 and 3.23). This transition takes places between leaves five and eight, which is also the transition zone for other phase-specific traits in this line (table 3.4). The cell shape factor is consistently lower in Oh43E than in Oh43. In Oh43E, the transition from a shape factor of 0.86 to 0.87 in the early vegetative leaves to a shape factor of 0.67 to 0.70 in late vegetative leaves takes place between leaves four and seven (figure 3.22). The greatest drop in value is between leaves five and six. This is about 1 to 2 leaves earlier than in Oh43, indicating that the leaf epidermal cell shape in Oh43E is also affected by the early flowering trait. This earlier transition in Oh43E coincides with changes in the phase-specific traits as discussed in the previous section. The morphological traits scored in the plants used for this experiment are consistent with previous results except for the position of the first glossy leaf and the first leaf with hairs. For unknown reasons, both were significantly lower in Oh43E than in Oh43.

In summary, the changes in leaf epidermal cell shape support the finding that the transition from the early vegetative phase to the late vegetative phase of development and reproductive maturaity are independent of each other in A632E, whereas the vegetative phase change and reproductive maturity are coordinately regulated in the Oh43.

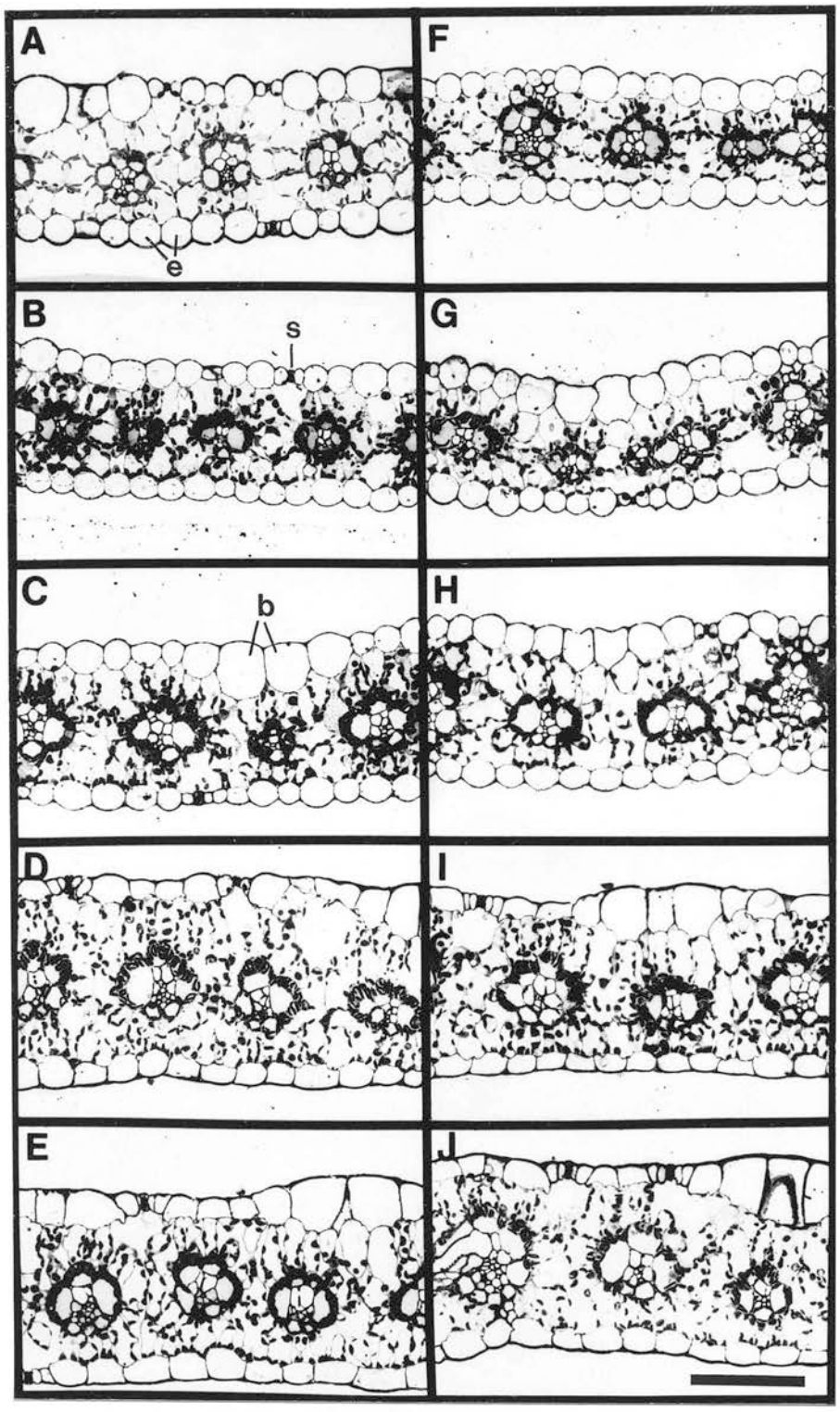

Figure 3.20 The cell shape of the of the abaxial leaf epidermis changes from round in cross section in early vegetative leaves to rectangular in late vegetative leaves in cross sections of A632 (A to E) and A632E leaves (F to J). The change occurs at the same leaf position in both genotypes. The sections are from leaf 4 (A, F), leaf 5 (B,G), leaf 6 (C,H) , leaf 7 (D,I), and leaf 8 (E,J). Scale bar equals 100µm.

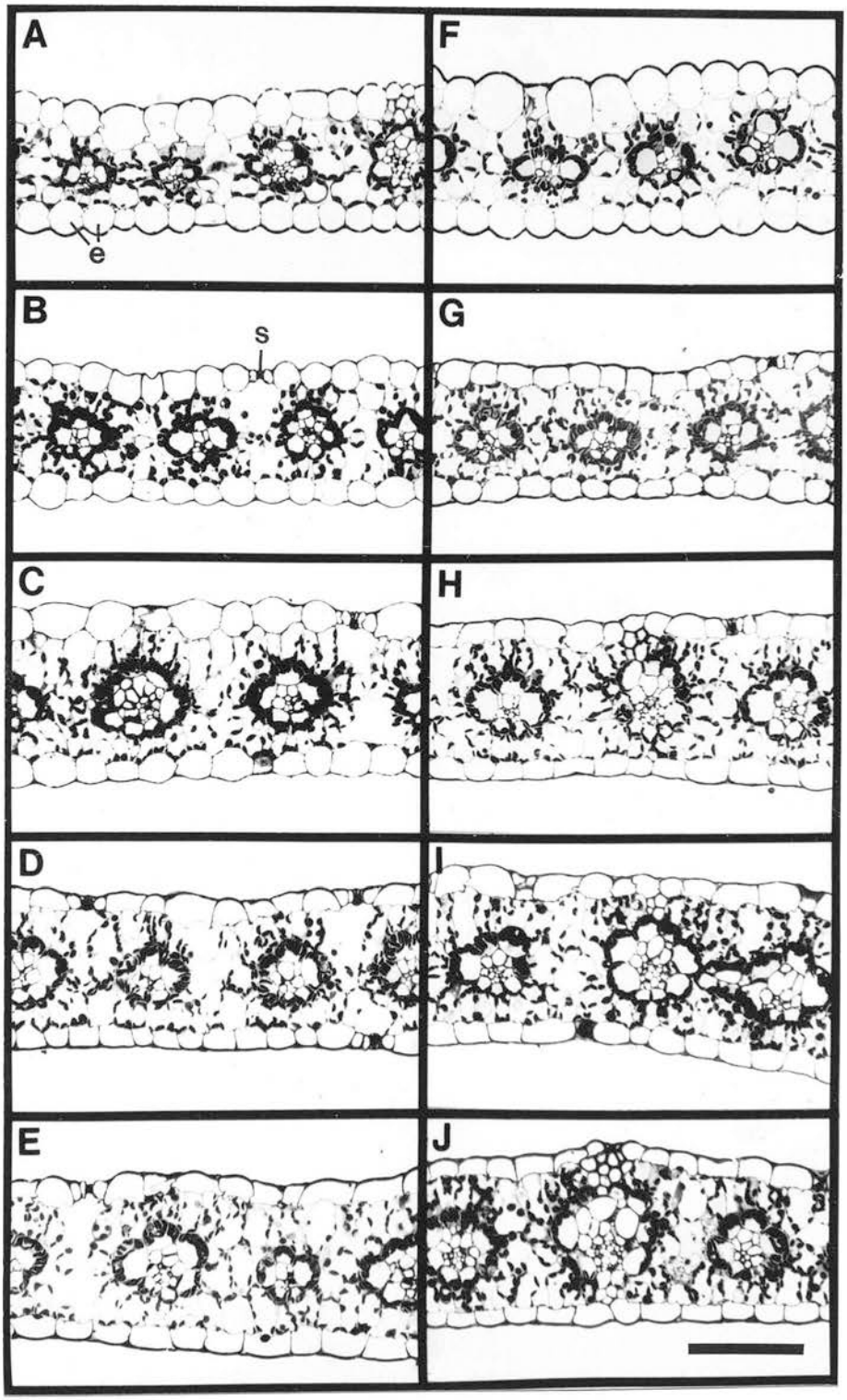

Figure 3.21 The cell shape of the of the abaxial leaf epidermis changes from round in cross section in early vegetative leaves to rectangular in late vegetative leavrs in cross sections of Oh43 (A to E) and Oh43E leaves (F to J). The change occurs at the same leaf position in both genotypes. The sections are from leaf 4 (A, F), leaf 5 (B,G), leaf 6 (C,H) , leaf 7 (D,I), and leaf 8 (E,J). Scale bar equals 100µm.

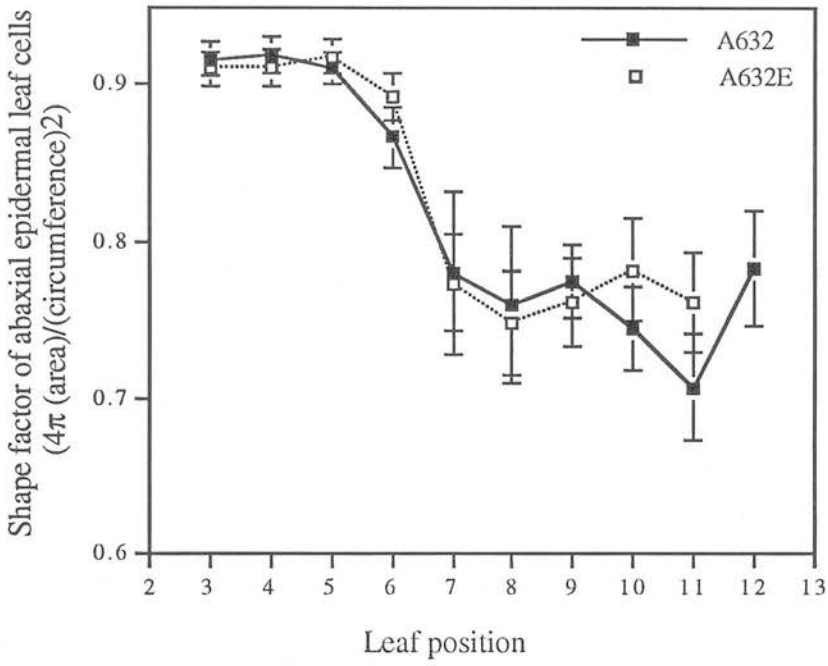

Figure 3.22 Shape of the abaxial epidermal leaf cells in transverse sections as a function of leaf position in A632 and A632E. All errors given as ±2 s.e.m.

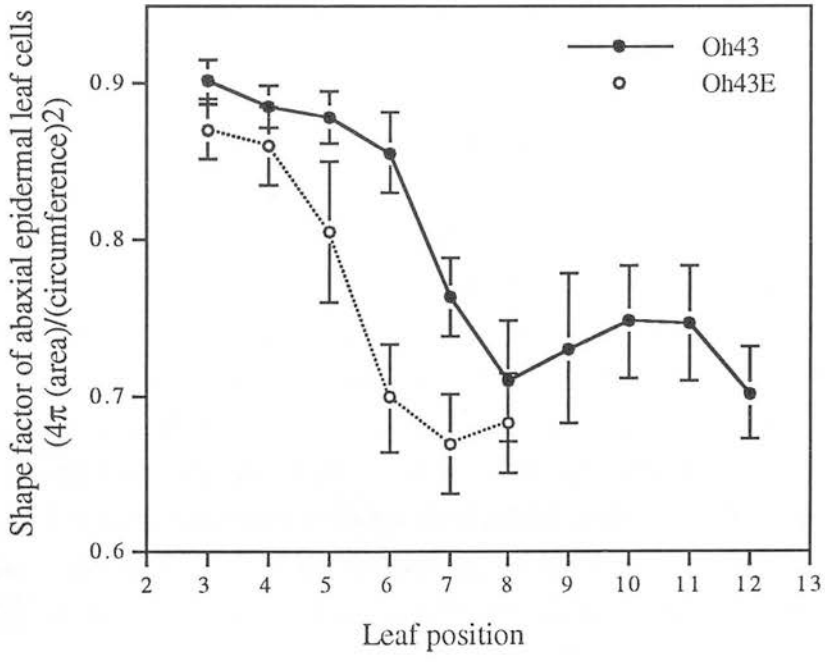

Figure 3.23 Shape of the abaxial epidermal leaf cells in transverse sections as a function of leaf position in Oh43 and Oh43E. All errors given as ±2 s.e.m.

Table 3.4 Morphological traits of the plants used for leaf sectioning (N is the number of plants scored. All errors given as ±2 s.e.m.).

	Last node with prop roots	First partially glossy leaf	First leaf with trichomes	Last partially glaucous leaf	Ear placement node	Total number of leaves	N
A632	7.0±0.0	7.0±0.0	5.2±0.4	10.8±0.4	11.8±0.4	17.8±0.4	5
A632E	6.6±0.5	**6.0±0.0**^{c,2}	5.6±0.5	10.2±0.7	**8.0±0.0**^{c,2}	**11.2±0.4**^{c,2}	5
Oh43	6.0±0.0	6.2±0.4	5.6±0.5	10.8±0.4	9.6±0.5	14.6±0.5	5
Oh43E	**4.8±0.4**^{c,2}	**5.0±0.0**^{c,2}	**5.0±0.0**^{a,1}	**7.0±0.0**^{c,2}	**6.0±0.0**^{c,2}	**8.8±0.4**^{c,2}	5

a, 1: significantly smaller than inbred line at $p<0.05$ (1-tailed *t* test, 1-tailed *U* test)
b, 2: significantly smaller than inbred line at $p<0.01$ (1-tailed *t* test, 1-tailed *U* test)
c, 3: significantly smaller than inbred line at $p<0.001$ (1-tailed *t* test, 1-tailed *U* test)
(a,b, and c indicate results from Student's *t* test; 1,2, and 3 indicate results from the Mann-Whitney *U* test)

3.1.2.3. Tissue staining

Another useful phase-specific trait is the staining pattern of leaves in toluidine blue O. This stain is metachromatic at low pH, a characteristic which has been used for differential staining of plant tissues (O'Brien et al., 1965; Sakai, 1973). Lignin, suberin, and some tannins can determine the coloration of plant tissues: most plant material stains purple, whereas lignified or suberised tissues stain turquoise (aqua). In maize, the adaxial epidermis of early vegetative maize leaves stains evenly purple, whereas the adaxial epidermis of late vegetative leaves has alternating strips of purple and aqua staining cells (Bongard-Pierce and Poethig, in preparation). The purple epidermal cells in these late leaves are the bulliform cells. In order to further examine whether different patterns of shoot development exist in A632; A632E and Oh43; Oh43E, successive leaves of each line were stained with toluidine blue O.

The results of this analysis confirm those reported in the previous two sections. The change from a leaf staining in a completely early vegetative fashion to one with a

completely late vegetative staining takes place over several leaves, similar to the changes in epicuticular wax. The epidermal staining pattern of tissues halfway along the length of the leaf is illustrated in figures 3.24 and 3.25. At this location, the transition from early vegetative to late vegetative staining occurs at leaf 7 in A632 and A632E. This transition takes place at leaf position 7 in Oh43 and between leaves 5 and 6 in Oh43E. The position at which these changes occur coincides with the changes in phase-specific characters scored in the stained plants (table 3.5). The photographs in figure 3.24 and 3.25 also show that macrohairs are generally absent in early vegetative leaves that stain completely purple, and present in leaves that have aqua-and-purple staining tissue. This result corroborates the previous findings that the vegetative phases of development are regulated independently of the reproductive maturity in A632;A632E, whereas the expression of the vegetative phases of development is correlated with the onset of reproductive maturity in Oh43;Oh43E. Given that the aqua coloration of tissue after staining usually, but not necessarily, is indicative of lignin (O'Brien et al., 1965; Sakai, 1973), this result also suggests that the epidermis of late vegetative leaves in maize may be lignified, whereas the epidermis of early vegetative leaves is not.

Table 3.5 Morphological traits of the plants used for the leaf tissue staining.

	Last node with prop roots	First partially glossy leaf	First leaf with trichomes	Last partially glaucous leaf	Ear placement node	Total number of leaves	N
A632	7.0±0.3	7.1±0.2	4.8±0.3	11.4±0.7	13.8±0.3	20.7±0.5	9
A632E	**6.2±0.3**^{c}	7.0±0.0	**5.1±0.2**^{a}	12.4±0.3*	**10.4±0.4**^{c}	**16.6±0.3**^{c}	9
Oh43	6.0±0.0	6.8±0.4	4.8±0.5	11.4±0.5	10.6±0.5	16.0±0.6	5
Oh43E	**4.4±0.5**^{c}	**5.8±0.4**^{b}	**4.0±0.0**^{b}	**9.0±0.9**^{b}	**6.6±0.5**^{c}	**11.4±1.2**^{c}	5

a: significantly smaller than inbred line at $p<0.05$ (1-tailed *t* test

b: significantly smaller than inbred line at $p<0.01$ (1-tailed *t* test)

c: significantly smaller than inbred line at $p<0.001$ (1-tailed *t* test)

*: significantly different from inbred line at $p<0.01$ in a 2-tailed *t* test

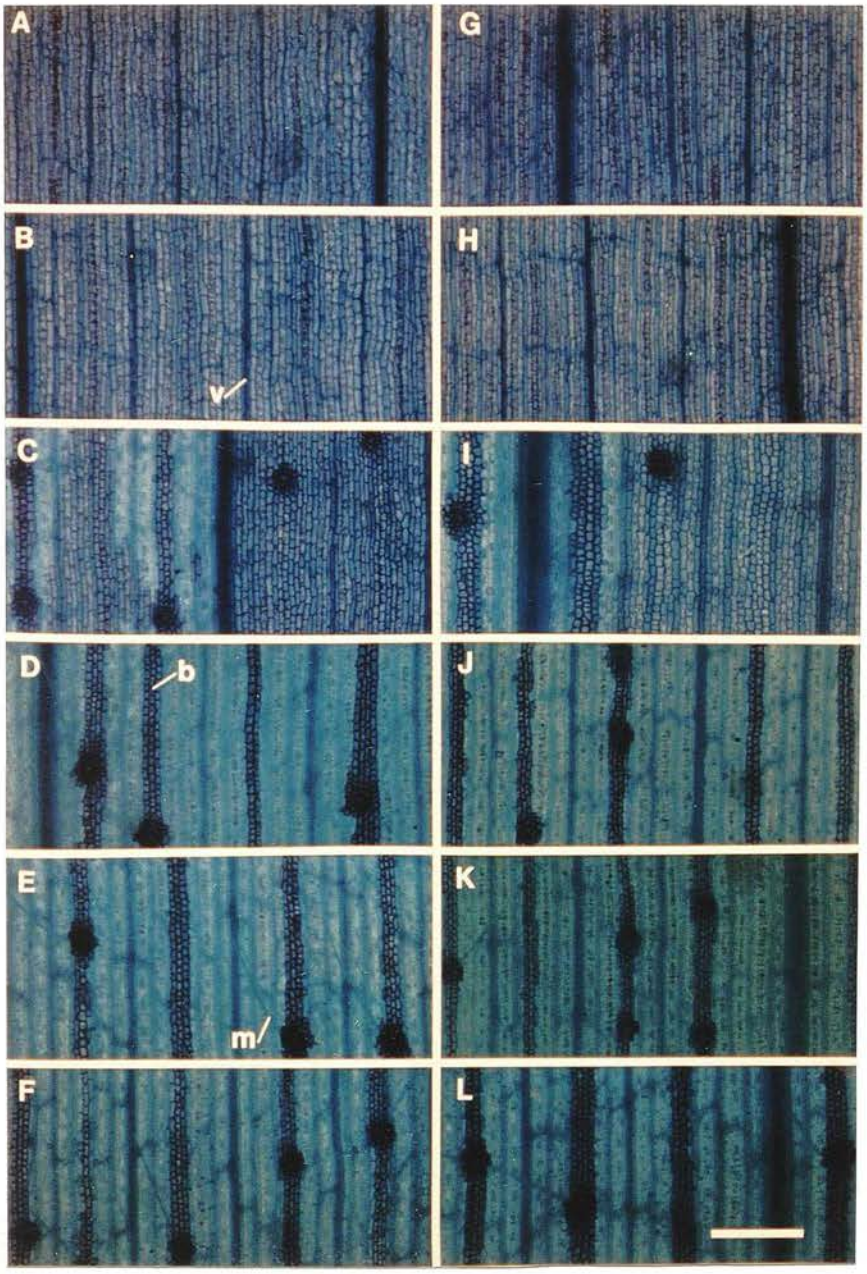

Figure 3.24 Phase-specific staining patterns of A632 (A to F) and A632E leaves (G to L) stained with toluidine blue O. The samples were taken halfway along the length of the leaf at leaf position 4 (A, G), leaf 6 (B, H), leaf 7 (C, I), leaf 8 (D, J), leaf 9 (E, K), and leaf 10 (F, L). Veins (v) are visible in all leaves, macrohairs (m) are formed in late vegetative leaves from bulliform cells (b). Scale bar equals 0.5cm.

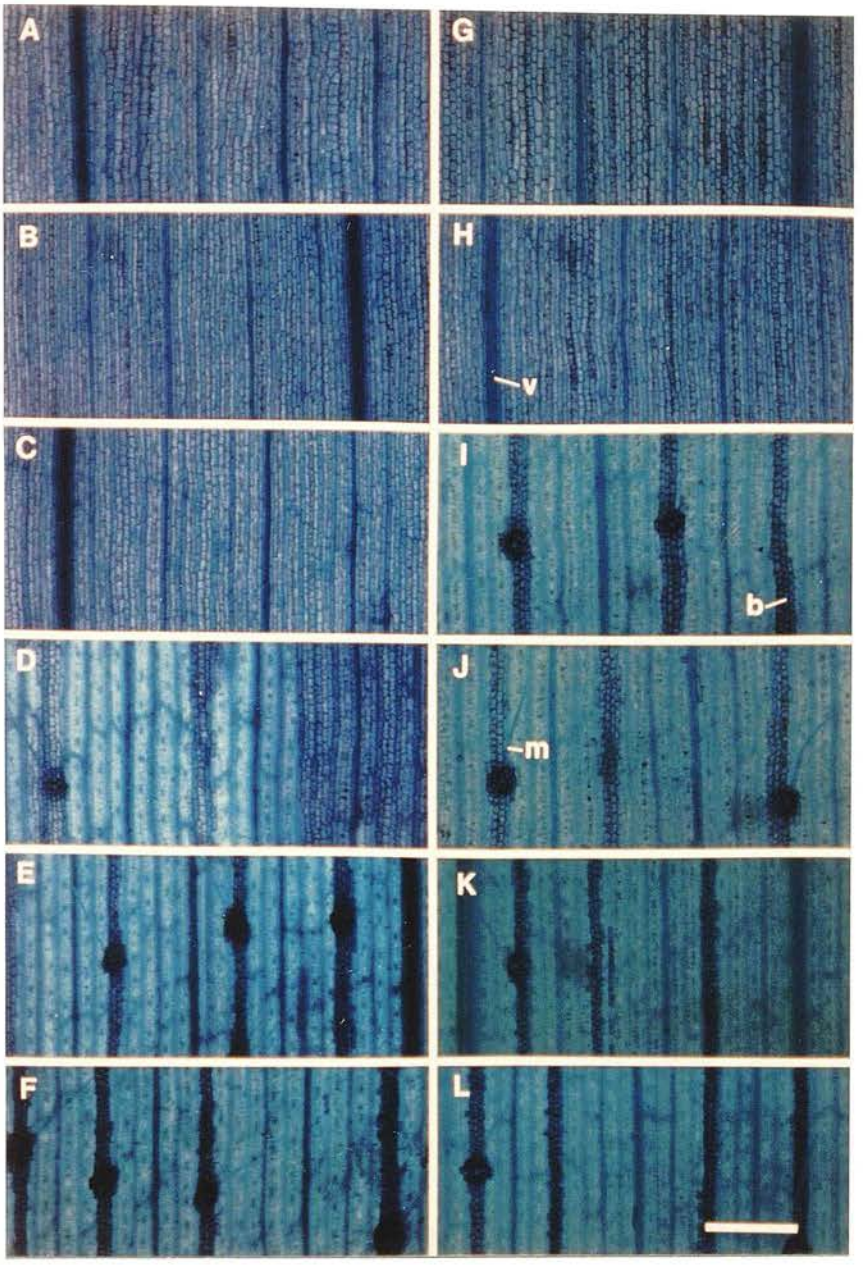

Figure 3.25 Phase-specific staining patterns of Oh43 (A to F) and Oh43E leaves (G to L) stained with toluidine blue O. The samples were taken halfway along the length of the leaf at leaf position 4 (A, G), leaf 5 (B, H), leaf 6 (C, I), leaf 7 (D, J), leaf 8 (E, K), and leaf 9 (F, L). Veins (v) are visible in all leaves, macrohairs (m) are formed in late vegetative leaves from bulliform cells (b). Scale bar equals 0.5cm.

3.1.2.4. Expression of a genetic marker, *Ragged1 (Rg1)*

Another way to investigate when the transition from the early vegetative to the late vegetative growth takes place is to assess the expression of mutant genes that are regulated in a phase-specific manner. One such mutation is the dominant *Ragged1* (*Rg1*)mutation which causes localised cytolysis of mesophyll cells, resulting in chlorotic spots and tearing of the leaf following leaf expansion (Mericle, 1950). In *Rg1* mutants early vegetative leaves are normal, whereas late vegetative leaves show the Ragged phenotype (Evans, personal communication). The Ragged phenotype may thus be used to indicate the beginning of the late vegetative phase of shoot development. This mutation is also interesting as a phase-specific marker in that its primary effect is in the mesophyll. All previously examined traits are expressed by the epidermis. In order to establish if the expression of *Ragged1* (*Rg1*) is modified by the early flowering trait in the pattern predicted by the previously examined traits, the mutation was crossed into the inbred lines and early derivatives, and families segregating *Rg1* were scored for the appearance of the Rg1 phenotype, glaucous leaves, and reproductive traits.

The results of this experiment are presented in table 3.6. In the families that were crossed to the early derivatives the TLN and the ear placement node are significantly lower than in those crossed to the inbred lines, indicating that the early flowering trait is effective also in these hybrid families. The position of the last partially glaucous leaf indicates that the same developmental pattern as observed earlier appears in these families: the position of this trait is the same in A632 and A632E, whereas it is significantly lower in Oh43E than in Oh43. This shows that the developmental pattern that was observed previously in the inbred lines and early derivatives is retained in these hybrid families. However, the position of the first leaf with a Rg1 phenotype was the same in A632 and A632E and in Oh43 and Oh43E, indicating that the expression of *Rg1* was not affected by the early flowering trait. Overall, the position of the first *Rg1* expression is somewhat less variable than TLN or ear placement node, as indicated by the small estimates of the standard error of the means.

The other two traits, the first leaf with glossy patches and leaf hairs, could not be reliably scored because of the distortion of leaf morphology caused by the *Rg1* mutation. Yet, it was evident that the expression of the Rg1 phenotype and of glossiness were associated. Beginning in the middle and the base of the leaf, the ragged area increased in subsequent leaves in the same fashion as the glossy patches. In the partially glaucous leaves, the glaucous areas were not ragged, and the ragged

areas extended over the whole leaf only in the leaves above the last partially glaucous leaf. This suggests that the Rg1 phenotype is expressed in the same manner as glossiness.

It is possible that the expression of *Rg1* is not altered by the early flowering trait in Oh43E for the same reasons that the first appearance of hair and glossiness were unaffected by the early flowering trait (section 3.1.2.1). It has been suggested in section 3.1.2.1 that the morphology of the first 4 to 5 basal leaves may be already specified by the time early flowering trait activity begins. This is consistent with the observation that *Rg1* is first expressed at the same leaf position where changes in leaf shape are first observed in Oh43E (leaf 5, section 3.1.1.4) and where hair and glossiness are first expressed. The alternative hypothesis that the onset of the late vegetative phase of development may not be linked to an early beginning of reproductive maturity is not consistent with the result that the production of hairs on the leaf sheath can be affected by the early flowering trait in Oh43E (section 3.1.2.1). Finally, variability resulting from the heterogeneity of these families may render any difference in *Rg1* expression between Oh43 and Oh43E undetectable. Whereas the variability is not excessive as seen by moderate standard error of means estimates, this point must be stressed in light of the results that will be described in section 3.1.4. Those results indicate that the inbred background determines at least in part if vegetative phase change and reproductive maturity are coordinately regulated. Adequate convergence to the inbred lines and the early derivatives is therefore desirable before the expression of *Rg1* can be assessed satisfactorily.

Table 3.6 Expression of the *Ragged1* mutation in normal and early-flowering plants (N is the number of plants scored. All errors given as ±2 s.e.m.).

	First ragged leaf	Last partially glaucous leaf	Ear node	Total leaf number	N
A632	5.7±0.2	8.0±0.2	13.3±0.6	18.5±0.7	17
A632E	5.7±0.3	8.0±0.4	**9.9±0.4**[c]	**14.4±0.6**[c]	9
Oh43	4.5±0.3	7.2±0.3	11.8±0.5	17.3±0.4	22
Oh43E	4.5±0.4	**6.6±0.3**[b]	**7.6±0.4**[c]	**12.4±0.5**[c]	19

b: significantly different from inbred line at $p<0.01$ (2-tailed *t* test)

c: significantly different from inbred line at $p<0.001$ (2-tailed *t* test)

3.1.3 Genetic analysis of the early flowering trait

The different patterns of vegetative development in A632;A632E and Oh43;Oh43E can be accounted for in either of two ways: firstly, A632E and Oh43E may each have received different genes from the donor parent Gaspé Flint. Those gene could be loci that condition the early flowering trait or closely linked modifiers of vegetative development. Alternatively, the inbreds A632 and Oh43 possess different modifiers of vegetative development, which become detectable in the presence of the early flowering trait. To study whether the first possibility applies, a genetic analysis of the early flowering trait in A632E and Oh43E was undertaken.

Time to maturity in maize is a quantitiative trait, with an unknown number of genes contributing to this trait. Estimates of the number of genes range from 2 to 20 loci, and most reports suggest 4 to 6 loci (Griesbrecht, 1969a,b; Hallauer, 1965; Mohamed, 1959). Shaver (1976) suggested that few, possibly two loci have been introgressed from Gaspé Flint into near-isogenic early flowering lines. The genetic basis for early flowering in Gaspé Flint and in the lines A632E and Oh43E is not known. Gaspé Flint, the donor line for the early flowering trait, is a very early flowering, genetically heterogeneous cultivar that may possess several loci conditioning early flowering. As a result of the convergence of the early flowering trait, different loci may have been introgressed into A632E and Oh43E.

In order to investigate the possibility that different genes conditioning early flowering were converged into A632E and Oh43E, it is necessary first to estimate the number of genes that condition the early flowering phenotype in each of the lines, and then to establish whether the two lines share the same early flowering genes. The former task can be achieved by analysing segregation frequencies in families segregating for the early flowering trait. The identity of the genes can be determined by molecular genetic techniques.

3.1.3.1. Estimate of the number of genes

The estimation of the number of loci controlling quantitative traits is difficult partly because continuous variation hinders the distinction of phenotypic classes. The presence of multiple loci, each with a different effect and expressivity, interaction between loci, and heterosis complicate matters further. Powers et al. (1950, 1955)

proposed the partition method to estimate of the number of loci regulating quantitative traits. For this method, large populations of parental lines (P1 and P2), single-cross hybrid families (F1), backcrosses of the F1 to the parental lines (B1 and B2), and families obtained from self-pollinating F1 plants (F2) are evaluated in several replicates over different environments to separate the environmental and genetic variances. The backcross and F2 populations are analysed by partitioning them into their component genotypes. Such a large experimental design was not employed in the present study. However, to achieve some estimate of the number of loci regulating the early flowering trait in A632E and Oh43E, F2 families segregating the early flowering trait that were derived from self-pollinated hybrid plants (IL/ED x self) were analysed.

As before, the families segregating the early flowering trait and the controls were evaluated for the total number of leaves they produced. The A632;A632E experiment was grown in the summer nursery (table 3.7), and three experiments involving Oh43;Oh43E were grown in different summer plantations or in the green house during the winter (table 3.8). The results are similar for A632E and Oh43E. Some effect of the environment on TLN is evident from the differences in TLN between inbreds grown in the three Oh43 experiments.

The total number of leaves in the F2 families were generally within the range expected from the inbred controls. This indicates that all factors conditioning fewer leaves were contributed by the early derivatives and all factors conditioning more leaves came from the inbred lines. However, in the F2 of A632 two plants with unexpectedly many leaves (TLN of 22 and 23 leaves) occurred, and the range of the TLN in all backcrosses extended slightly beyond that expected from the parents. This raises the possibility that some heterosis is present or that some loci conditioning fewer leaves were contributed by the inbred lines.

The genes conditioning early flowering were found to be incompletely dominant or additive in gene action. The means of the F1 families always fell between that of the parental inbreds. In A632, the means of the backcrosses were between those of the F1 and the inbreds and the means of the F1 and the F2 were not significantly different from each other. This suggests additive gene action for the loci segregating in A632. In the first Oh43 experiment, the means of the backcrosses were not clearly between the means of parental inbred and the F1. The means of the backcrosses were significantly lower than those of the later flowering parent, but they were also slightly lower and not significantly different from those of the earlier parent. This observation is within the

limits expected due to errors of random sampling if additive gene action is considered. Yet, other types of gene action and heterosis may also play a role.

As can be expected for a quantitative trait, no distinct phenotypic classes could be discerned. The frequency distribution of plants grouped by TLN in the F2 populations segregating the early trait describes a normal distribution. In such a case, the number of segregating loci can only be estimated based on the frequency of occurrence of the most extreme phenotypes. In these experiments, any plants having as many leaves as the earliest plants of the early derivative controls or fewer were considered extreme.

The observed numbers of plants were compare to the expected numbers in a χ^2 goodness-of-fit test. A statistical problem is encountered if the presence of several loci is considered and the expected number of plants with an extreme phenotype becomes too low to employ the χ^2 goodness-of-fit test. In such cases, a Poisson distribution approximation was performed and χ^2 was corrected for low numbers by using Yateses correction factor. All tests were considered significant at $p=0.05$. The fewest TLN of A632E plants was 15 leaves, thus only 2 plants of the total F2 population of 206 fell into the extremely early class. If three loci are segregating, .23 extreme plants can be expected. Both statistical tests show the best goodness-of-fit when 3 loci are assumed to segregate, but the presence of 4 loci cannot be excluded. For 3 loci tested, the χ^2 value corrected with the Yates constant is 0.163, $p>0.2$; and the Poisson distribution approximation (PDA) yields a $p\approx0.74$. Similarly, in the second Oh43 experiment, 4 plants out of 104 F2 plants have the extreme phenotype (9 leaves or fewer). Statistical analysis indicates the presence of 3 loci (expected number of plants 1.675, χ^2 value corrected with the Yates constant is 2.1978, $p>0.1$; PDA yields $p\approx0.36$). In the third Oh43 experiment, 4 extreme plants out of 148 were observed (10 leaves or fewer). This result is consistent with 2 or 3 loci segregating. When 2 loci are tested, the expected number of plants is 9.25, and the χ^2 is 3.178, $p>0.05$. For 3 loci tested, 2.31 extreme plant are expected. The χ^2 value corrected with the Yates constant is 0.619, $p>0.2$, and the PDA gives $p\approx0.44$.

These results indicate that there are 2 to 4 major loci conditioning early flowering in A632E and Oh43E, probably fewer. These estimates of the number of genes are very conservative, given that the calculations are based on the most extreme phenotype of the early derivatives, rather than their mean phenotype. This precaution was taken so that less early genotypes would not be counted in the most extreme class. If the mean TLN of the early derivative is considered the early flowering phenotype, the estimated number of loci is reduced to 1 to 2 major loci.

The above results are consistent with previous reports on the genetic control of early flowering. The estimate that only few genes controlling TLN segregate is consistent with an estimate by Shaver (1976) who suggested that no more than 2 to 3 such loci were introgressed into the early derivatives Wf9E, Hy2E, Oh41E, and 38-11E. He also reported heterosis effects in crosses between the inbred lines and their early derivatives, presumably conditioned by heterozygosity for the transferred chromosome segments. In all lines except Oh41, additive or incompletely dominant gene action was observed. Mohamed (1959) has found 2 to 3 loci conditioning early flowering that segregate in crosses between the inbred lines E.G.102 and E.G.205. Dominant gene action of the loci conditioning early flowering was found in these lines. Hallauer (1965) estimated 2 to 4 genes segregating in crosses between Oh45 and B14, and he observed additive gene action. Giesbrecht (1950, 1955) estimated 4 to 5 factors segregating in crosses between V3 and B14, and 4 factors between Mt42 and Wf9. In both studies, incomplete dominance of the genes conditioning early flowering was reported.

Table 3.7 Number of plants in each TLN group in the parental lines A632, A632E, the hybrid (F1), the F2 generation, and the backcross families segregating the early flowering trait.

TLN	14	15	16	17	18	19	20	≥21	total	mean TLN
A632					2	9	7	1	19	19.3±0.3
F1 x A632			1	8	44	52	19	2	126	18.7±0.2
Hybrid (F1)					7	8			15	18.5±0.3
F1 x A632E	1	3	7	28	28	3	3		73	17.4±0.3
A632E		1	17	10	1				29	16.3±0.3
F2		2	6	45	76	54	17	6†	206	18.2±0.2

†includes one plant with 22 leaves and one with 23 leaves

Table 3.8 Number of plants in each TLN group in the parental lines Oh43, Oh43E, the hybrid (F1), the backcross families, and the F2 populations segregating the early flowering trait trait. Plants for the first two experiments were grown in different summer plantations, and for the third experiment in the greenhouse in the winter.

TLN	8	9	10	11	12	13	14	15	16	17	≥18	total	mean TLN
Oh43								5	9	5	5	24	16.4±0.4
F1 x Oh43						7	12	11	12	5	3†	50	15.1±0.6
Hybrid (F1)							1	6	4			11	15.3±0.3
F1 x Oh43E		2	14	22	23	21	20	12	1			115	12.4±0.7
Oh43E				2	6	10	4	1				23	12.8±0.4
Oh43							3	1	3			7	15.0±0.5
Oh43E		3	5	2								10	9.9±0.3
F2 (summer)	1	3	28	18	24	13	10	4	3			104	11.7±0.7
Oh43							2	2	2			6	15.0±0.5
Hybrid (F1)							7	7				14	14.5±0.2
Oh43E			1	3	4							8	11.4±0.3
F2 (winter)		1	3	12	20	44	45	21	2			148	13.2±0.5

†includes one plant with 19 leaves

3.1.3.2. RFLP analysis

Having established that the early flowering trait in A632E and Oh43E is a quantitative trait likely to be conditioned by a very small number of major loci, the question arises as to whether the early flowering trait in these two lines is conditioned by the same loci. Shaver (1976) suggested that during the introgression of the early flowering trait from Gaspé Flint, the same number of loci was transferred to each early derivative, but that these loci may be different from one early derivative to another. If each line possesses a different array of genes conditioning early flowering, these differences may account for the distinct patterns of vegetative development observed in A632E and Oh43E. The aim of this experiment was to investigate whether A632E and Oh43E share transferred loci that condition early flowering.

Quantitative traits are difficult to study and select for because the identity of individual loci and their contribution to the trait cannot easily be established. In selecting for quantitative traits, breeders have long made use of polymorphic chromosomal markers, such as colour genes or isozyme loci (reviewed in: Dudley,

1993; Edwards et al., 1987). The use of restriction fragment length polymorphisms (RFLPs) has greatly increased the number of available markers and has allowed the development of linkage maps with a high degree of resolution. The mapping of quantitative trait loci using molecular marker has also benefitted from an elaboration of the analytical methods (Lander and Botstein, 1989; van Ooijen, 1992). Linked molecular markers can serve to identify quantitative trait loci as well as to assess the relative effect of each locus (Beckmann and Soller, 1988; Reiter et al., 1991; Stuber et al., 1992). Theory and results so far suggest that marker-assisted evaluation of breeding stock can speed conversions and aid selection (Cowan et al., 1990; Dekkers and Dentine; 1991; Dudley; 1993).

In maize, the time to reproductive maturity and several associated traits, such as days to pollen shed, plant height, or TLN, have been genetically dissected using isozyme and molecular markers (Abler et al., 1991; Koester et al., 1993; Phillips et al., 1992; Stuber et al., 1992). These studies typically analyse segregation of traits and markers in F2 or F3 families generated from crosses between unrelated inbred lines or between inbreds and near-isogenic, early-flowering lines, or in families derived from backcrosses between inbred lines. In the context of this study, a complete genetic dissection of reproductive maturity in A632;A632E and Oh43;Oh43E using molecular markers was not feasible. Instead, it was decided to investigate whether markers that have been associated with early flowering in other studies are also associated with early flowering in the lines used in the present study.

Depending on the trait that is used to assess reproductive maturity (e.g. days to anthesis, days to silking, plant height, TLN, grain moisture) and on the inbred lines studied, previous studies have identified several chromosome regions that are correlated with aspects of the early flowering phenotype (table 3.9). One region on the long arm of chromosome 8 near RFLP markers UMC12 and UMC89 is particularly interesting because it is consistently associated with early flowering in a variety of genetically diverse inbreds. Additionally, this region has a large effect on the early flowering phenotype. Koester et al. (1993) have studied the early flowering trait in two inbreds (Sc76, B73) and their early derivatives (Nc264, B73G) both of which were developed by introgressing the early flowering trait from Gaspé Flint. The strongest and most consistent associations of days to anthesis, days to silking, plant height, and TLN are with markers on chromosomes 1, 8, and 10. Stuber et al. (1992) reported markers on chromosome 8 to be associated with plant height, days to tassel, and grain moisture in crosses between B73 and Mo17. Association of days to silking and plant height with chromosome 8 has also been reported by Abler (1991). Phillips et al. (1992) reported

that in crosses between A662 and B73, days to pollen shed, days to silking, plant height, and node number were all associated with UMC12. This marker explained the largest portion of the phenotypic variance of each trait. A second marker on chromosome 9 was associated with days to pollen shed, days to silking, and node number. Moreover, days to pollen shed and days to silking were both associated with two markers on chromosomes 3 and 5, whereas two other markers (chromosomes 4 and 5) were associated with internode length and plant height. This indicates that each of these traits is regulated by several loci, and suggests common components of the traits: for example, of the three markers that are associated with plant height, one is also associated with node number and two with internode length. Only the region marked by UMC12 was found to be associated with all traits. Finally, UMC12 was also associated with days to pollen shed in 4 other lines (A679, A680, A681, N28E), but was not polymorphic in Oh43E (Phillips et al., 1992).

In order to examine whether any of these chromosome regions, particularly the region near UMC12, are associated with TLN in A632;A632E and Oh43;Oh43E, these lines were investigated for polymorphisms with RFLP markers, and plants from F2 and backcross populations studied for segregation of the polymorphisms. Since selective genotyping of the phenotypic extremes is effective in detecting important marker-trait associations (Koester et al., 1993), only the extremes of the segregating populations were sampled.

Several of the RFPL markers that have been associated with early flowering in other studies were tested in A632;A632E and Oh43;Oh43E (table 3.9). The markers that were chosen were those suggested by the results of Koester et al. (1993) or Phillips et al. (1992): BNL5.59 (1L), UMC119 (1L), UMC32 (3S), UMC39 (3L), NPI419 (6L), UMC12 (8L), NPI445 (10L). With nine different restriction enzyme digests, only UMC12 showed a polymorphism between the parental lines in the major band. In each of the three restriction enzyme-RFLP marker combinations that showed a polymorphism between A632;A632E and between Oh43;Oh43E, the restriction fragments in A632E and Oh43E were of the same size, whereas the fragments in A632 and Oh43 were of different sizes (figure 3.26). This indicates that this chromosome region is altered in the same way in A632E and Oh43E after introgression of the early flowering trait, suggesting that both lines now carry genetic material from Gaspé Flint in this region. A comparison between the sizes of the restriction fragments of all genotypes would be needed to ascertain that the polymorphic region on chromosome 8 was indeed introgressed from Gaspé Flint in both A632E and Oh43E, but no Gaspé Flint DNA was available at the time of the experiment.

When the plants with an extreme phenotype from the segregating families were genotyped with UMC12, a significant association between this marker and the TLN was detected. Segregation of the polymorphism was examined in digestions with EcoRI endonuclease (illustrated in figure 3.27) and with Kpn1 endonuclease. In the F2 progeny of A632/A632E plants, only 3 out of 22 chromosomes in the phenotypically late class were recombinant for UMC12 and only 2 out of 20 chromosomes in the early class were recombinant for this marker. In the case of Oh43, where backcross families were used for this experiment, none of the 8 late plants examined was recombinant for UMC12 (0 out of 8 chromosomes) and only 1 out of 13 early plants was recombinant for UMC12 (1 out of 13 chromosomes). The analysis was performed by calculating the exact binomial and using a one tailed test for each phenotypic class. In each class, the association in A632 was significant at $p<0.001$ (extremely late plants $p=0.000359$, extremely early plants $p=0.000382$) and in Oh43 at $p<0.01$ (extremely late plants $p=0.00390$, extremely early plants $p=0.00171$).

There is evidence suggesting that a major locus regulating flowering time and TLN is located in the chromosome region near UMC12 (table 3.9, Abler et al., 1991; Koester et al., 1993; Phillips et al., 1992; Stuber et al., 1992). The result that both early derivatives, A632E and Oh43E, are polymorphic in this region when compared to their inbred lines, but not for any of the other RFLP markers tested, indicates that this region is also the major factor determining early flowering and low TLN in the early derivatives. This observation would suggest that A632E and Oh43E share a major locus regulating flowering time, and probably also closely linked chromosome regions. Thus, the difference in the pattern of vegetative development between the lines is likely conditioned by factors residing elsewhere in the genome. However, these results are not conclusive: it is conceivable apart from the locus on 8L, there are different "early flowering" genes present in the two early derivatives, or that parts of the Gaspé Flint genome, linked to the "early flowering" locus on chromosome 8L, were retained in one early derivative and not the other. Alternatively, it is possible that the inbred backgrounds themselves carry or lack a modifying factor.

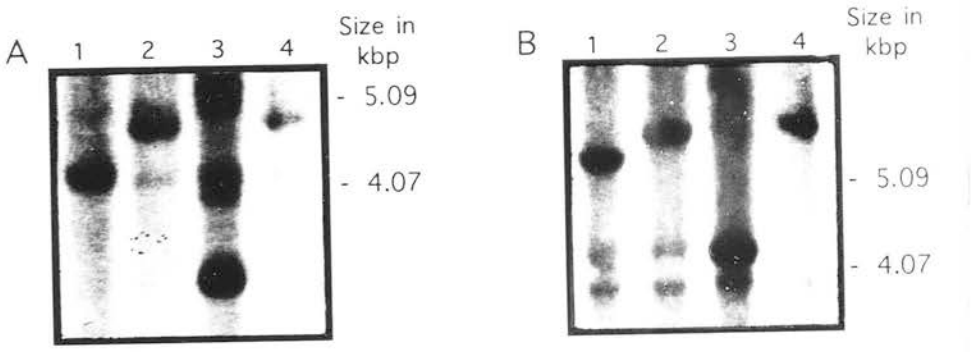

Figure 3.26 Southern blot of A632 (lane1), A632E (lane2), Oh43 (lane3), and Oh43E DNA (lane4) digested with A) Xba1 and B) Kpn1 endonucleases and probed with UMC12.

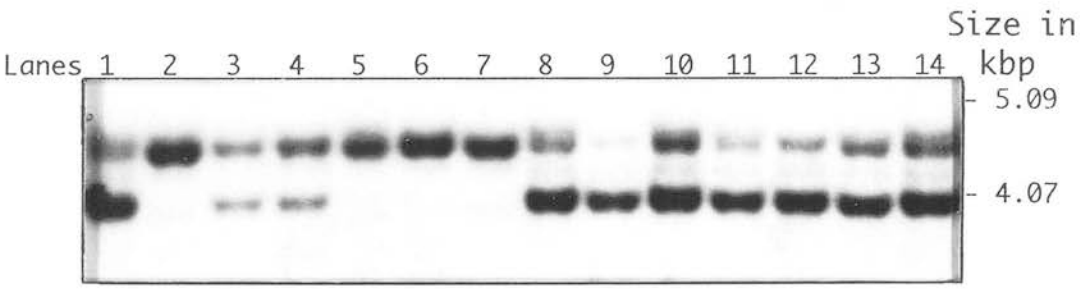

Figure 3.27 Southern blot of A632 (lane1), A632E (lane2), phenotypically early flowering plants (lanes 3 to 7), and phenotypically wild type plants (lanes 8 to 14), digested with EcoRI and probed with UMC12.

Table 3.9 List of chromosome regions determined to be significant for the expression of time to flowering, plant height and TLN in maize according to results in the cited references. The chromosome regions tested in this study are indicated (#).

		Abler et al., 1991	Bubeck, 1991[1]	Koester et al., 1993	Phillips et al., 1992	Stuber et al., 1992
1S	DAN			***		***[2]
1L #	DAN			***	**	
	SLK				**	
	PHT			***		***
	TLN			***		
3S #	DAN				*	
3L #	DAN					***
	SLK				**	
	PHT					***
	TLN			*		
4L	PHT				***	***
5L	DAN				**	
	SLK				**	
	PHT				***	
6L #	DAN			*		
	PHT			*		
8L #	DAN		**	***	***	***
	SLK	***	*		***	
	PHT	***		***	***	***
	TLN			***	***	
9L	DAN			*	*	
	SLK	***			*	
	PHT	***		*	*	***
	TLN				**	
10L #	DAN			***		
	SLK	**				
	PHT	***		**		***
	TLN			*		

Chromosome regions tested in this study.

*,**,*** Significant at $p<0.05$, 0.01, 0.001, respectively.

[1]as cited in Koester et at., 1993.

[2]Significance given in LOD score, all scores higher than 3.5.

DAN: Days to anthesis, SLK: Days to silking, PHT: Plant height, TLN: Total leaf no.

3.1.4 Does the genetic background determine the pattern of shoot development?

3.1.4.1. Additional inbred lines and early derivatives

The observed differences in vegetative development between A632; A632E and Oh43; Oh43E can be conditioned either by a factor that has been introgressed from Gaspé Flint (early flowering trait or a linked modifier) or by an unlinked modifier that is present in the genetic background of the inbreds. In the previous section 3.1.3, it was shown that both A632E and Oh43E have a polymorphism on chromosome 8L, a region frequently associated with a major factor regulating flowering time. Having been unable to resolve any other polymorphisms, attention was shifted to the question if the genetic background of the inbreds conditions the different patterns of shoot development. It should be possible to shed light on this problem by studying how common each pattern of vegetative development is in other early derivatives. In order to investigate this question vegetative development in several inbreds and their near-isogenic, early flowering lines was assessed.

A number of lines that differ in the pedigree of the inbred line and the direct origin of the early flowering trait were chosen for this experiment (table 2.1). Some inbred lines are akin in their parentage, as suggested by the material from which the inbreds have been generated (table 2.2). In some cases, the early derivatives were derived directly from Gaspé Flint in a series of crosses to the recurrent inbred parent (e.g. B73G, C123E, Mo17E, N28E, Nc264, Oh43E). In other lines the early flowering trait was first introduced into another inbred line, and then converged to the recipient inbred line. For example, A635E and H100E are both derived from A632E, which along with other lines derived its early flowering trait from Wf9E, which in turn received it from Gaspé Flint (Shaver, personal communication).

Seven inbreds and their respective early derivatives were compared (Table 3.10). Among the independently derived lines (A619E, B73G, C123E, Mo17E, N28E, Nc264) each type of pattern of vegetative development was observed in about half the cases: the transition from early vegetative to late vegetative growth was independent of reproductive maturity in C123E, Mo17E, and N28E, whereas it took place earlier in A619E, B73G and Nc264. If the pattern of vegetative development is determined by an introgressed modifier, this result would indicate that the linkage between the early flowering trait and the modifier is not very close, since the two can be unlinked within a

small number of backcrosses in half the lines. This would be contrary to the observation that the early flowering trait and the pattern of vegetative development do not segregate in families segregating for the early flowering trait (section 3. 1.2.1). If a modifier is part of the inbred genome, this would indicate that both patterns of vegetative development are frequent.

In all early derivatives tested here, the effect of the early flowering trait on reproductive maturity was similar: the TLN was reduced and the ear was placed at a lower position. The results indicate that related inbred lines also have a similar pattern of vegetative development. According to the published pedigrees, C123 and Mo17 are related, N28 is related to H100, A619 is related to Oh43, and A635 is related to A632 (table 2.2). B73 and Sc76 are not related to any of the studied lines. Overall, there was no effect of the early reproductive maturity on the vegetative phase change in C123E, Mo17E, N28E, and H100E. In those lines, only two traits were found at a significantly lower position in the early derivative than in the inbred line: leaf hairs in C123E and adventitious roots in N28E. A619E showed a pattern of vegetative development similar to that of Oh43E, as did B73G and Nc264. The notable exception was A635, in which the vegetative phase change took place at a lower leaf position in the presence of the early flowering trait. In the related line A632; A632E, the transition from early vegetative to late vegetative growth is not linked to reproductive maturity. This observation is particularly interesting because the early flowering trait in A635E was introgressed from A632E. H100E also received the early flowering trait from A632E, and its pattern of vegetative development is like that of A632E. In contrast to A635E, this is consistent with the inbred pedigree of H100 and with the source of the early flowering trait in H100E.

In summary, the results of this experiment, although not conclusive, suggest that the type of vegetative pattern observed in the early derivatives is conditioned by modifiers in the inbred background, and not by a gene that was introgressed from Gaspé Flint.

Table 3.10 Expression of vegetative and reproductive traits in several inbreds and near-isogenic, early-flowering lines. For the pedigree of the standard inbreds and the sources of the early flowering trait, see table 2.2 (N is the number of plants scored. All errors given as ±2 s.e.m.).

Inbred back-ground	Last node with prop roots	First partially glossy leaf	First leaf with hairs	Last partially glaucous leaf	Ear placement node	Total number of leaves	N
A619	8.4±0.5	7.2±0.2	4.1±0.4	11.2±0.2	10.9±0.2	16.3±0.4	12
A619E	**7.7±0.4**[a]	**6.7±0.4**[a]	**3.5±0.4**[a]	**9.0±0.0**[c]	**9.3±0.4**[c]	**14.5±0.5**[c]	6
A635	9.5±0.3	8.3±0.3	5.0±0.3	11.0±0.7	14.7±0.6	20.1±0.7	10
A635E	9.0±0.8	**7.1±0.3**[c]	**4.6±0.4**[a]	**9.7±0.9**[a]	**11.9±0.9**[c]	**17.7±1.3**[b]	7
B73	11.5±0.4	6.5±0.4	6.5±0.4	7.2±0.3	15.7±0.4	20.7±0.4	6
B73G	**9.9±0.5**[c]	**5.9±0.3**[a]	**5.6±0.4**[b]	**6.4±0.4**[b]	**12.1±0.3**[c]	**17.1±0.3**[c]	7
C123	7.3±0.2	5.0±0.0	4.5±0.3	6.0±0.0	11.5±0.3	15.7±0.3	15
C123E	7.1±0.2	5.0±0.0	**4.0±0.3**[a]	6.1±0.2	**10.1±0.2**[c]	**14.2±0.3**[c]	10
H100	11.3±0.5	7.0±0.0	6.4±0.3	9.0±0.2	15.1±0.4	21.9±1.0	13
H100E	10.7±0.7	7.0±0.0	6.3±0.7	8.7±0.7	**14.0±0.0**[a]	**19.3±0.7**[a]	3
Mo17	5.2±0.4	5.0±0.0	5.0±0.0	7.0±0.0	10.2±0.4	15.0±0.0	5
Mo17E	6.0±0.0*	5.0±0.0	5.0±0.0	7.0±0.0	**8.8±0.4**[c]	**13.8±0.4**[c]	5
N28	13.7±0.4	6.7±0.4	5.1±0.3	7.7±0.4	16.4±0.4	22.1±0.5	7
N28E	**11.6±0.4**[c]	6.9±0.3	5.3.±0.4	7.6±0.4	**13.7±0.4**[c]	**19.4±0.4**[c]	7
Sc76	10.0±0.4	7.1±0.2	3.4±0.3	9.0±0.3	17.9±0.5	23.4±0.5	10
Nc264	**9.1±0.3**[c]	**6.6±0.3**[b]	3.6±0.3*	**7.9±0.3**[c]	**13.6±0.3**[c]	**18.4±0.5**[c]	14

a: significantly smaller than standard inbred at $p<0.05$ (1-tailed *t* test)

b: significantly smaller than standard inbred at $p<0.01$ (1-tailed *t* test)

c: significantly smaller than standard inbred at $p<0.001$ (1-tailed *t* test)

*: Value is greater in the early flowering line than in the standard inbred, and was not tested with the 1-tailed *t* test.

3.1.4.2. Environmentally conditioned early flowering

Having established in section 3.1.2 that the presence of a genetically conditioned early flowering trait can modify vegetative development, the experiments in this section focus on whether the different response patterns of vegetative development between the two early derivatives are due to modifiers in the inbred background, or to the introgression of different genes from Gaspé Flint. This issue is studied by using photoperiodic induction to induce early flowering in the inbred lines, and studying the vegetative development in the photoinduced plants.

One of the best known environmental factors that can induce flowering in plants is day-length. The type of photoperiodic requirements for floral induction is species-specific. Photoperiodic induction in plants is thought to be a stepwise process. Day-length is most efficiently perceived by the leaves, generating a graft-transmissible floral signal of unknown nature (Bernier et al., 1981a). The sensitivity of the leaves depends on several factors, including the physiological age of the leaf and the position of the leaf on the stem. The floral signal is translocated from the leaves to the meristem, which becomes florally determined (Bernier et al., 1981a; Bernier, 1988; Evans, 1960; McDaniel et al., 1992).

Maize is a short day (SD) plant; most varieties grown in temperate climates nowadays are quantitative SD plants. These cultivars will flower under the long day (LD) conditions of the northern summer, yet the plants can still be induced to flower earlier when grown under short day conditions (Russel and Stuber, 1983). A few lines, including Oh43 and Gaspé Flint, are thought to be day-neutral, which means that their flowering time is largely independent of photoperiodic conditions (Francis et al., 1969; Hesket et al., 1969; Hunter et al., 1974; Russel and Stuber, 1983). In many species different environmental stimuli can interact to cause floral induction. In maize, there is, for instance, a significant interaction between day-length and temperature. Cool temperatures enhance the effectiveness of SD conditions (Coligado et al., 1975; Hunter et al., 1974, 1977). Photoinduction causes a reduction in leaf number similar to that described for the genetically conditioned early flowering. The advantage of environmentally induced early flowering is that it can be used to determine the effects of early flowering in genotypes lacking the early flowering trait. This provides a means to assess the effect of early flowering on vegetative development in a standard inbred line without the need to genetically alter the lines. Additionally, differences in the day-length requirements of A632, A632E, Oh43, and Oh43E may point to genotypic differences with regard to genes conditioning early flowering. Such differences could

potentially account for the two observed patterns of vegetative development. To investigate if the inbred background determines if vegetative phase change and reproductive maturity are coordinately regulated, plants of all four genotypes were grown under continuous SD conditions between 18 to 38 days after planting (DAP) (A632, A632E), 8 to 38 DAP (Oh43), and 8 to 23 DAP (Oh43E). These timing of these treatments was designed to ensure that photoinduction would occur in all SD sensitive lines. Oh43E was not used in the later SD treatments because tassel initiation is completed much earlier. In Oh43E, this usually occurs when 2 ligules and 4 leaves are visible. It is shown that all lines can be photoinduced and that the different patterns of vegetative development in A632E and Oh43E are specific to the inbred background.

Compared with the control plants grown under LD conditions, all plants grown under SD had significantly fewer leaves (*t* and *U* tests) (table 3.11). The TLN was reduced by SD conditions by an average of one (Oh43E) to three leaves (A632). All lines also had a significantly lower ear placement node under SD conditions, and as in the early derivatives, the ear placement node is proportionately reduced relative to the TLN (section 3.1.2.1). Althought the magnitude of the response varied between the lines and was smaller in the early derivatives than the inbred lines, all lines tested here respond to SD conditions. This indicates that while there are some genetic differences in the regulation of flowering between the lines, there is no qualitative change in the photoperiodic requirements of the early derivatives.

Previous reports suggest that Oh43 is a day-neutral line, which would make its photoperiodic requirements qualitatively different from the ones of A632 (Francis et al., 1969; Russel and Stuber, 1983). The present results demonstrate that Oh43 does not differ in its photoperiodic requirements from A632. It is possible that previous research failed to detect the SD requirements of Oh43 because growing conditions were not controlled sufficiently, and the response to photoinduction was eliminated. In fact, Russel and Stuber (1983) detected a lower TLN in photoinduced Oh43 plants, but still classified this line as day-neutral.

It is evident from table 3.11 that the pattern of vegetative development in photoinduced inbred line plants is the same as the one found when the early flowering trait is present. There was no change in the number of leaves expressing early vegetative epicuticular leaf wax in A632 plants grown under LD or SD. A632 produced 10.6±0.4 partially glaucous leaves under LD conditions, and 10.6±0.5 partially glaucous leaves under SD. Similarly, the expression of wax was unaffected by day-length in A632E (9.3±0.3 leaves under both LD and SD). The loss of

epicuticular wax occurred at an unexpectedly low leaf position in A632E. The reason for this is unclear, yet the phenomenon is considered unimportant in the present context, because there is no difference between A632E plants grown under LD or SD conditions. Loss of the early vegetative epicuticular wax takes place at a significantly lower node under SD conditions than under LD in Oh43 and Oh43E (table 3.11). Photoinduced early flowering thus causes the same type of changes in vegetative development in A632 and Oh43 as genetically induced early flowering. This strongly suggests that the genetic make-up of the background determines at least in part if vegetative phase change and reproductive maturity are coordinately regulated.

The experimental design employed here does not allow for a distinction of photoperiodic effects and effects caused by differences in the total amount of light received the the LD and SD grown plants. The SD treated plants can thus be expected to have received less light than the plants treated with LD. This problem could have been prevented by using SD conditions combined with night interruptions rather than LD treatments. Unfortunately, this was technically impossible with the available facilities. However, the questions in this study are whether and how the different genotypes respond to a given set of environmental conditions. The exact nature of the floral induction is not under investigation. Thus, differences in total light exposure between LD and SD treatments in this and the following experiments are just part of the environmental conditions that cause early flowering.

Table 3.11 The effect of continuous SD conditions on tassel initiation, ear placement, and expression of epicuticular wax in A632, A632E, Oh43 and Oh43E plants. All errors given as ±2 s.e.m.

Genotype	Treatment (DAP)	No. of emerged ligules*	No. of emerged leaves*	Last partially glaucous leaf	Ear placement node	Total leaf number
A632	LD			10.6±0.4	12.2±0.3	18.3±0.3
	SD (8-38)	0-5	2-7	10.6±0.5	**10.3±0.3**^{c,3}	**15.3±0.6**^{c,3}
A632E	LD			9.3±0.3	8.4±0.4	14.1±0.4
	SD (8-38)	0-5	2-8	9.3±0.3	**7.4±0.4**^{c,2}	**12.1±0.3**^{c,3}
Oh43	LD			9.8±0.3	9.3±0.3	14.0±0.4
	SD (8-38)	0-4	2-6	**9.1±0.3**^{b,1}	**7.5±0.3**^{c,3}	**11.7±0.3**^{c,3}
Oh43E	LD			7.5±0.4	5.6±0.5	9.8±0.7
	SD (8-38)	0-4	1.5-6	**6.9±0.2**^{b,1}	**5.1±0.2**^{a}	**8.7±0.3**^{b,1}

a, 1: significantly smaller than LD control at $p<0.05$ (1-tailed *t* test, 1-tailed *U* test)

b, 2: significantly smaller than LD control at $p<0.01$ (1-tailed *t* test, 1-tailed *U* test)

c, 3: significantly smaller than LD control at $p<0.001$ (1-tailed *t* test, 1-tailed *U* test)

a, b, c refer to results from *t* test, 1, 2, 3 refer to results from *U* test

*: before and after SD treatment

3.1.5 Placing the early flowering trait in a developmental pathway

3.1.5.1. *Teopod* and the early flowering trait

In the previous sections, it was shown that both the presence of the early flowering trait or photoinduction, can modify when the vegetative phase change occurs, depending on the inbred line. This suggests that the early flowering trait may be part of an independently regulated reproductive phase of development, and that the effect on the expression of the vegetative phase of development may be achieved indirectly via (a) modifier(s) present in the inbred background. If this is true, the early flowering trait should not be epistatic to genes regulating the expression of the vegetative phases of development. In order to test this hypothesis the expression of the early flowering trait and of mutations modifying the length of the early vegetative phase (*Teopod1* (*Tp1*) and *Teopod2* (*Tp2*)) was assessed in families segregating both traits.

Tp1 and *Tp2* are non-cell autonomous, gain-of function mutations that condition a prolonged expression of the early vegetative phase of development. Phytomers in late vegetative positions express early vegetative traits and vegetative structures are formed in the ear and tassel (Dudley and Poethig, 1991, Lindstrom, 1925; Poethig, 1988a, b; Weatherwax, 1929). The expression of all known early vegetative traits is modified in Tp mutants, suggesting that these genes play a role in the regulation of phase change. The mutations cause an increase in TLN, but no delay in flowering time. If the prolonged expression of the early vegetative phase of development does not interfere with the expression of the reproductive phase of development, as suggested by the results reported by Bassiri et al. (1992), the *Tp* mutations should not interfere with the expression of the early flowering trait. Thus, while the Tp plants will be expected to have a higher TLN than wild type, the range of TLN among wild type and Tp plants in a F2 family segregating the early flowering trait should be the same. Alternatively, if the transition to the reproductive phase of development is dependent on the termination of the early vegetative phase of development, the *Tp* mutations will suppress the expression of the early flowering trait, and the range in TLN will be smaller in the Tp plants than in the wild type plants. The effect of the early flowering trait on the Tp phenotype may depend on the aspects of phenotype that is considered. Results in *Tp2* and another mutation that causes a similar phenotype, *Corngrass1* (*Cg1*), suggest that environmental conditions that favour early flowering, that is, short photoperiods and cool temperatures, can partially normalise the mutant phenotype of the tassel (Bassiri et

al., 1992; Ritchings and Tracy, 1989). If this is a result of the increased ability to flower, a normalised tassel may also be expected in Tp2 plants that carry the early flowering trait.

The results for both inbred backgrounds (A632 and Oh43) and both *Tp* mutations show that *Tp* and the early flowering trait do not interfere with each other's expression (tables 3.12 and 3.13, figures 3.27, 3.28). In all families, Tp plants were easily distinguishable from their wild type sibs by the narrow leaves, increased number of nodes with adventitious roots, increased number of partially glaucous leaves, increased TLN, and the vegetatively transformed tassel; all these characters are typical of the Tp phenotype (Poethig, 1988a). Examples of the phenotype of Tp plants in inbred line and early derivative families are shown in figure 3.27 and 3.28.

The tassel morphology of Tp2 plants in A632 (*Tp2-E2*) or Oh43 (*Tp2*) families segregating the early flowering trait was not changed in the presence of the early flowering trait, indicating that increased potential to flower earlier does not alter the tassel morphology (table 3.14). This was not anticipated in the light of previous reports indicating that tassels of wild type plants that were environmentally induced to flower early tend to be smaller and bear fewer staminate spiklets (Hanway and Ritchie, 1985; Ritchings and Tracy, 1989). On the other hand, tassels of two of the dominant Tp-like mutations, *Tp2* and *Cg1*, produce more staminate flowers when environmentally induced by SD and cool temperatures (Bassiri et al., 1992; Ritchings and Tracy, 1989). Given that such normalisation of the tassel did not occur in *Tp2* in the presence of the early flowering trait, it is possible that the reported partial reversion in photoinduced Tp2 and Cg1 plants is not a result of changes in the reproductive ability.

Two results indicated that the early flowering trait was also expressed in a normal fashion (tables 3.12 and 3.13). Firstly, the mean TLN of Tp plants from F1 families (all plants heterozygous for the early flowering trait (IL/ED) and half the plants heterozygous for *Tp*) and from F2 families segregating both traits was similar, and lower than the corresponding TLN of Tp plants in the inbred lines (A632 or Oh43). The mean TLN of Tp plants from F1 families was also higher than the TLN of plants from early derivative-backcrossed families in all cases except in Tp2-E2 plants. This discrepancy is probably caused by the limited number of backcrosses to the early derivative: plants heterozygous for the early flowering trait are expected to segregate in a 1:1 ratio in this family. Secondly, the range of TLN in Tp plants from the F2 families was in all cases larger than the range of TLN in the wild type sibs. While a small

amount of heterosis cannot be excluded in these near-isogenic lines, this increase in range is more likely a result of the variability in TLN inherent to the Tp phenotype. This can best be confirmed in families segregating only *Tp*. The Tp plants always cover a greater range of TLN then their wild type sibs, and this increase is similar to the one seen in the F2 families.

In conclusion, the results indicate that the early flowering trait and Tp phenotypes are both expressed in a normal way in plants carrying both traits, suggesting that the early flowering trait and the *Tp* mutations act in different developmental pathways. This is consistent with the findings that the early flowering trait is part of a developmental pathway that regulates the reproductive phase of development, whereas the *Tp* genes are thought to regulate vegetative development (Bassiri et al., 1992; Poethig, 1988a).

Table 3.12 Expression of early flowering and *Tp1* in A632 and Oh43 recorded as mean TLN and the range of TLN (N is the number of plants scored. All errors are given as ±2 s.e.m.).

	wild type (+/+)			Teopod (*Tp1*/+)		
Genetic background	TLN	Range in TLN	N	Total number of nodes	Range in node number	N
A632	20.6±0.5	19-21	8	23.7±0.8	22-26	11
A632/A632E	18.6±0.4	18-20	11	20.6±0.6	19-21	8
A632E	17.8±0.6	16-20	14	21.0±1.5	18-22	6
F2*	19.2±0.3	16-21	52	21.8±0.5	19-27	53
F2	18.3±0.3	16-20	43	20.8±0.5	17-25	59
Oh43	14.9±0.4	14-16	13	17.1±0.3	15-19	44
O43/Oh43E	14.3±0.4	14-15	6	16.2±0.5	15-18	13
Oh43E	11.4±0.5	10-12	8	13.8±0.4	12-15	12
F2	13.2±0.2	10-15	60	15.2±0.2	12-18	62
F2	12.9±0.4	9-15	51	14.8±0.4	10-18	84
F2	13.8±0.3	12-16	32	16.0±0.2	13-20	62

*: F2 families were generated by crossing wild type and Tp siblings in families produced by the following cross: ED x *Tp*/+; IL

Table 3.13 Expression of early flowering and *Tp2-E2* in A632 and of *Tp2* in Oh43 recorded as mean TLN and the range of TLN (N is the number of plants scored. All errors are given as ±2 s.e.m.).

	wild type (+/+)			Teopod (*Tp2*/+)		
Genetic background	TLN	Range in TLN	N	Total no. of nodes	Range in node number	N
A632	20.0±0.4	19-21	7	22.1±0.6	21-23	9
A632/A632E	18.0±0.3	17-19	11	20.0±0.4	19-21	7
A632E	16.25±0.5	16-18	4	18.0±0.8	17-19	4
F2	17.8±0.4	15-21	47	19.9±0.4	17-23	49
Oh43	17.8±0.4	17-19	13	22.1±1.2	18-23	8
O43/Oh43E	14.6±0.5	13-15	9	20.9±1.0	18-22	8
Oh43E	12.0±0.5	10-14	19	16.3±1.0	14-18	7
F2	13.5±0.3	10-17	72	18.7±0.6	13-23	76

Table 3.14 Effect of early flowering on the tassel morphology of Tp2 plants in F2 families segregating *Tp2-E2* and the early flowering trait in A632 and *Tp2* and the early flowering trait in Oh43 (N is the number of plants scored. All errors are given as ±2 s.e.m.).

Total number of nodes	Tassel score (Tp2-E2; A632)	N	Tassel score (Tp2; Oh43)	N
13	-	-	7.0±0.0	4
14	-	-	7.0±0.0	6
15	-	-	5.0±4.0	2
16	-	-	7.0	1
17	3.3±0.7	3	7.0±0.0	3
18	3.7±0.7	3	7.0±0.9	9
19	3.3±0.4	14	7.4±0.4	20
20	3.3±0.3	14	6.8±0.6	14
21	3.5±0.3	10	6.9±1.0	11
22	3.0±0.0	3	6.3±1.3	3
23	3.5±1.0	2	7.7±0.7	3

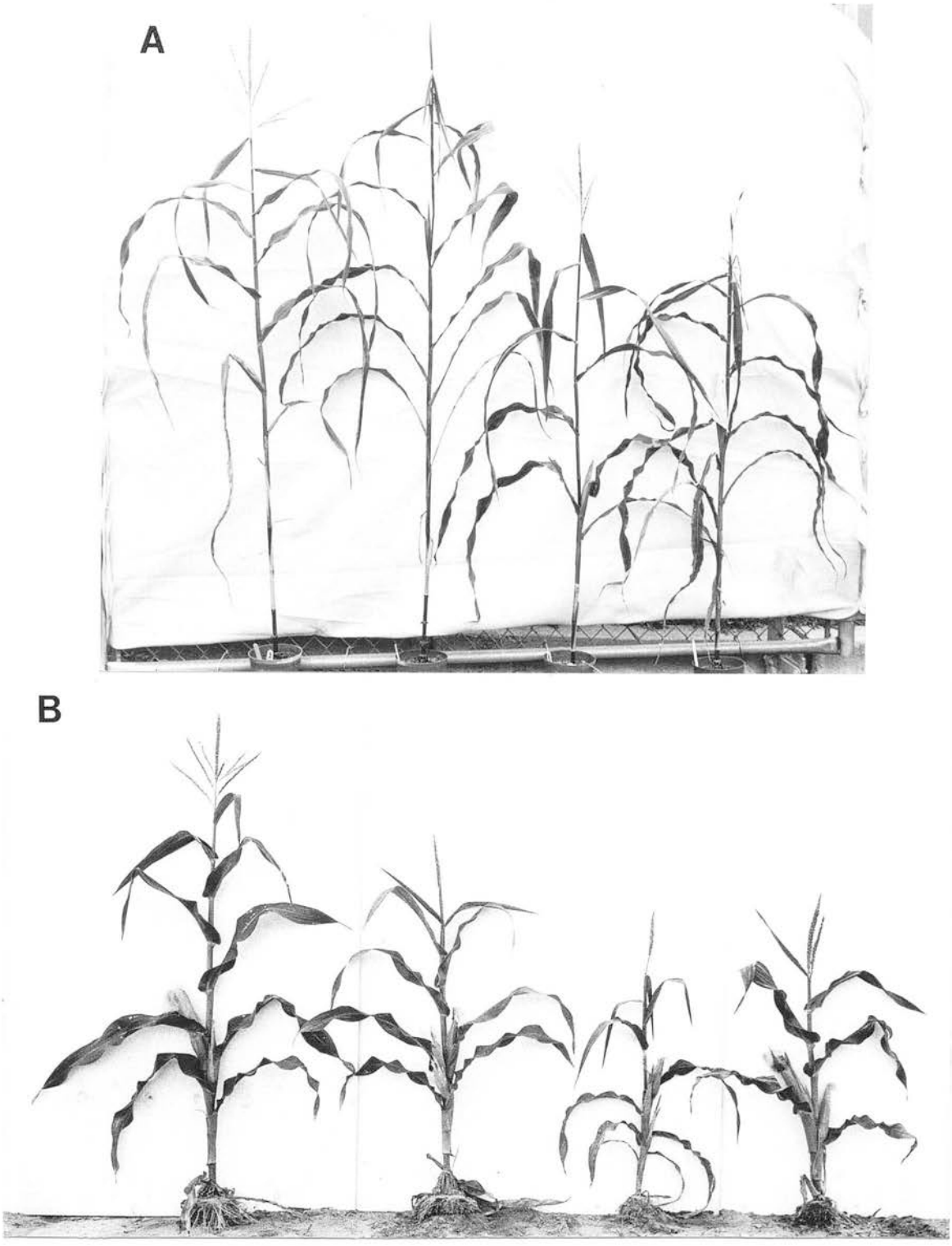

Figure 3.28 Phenotype of wild type and Tp1 plants in the absence or presence of the early flowering trait. (A, from left to right) A632 -- *Tp1*/+; A632 -- A632E -- *Tp1*/+; A632E(2) and (B) Oh43 -- *Tp1*/+; Oh43 -- Oh43E -- *Tp1*/+; Oh43E(3).

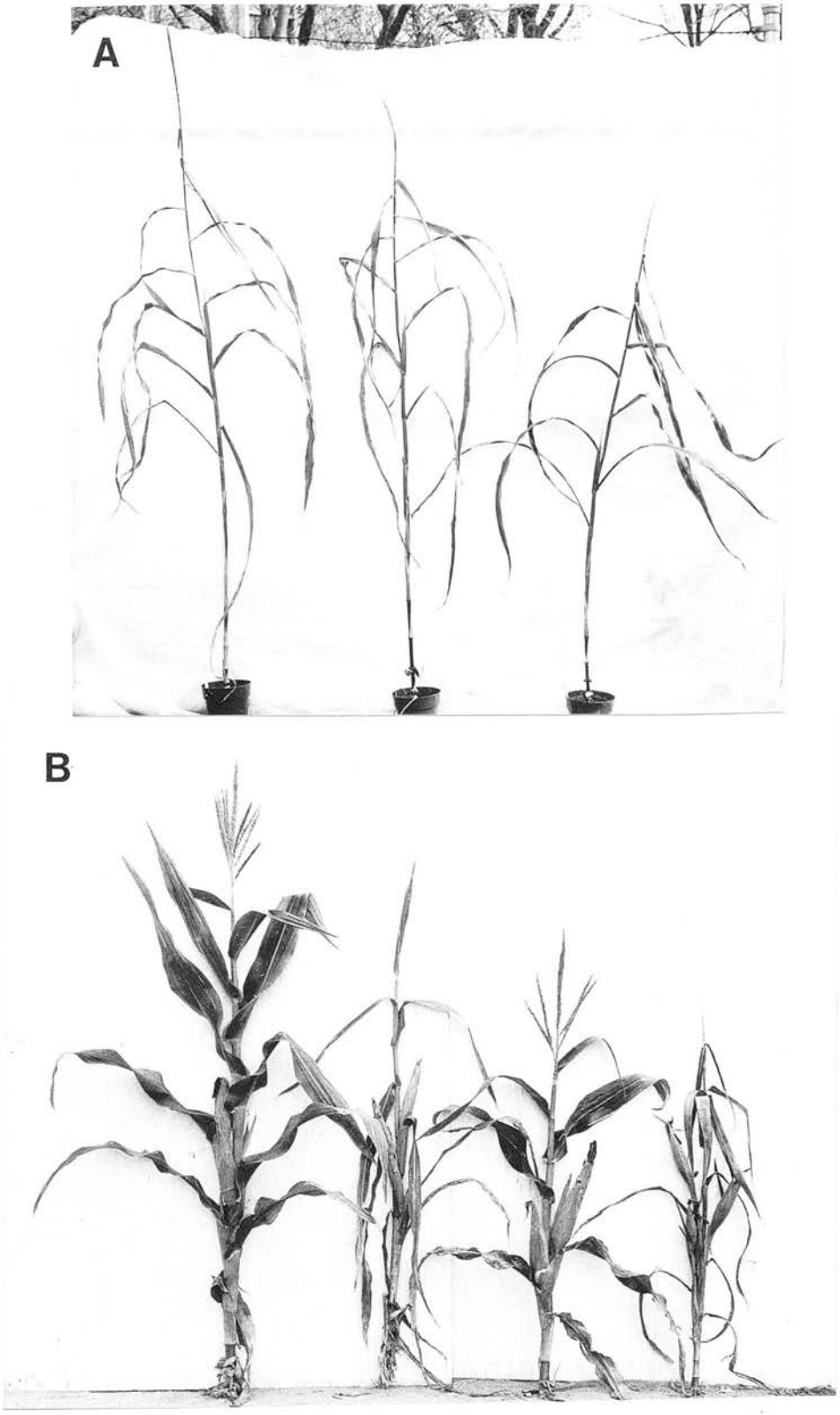

Figure 3.29 Phenotype of wild type and Tp2 plants in the absence or presence of the early flowering trait. (A, from left to right) *Tp2*/+; A632 -- *Tp2*/+; A632/A632E -- *Tp2*/+; A632E(2) and (B) Oh43 -- *Tp2*/+; Oh43 --*Tp2*/+; Oh43E(4) -- Oh43E.

3.1.5.2 The onset of the reproductive phase of development

Given that the early flowering trait and the ability to be photoinduced can be assumed to play a role in the developmental pathway that regulates flowering, it should be possible to place these two traits relative to each other in that pathway. The ability to perceive and respond to the photoinduction marks an early part of the reproductive phase of development. If the early flowering trait act prior to the photoperiodic requirement in the regulation of flowering, the photoperiodically sensitive period should be expressed earlier. This possibility is raised by result reported earlier: the changes in leaf shape reported in section 3.1.1.4 indicate that the early flowering trait is active early in shoot development. The activity of the early flowering trait is detectable through its effect on leaf shape no later than leaf 7 in A632E and leaf 5 in Oh43E. In order to determine when plants are able to perceive and respond to photoinduction, and to test whether this ability is expressed at an earlier time in the presence of the early flowering trait, plants of the four genotypes, A632, A632E, Oh43, and Oh43E, were grown under SD conditions at different times of shoot development. The plants used for the experiment described in section 3.1.4.2 were used as controls in this experiment.

Figure 3.30 summarises the results of the experiments grown in winter 1992/3. In the first experiment figure 3.30A), the strongest photoinduction in A632 occurred at 28 to 33 DAP (3 to 4 entirely emerged leaves and 6 to 7 leaves visible) (see appendix for details). A632 plants photinduced during this period produced significantly few leaves and formed the ear at a significantly lower position (appendix). Some induction was also detected in plants exposed to SD conditions at 23 to 28 and at 33 to 38 DAP, but this is reflected only in a change in TLN. In the same experiment, a small but significant reduction in TLN could be seen in A632E when exposed to SD conditions at 18 to 23 DAP (2 to 3 entirely emerged leaves and 4 to 5 leaves visible). This experiment suggests that A632E is sensitive to photoinduction between 18 and 23 DAP. This period is earlier than in A632, where it occured at around 28 to 33 DAP.

Oh43 plants were found to be sensitive to SD conditions at 23 to 28 DAP (2 to 3 entirely emerged leaves and 4.5 to 5.5 leaves visible) and at 28 to 33 DAP (3 to 4 entirely emerged leaves and 5.5 to 6.5 leaves visible) (figure 3.30B, appendix). In Oh43E, a significant reduction in TLN occurred in plants exposed to SD conditions at 8 to 13 DAP (0 to 1 entirely emerged leaves and 1 to 3 leaves visible). These results show that the photosensitive requirement is expressed earlier in the early derivatives than in the inbred lines, indicating that the reproductive phase of development does

indeed begins earlier in these genotypes. This suggests that the early flowering trait is either upstream of the photoperiodic requirement in the regulation of flowering, or is in a completely separate pathway that interacts with the photoperiod pathway.

The results also suggest that the reproductive phase of development, as described by the ability to perceive and respond to SD conditions, begins at least two leaves before changes in the leaf shape can be detected. In most plants, the photoperiod stimulus is best perceived by the leaves that are exposed to light. The leaves then produce a graft-transmissible floral signal that is translocated to the meristem. The meristem becomes florally determined when it is competent to do so and the threshold for the floral signal from the leaves is surpassed (Bernier et al., 1981a; Bernier, 1988; Evans, 1960; McDaniel et al., 1992). This model is thought to be generally true for all plants, although it has not been confirmed in maize, where grafting is not possible. If floral induction in maize is regulated by a factor produced by a photoinduced leaf, then this leaf or leaves must be one of the leaves visible on a plant at the end of an effective SD treatment.

In the experiments described here, the last leaf emerging from the whorl during the earliest effective SD treatment would be the youngest possible photoinduced leaf. The youngest visible leaf exposed during the sensitive period are leaves 6 to 7 in A632, leaf 5 for the tassel initiation in A632E, leaves 5 to 6 in Oh43, and leaf 3 in Oh43E. This indicates that the perception of the photoperiod stimulus and the production of the floral signal, both early components of the reproductive programme, occur at least two leaves before changes in leaf shape become apparent.

This also would suggest that the leaves that perceive the photoperiodic stimulus are early vegetative leaves. Leaves are usually completely glaucous until leaf 5 in A632E plant, and leaf 4 in Oh43E. Hairs generally appear on the leaf blade at leaf position 4 to 5 in A632E and at leaf 3 to 4 in Oh43E (see section 3.1.2.1). This indicates that the ability to produce a floral stimulus begins during and overlaps with the early vegetative phase of development. This is consistent with results from *Pisum*, where genes that regulate reproductive maturity are expressed as early as in the cotyledons (Murfet 1973b).

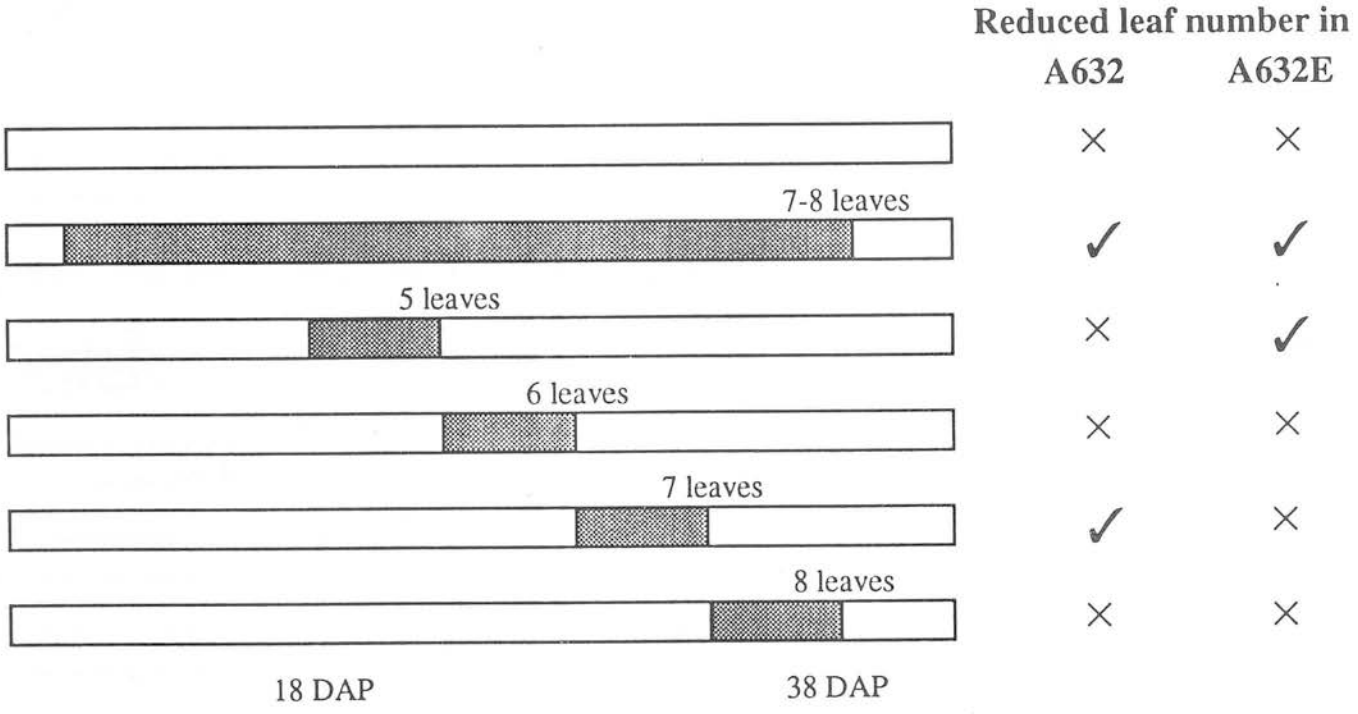

Figure 3.30A Photoperiodic sensitivity in A632 and A632E as measured by reduction in TLN. LD conditions are indicated by open boxes, SD conditions by shaded boxes. The time when a SD treatment was given is indicated in days after planting (DAP) at the bottom of the boxes. The number of leaves above the boxes indicates the number of the last leaves showing in the whorl at the end of the SD treatment. The effective SD treatments are indicated for each geneotype on the right of the diagram . When more than one SD treatment was effective in reducing TLN, the most effective treatment was determined by measuring the effect on the ear placement node (see Appendix).

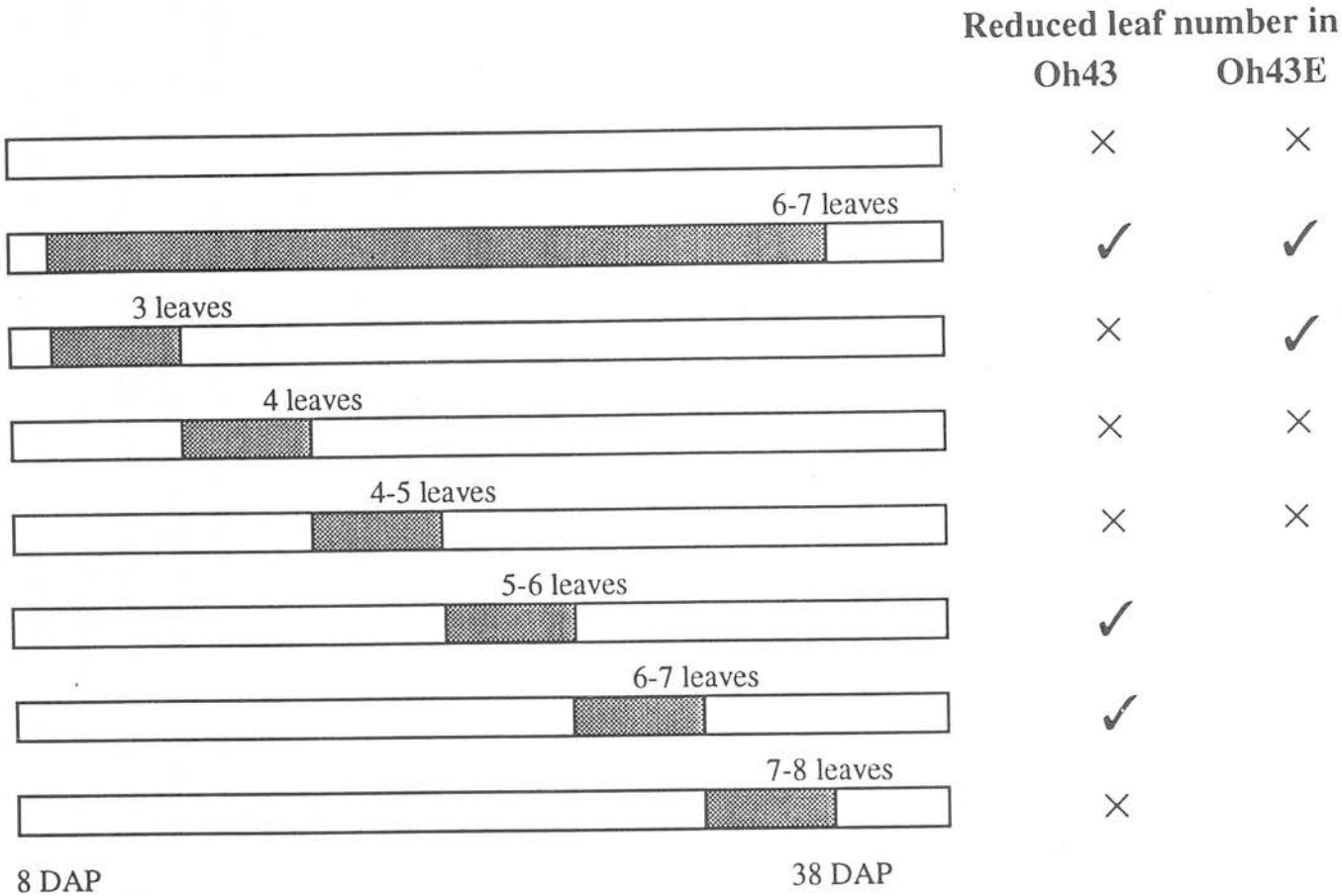

Figure 3.30B Photoperiodic sensitivity in Oh43 and Oh43E as measured by reduction in TLN. For explanation see figure 3.30A.

3.2 Late flowering mutants

Another approach to studying the relationship between the vegetative and the reproductive phases of shoot development is to examine the vegetative development in plants that are delayed in flowering. In accordance with the findings reported for early flowering plants, late flowering generally causes an increase in TLN and plant height. As before, three different patterns of vegetative development are possible in late flowering plants (see section 1.4): 1-Only the length of the late vegetative phase of development is increased. The early vegetative phase would in this case be regulated independently. 2-Both the early vegetative and the late vegetative phases of development are increased in length. This would indicate that the vegetative phases and the reproductive phase of development are coordinately regulated. 3-Only the length of the early vegetative phase is increased, also indicating coordinate regulation between vegetative phase change and reproductive maturity.

Two previously described genes causing late flowering in maize, *Leafy1* (*Lfy1*) and *indeterminate* growth habit (*id1*) , as well as two newly identified, ethyl methane sulfonate-induced mutations, *delayed flowering* (*dlf*) (isolated by M.G.Neuffer, personal communication) and *late flowering* (*lft*) (mutagenesis by E.H.Coe, screened in the summer plantations in New Jersey), were used to study patterns of vegetative development in late flowering maize plants.

3.2.1 *Leafy1*

Leafy1 (*Lfy1*) is a dominant mutation that was first identified in 1971 (Shaver, 1983). Its primary effect is a delay in the transition from a vegetative shoot apex to the tassel, resulting in an increased number of leaves between the ear and the tassel, an increased TLN and an increase in the number of days to anthesis. The ear placement node is generally at a slightly higher position in Lfy1 plants than in their wild type sibs. The expression of *Lfy1* is strongly affected by the inbred background, suggesting the presence of several modifiers. The early flowering trait is evidently one such modifier. It can partially suppress the Lfy1 phenotype: in Lfy1 plants carrying the early flowering trait, the ear is located at the same position as in wild type plants, and the number of leaves between the ear and the tassel, while still not normal, is reduced (Shaver, 1983).

The location of *Lfy1* in the genome is not known, and mapping experiments are currently under way.

In order to further study the effect of the *Lfy1* mutation, a phenotypic characterisation was carried out in A632 families segregating *Lfy1*. The observation reported by Shaver (1983) regarding TLN, ear position and the number of leaves between the ear and the tassel for the expression of Lfy1 in A632 were confirmed (figure 3.31, table 3.15). Lfy1 caused a significant increase in the TLN and the number of leaves between the ear and the tassel as well as a slightly, but significantly higher ear placement node. In the presence of the early flowering trait, both in plants heterozygous for the early flowering trait and in families backcrossed three times to A632E, the Lfy1 phenotype was partially suppressed (table 3.15). The TLN and the ear placement node in plants heterozygous for *Lfy1* and the early flowering trait was slightly but significantly lower to that of wild type Lfy1 plants and the number of leaves between the ear and the tassel was reduced, although the Lfy1 phenotype was clearly present. These results indicate that *Lfy1* and the early flowering trait are expressed in an additive fashion, which suggests that they act in different developmental pathways: the early flowering trait is part of a reproductive pathway, whereas *Lfy1* may be part of a vegetative pathway. This finding is supported by observations that indicate that the *Lfy1* and *Tp2* mutations act synergistically. Plants heterozygous for both *Lfy1* and *Tp2* in A632 were found to produce a very large number of leaves, a very high ear placement node, many more partially glaucous leaves and many nodes with adventitious roots (table 3.15). The increase in TLN is more than could be expected if both mutations were expressed additively. This suggests that the two mutations affect the same developmental pathway, that is, a pathway regulating vegetative development. However, the number of seeds available for this experiment was very small and further study is needed.

Finally, the effect of *Lfy1* on the vegetative phases of development was studied by assessing phase-specific traits in families segregating Lfy1 plants (table 3.15). The last partially glaucous leaf is representative of the vegetative leaf traits; data for the first partially glossy leaf and the first leaf with hairs are presented in the appendix. The position of none of these traits was affected by the *Lfy1* mutation, suggesting that *Lfy1* does not affect the vegetaive phase change. Whether this is true *per se* or only in A632 could not as yet be determined because the mutation had not been converged to Oh43. Convergence is currently under way to examine this question.

Table 3.15 Phase specific traits in families segregating only *Lfy1* or *Lfy1* and *Tp2*. (N is the number of plants scored. All errors are gives as ± 2 s.e.m.).

Genotype	wild type					*Lfy1+*				
	Prop roots	Last part. glaucous leaf	Ear node	Total number of leaves	N	Prop roots	Last part. glaucous leaf	Ear node	Total number of leaves	N
A632	9.6±0.6	9.9±0.4	14.1±0.3	20.1±0.4	13	10.0±0.7	10.2±0.3	15.7±0.8	29.6±1.7	9
A632	9.5±0.5	10.2±0.2	13.9±0.3	19.8±0.2	11	9.9±0.5	10.1±0.4	16.0±0.5	31.3±1.4	15
A632 A632E	9.9±0.2	9.3±0.3	**12.6±0.4**[c]	**18.3±0.5**[c]	9	9.7±0.4	9.5±0.3	**14.1±0.5**[b]	**27.7±1.3**[b]	11
A632E (3)	9.4±0.4	9.6±0.4	**11.8±0.5**[c]	**16.8±0.5**[c]	8	9.2±0.5	9.7±0.3	**12.5±0.4**[c]	**22.3±0.8**[c]	11
A632E (3)	8.9±0.3	9.9±0.5	**10.9±0.5**[c]	**16.6±0.4**[c]	7	9.2±0.5	9.7±0.4	**12.2±0.5**[c]	**23.3±1.0**[c]	12
A632E (3)	8.3±0.6	9.4±0.5	**10.2±0.3**[c]	**15.3±0.6**[c]	12	8.6±0.4	9.7±0.4	**11.4±0.6**[c]	**21.7±1.1**[c]	7
wilf type	9.3±0.7	8.3±0.7	13.7±0.7	19.3±0.7	3	-	-	-	-	-
Tp2/+	**19.8±2.2**	*	**23.8±1.3**	**27.0±0.8**	4	**23.0±3.1**	**38.5±4.1**[1]	**41.0±2.0**	**50.3±1.8**	3

b: significantly smaller than same genotype lacking the EFT at p≤0.01
c: significantly smaller than same genotype lacking the EFT at p≤0.001
*: Epicuticular wax not scored in these plants. Wax is typically expressed much longer in Tp2 plants than in wild type sibs.
1: Epicuticular wax could be scored only on 2 plants.

Figure 3.31: Field-grown plants heterozygous for *Lfy1* and wild type siblings in A632: wild type, Lfy1 plant, Lfy1 plant in A632E, wild type sibling in A632E (from left to right). Note that the ears are approximately at the same positions, whereas the tassels are produced much later in the Lfy1 plants.

3.2.2 *Indeterminate growth1* (*id1*)

The recessive *indeterminate growth* (*id1*) mutation was first reported by Singleton (1946) and is located on chromosome 1L-104±. It causes a severe increase in the TLN, usually delaying flowering so much that no tassels are produced in field grown plants. Photoinduced id1 plants grown for an extended period can produce tassels, but ears are generally not produced.

In order to investigate the effect of *id1* on vegetative development, families segregating the *id1* mutation in an unknown genetic background (+/*id1* x self) were scored for several phase-specific traits. The field grown id1 plants produced no ears and died before a tassel was initiated, so the TLN in these plants indicates the last visible leaf. The TLN and the number of nodes with prop roots are significantly higher in the id1 plants (*id1/id1*) than in their wild type sibs (+/+ and +/*id1*) (table 3.16). In contrast, the position of the last partially glaucous leaf is only slightly, but significantly higher in the id1 plants. The increase in the number of partially glaucous leaves is not proportionate to the increase in TLN. The expression of leaf hairs and the position of the first partially glossy leaf were unchanged. Thus it is concluded that the expression of the early vegetative phase it is not strictly coupled with the reproductive phase of development in id1 plants. The mutation is being converged into several inbred lines for further study.

Table 3.16 Expression of phase-specific traits in id1 plants and their wild type siblings in a family segregating *id1* (N is the number of plants scored. All errors are gives as ± 2 s.e.m.).

	Nodes with prop roots	First part. glossy leaf*	First leaf with hair*	Last part. glaucous leaf	Ear node	Total number of leaves	N
wild type	8.7±0.3	7.0±0.3	6.9±0.5	7.6±0.5	12.8±0.4	18.5±0.5	33
id1 plants	**18.4±3.0**^{c}	7.0±0.0	6.7±0.7	**8.2±0.3**^{a}	-	**31.0±1.6**^{c}	13

*: N=3 in *id1* and N=9 in wild type plants.

a: significantly different from wild type at $p<0.05$ (2-tailed *t* test)

c: significantly different from wild type at $p<0.001$ (2-tailed *t* test)

3.2.3 *Delayed flowering (dlf)*

This recessive mutation has recently been identified by Dr. M.G. Neuffer (pers. comm.), who kindly provided the seed. The mutation is little characterised and has preliminarily been named *delayed flowering* (*dlf*). To study the effect of *dlf* on the vegetative phases of development, phase-specific traits were scored in families that were either homozygous or heterozygous for *dlf*, and in wild type plants from families segregating the mutation. These families are B73;Mo17 hybrids in their genetic back ground. The results indicate that the TLN, the ear placement node and the number of nodes with prop roots were significantly higher in the dlf plants than in the wild type plants, whereas the position of the first leaf with hair, of the first partially glossy leaf and of the last partially glaucous leaf were unaffected (table 3.17, figure 3.32). Thus, it is concluded that the transition from early vegetative growth to late vegetative growth is not modified by the *dlf* mutation. Convergence of this mutation into other inbreds for further study and allelism tests with other late flowering mutations have been initiated.

Table 3.17 Expression of phase-specific traits in dlf plants and their wild type siblings in a family segregating *dlf* (N is the number of plants scored. All errors are gives as ± 2 s.e.m.).

	Last node with prop roots	First part. glossy leaf	First leaf with hair	Last part. glaucous leaf	Ear node	Total number of leaves	N
wild type	9.4±0.4	6.7±0.3	4.8±0.4	9.1±0.2	14.4±0.5	19.8±0.6	9
dlf	**10.2±0.4**[a]	6.2±0.4	4.6±0.5	9.6±0.5	**16.2±0.4**[c]	**23.4±0.5**[c]	5

a: significantly different from wild type at $p<0.05$ (2-tailed *t* test)

c: significantly different from wild type at $p<0.001$ (2-tailed *t* test)

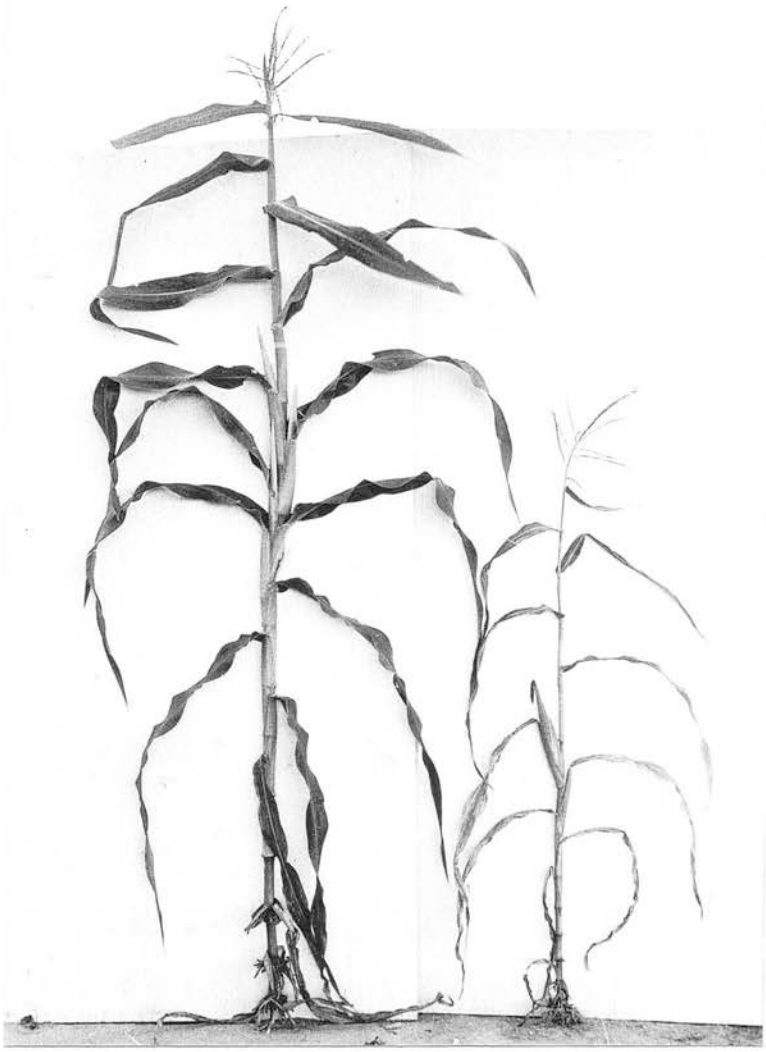

Figure 3.32 Field-grown dlf plant and a wild type sibling from a family segregating *dlf*.

3.2.4 *Late flowering (ltf)*

This mutation has recently been identified in plants coming out of a EMS pollen mutagenesis in A632 (provided by E.H. Coe) and has been named *late flowering* (*ltf*) pending the results from allelism tests. The lft plants have an increased TLN and flower about six weeks later than wild type plants. The tassels of lft plants are very large and have leafy structures that always subtend tassel branches (figures 3.33 and 3.34). The ears tend to bear pistillate spiklets only in the basal half, whereas the distal half carries staminate spiklets. M3 families homozygous for the mutation or segregating *ltf* and an A632 family were scored for the phase-specific traits. Although the position of prop roots, of the last glaucous leaf, and of the ear were slightly but significantly different between A632 plants and the wild type sibs of the segregating families, the plants were similar, indicating no or little negative effect of the mutagenesis. The results indicate that the TLN, the ear placement node, and the number of nodes with prop roots were significantly increased in the ltf1 plants, whereas the number of partially glaucous leaves and of leaves with hair were unchanged (table 3.18). The number of partially glossy leaves is slightly smaller in ltf plants than in wild type plants, a change that is not significant in Mann-Whitney *U*-test. This indicates that the change from the early vegetative to the late vegetative phase of development was not affected by the presence of *ltf*. However, this experiment has been carried out only in A632, in which vegetative and reproductive development are also uncoupled in early flowering plants. In order to characterise this mutation further, *ltf* is currently being converged into other inbreds including Oh43.

Table 3.18 Expression of phase-specific traits in A632 plants and in ltf plants and their wild type siblings in a M3 family segregating *ltf* in A632 (N is the number of plants scored. All errors are gives as ± 2 s.e.m.).

	Last nodes with prop roots	First part. glossy leaf	First leaf with hair	Last part. glaucous leaf	Ear node	Total number of leaves	N
A632	**9.1±0.1**^{c}	7.0±0.1	4.9±0.2	**9.9±0.2**^{b}	**13.8±0.3**^{c}	19.6±0.3	16
wild type	9.9±0.2	7.0±0.0	4.7±0.2	9.3±0.2	14.9±0.4	20.1±0.5	16
ltf	**14.7±0.7**^{c}	**6.7±0.7**^{a}	4.7±0.7	9.3±0.7	**21.7±0.7**^{c}	**28.0±0.0**^{c}	3

a: significantly different from wild type at $p<0.05$ (2-tailed *t* test), not significant in 2-tailed *U* test

b: significantly different from wild type at $p<0.01$ (2-tailed *t* test)

c: significantly different from wild type at $p<0.001$ (2-tailed *t* test and 2-tailed *U*-test)

a, b, c refer toresults from *t* test; 1, 2, 3 refer toresults from *U* test

Figure 3.33 Field-grown ltf plant and wild type sibling from a family segregating *ltf*. Note the enlarged tassel of the ltf plant.

Figure 3.34 Tassel morphology of ltf and wild type plants. The ltf tassel is larger and has leaves subtending many of the tassel branches.

3.2.5 Summary

In summary, the transition from early vegetative growth to late vegetative growth was uncoupled from reproductive development in all of the late flowering mutations. This suggests that only certain parts of the reproductive phase of development affect the change from the early vegetative to the late vegetative phase of shoot development. It is possible that for the type of coupling of the vegetative and reproductive development studied here, activity is required early in shoot development. It may be that the genes conditioning late flowering act late in shoot development, as is suggested by the phenotype of Lfy1, whereas the early flowering trait is active early. Given the presence of modifiers of the early flowering trait in inbred lines, a better understanding of the relationship between the late flowering mutations and vegetative development requires a better characterisation of these mutants in different inbreds.

CHAPTER 4

CONCLUDING DISCUSSION AND POSSIBILITIES FOR FUTURE RESEARCH

4.1 Heterochrony in plant development

All organisms go through distinct phases of development during ontogenesis. The correct timing of developmental events is important for proper growth and development of an individual. Changes in the relative timing of developmental events or in the rate of developmental processes, a phenomenon called heterochrony, are thought to play an important role in evolutionary changes of species (Gould, 1977, 1982; Raff and Wray, 1989; Takhtajan, 1972). Heterochrony is most frequently considered when a shift of vegetative (somatic) events in development relative to reproductive maturity is observed. In animals, developmental phases define distinct periods in the life of the whole individual, whereas in plants developmental phases are episodes in the life of the apical meristem. Since changes in time and position occur simultaneously in plant growth, it is extremely difficult to distinguish whether variation of developmental events along the axis of a plant is heterochronic (changes in temporal information) or as homeotic (changes in positional information) in nature.

Changes in the relative timing of developmental events are considered a major source of phenotypic variation in evolution. The role of heterochrony in plant evolution has been considered for various aspects of plant development, including leaf development and flower development (Guerrant, 1988; Jones, 1992, 1993; Kellog, 1990; Lord et al., 1989; McLellan, 1990, 1993). In maize shoot development, heterochronic mutations that alter the timing of the early vegetative phase (*Teopod* mutations) can significantly alter the appearance of the shoot (Poethig, 1988a). The results of the present study show that the shoot phenotype can also modified by changes in reproductive maturity relative to vegetative phase change. Given the distinct phenotypic character of phytomers produced during each phase of shoot development, a question arises as to the functional significance and possibly selective advantage of the vegetative phases of shoot development. While it has been suggested that the functional value of the vegetative phase of development lies in the prevention of precocious flowering (von Denffer, 1950), the early vegetative phase and the late vegetative phase may each have another adaptive significance. It is possible that the change from one vegetative phase to another allows the plant to change its vegetative phenotype in preset ways at ontogenetically predetermined times. This may be adaptive when predictable environmental changes occur throughout the season. Little research has been directed towards uncovering adaptive features of each of the vegetative phases of shoot development, yet some features of one or the other vegetative phase of development may hold selective advantages. In English ivy, for instance, a change in

leaf form from a shade leaf type to a sun leaf type takes places at the transition from early vegetative to late vegetative growth regardless of light exposure (Bauer and Bauer, 1980). Presumably, the late vegetative leaves are more likely to be exposed to high light conditions as the late vegetative growth normally occurs high up on trees. Phase-specific resistance against pests or pathogens has been reported for several species. In *Pinus radiata*, rooted cuttings from the late vegetative phase of development were found to be more resistant to western gall rust, but more susceptible to feeding by hares than the early vegetative growth (Libby and Hood, 1976; Zagory and Libby, 1985). Zagory and Libby (1985) suggest that a feeding repellent may be present in the early vegetative growth because feeding damage in the rooted late vegetative clones occurred although the early vegetative needles were greener, more abundant, and less stiff. The authors suggest that such a trait would be adaptive because normally early vegetative growth is more accessible to hares. In *Populus angustifolia* , Kearsley and Whitham (1989) reported selective colonisation of early vegetative leaves by a leaf-feeding beetle, and of late vegetative growth by a gall-forming aphid. In both cases growth and survival of the pests was better in the preferred environment, suggesting the presence of pest-specific and phase-specific resistance. The authors suggest that developmental changes in resistance can occur rapidly within one plant and are important components in determining the distribution of plant pests. In maize, there is also some evidence of potentially adaptive, phase-specific features. Preliminary observations, made in field-grown and greenhouse-grown plants of different genotypes and in different years, indicate that feeding damage by thrips occurs preferentially on early vegetative tissues, whereas late vegetative leaves appear resistant to attack (appendix). Variation in the relative timing of the two vegetative phases of shoot development may thus be adaptive under certain environmental conditions.

4.2 A model of shoot development

Heterochronic expression of developmental events requires independence in the regulation of these events, and one of the most important questions in the study of development is the extent to which developmental events are independent of each other. Events that are regulated completely independently of each other can be shifted relative to each other, whereas events that are causally related have to be expressed in a predetermined order. In maize shoot development, the relative timing of the phases of development can be shown to change. Phytomers that are produced during the transition from early vegetative to late vegetative growth usually express traits

characteristic of both phases. Characters specific to one or the other phase are generally located in distinct regions of these transition phytomers. This observation and the existence of mutations that specifically modify the expression of one phase of development, such as the *Teopod* mutations, suggest that the two vegetative phases of development are regulated by at least two independent developmental programmes that interact and specify the vegetative character of the shoot in a combinatorial fashion.

Contrary to the assumption of the traditional model of shoot development that changes in the vegetative morphology are correlated with changes in the reproductive maturity, findings in maize shoot development point to the independent regulation of vegetative phase change and reproductive maturation This is suggested not only by the results presented in this study for A632, A632E, but for several other inbred lines and late flowering mutants as well. The timing of the photoperiod-sensitive period of the *Teopod* mutants also indicates that the prolonged expression of the early vegetative phase has no or only a very small effect on the onset of reproductive maturity (Bassiri et al., 1992). On the other hand, the transition from early vegetative growth to late vegetative growth is linked to reproductive maturation in Oh43 and several other lines. These findings suggest that the phases of shoot development are regulated largely independently, but that they can interact. Specifically, reproductive maturity and vegetative phase change can in some cases be coordinately regulated. The presence of both types of patterns, independence and coordinate regulation of the vegetative phase change and reproductive maturity, suggests a developmental plasticity that may hold evolutionary advantages for the species.

The findings in maize so far favour a model of shoot development in which there are at least three discrete phases of development. A question remains as to how the vegetative phases and the reproductive phase interact to produce a normally developed shoot. Von Denffer (1950) suggests that flowering is a primary function of a plant, and that an extended vegetative phase serves to suppress the inherent ability to flower in order to improve the fitness of the plant. This author points out that in lower plants, such as *Chlamydomonas eugametos*, meiosis immediately follows the formation of the zygote, whereas in phylogenetically more advanced plants, the sporophytic phase is emphasised. Higher plants generate vegetative growth for extended periods of time and reproductive maturation is delayed. Von Denffer (1950) also cites the not infrequent occurrence of precocious flowering as an indication that higher plants can enter reproductive maturity very early on and do not have to rely on floral stimuli to be produced later in shoot development. Flowering in higher plants would thus be promoted not only by an increased ability to flower, but by a decrease in the

suppression of reproductive maturation. Von Denffer's hypothesis is supported by the existence of the *embryonic flower* mutations in *Arabidopsis thaliana* (Sung et al., 1992). If these recessive alleles are loss-of-function mutations, the wild type allele would function to promote vegetative growth and to suppress reproductive development.

It is unclear, however, if there is a vegetative stimulus that can suppress flowering directly, and whether such stimulus would be part of any of the vegetative phases of development, or whether flowering is suppressed in an independent pathway and leaves are produced until flowering occurs. Results in *Teopod* mutants and in a variety of inbred lines indicate that early vegetative growth *per se* is not antagonistic to reproductive maturation, although the phenotype of the *Teopod* mutants clearly suggests that the prolonged expression of the early vegetative phase can greatly alter floral differentiation, that is modify the identity of the reproductive phytomers (Bassiri et al., 1992; Poethig, 1988a; Poethig and Passas, 1993). The findings that early vegetative leaves are apparently involved in photoinduction and that the timing of the transition from early vegetative to late vegetative growth is unchanged in A632E and in photoinduced A632 plants also suggest that early vegetative growth has no negative effect on the reproductive phase of development.

However, the results presented here for plants carrying the early flowering trait or a late flowering mutation indicate that the number of nodes with prop roots is correlated with flowering time, and not with the early vegetative traits. This suggests that this trait may not be specific to the early vegetative phase of development as previously thought (Poethig, 1988a, 1990), but instead correlated with total leaf number. This observation is consistent with the results from the *Teopod* mutations. Poethig (1988a) reported that in Tp2 plants, the number of additional vegetative phytomers is approximately the same as the number of additional nodes with prop roots, whereas many more phytomers with epicuticular wax are produced. The association of adventitious roots (or rooting ability) with the non-flowering (juvenile) phase in the traditional model of shoot development and the finding that roots negatively affect floral determination and differentiation are also consistent with this assumption (Doorenbos, 1965; Geneve et al., 1988; McDaniel, 1980; Schwabe and Al-Doori, 1973; Smith and McDaniel, 1992; Robbins, 1957b, 1961; Steele et al., 1990; Stein and Fosket, 1969).

Based on these observations it is possible to suggest a working model of maize shoot development. A model for the regulation of phase change during the floral induction process in *Arabidopsis thaliana* has recently been proposed (Schultz and Haughn, 1993). Phase change is suggested to take place only in the meristem. The

authors propose that phase change occurs as a result of gradual changes in the activity of a mechanism controlling phase switching. The authors suggest that a set of regulatory genes, the floral induction process genes, regulates the activity of the mechanism controlling phase switching. A similar model based on a single gradient that regulates the expression of different phases could also be proposed for maize shoot development. The question would then be how independent expression of the phases is achieved.

In the model preferred here, three largely independently regulated phases of development are assumed, and the way in which coordinate regulation of the phase takes place is studied. This model is proposed as a working model that is distinct from the traditional model of shoot development, and that is expected to change and improve as new observations are made. In figure 4.1, the two vegetative phases and the reproductive phase of development are depicted as triangles which represent the expression of each developmental programme. Developmental events occurring at the base of the plants are depicted at the bottom of the diagramme, and apical growth at the top. The observed phase-specific traits are schematically presented to the right of the diagrammme. The two vegetative phases of development overlap, producing several transition leaves with traits from both phases. The overall length of the vegetative phase is determined by the time of tassel initiation (or *vice versa*): vegetative growth and reproductive growth are presumed to be mutually exclusive under normal circumstances (indicated by the inhibitory symbols and the question mark at the top).

The effect of the onset of the reproductive phase of development on the vegetative phase change is indicated by the inhibitory symbol at the bottom. This link is not obligatory, and is expressed only in certain genotypes such as Oh43. It is clearly tied to changes in the reproductive development, but it is unclear whether it is an integral part of the reproductive phase of development or a separate pathway. The time of expression of this link is also uncertain, indicated by the vertical arrows (open arrow heads).

The earliest measurable trait of the reproductive phase is the sensitivity to photoinduction; in the diagramme, this event is placed at the bottom tip of the triangle representing the reproductive phase. The early flowering trait acts prior to the appearance of photoperiodic sensitivity and affects the whole reproductive phase of development. Thus, the triangle representing the expression of reproductive development would move downwards (to an earlier time) in plants carrying the early flowering trait. The reproductive phase of development, as represented by the early

flowering trait and by photoperiodic sensitivity, begins early during shoot development and overlaps in its expression with both vegetative phases of development. Following photoinduction, reproductive traits are expressed earlier. This effect probably results from an increase in the expression of the reproductive programme, which can be represented in the diagramme as a broadening and shortening of the reproductive triangle. Assuming that the position of the ear and the tassel and the position of the transition from early vegetative to late vegetative growth are regulated by the expression of this programme, this would explain the expression of the phase-specific traits at a lower position on the shoot.

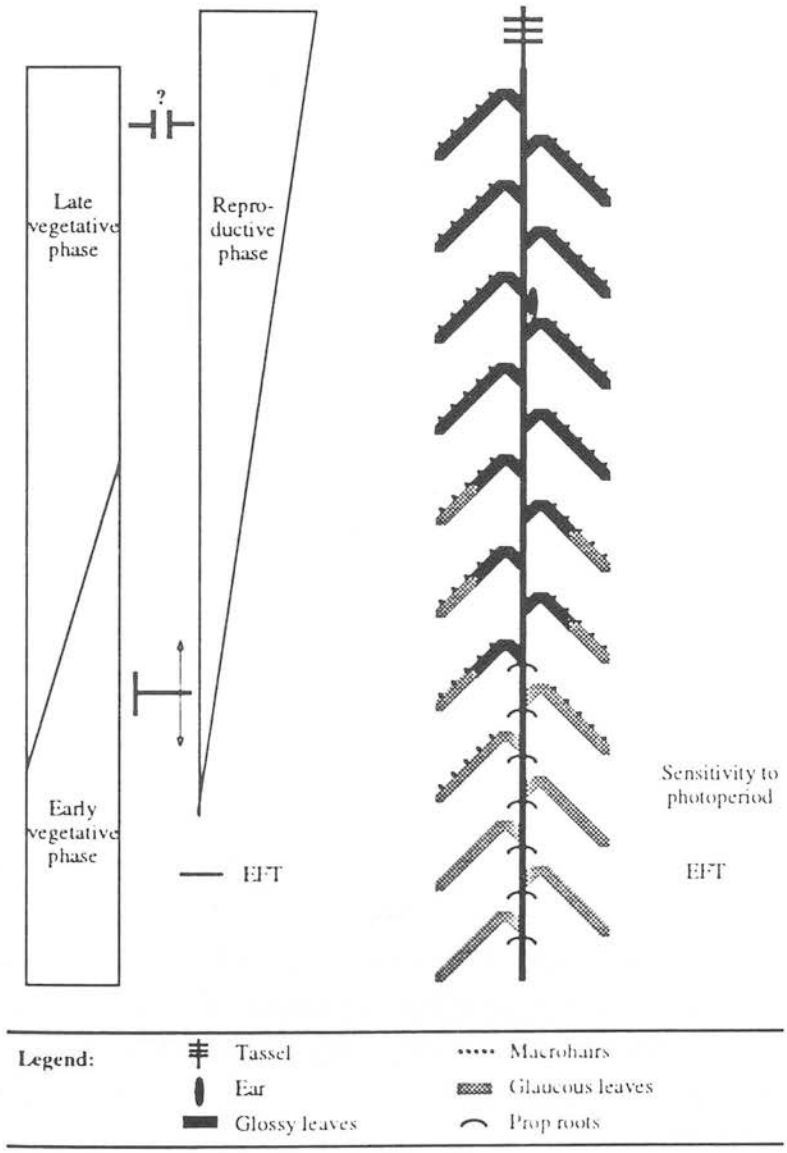

Figure 4.1: Proposed working model of shoot development in maize. For explanations see main text.

4.3 Possibilities for future work

Given that so far none of the late flowering mutations in maize has been shown to modify vegetative phase change, these mutations may act late in shoot development. Based on the model presented in figure 4.1, the question may be asked whether these mutations act in a vegetative or in a reproductive progamme. *Lfy1* for example appears to interact synergistically with *Tp2*, but is expressed in an additive fashion with the early flowering trait, suggesting that the gene may be part of the vegetative phase of development. Further work is needed to establish the placement of these late flowering mutations in the regulation of either the vegetative or the reproductive phases of shoot development.

At present our knowledge of the regulation of shoot development is very limited, and any model will be far from comprehensive. Our understanding of the developmental processes would greatly benefit from a better characterisation of known mutations and from the identification, isolation, and characterisation of additional genes regulating shoot development. The large number of genes affecting flowering time and floral differentiation in *Arabidopsis thaliana*, for instance, indicates that an array of genes can be expected to control each phase of shoot development (see section 1.2.1). Regulation of flowering in maize may be studied by characterising the late flowering mutations and by genetically dissecting the early flowering trait and identifying and characterising the genes contributing to it. Whereas the genetic analysis of flowering is not very advanced in maize compared to other species, several genes regulating vegetative development are known in maize. Double mutant analyses with these mutations and genes regulating flowering time can serve to place the gene that regulate flowering time in a vegetative or reproductive pathway.

A newly identified mutation, called *glossy-early flowering1* (*gef1*), is likely to be very useful in the study of shoot development. In *gef1* mutants, vegetative phase change occurs very early and the plants flower earlier than wild type plants.

An attempt may be made to isolate the gene(s) that condition the coordinate regulation of the vegetative phase change and reproductive maturity in Oh43 (or lack thereof in A632). One way to accomplish this would be to carry out a mutagenesis in A632E and Oh43E and look for a modified vegetative phase change. Alternatively, one could try to converge the trait from Oh43E to A632E, and *vice versa*.

The identification and phenotypic characterisation of any gene regulating shoot development greatly depends on the availability of phase-specific markers. Further

markers, particularly makers specific to the late vegetative phase , markers expressed in the mesophyll and markers expressed early during the reproductive phase of development would be valuable. A search for phase-specific molecular markers is currently under way in the laboratory of Prof. S. Poethig.

As discussed earlier, some traits that are expressed phase-specifically may have an adaptive value. A better understanding of this aspect of shoot development is desirable to place the analysis of phase change in maize into the context of heterochrony, plant evolution and plant breeding. Abedon and Tracy (personal communication) have investigated phase-specific resistance using the *Cg1* mutation, and their results suggest that late vegetative maize leaves are more resistant to common rust (*Puccinia sorghi* Schw.) than early vegetative leaves. Preliminary results suggest that early vegetative leaves are also more susceptible to thrip damage (see appendix); this could be confirmed by feeding trials and the mechanism of resistance could be investigated. Likely factor for resistance to thrips include the composition of the epicuticular wax and the lignification of the epidermal cell walls.

Additionally, it has become clear that it is very important to establish the separate identity of the two vegetative phases. The two phase-specific types of vegetative leaves have not as yet been generally recognised as distinct types of structures, and this distinction has been criticised. Structures of the plant shoot that are generally recognised as being distinct, such as bracts, sepals, petals etc., are defined by their position, appearance and function. It can be argued that the leaves formed during each of the vegetative phases can be distinguished by the same criteria, and thus rightly deserve to be named separately. It may thus be worthwhile in the future to make a case for the clear distinction of the early vegetative and late vegetative part of the shoot and to name the vegetative phases in a less descriptive fashion.

In order to better understand shoot development it is important to determine how and when phase change takes place. So far, it has been suggested that the presence of transition leaves can be explained by a combinatorial expression of the early vegetative and late vegetative phase of development. In this concept, the expression of the early vegetative phase would slowly decrease and the expression of the late vegetative phase slowly increase.

Alternatively, the vegetative phase change could be abrupt and affect the meristem and developing leaf primordia equally. Only immature tissues in the primordia that are not yet committed to one particular phase of development would be affected by this

shift. Using photoperiod to induce vegetative or reproductive growth in *Impatiens balsamina*, Battey and Lyndon (1988) have shown that developing primordia exposed a change in photoperiod go on to form transitional structures that express both vegetative and reproductive traits. A similar basipetal effect on immature phytomers has been proposed by Hempel and Feldman (1994) for the change from vegetative to reproductive growth in *Arabidopsis thaliana*. The pattern in which phase-specific traits are expressed in transition leaves in maize is consistent with such a model. According to Sharman (1942), the leaf tip matures first in maize. The tip is also the part of the leaf that retains the glaucous appearance of early vegetative leaves. The possibility that an abrupt shift from the early vegetative to the late vegetative phase of development occurs in maize may be investigated using *in vitro* rejuvenation of shoot tips. Some experiments of this kind have been carried out, and the preliminary results suggest that the phase-specific character of maize leaves is indeed acquired during leaf organogenesis, and not at the time when the primordia are formed. Further research in this area is needed to understand how vegetative phase change proceeds.

REFERENCES

Abbe, E. C. and B. O. Phinney. (1951). The growth of the shoot apex in maize: external features. *American Journal of Botany*, **38**, 737-744.

Abbe, E. C., B. O. Phinney and D. F. Baer. (1951). The growth of the shoot apex in maize: internal features. *American Journal of Botany*, **38**, 744-751.

Abbe, E. C., L. F. Randolph and J. Einset. (1941). The developmental relationship between shoot apex and growth pattern of leaf blade in diploid maize. *American Journal of Botany*, **28**, 778-784.

Abler, B. S. B., M. D. Edwards and C. W. Stuber. (1991). Isoenzymatic identification of quantitative trait loci in crosses of elite maize inbreds. *Crop Science*, **31**, 267-274.

Alexander, K. G. and M. H. Miller. (1991). The effect of soil aggregate size on early growth and shoot-root ratio of maize (*Zea mays* L.). *Plant and Soil*, **138**, 189-194.

Allsopp, A. (1967). Heteroblastic development in vascular plants. *Advances in Morphogenesis*, **6**, 127-171.

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

Araki, T. and Y. Komeda. (1993). Analysis of the role of the late-flowering locus, *GI* in the flowering of *Arabidopsis thaliana*. *The Plant Journal*, **3**, 231-239.

Arnold, C. Y. (1969). Environmentally induced variations of sweet corn characteristics as they relate to the time required for development. *J. Amer. Soc. Hort. Sci.*, **94**, 115-118.

Ashby, E. (1948). Studies in the morphogenesis of leaves II. The area, cell size and cell number of leaves of *Ipomoea* in relation to their position on the shoot. *New Phytologist*, **47**, 177-195.

Ashby, E. (1950). Studies in the morphogenesis of leaves VI. Some effects of length of day upon leaf shape in *Ipomoea caerulea*. *New Phytologist*, **49**, 375-387.

Ashby, E. and E. Wangermann. (1950). Studies in the morphogenesis of leaves IV. Further observations on area, cell size and cell number of leaves of *Ipomoea* in relation to their position on the shoot. *New Phytologist*, **49**, 23-35.

Ausubel, F. M., R. Brent, R. E. Kington, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl. (Ed.) (1989). *Current protocols in molecular biology*. New York: John Wiley & Sons.

Bagnall, D. J. (1992). Light quality and vernalization interact in controlling late flowering in *Arabidopsis* ecotypes and mutants. *Annals of Botany*, **71**, 75-83.

Baker, E. A. (*1974*). The influence of environment on leaf wax development in *Brassica oleracea* var. *gemmifera*. *New Phytology*, **73**, 955-966.

Banks, M. S. (1979). Plant regeneration from callus from two growth phases of English ivy, *Hedera helix* L. *Zeitschrift für Pflanzenphysiologie*, **92**, 349-353.

Bassiri, A., E. E. Irish and R. S. Poethig. (1992). Heterochronic effects of *Teopod 2* on the growth and photosensitivity of the maize shoot. *The Plant Cell*, **4**, 497-504.

Battey, N. H. and R. F. Lyndon. (1988). Determination ans differentiation of leaf and petal primordia in *Impatiens balsamina*. *Annals of Botany*, **61**, 9-16.

Bauer, H. and U. Bauer. (1980). Photosynthesis in leaves of the juvenile and adult phase of ivy (Hedera helix). *Physiologia Plantarum*, **49**, 366-372.

Beckmann, J. S. and M. Soller. (1988). Detection of linkage between marker loci and loci affecting quantitative traits in crosses between segregating populations. *Theoretical and Applied Genetics*, **76**, 228-236.

Bennett, M. D. (1972). Nuclear DNA content and minimum generation time in herbaceous plants. *Proceedings of the Royal Society of London B*, **181**, 109-135.

Bernier, G. (1988). The control of floral evocation and morphogenesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **39**, 175-219.

Bernier, G., A. Havelange, C. Houssa, A. Petitjean and P. Lejeune. (1993). Physiological signals that induce flowering. *The Plant Cell*, **5**, 1147-1155.

Bernier, G., J.-M. Kinet and R. M. Sachs. (1981a). *The physiology of flowering, Volume 1*. Boca Raton, FL: CRC Press.

Bernier, G., J.-M. Kinet and R. M. Sachs. (1981b). *The physiology of flowering, Volume 2*. Boca Raton, FL: CRC Press.

Bianchi, A. and G. Marchesi. (1960). The surface of the leaf in normal and glossy maize seedlings. *Zeitschrift für Vererbungslehre*, **91**, 214-219.

Bianchi, G., P. Avato and F. Salamini. (1984). Surface waxes from grain, leaves, and husks of maize (*Zea mays* L.). *Cereal Chemistry*, **61**(1), 45-47.

Blaker, T. W. and R. I. Gresyon. (1988). Developmental variation of leaf surface wax of maize, *Zea mays*. *Canadian Journal of Botany*, **66**, 839-846.

Bleeker, A. B., M. A. Estelle, C. Somerville and H. Kende. (1988). Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science*, **241**, 1086-1089.

Borchert, R. (1976a). The concept of juvenility in woody plants. *Acta Horticulturae*, **56**, 21-35.

Borchert, R. (1976b). Differences in shoot growth patterns between juvenile and adult trees and their interpretation based on systems analysis of trees. *Acta Horticulturae*, **56**, 123-130.

Brink, R. A. (1962). Phase change in higher plants and somatic cell heredity. *Quarterly Review of Botany*, **37**(1), 1-22.

Brink, R. A. and P. H. Senn. (1931). Heritable Characters in maize. XL. *Ragged*, a dominant character, linked with *a1 ts4* and *d1*. *Journal of Heredity*, **22**, 155-161.

Bullock, D. and A. L. Rayburn. (1991). Genome size variation in Southwestern US Indian maize populations may be a function of effective growing season. *Maydica*, **36**, 247-250.

Cao, W. and D. N. Moss. (1989). Temperature effect on leaf emergence and phyllochron in wheat and barley. *Crop Science*, **29**, 1018-1021.

Chase, S. S. and D. K. Nanda. (1967). Number of leaves and maturity classification in *Zea mays* L. *Crop Science*, **7**, 431-432.

Chaudhury, A. M., S. Letham, S. Craig and E. S. Dennis. (1993). amp1 - a mutant with high cytokinin levels and altered embryonic pattern, faster vegetative growth, constitutive photomorphogenesis and precocious flowering. *The Plant Journal*, **4**, 907-916.

Chory, J., P. Nagpal and C. A. Peto. (1991). Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in *Arabidopsis*. *The Plant Cell*, **3**, 445-459.

Coe, E. H. J., M. G. Neuffer and D. A. Hoisington. (1988). The genetics of corn. In Sprague, G. F. and J. W. Dudley (Ed.), *Corn and Corn Improvement* (pp. 81-258). Madison, WI: American Society of Agronomy.

Coligado, M. C. and D. M. Brown. (1975). Response of corn (*Zea mays*) in the pre-tassel initiation period to temperature and photoperiod. *Agricultural Meteorology*, **14**, 357-367.

Comfort, A. (1964). *Ageing, the biology of senescence*. London: Routledge & Kegan Paul.

Conway, L. J. and R. S. Poethig. (1993). Heterochrony in plant development. *Developmental Biology*, **4**, 65-72.

Cowan, C. M., M. R. Dentine, R. L. Ax and L. A. Schuler. (1990). Structural variation around prolactin gene linked to quantitative traits in an elite Holstein sire family. *Theoretical and Applied Genetics*, **79**, 577-582.

Cross, H. Z. (1990). Selecting for rapid leaf expansion in early-maturing maize. *Crop Science*, **30**, 1029-1032.

Crow, J. F. (1976). *Genetics notes*. 7th. Minneapolis: Burgess.

Dekkers, J. C. M. and M. R. Dentine. (1991). Quantitative genetic variance associated with chromosomal markers in segregating populations. *Theoretical and Applied Genetics*, **81**, 212-220.

Dellaporta, S. L., J. Wood and J. B. Hicks. (1983). A plant minipreparation: Version 2. *Plant Molecular Biology Reporter*, **1**, 19-21.

Demotes-Mainard, S. and S. Pellerin. (1992). Effect of mutual shading on the emergence of nodal roots and the root/shoot ratio of maize. *Plant and Soil*, **147**, 87-93.

Denffer, D. v. (1950). Blühhormon oder Blühhemmung? Neue Gesichtspunkte zur Physiologie der Blütenbildung. *Die Naturwissenschaften*, **37**, 296-301.

Domoney, C. and J. N. Timmis. (1980). Ribosomal RNA gene redundancy in juvenile and mature ivy (*Hedera helix*). *Journal of Experimental Botany*, **31**, 1093-1100.

Donald, R. G., B. D. Kay and M. H. Miller. (1987). The effect of soil aggregate size on early shoot and root growth of maize (*Zea mays* L.). *Plant and Soil*, **103**, 251-259.

Doorenbos, J. (1954). "Rejuvenation" of *Hedera helix* in graft combinations. *Proceedings of Koniglijke Nederlandse Akademie van Wetenschappen - Series C*, **57**, 99-102.

Doorenbos, J. (1965). Juvenile and adult phases in woody plants. In Ruhlard, W. (Ed.), *Encyclopedia of Plant Physiology* (pp. 1222-1235). Berlin: Springer.

Dudley, J. W. (1993). Molecular markers in plant improvement: Manipulation of genes affecting quantitative traits. *Crop Science*, **33**, 660-668.

Dudley, M. and R. S. Poethig. (1991). The effect of a heterochronic mutation, *Teopod2*, on the cell lineage of the maize shoot. *Development*, **111**, 733-739.

Dunberg, A. (1977). Juvenility, maturation, ageing and rejuvenation in woody plants. *Vegetative propagation of forest trees, physiology and practice* (pp. 55-64). Uppsala: Institute for Forest Improvement, Swedish University of Agricultural Sciences.

Duncan, W. G. and J. D. Hesketh. (1968). Net photosynthetic rates, relative leaf growth rates, and leaf numbers of 22 races of maize growth at eight temperatures. *Crop Science*, **8**, 670-674.

Edwards, M. D., C. W. Stuber and J. F. Wendel. (1987). Molecular-marker-facilitated investigations of quanitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics*, **116**, 113-125.

Eik, K. and J. J. Hanway. (1965). Some factors affecting development and longevity of leaves of corn. *Agronomy*, **57**, 7-12.

Evans, L. T. (1969). *The induction of flowering*. Melbourne, Australia: MacMillan.

Evans, M. M. S., H. J. Passas and R. S. Poethig. (1994). Heterochronic effects of *glossy15* mutations on epidermal cell identity in maize. **(in press)**,

Faboya, O. O. P., J. I. Okogun and D. R. Goddard. (1980). Dependence of the hydrocarbon constituents of the leaf waxes of *Khaya* species on leaf age. *Phytochemistry*, **19**, 1226-1227.

Feinberg, A. P. and B. Vogelstein. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry*, **132**, 6-13.

Fortanier, E. J. and H. Jonkers. (1976). Juvenility and maturity of plants as influenced by their ontogenetical and physiological ageing. *Acta Horticulturae*, **56**, 37-44.

Franich, R. A., L. G. Wells and J. R. Barnett. (1977). Variation with tree age of needle cuticle topography and stomatal structure in *Pinus radiata* D. Don. *Annals of Botany*, **41**, 621-626.

Frydman, V. M. and P. F. Wareing. (1974). Phase change in *Hedera helix* L. *Journal of Experimental Botany*, **25**(85), 420-429.

Galinat, W. C. (1954a). *Corn Grass*: I. *Corn Grass* as a prototype or a false progenitor of maize. *The American Naturalist*, **88**, 101-104.

Galinat, W. C. (1954b). *Corn Grass*: II. Effect of the *Corn Grass* gene on the development of the maize inflorescence. *American Journal of Botany*, **41**, 803-806.

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

Gallagher, J. N. (1979). Field studies of cereal leaf growth. I. Initiation and expansion in relation to temperature and ontogeny. *Journal of Experimental Botany*, **30**, 625-636.

Geneve, R. L., W. P. Hackett and B. T. Swanson. (1990). Root initiation in debladed petioles from juvenile and mature English ivy in response to inhibitors of ethylene biosynthesis and action. *Journal of the American Society of Horticultural Science*, **115**, 128-131.

Giesbrecht, J. (1960a). The inheritance of maturity in maize. *Can. J. Plant Sci.*, **40**, 490-499.

Giesbrecht, J. (1960b). The inheritance of timing of silking and pollen shedding in maize. *Canadian Journal of Genetics and Cytology*, **1**, 329-338.

Goodin, J. R. (1965). Anatomical changes associated with juvenile-to-mature growth phase transition in *Hedera*. *Nature*, **208**, 504-505.

Goto, N., T. Kumagai and M. Koornneef. (1991). Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long-day plant. *Physiologia Plantarum*, **83**, 209-215.

Gould, K. S. (1993). Leaf heteroblasty in *Pseudopanax crassifolius*: Functional significance of leaf morphology and anatomy. *Annals of Botany*, **71**, 61-70.

Gould, S. J. (1977). *Ontogeny and phylogeny*. Cambridge, MA: Belknap Press/Harvard University Press.

Gould, S. J. (1982). Changes in developmental timing as a mechanism of macroevolution. In Bonner, J. T. (Ed.), *Evolution and development* (pp. 333-346). New York, NY: Springer.

Greenwood, M. S. (1984). Phase change in loblolly pine: shoot development as a function of age. *Physiologia Plantarum*, **61**, 518-522.

Greenwood, M. S., C. A. Hopper and K. W. Hutchison. (1989). Maturation in larch. I. Effect of age on shoot growth, foliar characteristics, and DNA methylation. *New Phytologist*, **90**, 406-412.

Greyson, R. I., D. B. Walden and W. J. Smith. (1982). Leaf and stem heteroblasty in Zea. *Botanical Gazette*, **143**, 73-78.

Guerrant, E. O. (1988). Heterochrony in plants: The intersection of evolution, ecology and ontogeny. In McKinney, M. L. (Ed.), *Heterochrony in evolution: A molecular approach* (pp. 111-133). New York and London: Plenum.

Guzmán, P. and J. R. Ecker. (1990). Exploiting the triple reponse of *Arabidopsis* to identify ethylene-related mutants. *The Plant Cell*, **2**, 513-523.

Habermann, H. M. and D. B. Sekulow. (1972). Development and aging in *Helianthus annuus* L. Effects of the biological *milieu* of the apical meristem on patterns of development. *Growth*, **36**, 339-349.

Hackett, W. P. (1976). Control of phase change in woody plants. *Acta Horticulturae*, **56**, 143-154.

Hackett, W. P. (1983). Phase change and intra-clonal variability. *Horticultural Science*, **18**(6), 840-844.

Hackett, W. P. (1985). Juvenility, maturation, and rejuvenation in woody plants. *Horticultural Reviews*, **7**, 109-155.

Hackett, W. P., R. E. Cordero and C. Srinivasan. (1987). Apical meristem characteristics and activity in relation to juvenility in *Hedera*. In Atherton, J. G. (Ed.), *Manipulation of flowering* (pp. 93-99). London: Butterworths.

Hackett, W. P. and J. R. Murray. (1992). Maturation and rejuvenation in woody plants. *Acta Horticulturae*, **314**, 195-203.

Hallauer, A. R. (1965). Inheritance of flowering in maize. *Genetics*, **52**, 129-137.

Hanway, J. J. and S. W. Ritchie. (1985). Introduction. In Halevy, A. H. (Ed.), *Handbook of flowering* (pp. 525-541). Boca Raton, FL: CRC Press.

Haupt, W. and E. Nakamura. (1970). Vernalisation bei einer frühblühenden Sorte von *Pisum sativum. Zeitschrift für Pflanzenphysiologie*, **62**, 270-275.

Hay, R. K. M. and R. Delécolle. (1989). The setting of rates of wheat plants at crop emergence: Influence of the environment on rates of leaf appearance. *Annals of Applied Biology*, **115**, 333-341.

Hayman, A. R., N. B. Perry and R. T. Weavers. (1986). Juvenile-adult chemical dimorphism in foliage of *Dacrydium biforme*. *Phytochemistry*, **25**(1), 649-653.

Hempel, F. D. and L. J. Feldman. (1994). Bi-directional inflorescence development in *Arabidopsis thaliana*: acropetal initiation of flowers and basipetal initiation if paraclades. *Planta*, **192**, 276-286.

Henderson, C. B. (1984). *Maize research and breeders manual no. X: Inbreds, breeding stocks, maize investigations and academic research personnel.* Champaign, IL: Illinois Foundation Seeds.

Hesketh, J. D., S. S. Chase and D. K. Nanda. (1969). Environmental and genetic modifiation of leaf number in maize, sorghum, and Hungarian millet. *Crop Science*, **9**, 460-463.

Heuser, C. W. (1976). Juvenility and rooting cofactors. *Acta Horticulturae*, **56**, 251-261.

Hoagland, D. R. and D. J. Arnon. (1950). The water culture method for growing plants without soil. *Calif. Agric. Exp. Station Circular*, **347**,

Holst, M. J. (1961). Experiments with flower promotion in *Picea Glauca* (Moench) voss. and *Pinus resinosa* Ait. *Recent Advances in Botany*, **6**, 1654-1658.

Hook, G. R. and W. Rasband. (1987). MacMeasure: A low-cost, easy-to-operate quantitative morphometric system for the Macintosh computer. 45th Annual Meeting of the Electron Microscopy Society of America.

Huang, L.-C., S. Lius, B.-L. Huang, T. Murashige, E. F. M. Mahdi and R. V. Gundy. (1992). Rejuvenation of *Sequoia sempervirens* by repeated grafting of shoot tips onto juvenile rootstocks *in vitro*. *Plant Physiologist*, **98**, 166-173.

Hubbard, J. E. (1951). *Leaf patterns in mature embryos of inbred lines of corn*. M. Sc. University of Illinois.

Hunter, R. B., L. A. Hunt and L. W. Kannenberg. (1974). Photoperiod and temperature effects on corn. *Canadian Journal of Plant Science*, **54**, 71-78.

Hunter, R. B., M. Tollenaar and C. M. Breuer. (1977). Effects of photoperiod and temperature of vegetative and reproductive growth of a maize (Zea mays) hybrid. *Canadian Journal of Plant Science*, **5**, 1127-1133.

Hutchison, K. W., C. D. Sherman, J. Weber, S. Schiller-Smith, P. B. Singer and M. S. Greenwood. (1990). Maturation in larch. II. Effects of age on photosynthesis and gene expression in developing foliage. *Plant Physiologist*, **94**, 1308-1315.

Jackson, D. I. and G. B. Sweet. (1972). Flower initiation in temperate woody plants. *Horticultural Abstracts*, **42**, 9-24.

Jacobsen, S. E. and N. E. Olszewski. (1993). Mutations in the SPINDLY locus of *Arabidopsis* alter gibberellin signal transduction. *The Plant Cell*, **5**, 887-896.

Johansen, D. A. (1940). *Plant microtechnique*. New York and London: McGraw-Hill.

Jones, C. S. (1992). Comparative ontogeny of a wild cucurbit and its derived cultivar. *Evolution*, **46**, 1827-1847.

Jones, C. S. (1993). Heterochrony and heteroblastic leaf development in two subspecies of *Cucurbita argyrosperma* (Cucurbitaceae). *American Journal of Botany*, **80**, 778-795.

Kearsley, M. J. C. and T. G. Whitham. (1989). Developmental changes in resistance to herbivory: Implications for individuals and populations. *Ecology*, **70**(2), 422-434.

Kellog, E. A. (1990). Ontogenetic studies of florets in *Poa* (Gramineae): allometry and heterochrony. *Evolution*, **44**, 1978-1989.

Kessler, B. and S. Reches. (1977). Structural and functional changes of chromosomal DNA during aging and phase change in plants. *Chromosomes Today*, **6**, 237-246.

Kiesselbach, T. A. (1949). *The structur and reproduction of corn*. Agric. Exp. Sta., University of Nebraska College of Agriculture: University of Nebraska Press, reprinted 1980.

Kinet, J.-M., R. M. Sachs and G. Bernier. (1985). *The physiology of flowering, Volume 3*. Boca Raton, FL: CRC Press.

Koester, R. P., P. H. Sisco and C. W. Stuber. (1993). Identification of quantitative trait loci controlling days to flowering and plant height in two near isogenic lines of maize. *Crop Science*, **33**, 1209-1216.

Kolattukudy, P. E. (1970). Biosynthesis of cuticular lipids. *Annual Review of Plant Physiology*, **21**, 163-192.

Kolattukudy, P. E. (1980). Cutin, suberin, and waxes. In Stumpf, P. K. and E. E. Conn (Ed.), *The biochemistry of plants: a comprehensive treatise* (pp. 571-645). New York: Academic Press.

Koornneef, M., C. J. Hanhart and J. H. van der Veen. (1991). A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Molecular and General Genetics*, **229**, 57-66.

Koornneef, M. and J. H. van der Veen. (1980). Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. *Theoretical and Applied Genetics*, **58**, 257-263.

Kurtz, E. B., Jr. (1950). The relation of the characteristics and yield of wax to plant age. *Plant Physiology*, **25**, 269-278.

Laibach, F. (1951). Über sommer- und winterannuelle Rassen von *Arabidopsis thaliana* (L.) Heynh. Ein Beitrag zur Ätiologie der Bütenbildung. *Beiträge zur Biologie der Pflanzen*, **28**(3), 173-210.

Lander, E. S. and D. Botstein. (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, **121**, 185-199.

Ledin, R. B. (1954). The vegetative shoot apex of *Zea mays*. *American Journal of Botany*, **41**, 11-17.

Libby, W. J. and J. V. Hood. (1976). Juvenility in hedged Radiata pine. *Acta Horticulturae*, **56**, 91-98.

Lindstrom, E. W. (1925). Heritable characters of maize. XVI. A new dominant hereditary character - *Teopod**. *Journal of Heredity*, **16**, 135-140.

Longman, K. A. and P. F. Wareing. (1959). Early induction of flowering in birch seedlings. *Nature*, **184**(4704), 2037-2038.

Lord, E. M., K. J. Eckard and W. Crone. (1989). Development of the dimorphic anthers in *Collomia grandiflora*; evidence for heterochrony in the evolution of the cleistogamous anther. *Journal of evolutionary biology*, **2**, 81-93.

Lord, E. M. and J. P. Hill. (1987). Evidence for heterochrony in the evolution of plant form. In Raff, R. A. and E. C. Raff (Ed.), *Development as an evolutionary process* (pp. 47-70). New York, NY: Alan R. Liss.

Lorenzoni, C. and F. Salamini. (1975). Glossy mutants of maize V. Morphology of the epicuticular waxes. *Maydica*, **20**, 5-19.

Martin, E. M. and W. M. Harris. (1976). Adventitious root development from the coleoptilar node in *Zea mays* L. *American Journal of Botany*, **63**, 890-897.

Martinez-Zapater, J. M. and C. R. Somerville. (1990). Effect of light quality and vernalization on late-flowering mutants of *Arabidopsis thaliana*. *Plant Physiology*, **92**, 770-776.

MBS Inc. (1988). *Seedman's Handbook, Corn.* 15th. Ames, IA: MBS, Inc.

McCall, R. B. (1990). *Fundamental statistics for behavioral sciences.* 5th. San Diego, CA: Harcourt Brace Jovanovich.

McDaniel, C. N. (1980). Influence on leaves and roots on meristem development in *Nicotiana tabacum* L. cv. Wisconsin 38. *Planta*, **148**, 462-467.

McDaniel, C. N. (1984). Shoot meristem development. In Barlow, P. W. and C. J. Carr (Ed.), *Positional control in plant development* (pp. 319-347). Cambridge: Cambridge University Press.

McDaniel, C. N. (1992). Determination to flower in *Nicotiana. Current Topics in Developmental Biology*, **27**, 1-37.

McDaniel, C. N. and F. C. Hsu. (1976). Positional information in relation to aging. *Acta Horticulturae*, **56**, 291-298.

McDaniel, C. N., S. R. Singer and S. M. E. Smith. (1992). Developmental states associated with the floral transition. *Developmental Biology*, **153**, 59-69.

McLellan, T. (1990). Development of differences of leaf shape in *Begonia dregei* (Begoniaceae). *American Journal of Botany*, **77**, 323-337.

McLellan, T. (1993). The roles of heterochrony and heteroblasty in the diversification of leaf shapes in *Begonia dregei* (Begoniaceae). *American Journal of Botany*, **80**, 796-804.

McMurphy, L. M. and A. L. Rayburn. (1991). Lack of relationship between relative maturity and genome size in hybrid maize. *Crop Science*, **31**, 63-67.

Mericle, L. W. (1950). The developmental genetics of the *Rg* mutant in maize. *American Journal of Botany*, **37**, 100-116.

Mohamed, A. H. (1959). Inheritance of quantitative characters in *Zea mays*. I. Estimation of the number of genes controlling the time of maturity. *Genetics*, **44**, 713-724.

Morgan, D. L. and E. L. McWilliams. (1976). Juvenility as a factor in propagating *Quercus virginiana* Mill. *Acta Horticulturae*, **56**, 263-268.

Mullins, M. G., Y. Nair and P. Sampet. (1979). Rejuvenation *in vitro* induction of juvenile characters in an adult clone of *Vitis vinifera* L. *Annals of Botany*, **44**, 623-627.

Murfet, I. C. (1971a). Flowering in *Pisum:* reciprocal grafts between known genotypes. *Australian Journal of Biological Sciences*, **24**, 1089-1101.

Murfet, I. C. (1971b). Flowering in *Pisum.* Three distinct phenotypic classes determined by the interaction of a dominant late gene. *Heredity*, **26**, 243-257.

Murfet, I. C. (1971c). Flowering in *Pisum.* A three gene system. *Heredity*, **27**, 93-110.

Murfet, I. C. (1973a). Flowering in *Pisum. Hr*, a gene for high response to photoperiod. *Heredity*, **31**, 157-164.

Murfet, I. C. (1973b). Flowering in *Pisum*. The effect of cotyledon removal on genotypes *lf E Sn hr* and *lf e Sn hr*. *Australian Journal of Biological Sciences*, **26**, 669-673.

Murfet, I. C. (1973c). Flowering in *Pisum*. Evidence that the*Sn* gene controls a graft-transmissible inhibitor. *Australian Journal of Biological Sciences*, **26**, 675-677.

Murfet, I. C. (1975). Flowering in *Pisum*: Multiple alleles at the *lf* locus. *Heredity*, **35**, 85-98.

Murfet, I. C. (1977). The physiological genetics of flowering. In Sutcliffe, J. F. and J. S. Pate (Ed.), *The physiology of the garden pea* (pp. 385-430). London: Academic Press.

Murfet, I. C. (1982). Flowering in the garden pea: Expression of the gene *Sn* in the field and use of multiple characters to detect segregation. *Crop Science*, **22**, 923-926.

Murfet, I. C. (1989). Flowering genes in *Pisum*. In Lord, E. M. and G. Bernier (Ed.), *Plant reproduction: from floral induction to pollination* Rockville, MD: American Society of Plant Physiologists.

Murfet, I. C. and J. B. Reid. (1986). Flowering in *Pisum*. Gibberellins and the flowering genes. *Journal of Plant Physiology*, **127**, 23-29.

Napp-Zinn, K. (1962). Über die genetischen Grundlagen des Vernalisationsbedürfnisses bei *Arabidopsis thaliana*. *Zeitschrift für Vererbungslehre*, **93**, 154-163.

Napp-Zinn, K. (1987). Vernalisation - Environmental and genetic regulation. In Atherton, J. G. (Ed.), *Manipulation of flowering* (pp. 123-132). London: Butterworths.

Nienstaedt, H. (1961). Induction of early flowering: A critical review of recent literature. *Recent Advances in Botany*, **6**, 1658-1662.

O'Brien, T. P., N. Feder and M. E. McCully. (1965). Polychromatic staining of plant cell walls by Toluidine Blue O. *Protoplasma*, **59**, 368-373.

O'Brien, T. P. and M. E. McCully. (1981). *The study of plant structure - principles and selected methods*. Melbourne, Australia: Termarcarphi Pty.

Ooijen, J. W. v. (1992). Accuracy of mapping quantitative trait loci in autogamous species. *Theoretical and Applied Genetics*, **84**, 803-811.

Pharis, R. P., S. D. Ross, R. L. Wample and J. N. Owens. (1976). Promotion of flowering in conifers of the *Pinaceae* by certain of the Gibberellins. *Acta Horticulturae*, **56**, 155-162.

Phillips, R. L., T. S. Kim, S. M. Kaeppler, S. N. Parentoni, D. L. Shaver, R. E. Stucker and S. J. Openshaw. (1992). Genetic dissection of maturity using RFLPs. *Proceedings of the Annual Corn and Sorghum Industry Research Conference*, **47**, 135-150.

Poethig, R. S. (1984). Maize - the plant and its parts. In Sheridan, W. F. (Ed.), *Maize for biological research* (pp. 9-18). Grand Forks, ND: Plant Molecluar Biology Association, University Press, University of North Dakota.

Poethig, R. S. (1988a). A non-cell-autonomous mutation regulating juvenility in maize. *Nature*, **336**(6194), 82-83.

Poethig, R. S. (1988b). Heterochronic mutations affecting shoot development in maize. *Genetics*, **119**, 959-973.

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

Poethig, R. S. and H. J. Passas. (1993). Phase change in inbred and exotic lines of maize. *Maize Genetics Cooperation Newsletter*, **67**, 92.

Polito, V. S. and V. Alliata. (1981). Growth of calluses derived from shoot apical meristems of adult and juvenile English ivy (*Hedera helix* L.). *Plant Science Letters*, **22**, 387-393.

Powers, L. (1955). Components of variance method and partitioning method of genetic analysis applied to weight per fruit of tomato hybrid and parental populations. *Technical Bulletin* **1131.** USDA, Washington, D.C.

Powers, L., L. F. Locke and J. C. Garrett. (1950). Partitioning method of genetic analysis applied to quantitative characters of tomato crosses. *Technical Bulletin* **998.** USDA, Washington, D.C.

Price, H. J. (1988). Nuclear DNA content variation within angiosperm species. *Evolutionary Trends in Plants*, **2**, 53-60.

Raff, R. A. and T. C. Kaufman. (1983). *Embryos, genes and evolution*. New York, NY: Macmillan.

Raff, R. A. and G. A. Wray. (1989). Heterochrony: developmental mechanisms and evolutionary results. *Journal of Evolutionary Biology*, **2**, 409-434.

Rayburn, A. L. and J. A. Auger. (1990). Genome size variation in *Zea mays* ssp. *mays* adapted to different altitudes. *Theoretical and Applied Genetics*, **79**, 470-474.

Rayburn, A. L., H. J. Price, J. D. Smith and J. R. Gold. (1985). C-band heterochromatin and DNA content in *Zea mays*. *American Journal of Botany*, **72**, 1610-1617.

Reed, J. W., P. Nagpal, D. S. Poole, M. Furuya and J. Chory. (1993). Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. *The Plant Cell*, **5**, 147-157.

Reicosky, D. A. and J. W. Hanover. (1976). Seasonal changes in leaf surface waxes of *Picea pungens*. *American Journal of Botany*, **63**, 449-456.

Reid, J. B. (1980). Apical senescence in *Pisum*. A direct or indirect role for the flowering genes? *Annals of Botany*, **45**, 195-201.

Reid, J. B. (1986). Gibberellin mutants. In Blonstein, A. D. and P. J. King (Ed.), *A genetic approach to Plant Biochemistry* (pp. 1-34). Wien: Springer.

Reid, J. B., P. J. Dalton and I. C. Murfet. (1977). Flowering in *Pisum*. Does gibberellic acid directly influence the flowering process? *Australian Journal of Plant Physiology*, **4**, 479-483.

Reid, J. B. and I. C. Murfet. (1975). Flowering in *Pisum*: the sites and possible mechanisms of the vernalisation response. *Journal of Experimental Botany*, **26**, 860-867.

Reiter, B. S., J. C. Coors, M. R. Sussman and W. H. Gabelman. (1991). Genetic analysis of tolerance to low-phosphorus stress in maize using restriction fragment length polymorphisms. *Theoretical and Applied Genetics*, **82**, 561-568.

Ritchings, B. W. and W. F. Tracy. (1989). Day length, temperature, and the expression of the *Corn grass* gene in maize (*Zea mays* L.). *Journal of Heredity*, **80**(4), 324-327.

Robbins, W. J. (1957a). Gibberellic acid and the reversal of adult *Hedera* to a juvenile state. *American Journal of Botany*, **44**, 743-746.

Robbins, W. J. (1957b). Physiological aspects of aging. *American Journal of Botany*, **44**, 289-294.

Robbins, W. J. (1961). Juvenility and the induction of flowering. *Recent Advances in Botany*, **6**, 1647-1652.

Robinson, L. W. and P. F. Wareing. (1969). Experiments on the juvenile-adult phase change in some woody species. *New Phytologist*, **68**, 67-78.

Rogler, C. E. and W. P. Hackett. (1975a). Phase change in *Hedera helix*: Induction of the mature to juvenile phase change by Gibberellin A_3. *Physiologia Plantarum*, **34**, 141-147.

Rogler, C. E. and W. P. Hackett. (1975b). Phase change in *Hedera helix*: Stabilization of the mature form with abscisic acid and growth retardants. *Physiologia Plantarum*, **34**, 148-152.

Russell, W. K. and C. W. Stuber. (1983). Effects of photoperiod and temperatures on the duration of vegetative growth in maize. *Crop Science*, **23**, 847-850.

Sahai, P. N. and A. C. Chibnall. (1932). Wax metabolism in the leaves of Brussels sprout. *Biochemical Journal*, **26**(1), 403-412.

Sakai, W. S. (1973). Simple method for differential staining of paraffin embedded plant material using Toluidine Blue O. *Stain Technology*, **48**(5), 247-249.

Sambrook, J., E. F. Fritsch and T. Maniatis. (1989). *Molecular cloning*. 2nd. Cold Spring Harbor: Cold Spring Harbor Press.

Schaffalitzky de Muckadell, M. (1954). Juvenile stages in woody plants. *Physiologia Plantarum*, **7**, 782-796.

Schäffner, K.-H. and W. Nagl. (1979). Differential DNA replication involved in transition from juvenile to adult phase in *Hedera helix* (*Araliaceae*). *Pl. Syst. Evol. Suppl.*, **2**, 105-110.

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

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

Schwabe, W. W. (1976). Applied aspects of juvenility and some theoretical considerations. *Acta Horticulturae*, **56**, 45-56.

Schwabe, W. W. and A. H. Al-Doori. (1973). Analysis of a juvenile-like condition affecting flowering in the black currant (*Ribes nigrum*). *Journal of Experimental Botany*, **24**, 969-981.

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

Sharman, B. C. (1942). Developmental anatomy of the shoot of Zea mays L. *Annals of Botany*, **6**, 245-282.

Shaver, D. (1983). Genetics and breeding of maize with extra leaves above the ear. *Proceedings of the Annual Corn and Sorghum Industry Research Conference*, **38**, 161-180.

Shaver, D. L. (1967). Perennial maize. *Journal of Heredity*, **58**, 271-273.

Shaver, D. L. (1976). Conversions for earliness in maize inbreds. *Maize Genetics Cooperation Newsletter*, **50**, 20-23.

Singleton, W. R. (1946). Inheritance of indeterminate growth in maize. *Journal of Heredity*, **37**, 61-64.

Singleton, W. R. (1951). Inheritance of *Corn Grass* a macromutation in maize, and its possible significance as an ancestral type. *The American Naturalist*, **85**, 81-96.

Sisco, P. H., M. M. Goodman and D. L. Thompson. (1989). Registration of NC264 parental line of maize. *Crops Science*, **29**, 248.

Smith, D. L., K. Kelly and A. D. Krikorian. (1989). Ethylene-associated phase change from juvenile to mature phenotype of daylily (*Hemerocallis*) in vitro. *Physiologia Plantarum*, **76**, 466-473.

Smith, S. M. E. and C. N. McDaniel. (1992). The *maryland mammoth* allele and rooting both perturb the fate of florally determined apices in *Nicotiana tabacum*. *Developmental Biology*, **153**, 176-184.

Southern, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology*, **98**, 503-517.

Spurr, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research*, **26**, 31-43.

Steele, M. J. (1987). *Morphological and physiological changes with age for Sitka spruce, and their development as indices of physiological age*. Ph.D. University of Edinburgh.

Steele, M. J., M. P. Coutts and M. M. Yeoman. (1989). Developmental changes in Sitka spruce as indices of physiological age. I. Changes in needle morphology. *New Phytologist*, **113**, 367-375.

Steele, M. J., M. P. Coutts and M. M. Yeoman. (1990). Developmental changes in Sitka spruce as indices of physiological age. II. Rooting of cuttings and callusing of needle explants. *New Phytologist*, **114**, 111-120.

Stein, O. L. and E. B. Fosket. (1969). Comparative developmental anatomy of shoots of juvenile and adult *Hedera helix*. *American Journal of Botany*, **56**, 546-551.

Stephens, S. G. (1944a). The genetic organisation of leaf-shape development in the genus *Gossypium*. *Journal of Genetics*, **46**, 28-51.

Stephens, S. G. (1944b). A genetic survey of leaf shape in new world cottons - a problem in critical identification of alleles. *Journal of Genetics*, **46**, 313-330.

Stephens, S. G. (1944c). The modifier concept. A developmental ananlysis of leaf-shape 'modification' in new world cotton. *Journal of Genetics*, **46**, 331-344.

Stevenson, J. C. and M. M. Goodman. (1972). Ecology of exotic races of maize. I. Leaf number and tillering of 16 races under 4 temperatures and 2 photoperiods. *Crop Science*, **12**, 864-868.

Stoutemyer, V. T. (1964). Juvenility and flowering potential in woody plants. *American Horticultural Magazine*, **43**, 161-167.

Stoutemyer, V. T. and O. K. Britt. (1961). Effects of temperature and grafting on vegetative growth phases of Algerian ivy. *Nature*, **189**(4767), 854-855.

Stoutemyer, V. T. and O. K. Britt. (1965). The behavior of tissue cultures from English and Algerian ivy in different growth phases. *American Journal of Botany*, **52**, 805-810.

Stuber, C. W., S. E. Lincoln, D. W. Wolff, T. Helentjaris and E. S. Lander. (1992). Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics*, **132**, 823-839.

Sung, Z. R., A. Belachew, B. Shunong and R. Bertrand-Garcia. (1992). *EMF*, an *Arabidopsis* gene required for vegetative shoot development. *Science*, **258**, 1645-1647.

Sussex, I. (1976). Phase change: Physiological and genetic aspects. *Acta Horticulturae*, **56**, 275-280.

Sutter, E. and R. W. Langhans. (1982). Formation of epicuticular wax and its effect on water loss in cabbage plants regenerated from shoot-tip culture. *Canadian Journal of Botany*, **60**, 2896-2902.

Takhtajan, A. (1972). Patterns of ontogenic alterations in the evolution of higher plants. *Phytomorphology*, **22**, 164-171.

Teyker, R. H. (1992). Seedling responses to band applied NH_4OH rates and to N form in two maize hybrids. *Plant and Soil*, **144**, 289-295.

Thiagarajah, M. R. and L. A. Hunt. (1982). Effects of temperature on leaf growth in corn (*Zea mays*). *Canadian Journal of Botany*, **60**, 1647-1652.

Thiagarajah, M. R., L. A. Hunt and J. D. Mahon. (1981). Effects of position and age on leaf photosynthesis in corn (*Zea mays*). *Canadian Journal of Botany*, **59**, 28-33.

Thompson, G. A., Jr. (1980). Plant lipids of taxonomic significance. In Bell, E. A. and B. V. Charlwood (Ed.), *Encyclopedia of plant physiology* (pp. 536-553). New York: Springer.

Tito, C. M., L. Poggio and C. A. Naranjo. (1991). Cytogenetic studies in the genus *Zea* 3. DNA content and heterochromatin in species and hybrids. *Theoretical and Applied Genetics*, **83**, 58-64.

Tollenaar, M. and R. B. Hunter. (1983). A photoperiod and temperature sensitive period for leaf number of maize. *Crop Science*, **23**, 457-460.

Tollenaar, M., J. F. Muldoon and T. B. Daynard. (1984). Difference in rates of leaf appearance among maize hybris and phases of development. *Canadian Journal of Plant Science*, **64**, 759-763.

Troyer, A. F. (1990). Selection for early flowering in corn: Three adapted synthetics. *Crop Science*, **30**, 896-900.

Troyer, A. F. and J. R. Larkins. (1985). Selection for early flowering in corn: 10 late synthetics. *Crop Science*, **25**, 695-697.

Trump, B. F., E. A. Smuckler and E. P. Benditt. (1961). A method for staining epoxy sections for light microscopy. *Journal of Ultrastructural Research*, **5**, 343-348.

Tulloch, A. P. (1973). Composition of leaf surface waxes of *Triticum* species: Variation with age and tissue. *Phytochemistry*, **12**, 2225-2232.

Veen, B. W., M. v. Noordwijk, P. d. Willigen, F. R. Boone and M. J. Kooistra. (1992). Root-soil contact of maize, as measured by a thin section technique III. Effects on shoot growth, nitrate and water uptake efficiency. *Plant and Soil*, **139**, 131-138.

Vieitez, E. and A. M. Vieitez. (1976). Juvenility factors related to the rootability of chestnut cuttings. *Acta Horticulturae*, **56**, 269-274.

Vig, J. C. and P. Limberg. (1986). Die Wirkung der Photoperiode auf die Ausbildung des Maiskolbens. *Landwirtschaftliche Forschung*, **39**, 179-188.

Wallerstein, I. and W. P. Hackett. (1989). Inflorescence induction and initiation in *Hedera helix*. *Israel Journal of Botany*, **38**, 71-83.

Wareing, P. F. (1959). Problems of juvenility and flowering in trees. *Journal of the Linnean Society, Botany*, **56**, 282-289.

Wareing, P. F. (1961). Juvenility and induction of flowering. *Recent Advances in Botany*, **6**, 1652-1654.

Wareing, P. F. (1987). Juvenility and cell determination. In Atherton, J. G. (Ed.), *Manipulation of flowering* (pp. 83-92). London: Butterworths.

Wareing, P. M. and V. M. Frydman. (1976). General aspects of phase change, with special reference to *Hedera helix* L. *Acta Horticulturae*, **56**, 57-69.

Warrington, I. J. and E. T. Kanemasu. (1983). Corn growth response to temperature and photoperiod. II. Leaf-initiation and leaf-appearance rates. *Agronomy*, **75**, 755-761.

Weatherwax, P. (1929). The morphological nature of Teopod corn. *Journal of Heredity*, **20**, 325-330.

Whaley, W. G. and J. H. Leech. (1950). The developmental morphology of the mutant *"Corn Grass". Bulletin of the Torrey Botanical Club*, **77**, 274-286.

Wilkinson, R. E. and M. J. Kasperbauer. (1972). Epicuticular alkane content of tobacco as influenced by photoperiod, temperature and leaf age. *Phytochemistry*, **11**, 2439-2442.

Wilson, R. N., J. W. Heckman and C. R. Somerville. (1992). Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. *Plant Physiology*, **100**, 403-408.

Zagory, D. and W. J. Libby. (1985). Maturation-related resistance of *Pinus radiata* to Western gall rust. *American Phytopathological Society*, **75**, 1443-1447.

Zagotta, M. T., S. Shannon and C. Jacobs. (1992). Early-flowering mutants of *Arabidopsis thaliana. Australian Journal of Plant Physiology*, **19**, 411-418.

Zeevaart, J. A. D. (1976). Physiology of flower formation. *Annual Review of Plant Physiology*, **27**, 321-348.

Zeevaart, J. A. D. (1983). Gibberellins and flowering. In Crozier, A. (Ed.), *The biochemistry and physiology of gibberellins* (pp. 333-374). New York, NY: Praeger.

Zeller, R. A. and E. G. Carmines. (1978). *Statistical analysis of social data*. Chicago: Rand McNally College Publishing.

Zimmerman, R. H. (1972). Juvenility and flowering in woody plants: A review. *HortScience*, **7**, 447-455.

Zimmerman, R. H. (1973). Juvenility and flowering of fruit trees. *Acta Horticulturae*, **34**, 139-142.

Zimmerman, R. H., W. P. Hackett and R. P. Pharis. (1985). Hormonal aspects of phase change and precocious flowering. In Pharis, R. P. and D. M. Reid (Ed.), *Hormonal regulation of development III; Encyclopedia of Plant Physiology New Series* (pp. 79-115). Berlin: Springer Verlag.

APPENDIX

Phase specific insect damage

Phase-specific resistance to pests and pathogens has been reported in several plant species (Kearsley and Whitham, 1989; Libby and Hood, 1976; Zagory and Libby, 1985). It has been suggested that phase-specific resistance is adaptive, can occur rapidly within one plant and is an important component determining the distribution of plant pests (Kearsley and Whitham, 1989).

In this section, a casual observation of phase-specific insect resistance in maize is reported. Although this phenomenon has not be fully investigated, observation of differential resistance have been made repeatedly in different years, genotypes, and environments. In plants that are prey to thrips (Thysanoptera), feeding damage appears as small yellow spots on the leaf where cells have been pierced and fed on. It was noticed that feeding damage was concentrated in early vegetative leaves and in the glaucous parts of transition leaves. An example of such damage is presented in figure A1. The areas of waxy bloom and feeding damage also coincide with the completely purple staining pattern in leaves stained with toluidine blue O. This suggests that thrips preferentially feed on early vegetative leaves and tissues of maize.

The determining factors for this feeding preference are not known. Epidermal cell wall lignification, suggested by the aqua staining of late vegetative leaves, may act as a physical barrier. Different components of the leaf surface may act as feeding attractants or deterrents. While the primary function of the cuticle is believed to be its role as a physical barrier to water loss, its chemical composition may play a role in pest and pathogen resistance. Several compounds of leaf surface extracts have been reported to stimulate probing, feeding, aggregation, oviposition or other potentially harmful activities in insects (see: Jeffree, 1986; Kolattukudy, 1980; Städler, 1986; Woodhead and Chapman, 1986). In most insect-plant combinations investigated, short-chain wax compounds tend to deter damaging insect behaviour, whereas long-chain coumpounds tend to stimulate such behaviour (Woodhead and Chapman, 1986). The amount and composition of cuticular waxes is known to change from the early vegetative to the late vegetative phase of development (Blaker and Greyson, 1988). Bianchi et al. (1989) reported that in leaves of mature plants (12 to 14 leaves), the largest fraction of the leaf wax esters are short-chain esters (C_{44} and C_{46}), whereas in seedling leaves (up to 5 leaves) long-chain esters prevail (C_{54} and C_{56}). A study of thrips behaviour in relation to wax extracts from different maize leaves could reveal whether wax composition is a factor in the feeding preference of thrips on maize. However, a large number of other

physical or biochemical factors may be involved in this phase-specific resistance, and further reseach is needed to understand this phenomenon.

Figure A1: Consecutive transition leaves of a field-grown plant showing thrip feeding damage that is localised at the tip of the leaf in tissues that express early vegetative traits.

Table A1: Total leaf number and ear placement node in A632, A632/A632E, and A632E plants.

Genotype	A632		A632/A632E		A632E	
Trait	EAR	TLN	EAR	TLN	EAR	TLN
Plant no. 1	13	18	13	19	11	16
2	14	19	12	18	11	17
3	14	20	13	18	11	16
4	14	19	13	18	11	17
5	14	19	13	18	11	16
6	14	19	13	19	11	16
7	14	20	12	18	10	16
8	14	19	13	19	11	16
9	14	20	12	18	10	15
10	14	19	13	18	10	16
11	14	20	13	19	10	16
12	13	19	13	19	11	16
13	13	18	13	19	10	16
14	14	20	13	19	11	17
15	14	19	13	19	11	17
16	13	19			11	17
17	14	20			11	17
18	14	20				
mean	13.78	19.28	12.8	18.53	10.71	16.29
s.e.m.	0.101	0.158	0.107	0.133	0.114	0.143

Table A2: Total leaf number (TLN) as a function of time to flowering (in days to anthesis (DAN)) in Oh43

	Oh43		Oh43/Oh43E		Oh43E		Family segregating the early flowering trait			
	DAN	TLN	DAN	TLN	DAN	TLN	DAN (12 TLN)	DAN (13 TLN)	DAN (14 TLN)	DAN (15 TLN)
Pl.no.1	62	14	59	13	55	9	61	62	62	62
2	62	14	60	13	55	9	55	61	60	64
3	62	14	59	13	55	9	57	58	63	58
4	61	14	61	13	54	10	56	59	60	59
5	64	15	57	14	56	10	60	61	58	62
6	61	14	61	14	54	10	58	56	61	63
7	61	15	59	13	56	10	55	60	64	66
8	64	15	57	12	50	10	69	62	65	62
9	61	15			56	11	59	60	61	63
10	60	14			55	11	59	60	62	62
11							57	60	60	64
12							60	57	60	63
13							57	57	56	63
14							55	61	58	61
15							56	57	60	64
16								54	60	61
17								63	57	60
18								54	58	64
19								56	60	68
20								56	54	60
21								57	58	60
22								59	60	62
23								60	62	61
24								58	61	63
25								59	62	60
26								58	59	63
27								59	63	59
28								58	57	
29								57	62	
30								56	57	
31									64	
32									61	
33									64	
34									63	
35									62	
36									54	
37									57	
mean	61.80	14.40	59.13	13.13	54.6	9.9	58.27	58.50	60.14	62.11
s.e.m.	0.42	0.16	0.55	0.23	0.56	0.23	0.92	0.42	0.45	0.42
st. dev.	1.32	0.52	1.55	0.64	1.78	0.74	3.56	2.30	2.72	2.21

Leaf initiation

The smallest primordium was scored as a decimal based on its size.

.0: no new primordium visible, and previous primordium wraps around apex

.2: primordium is a small bump on the side of the apex

.4: primordium is a distinct bump on the side of the apex

.6: small groove visible between primordium and the apex

.8: groove between primordium and apex extends around sides of the apex, primordium has a tip

1.0: primordium has a distinct tip and wraps around apex

Table A3: Leaf inititation and emergence in A632

DAP	5		11		17		23		29		35	
No. of leaves	visible	initiated	visible	initiated	visible	initiated	visible	initiated	visible	initiated	visible	initiated
Plant no. 1	3	7.6	2	9.6	4	10.4	5	12	6	13	7	13.8
2	3	7.8	2	9	4	10.8	5	11.2	6	12.8	7	13.8
3	3	7.6	2	9.2	5	10.6	5	11	6	12.4	7	14
4	3	7.8	2	9.2	5	10.8	5	11.6	6	12.8	7	13
5	3	7.4	2	8.8	4	10.2	5	12	6	12.8		
6	3	7.8	4	9.6	4	10.6	5	12	6	12.6		
7	3	7.8	4	9.4	4	10.8	5	12	6	12.6		
8			4	9.4	4	10.2	6	12.4	6	12.8		
9			4	9.8	5	10.4			6	12.2		
10			4	9.2	4	10.6			6	12.6		
11			4	9	4	10.4						
mean	3.00	7.69	3.09	9.29	4.27	10.53	5.13	11.78	6.00	12.66	7.00	13.65
s.e.m.	0.00	0.06	0.31	0.09	0.14	0.07	0.13	0.17	0.00	0.07	0.00	0.22
st. dev.	0.00	0.16	1.04	0.30	0.47	0.22	0.35	0.47	0.00	0.23	0.00	0.44

Table A4: Leaf inititation and emergence in A632E

DAP	5		11		17		23		29		35	
No. of leaves	visible	initiated	visible	initiated	visible	initiated	visible	initiated	visible	initiated	visible	initiated
Plant no. 1	3	7.8	4	9.8	5	10.4	4	9	6	13.2	7	13
2	3	7.8	4	9.2	5	11	5	11.4	5	11	7	13
3	3	7.6	4	9.8	4	9	5	10.2	6	11.6	6	12.8
4	3	7.6	4	9.4	4	9.8	4	9.2	6	10.6		
5	3	7.8	4	9.6	4	10	5	11	6	13		
6			4	9.4	4	9.4	5	10.2	7	14.2		
7			4	9.4	4	10	6	12.6	6	12.8		
8			4	9.4	4	9.6	6	13	6	12.8		
9			4	9.4	4	10.4			5	11		
mean	3.00	7.72	4.00	9.49	4.17	9.96	5.00	10.83	5.89	12.24	6.67	12.93
s.e.m.	0.00	0.05	0.00	0.07	0.15	0.20	0.27	0.52	0.20	0.41	0.33	0.07
st. dev.	0.00	0.11	0.00	0.20	0.44	0.60	0.76	1.46	0.60	1.23	0.58	0.12

Table A5: Leaf initiation in Oh43

DAP	1	3	5	7	9	11	13	15	17	20	23	26	29
Plant no. 1	5.2	5.4	6.8	7.6	8.8	9	10	11.2	11	13.2	15.2	16.6	16+Tassel
2	5	5.4	6.4	7.8	8.6	8.8	9.8	11.2	11.2	13	13.8	15.8	16+Tassel
3	5.4	5.8	6.2	7.6	8.2	9	9.8	11	10.6	12.8	15.2	17.2	16+Tassel
4	5.4	5.6	6.4	7.4	8.8	8.8	9.8	10.8	11.2	12.2	14.8		16+Tassel
5	5.6	5.8	6.2	7.6	8	9.2	10	10.4	11.4	12.4	14.2	16+Tassel	16+Tassel
6	5.2			7.2	8.6	9	10.2	10.8	11.4	12.2	13.4	16+Tassel	
7	5.4			7.4	8.2	9	9.6	11		12.8	14.6		
8	5.6			7			9.4	11.2					
Mean	5.35	5.60	6.40	7.45	8.46	8.97	9.83	10.95	11.13	12.66	14.46	16.32	
s.e.m.	0.07	0.09	0.11	0.09	0.12	0.05	0.09	0.10	0.12	0.15	0.26	0.26	
st. dev.	0.21	0.20	0.24	0.26	0.32	0.14	0.25	0.28	0.30	0.40	0.69	0.58	

Table A6: Leaf initiation in Oh43E

DAP	1	3	5	7	9	11	13	15	17
Plant no. 1	5	5.4	6.8	8.2	8.6	9.8	9.6	12.2	12+Tassel
2	5	5.4	6.6	7.8	9.4	9.4	10	12.2	12+Tassel
3	5	6	6.8	7.8	9.2	9.6	10.2	12.4	13+Tassel
4	5	5.6	6.4	7.8	9.4	9.4	10.2		
5	5		6.4	7.6	8.8	9.4	10	12+Tassel	
6				7.8	8.6			12+Tassel	
7				7.8			11	13+Tassel	
8				7.6					
Mean	5	5.6	6.6	7.8	9	9.52	10.167	12.3	
s.e.m.	0.00	0.14	0.09	0.07	0.15	0.08	0.19	0.15	
st. dev.	0.00	0.28	0.20	0.19	0.38	0.18	0.46	0.37	

Root experiment

Table A7: Root experiment in Oh43

PLANT No.	DAP	V	L	Lvs. init.	FWS	DWS	FWP	DWP	FWR	DWR	FWPR	DWPR	FW S/PR	DW S/PR	LeP	LeR	LePR
1	6	0	0	6.00	35.00	5.00	3.00	0.00	23.00	0.00	26.00	0.00	1.35	3.00	0.90	3.30	4.20
2	6	0	0	6.40	76.00	8.00	9.00	5.00	63.00	0.00	72.00	5.00	1.06	1.60	6.20	2.90	9.10
3	6	0	0	6.20	116.00	9.00	6.00	3.00	64.00	0.00	70.00	3.00	1.66	3.00	12.10	18.00	30.10
4	6	0	0	6.80	159.00	16.00	24.00	4.00	56.00	1.00	80.00	5.00	1.99	3.20	13.80	24.40	38.20
5	6	0	0	6.80	170.00	5.00	70.00	6.00	81.00	6.00	151.00	12.00	1.13	0.42	14.60	30.30	44.90
mean				6.44	111.20	8.60	22.40	3.60	57.40	1.40	79.80	5.00	1.43	2.24	9.52	15.78	25.30
s.e.m.				0.16	25.31	2.01	12.44	1.03	9.53	1.17	20.15	1.97	0.17	0.54	2.61	5.53	8.00
6	10	1	3	8.00	640.00	71.00	177.00	13.00	272.00	18.00	449.00	31.00	1.43	2.29	70.06	99.25	169.31
7	10	1	3	8.00	510.00	64.00	76.00	5.00	181.00	13.00	257.00	18.00	1.98	3.56	38.62	70.06	108.68
8	10	1	3	8.80	1009.00	95.00	485.00	29.00	244.00	15.00	729.00	44.00	1.38	2.16	145.06	115.42	260.48
9	10	1	3	8.20	786.00	76.00	184.00	13.00	360.00	21.00	544.00	34.00	1.44	2.24	64.67	138.77	203.44
10	10	1	3	8.20	753.00	77.00	172.00	15.00	330.00	19.00	502.00	34.00	1.50	2.26	71.41	116.77	188.17
mean				8.24	739.60	76.60	218.80	15.00	277.40	17.20	496.20	32.20	1.55	2.50	77.96	108.05	186.02
s.e.m.				0.15	82.93	5.14	69.43	3.90	31.64	1.43	76.14	4.18	0.11	0.26	17.79	11.39	24.61
11	13	2	4	9.20	1289.00	113.00	284.00	16.00	756.00	38.00	1040.00	54.00	1.24	2.09	72.24	219.02	291.26
12	13	2	4	9.40	1667.00	126.00	442.00	9.00	510.00	22.00	952.00	31.00	1.75	4.06	121.47	171.63	293.10
13	13	2	4	9.60	1637.00	134.00	529.00	49.00	662.00	30.00	1191.00	79.00	1.37	1.70	110.43	237.88	348.31
14	13	2	4	9.40	1555.00	132.00	351.00	25.00	560.00	26.00	911.00	51.00	1.71	2.59	83.74	177.61	261.35
15	13	2	4	9.40	1702.00	142.00	498.00	23.00	555.00	28.00	1053.00	51.00	1.62	2.78	109.97	184.05	294.02
mean				9.40	1570.00	129.40	420.80	24.40	608.60	28.80	1029.40	53.20	1.54	2.65	99.57	198.04	297.61
s.e.m.				0.06	74.33	4.83	45.66	6.76	44.46	2.65	48.37	7.64	0.10	0.40	9.23	12.92	14.07
16	18	3	5	12.20	3887.00	341.00	805.00	48.00	1363.00	62.00	2168.00	110.00	1.79	3.10	172.73	400.65	573.38
17	18	3	5	12.60	4877.00	407.00	1545.00	87.00	1358.00	62.00	2903.00	149.00	1.68	2.73	293.51	445.45	738.96
18	18	3	5	12.20	4524.00	398.00	1299.00	74.00	1327.00	59.00	2626.00	133.00	1.72	2.99	190.91	415.58	606.49
19	18	3	5	12.20	4535.00	402.00	1293.00	71.00	1556.00	71.00	2849.00	142.00	1.59	2.83	233.77	445.45	679.22
20	18	3	5	12.40	4939.00	523.00	1013.00	57.00	1586.00	75.00	2599.00	132.00	1.90	3.96	213.64	474.68	688.31
mean				12.32	4552.40	414.20	1191.00	67.40	1438.00	65.80	2629.00	133.20	1.74	3.12	220.91	436.36	657.27
s.e.m.				0.08	186.91	29.71	128.07	6.80	54.85	3.06	129.79	6.58	0.05	0.22	20.87	12.92	29.78

PLANT No.	day	V	L	Lvs. init.	FWS	DWS	FWP	DWP	FWR	DWR	FWPR	DWPR	FW S/PR	DW S/PR	LeP	LeR	LePR
21	23	4	6	14.20	6510.00	634.00	1611.00	86.00	4315.00	189.00	5926.00	275.00	1.10	2.31	281.18	574.33	855.51
22	23	4	6	12.80	4330.00	421.00	1046.00	58.00	3120.00	152.00	4166.00	210.00	1.04	2.00	159.13	485.93	645.06
23	23	4	7	14.60	7930.00	694.00	2194.00	110.00	3364.00	145.00	5558.00	255.00	1.43	2.72	337.64	526.43	864.07
24	23	4	6	12.80	4430.00	434.00	534.00	38.00	4175.00	210.00	4709.00	248.00	0.94	1.75	91.25	531.56	622.81
25	23	4	7	16.20	11610.00	775.00	4016.00	211.00	2732.00	133.00	6748.00	344.00	1.72	2.25	350.19	557.79	907.98
mean				14.12	6962.00	591.60	1880.20	100.60	3541.20	165.80	5421.40	266.40	1.25	2.21	243.88	535.21	779.09
s.e.m.				0.63	1343.20	70.66	601.69	30.19	305.29	14.48	453.69	22.07	0.14	0.16	50.97	15.10	60.03
26	27	6	8	17.00	24420.00	1659.00	7676.00	325.00	3819.00	162.00	11495.00	487.00	2.12	3.41	563.85	513.67	1077.52
27	27	6	9	19.00	24750.00	1824.00	8674.00	399.00	4104.00	186.00	12778.00	585.00	1.94	3.12	682.55	541.19	1223.74
28	27	6	8	20.00	26580.00	1683.00	6606.00	393.00	3495.00	148.00	10101.00	541.00	2.63	3.11	463.49	515.83	979.32
29	27	5	8	18.20	23130.00	1611.00	6702.00	275.00	4027.00	160.00	10729.00	435.00	2.16	3.70	476.98	599.46	1076.44
30	27	5	8	18.20	18770.00	1550.00	3042.00	165.00	5767.00	258.00	8809.00	423.00	2.13	3.66	343.17	675.00	1018.17
31	27	6	8	19.40	24770.00	1679.00	2919.00	167.00	7840.00	305.00	10759.00	472.00	2.30	3.56	253.06	745.14	998.20
mean				18.63	23736.67	1667.67	5936.50	287.33	4842.00	203.17	10778.50	490.50	2.21	3.43	463.85	598.38	1062.23
s.e.m.				0.43	1090.60	37.39	983.83	42.68	681.01	26.00	543.20	25.48	0.10	0.11	62.45	38.59	36.26

Abbreviations:

Developmental stage: V: Number of visible ligules L: Number of visible leaves Lvs. init.: Number of leaves initiated
T: Tassel

Parameter measured as: FW..: Fresh weight of.. in mg DW..: Dry weight of.. in mg Le..: Length of.. in cm

Plant parts: S: Shoot (e.g. FWS: Fresh weight of the shoot) P: Prop roots
R: Seminal roots PR: Prop roots and seminal roots S/PR: Shoot-to-total-root ratio

Table A8: Root experiment in Oh43E (Abbreviations as in previous table)

PLANT No.	day	V	L	Lvs. init.	FWS	DWS	FWP	DWP	FWR	DWR	FWPR	DWPR	FW S/PR	DW S/PR	LeP	LeR	LePR
1	6	0	0	6	84.00	7.00	7.00	0.00	55.00	3.00	62.00	3.00	1.35	2.33	0.90	3.30	4.20
2	6	0	1	6.8	229.00	20.00	54.00	5.00	56.00	3.00	110.00	8.00	2.08	2.50	6.20	2.90	9.10
3	6	1	2	7.2	335.00	25.00	59.00	2.00	113.00	7.00	172.00	9.00	1.95	2.78	12.10	18.00	30.10
4	6	1	2	7.4	440.00	38.00	77.00	4.00	159.00	10.00	236.00	14.00	1.86	2.71	13.80	24.40	38.20
5	6	1	3	7.4	417.00	38.00	55.00	4.00	135.00	11.00	190.00	15.00	2.19	2.53	14.60	30.30	44.90
mean				6.96	301.00	25.60	50.40	3.00	103.60	6.80	154.00	9.80	1.89	2.57	9.52	15.78	25.30
s.e.m.				0.26	65.65	5.85	11.62	0.89	20.94	1.69	30.61	2.18	0.14	0.08	2.61	5.53	8.00
6	10	2	4	9.6	1167.00	131.00	269.00	15.00	427.00	23.00	696.00	38.00	1.68	3.45	101.50	151.80	253.29
7	10	2	4	10+T	1380.00	125.00	298.00	19.00	617.00	36.00	915.00	55.00	1.51	2.27	100.15	166.62	266.77
8	10	2	4	10.2	1272.00	116.00	361.00	19.00	658.00	31.00	1019.00	50.00	1.25	2.32	103.29	199.40	302.69
9	10	2	4	10.2	1565.00	135.00	340.00	17.00	923.00	41.00	1263.00	58.00	1.24	2.33	95.66	232.63	328.29
10	10	2	4	9.4	1028.00	91.00	142.00	17.00	542.00	26.00	684.00	43.00	1.50	2.12	52.54	167.07	219.61
mean					1282.40	119.60	282.00	17.40	633.40	31.40	915.40	48.80	1.44	2.50	90.63	183.50	274.13
s.e.m.					91.52	7.83	38.49	0.75	82.37	3.26	107.99	3.71	0.08	0.24	9.60	14.54	18.99
11	13	3	5	11+T	2165.00	167.00	510.00	22.00	938.00	39.00	1448.00	61.00	1.50	2.74	102.61	279.29	381.90
12	13	3	5	10+T	3070.00	239.00	376.00	18.00	1616.00	37.00	1992.00	55.00	1.54	4.35	86.50	380.98	467.48
13	13	3	5	11.4	2691.00	227.00	618.00	27.00	1096.00	24.00	1714.00	51.00	1.57	4.45	141.72	319.79	461.50
14	13	3	5	9+T	3060.00	246.00	659.00	30.00	1350.00	52.00	2009.00	82.00	1.52	3.00	124.23	335.89	460.12
15	13	3	5	12+T	3059.00	241.00	608.00	30.00	1334.00	57.00	1942.00	87.00	1.58	2.77	130.21	341.41	471.63
mean					2809.00	224.00	554.20	25.40	1266.80	41.80	1821.00	67.20	1.54	3.46	117.06	331.47	448.53
s.e.m.					176.39	14.59	50.82	2.36	116.33	5.84	107.19	7.28	0.01	0.39	9.94	16.48	16.78
16	18	4	6	12+T	6066.00	529.00	1486.00	88.00	1438.00	70.00	2924.00	158.00	2.07	3.35	258.44	425.32	683.77
17	18	4	6	11+T	7918.00	705.00	2350.00	135.00	1826.00	89.00	4176.00	224.00	1.90	3.15	376.62	481.17	857.79
18	18	4	6	11+T	7263.00	660.00	1714.00	111.00	1873.00	90.00	3587.00	201.00	2.02	3.28	344.81	490.26	835.06
19	18	4	6	12+T	7162.00	642.00	2243.00	117.00	1586.00	66.00	3829.00	183.00	1.87	3.51	374.03	418.83	792.86
20	18	4	6	10+T	6836.00	605.00	1472.00	101.00	1457.00	78.00	2929.00	179.00	2.33	3.38	288.31	455.19	743.51
mean					7049.00	628.20	1853.00	110.40	1636.00	78.60	3489.00	189.00	2.04	3.33	328.44	454.16	782.60
s.e.m.					302.11	29.56	186.85	7.87	91.11	4.85	247.99	11.10	0.08	0.06	23.64	14.34	31.47

PLANT No.	day	V	L	Lvs. init.	FWS	DWS	FWP	DWP	FWR	DWR	FWPR	DWPR	FW S/PR	DW S/PR	LeP	LeR	LePR
21	23	4	7	8+T	8440.00	781.00	5569.00	247.00	2224.00	102.00	7793.00	349.00	1.08	2.24	662.74	303.99	966.73
22	23	4	7	9+T	10900.00	1012.00	3030.00	169.00	6644.00	276.00	9674.00	445.00	1.13	2.27	314.83	740.30	1055.13
23	23	4	7	9+T	9100.00	836.00	1873.00	113.00	5905.00	291.00	7778.00	404.00	1.17	2.07	233.84	673.57	907.41
24	23	4	7	9+T	5570.00	585.00	3191.00	182.00	3311.00	179.00	6502.00	361.00	0.86	1.62	423.76	406.65	830.42
25	23	4	7	8+T	8650.00	841.00	2077.00	116.00	5569.00	269.00	7646.00	385.00	1.13	2.18	285.74	695.25	980.99
mean					8532.00	811.00	3148.00	165.40	4730.60	223.40	7878.60	388.80	1.07	2.08	384.18	563.95	948.14
s.e.m.					858.08	68.52	657.64	24.63	837.90	36.14	509.41	16.97	0.06	0.12	76.24	87.37	37.68
26	27	6	9	10+T	36310.00	2642.00	1103.00	497.00	5679.00	223.00	6782.00	720.00	5.35	3.67	824.46	636.15	1460.61
27	27	5	8	9+T	25970.00	2040.00	7599.00	357.00	4981.00	316.00	12580.00	673.00	2.06	3.03	544.96	597.30	1142.27
28	27	5	9	11+T	40700.00	2996.00	12079.00	579.00	6683.00	304.00	18762.00	883.00	2.17	3.39	685.25	691.73	1376.98
29	27	5	8	9+T	27950.00	2085.00	7029.00	320.00	6450.00	293.00	13479.00	613.00	2.07	3.40	481.83	752.70	1234.53
30	27	6	9	11+T	37710.00	2506.00	10963.00	484.00	5377.00	205.00	16340.00	689.00	2.31	3.64	595.14	627.52	1222.66
31	27	6	10	13+T	40630.00	2887.00	11144.00	502.00	7468.00	296.00	18612.00	798.00	2.18	3.62	751.08	750.54	1501.62
mean					34878.33	2526.00	8319.50	456.50	6106.33	272.83	14425.83	729.33	2.69	3.46	647.12	675.99	1323.11
s.e.m.					2610.25	162.87	1666.95	40.00	377.96	19.03	1850.42	39.48	0.53	0.10	52.97	26.97	58.97

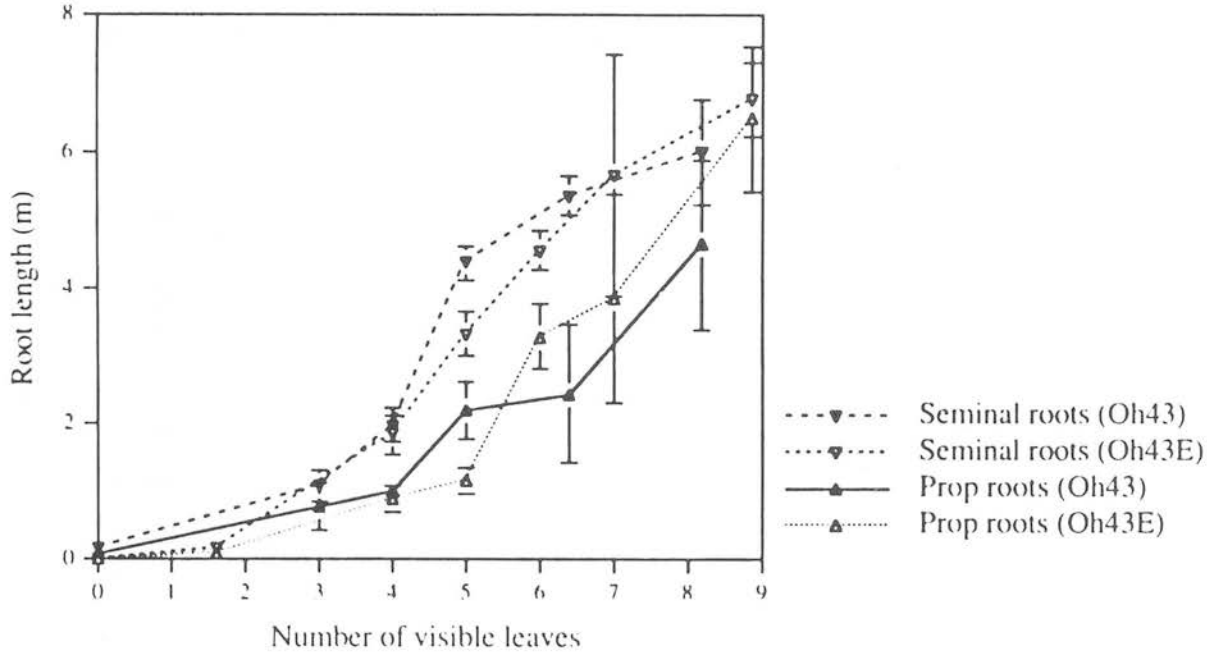

Figure A2: Length of prop roots and seminal roots as a function of developmental state (number of visible leaves) in Oh43 and Oh43E. All errors are given as ±2 s.e.m.

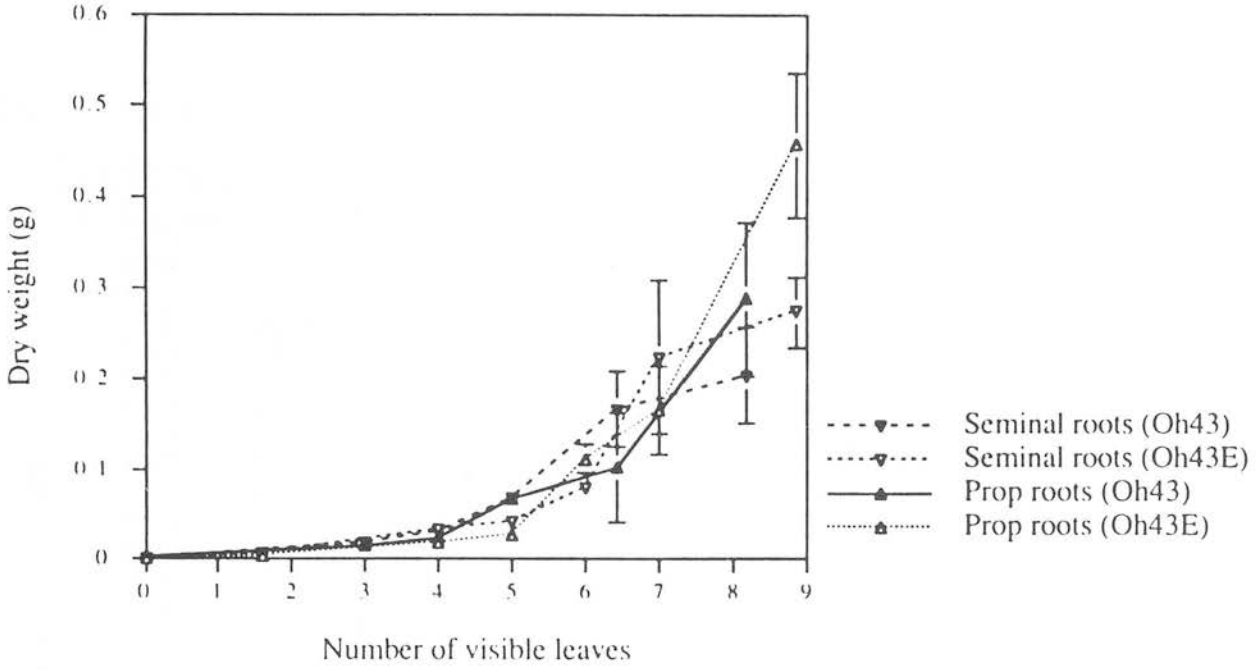

Figure A3: Dry weight of prop roots and seminal roots as a function of developmental state (number of visible leaves) in Oh43 and Oh43E. All errors are given as ±2 s.e.m.

Leaf form measurements

Table A9: Leaf length in A632 plants (cm)

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.
Leaf no. 1	4.20	5.10	4.40	4.30	3.50	4.60	2.70	3.60	4.10	4.90	4.30	4.15	0.20
2	10.30	11.30	10.50	10.30	9.90	9.50	9.40	9.60	11.80	11.10	10.40	10.37	0.23
3	19.60	18.40	18.60	18.60	20.60	20.00	18.10	17.10	21.20	21.00	20.80	19.45	0.41
4	26.20	25.20	25.50	32.50	29.20	26.10	26.50	25.00	31.50	28.70	27.40	27.62	0.77
5	33.80	35.20	34.40	32.50	39.40	34.40	36.20	33.60	40.80	40.00	37.80	36.19	0.86
6	48.70	49.80	45.30	44.10	52.50	47.50	52.70	49.40	55.40	54.50	54.70	50.42	1.16
7	61.10	61.60	57.00	54.50	63.60	60.60	65.70	65.10	66.50	67.90	69.00	62.96	1.36
8	74.20	71.10	67.50	72.90	71.00	69.20	75.40	77.50	75.30	77.80	77.80	73.61	1.07
9	76.80	78.20	75.80	75.10	75.60	73.70	78.80	81.10	82.10	81.90	82.90	78.36	0.97
10	73.30	76.50	75.00	74.40	71.70	72.30	80.80	78.10	83.70	80.60	79.40	76.89	1.18
11	69.10	74.70	72.60	60.00	67.40	71.50	80.50	75.70	77.20	72.80	75.50	72.45	1.67
12	62.70	69.50	67.80	54.30	60.40	65.90	73.00	65.20	71.30	65.90	67.20	65.75	1.58
13	54.90	63.10	61.50	48.40	55.40	57.70	67.00	60.40	64.50	59.60	60.30	59.35	1.55
14	49.60	59.10	54.90	44.40	51.40	53.20	62.90	56.10	56.70	54.10	55.30	54.34	1.47
15	45.20	55.60	51.00	38.70	47.70	47.90	57.90	51.90	50.30	48.30	50.30	49.53	1.54
16	40.70	48.40	44.80	34.50	41.70	43.10	48.20	45.40	45.10	41.50	41.40	43.16	1.18
17	34.20	42.90	38.40	32.80	29.90	31.40	43.40	37.30	37.40	36.40	31.40	35.95	1.36
18	23.80	28.70	28.80	23.20				31.30		27.00		27.13	1.28

Table A10: Leaf length in A632E plants (cm)

Leaf no.	Plant no.1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	4.70	6.80	4.30	5.20	5.60	5.30	5.50	5.60	6.60	5.80	6.30	5.65	0.21	0.69
2	13.80	14.70	13.80	13.20	14.90	13.20	13.50	14.30	14.80	13.40	13.90	13.95	0.19	0.63
3	24.60	26.50	21.10	24.60	27.20	26.40	24.50	23.30	26.50	22.60	25.40	24.79	0.57	1.88
4	32.60	37.80	29.90	32.50	36.70	38.70	35.80	31.70	36.00	29.90	35.90	34.32	0.94	3.11
5	43.20	47.80	41.00	43.80	43.90	51.20	46.70	42.30	46.90	37.70	45.60	44.55	1.10	3.65
6	58.50	62.40	53.30	59.60	53.30	64.70	59.50	60.90	58.80	53.70	56.00	58.25	1.15	3.80
7	67.00	71.90	56.60	70.40	62.00	73.90	66.30	70.40	64.90	60.00	65.70	66.28	1.59	5.27
8	65.70	76.30	53.50	72.90	66.40	77.60	70.70	71.50	66.70	61.90	71.40	68.60	2.07	6.85
9	62.60	75.10	48.20	69.00	63.00	76.50	68.50	68.10	63.00	56.10	69.60	65.43	2.46	8.16
10	55.80	67.00	43.40	65.40	58.30	68.40	63.20	61.50	58.10	50.60	61.70	59.40	2.23	7.40
11	52.10	61.70	35.10	58.20	53.30	61.80	58.70	54.70	54.00	42.50	58.00	53.65	2.47	8.18
12	45.60	55.10		51.70	47.10	55.70	54.80	48.40	48.30	33.50	53.40	49.36	2.10	6.64
13	39.60	49.80		41.20	37.80	51.00	51.00	35.30	43.00		47.80	44.06	2.00	6.00
14	31.50	44.70				45.60	46.00		35.00		42.90	40.95	2.51	6.16
15		37.90				34.70	34.10				33.90	35.15	0.93	1.86
16		26.30										26.30		

Table A11: Leaf lengths in Oh43 (cm)

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	3.50	2.90	4.90	4.10	5.10	3.40	4.30	4.30	4.10	3.20	2.70	3.86	0.24	0.79
2	12.80	10.40	14.60	13.10	15.60	11.70	12.40	12.90	14.00	7.40	9.10	12.18	0.73	2.42
3	24.60	21.70	23.80	21.50	27.50	19.30	21.10	20.30	24.20	15.20	16.10	21.39	1.10	3.65
4	33.80	31.10	32.70	28.80	36.50	27.40	29.60	30.10	31.80	21.50	24.50	29.80	1.28	4.23
5	35.60	42.20	47.70	40.70	49.00	41.20	40.90	45.30	44.60	35.00	39.10	41.94	1.35	4.48
6	59.80	52.00	59.00	52.80	61.70	56.70	56.70	59.50	60.50	51.60	55.20	56.86	1.08	3.57
7	68.20	64.70	70.90	66.00	74.00	72.70	68.00	72.70	72.10	66.90	72.40	69.87	0.96	3.20
8	78.70	76.10	79.70	79.70	83.10	88.30	79.30	85.50	83.10	83.10	87.60	82.20	1.17	3.87
9	81.50	77.40	78.90	76.50	79.20	85.90	80.40	86.30	78.30	84.20	89.60	81.65	1.28	4.25
10	76.70	74.10	74.90	73.50	72.90	77.40	74.60	84.10	69.50	76.30	78.70	75.70	1.13	3.73
11	70.90	66.40	67.50	68.70	62.90	69.10	66.30	74.80	55.90	66.30	71.60	67.31	1.49	4.95
12	65.70	56.30	61.50	63.90	53.90	57.90	55.40	66.90	47.30	55.00	62.20	58.73	1.78	5.89
13	56.20	45.60	52.70	55.30	47.10	44.50	46.70	58.30	34.60	42.50	46.20	48.15	2.09	6.94
14	46.10	33.70	35.70	38.00	34.90	20.40	32.20	38.50	16.30	20.60	21.60	30.73	2.87	9.50
15	31.50	17.80	14.20	18.90	15.10		16.70	23.00				19.60	2.26	5.98

Table A12: Leaf length in Oh43E plants (cm)

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	5.50	4.50	3.60	Plant	4.20	3.50	4.80	3.40	4.40	5.00	3.60	4.25	0.23	0.72
2	16.40	14.40	12.40	died	13.60	12.30	13.50	11.70	14.60	15.30	10.90	13.51	0.54	1.71
3	29.10	26.30	22.40		24.70	21.10	25.50	21.20	23.90	28.20	18.30	24.07	1.07	3.38
4	40.00	38.70	33.40		36.50	32.80	36.10	30.90	34.30	39.50	25.10	34.73	1.43	4.53
5	53.20	54.70	50.20		54.00	52.70	51.10	50.60	52.40	51.90	39.30	51.01	1.38	4.36
6	62.60	68.00	66.20		66.90	66.90	66.20	63.60	65.20	63.70	55.60	64.49	1.13	3.56
7	65.50	74.60	76.00		70.40	77.70	73.90	73.20	84.50	68.30	65.70	72.98	1.84	5.82
8	62.10	69.50	70.70		63.50	70.60	70.60	65.60	67.30	64.40	70.10	67.44	1.05	3.31
9	53.00	56.70	59.20		51.30	59.50	63.50	55.70	59.30	57.40	60.00	57.56	1.13	3.57
10	39.40	40.20	47.10		34.20	47.10	48.80	45.30	47.70	32.40	55.90	43.81	2.27	7.19
11	29.50		36.20			36.10	28.30	34.60	35.20	22.80	36.80	32.44	1.78	5.04
12						16.50		17.30			26.90	20.23	3.34	5.79

Table A13: Leaf width in A632 plants (cm)

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	1.20	0.90	0.90	1.00	1.10	1.10	0.80	0.80	0.90	0.80	1.10	0.96	0.09	0.14
2	1.00	1.10	0.90	0.80	1.10	1.00	1.10	1.00	1.10	1.00	1.10	1.02	0.06	0.10
3	1.00	1.10	1.20	1.10	1.10	1.00	1.10	1.20	1.20	1.20	1.20	1.13	0.05	0.08
4	1.50	1.50	1.60	2.20	1.60	1.60	1.60	1.70	1.60	1.80	1.80	1.68	0.12	0.20
5	1.90	2.10	2.40	2.20	2.40	2.20	2.20	2.20	2.50	2.70	2.40	2.29	0.13	0.22
6	2.60	2.80	2.70	2.80	2.90	2.70	2.80	2.80	3.10	3.00	2.90	2.83	0.09	0.14
7	3.00	3.50	3.60	3.30	3.60	3.30	3.40	3.10	3.60	3.90	3.60	3.45	0.16	0.26
8	4.20	4.10	4.20	4.40	3.70	4.10	4.40	4.20	4.00	4.40	4.40	4.19	0.13	0.22
9	5.30	4.80	5.10	4.80	4.30	4.90	4.90	4.80	4.60	5.10	5.20	4.89	0.17	0.28
10	5.20	5.00	5.70	5.00	4.40	5.00	4.80	5.00	4.80	5.10	5.40	5.04	0.20	0.34
11	5.00	5.40	5.60	5.20	4.40	5.20	5.00	5.00	5.00	5.20	5.10	5.10	0.18	0.30
12	5.20	5.40	5.70	5.20	4.40	5.50	5.30	5.30	5.30	5.40	5.30	5.27	0.19	0.32
13	4.50	5.40	5.90	5.00	4.40	5.10	5.10	4.80	5.40	5.10	4.90	5.05	0.25	0.42
14	4.10	4.80	5.40	4.70	4.00	4.70	4.70	4.40	5.00	4.80	4.60	4.65	0.24	0.39
15	3.30	4.10	4.70	4.00	3.50	4.00	3.90	3.80	4.40	4.30	4.00	4.00	0.24	0.39
16	3.00	3.60	4.20	3.50	2.90	3.20	3.60	3.20	3.80	3.60	3.30	3.45	0.23	0.38
17	2.60	2.90	3.30	2.80	2.40	2.80	2.90	2.70	3.00	2.90	2.70	2.82	0.14	0.23
18	2.40	2.60	2.90	2.50				2.30		2.40		2.52	0.17	0.21

Table A14: Leaf width in A632E plants (cm)

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	1.30	1.10	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.00	1.25	0.03	0.10
2	1.30	1.40	1.30	1.30	1.20	1.30	1.30	1.00	1.30	1.30	1.30	1.27	0.03	0.10
3	1.40	1.40	1.30	1.40	1.30	1.30	1.40	1.30	1.40	1.20	1.40	1.35	0.02	0.07
4	2.00	2.50	1.70	1.90	1.90	2.20	2.20	1.80	2.20	1.70	2.20	2.03	0.08	0.25
5	2.70	3.20	2.30	2.90	2.60	3.20	3.20	2.40	3.10	2.10	3.10	2.80	0.12	0.40
6	3.10	4.40	2.80	3.20	3.30	4.10	4.10	3.00	4.00	2.40	4.00	3.49	0.20	0.65
7	4.20	4.50	3.80	4.00	3.60	4.40	4.30	3.70	4.30	3.30	4.00	4.01	0.11	0.38
8	5.00	5.00	4.20	5.30	4.20	4.70	4.60	5.10	5.00	4.00	4.40	4.68	0.13	0.43
9	5.60	5.70	3.80	5.80	5.00	4.40	5.10	5.40	5.50	3.90	5.60	5.07	0.22	0.72
10	5.30	5.80	3.10	5.70	5.40	5.60	5.30	5.10	5.40	3.50	5.90	5.10	0.28	0.93
11	4.80	5.40	2.80	5.00	5.00	5.40	4.90	4.70	5.40	3.40	5.40	4.75	0.26	0.86
12	3.80	5.20		4.30	3.90	4.70	4.60	4.10	4.90	2.60	5.00	4.31	0.24	0.76
13	3.30	4.30		3.50	3.30	4.20	4.00	3.30	3.80		4.20	3.77	0.14	0.42
14	2.90	4.00				3.90	3.30		3.40		3.80	3.55	0.17	0.42
15		3.10				3.00	2.90				3.00	3.00	0.04	0.08
16		2.60										2.60		

Table A15: Leaf width in Oh43 plants (cm)

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	1.90	1.70	1.90	1.70	1.80	1.90	1.80	1.80	1.80	1.80	1.70	1.80	0.02	0.08
2	1.80	1.80	1.70	1.60	1.60	1.70	1.50	1.70	1.60	1.80	1.60	1.67	0.03	0.10
3	1.70	1.70	1.70	1.50	1.90	1.70	1.80	1.90	1.80	1.60	1.70	1.73	0.04	0.12
4	3.10	2.80	3.10	2.70	3.70	2.70	2.70	3.00	2.80	2.50	2.50	2.87	0.10	0.34
5	3.30	3.80	3.70	3.30	4.30	3.30	4.40	3.70	3.70	3.10	2.90	3.59	0.14	0.47
6	5.30	4.50	4.80	4.10	4.60	4.20	4.80	4.80	4.20	4.00	3.90	4.47	0.13	0.43
7	6.10	5.40	5.90	5.70	5.70	5.40	5.60	6.00	5.40	4.60	5.00	5.53	0.13	0.44
8	7.00	6.00	6.90	6.40	7.00	6.40	6.50	7.10	6.20	6.00	5.90	6.49	0.13	0.45
9	8.00	7.30	6.90	7.50	7.30	6.70	7.10	7.40	6.70	6.50	6.10	7.05	0.16	0.53
10	8.00	7.50	7.20	7.50	6.90	7.10	6.50	7.80	6.70	6.50	7.10	7.16	0.15	0.50
11	8.00	7.40	7.40	7.60	7.20	7.30	7.30	7.80	7.10	6.80	7.20	7.37	0.10	0.33
12	8.00	7.30	7.60	7.60	7.20	7.10	7.30	7.50	6.40	6.40	6.80	7.20	0.15	0.50
13	7.40	6.20	6.20	7.00	6.50	5.70	6.40	6.80	4.70	5.20	5.50	6.15	0.24	0.81
14	6.20	4.90	4.90	5.50	5.50	3.40	4.60	5.50	2.60	3.20	3.10	4.49	0.37	1.21
15	4.40	2.80	2.80	3.30	3.00		2.70	3.50				3.21	0.23	0.60

Table A16: Leaf width in Oh43E plants (cm)

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	1.90	1.80	1.70	Plant	1.80	2.10	1.90	1.70	1.70	1.90	2.10	1.86	0.05	0.15
2	1.80	1.60	1.60	died	1.60	1.90	1.70	1.60	1.60	1.70	1.90	1.70	0.04	0.12
3	2.30	2.10	1.90		2.00	1.90	2.00	1.80	2.00	2.20	1.80	2.00	0.05	0.16
4	4.50	3.90	3.10		3.70	3.60	3.50	3.50	3.60	3.90	2.80	3.61	0.15	0.46
5	6.30	5.00	4.60		5.20	4.60	5.40	4.50	4.90	5.30	3.90	4.97	0.20	0.65
6	7.00	5.90	5.30		6.20	5.30	6.20	5.20	5.80	6.00	5.20	5.81	0.18	0.58
7	7.30	7.10	6.70		7.30	7.10	7.30	6.70	7.00	6.90	6.30	6.97	0.10	0.33
8	7.80	7.60	7.00		8.40	7.50	8.00	7.40	8.10	7.40	7.30	7.65	0.13	0.42
9	7.10	6.80	6.30		7.10	6.50	8.00	6.30	7.50	7.10	7.40	7.01	0.17	0.55
10	5.00	4.80	4.60		4.40	5.00	5.70	5.00	5.20	4.60	6.40	5.07	0.19	0.59
11	3.60		3.70			3.70	3.90	4.10	4.10	3.00	4.80	3.86	0.18	0.52
12						2.20		2.10			3.60	2.63	0.48	0.84

Table A17: Leaf length-to-width ratio in A632 plants

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	3.50	5.67	4.89	4.30	3.18	4.18	3.38	4.50	4.56	6.13	3.91	4.38	0.28	0.92
2	10.30	10.27	11.67	12.88	9.00	9.50	8.55	9.60	10.73	11.10	9.45	10.28	0.38	1.26
3	19.60	16.73	15.50	16.91	18.73	20.00	16.45	14.25	17.67	17.50	17.33	17.33	0.51	1.69
4	17.47	16.80	15.94	14.77	18.25	16.31	16.56	14.71	19.69	15.94	15.22	16.51	0.45	1.51
5	17.79	16.76	14.33	14.77	16.42	15.64	16.45	15.27	16.32	14.81	15.75	15.85	0.31	1.02
6	18.73	17.79	16.78	15.75	18.10	17.59	18.82	17.64	17.87	18.17	18.86	17.83	0.28	0.93
7	20.37	17.60	15.83	16.52	17.67	18.36	19.32	21.00	18.47	17.41	19.17	18.34	0.47	1.56
8	17.67	17.34	16.07	16.57	19.19	16.88	17.14	18.45	18.83	17.68	17.68	17.59	0.29	0.95
9	14.49	16.29	14.86	15.65	17.58	15.04	16.08	16.90	17.85	16.06	15.94	16.07	0.32	1.07
10	14.10	15.30	13.16	14.88	16.30	14.46	16.83	15.62	17.44	15.80	14.70	15.33	0.38	1.25
11	13.82	13.83	12.96	11.54	15.32	13.75	16.10	15.14	15.44	14.00	14.80	14.25	0.39	1.29
12	12.06	12.87	11.89	10.44	13.73	11.98	13.77	12.30	13.45	12.20	12.68	12.49	0.29	0.97
13	12.20	11.69	10.42	9.68	12.59	11.31	13.14	12.58	11.94	11.69	12.31	11.78	0.30	1.01
14	12.10	12.31	10.17	9.45	12.85	11.32	13.38	12.75	11.34	11.27	12.02	11.72	0.35	1.17
15	13.70	13.56	10.85	9.68	13.63	11.98	14.85	13.66	11.43	11.23	12.58	12.47	0.47	1.56
16	13.57	13.44	10.67	9.86	14.38	13.47	13.39	14.19	11.87	11.53	12.55	12.63	0.44	1.47
17	13.15	14.79	11.64	11.71	12.46	11.21	14.97	13.81	12.47	12.55	11.63	12.76	0.39	1.28
18	9.92	11.04	9.93	9.28				13.61		11.25		10.84	0.63	1.55

Table A18: Leaf length-to-width ratio in A632E plants

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	3.62	6.18	3.62	4.00	4.31	4.08	4.23	4.31	5.08	4.46	6.30	4.56	0.28	0.92
2	10.62	10.50	10.62	10.15	12.42	10.15	10.38	14.30	11.38	10.31	10.69	11.05	0.38	1.26
3	17.57	18.93	16.23	17.57	20.92	20.31	17.50	17.92	18.93	18.83	18.14	18.44	0.40	1.34
4	16.30	15.12	17.59	17.11	19.32	17.59	16.27	17.61	16.36	17.59	16.32	17.02	0.33	1.10
5	16.00	14.94	17.83	15.10	16.88	16.00	14.59	17.63	15.13	17.95	14.71	16.07	0.39	1.30
6	18.87	14.18	19.04	18.63	16.15	15.78	14.51	20.30	14.70	22.38	14.00	17.14	0.86	2.84
7	15.95	15.98	14.89	17.60	17.22	16.80	15.42	19.03	15.09	18.18	16.43	16.60	0.40	1.31
8	13.14	15.26	12.74	13.75	15.81	16.51	15.37	14.02	13.34	15.48	16.23	14.69	0.40	1.33
9	11.18	13.18	12.68	11.90	12.60	17.39	13.43	12.61	11.45	14.38	12.43	13.02	0.51	1.70
10	10.53	11.55	14.00	11.47	10.80	12.21	11.92	12.06	10.76	14.46	10.46	11.84	0.40	1.33
11	10.85	11.43	12.54	11.64	10.66	11.44	11.98	11.64	10.00	12.50	10.74	11.40	0.24	0.79
12	12.00	10.60		12.02	12.08	11.85	11.91	11.80	9.86	12.88	10.68	11.57	0.28	0.90
13	12.00	11.58		11.77	11.45	12.14	12.75	10.70	11.32		11.38	11.68	0.19	0.58
14	10.86	11.18				11.69	13.94		10.29		11.29	11.54	0.52	1.26
15		12.23				11.57	11.76				11.30	11.71	0.20	0.39
16		10.12										10.12		

Table A19: Leaf length-to-width ratio in Oh43 plants

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	1.84	1.71	2.58	2.41	2.83	1.79	2.39	2.39	2.28	1.78	1.59	2.14	0.13	0.42
2	7.11	5.78	8.59	8.19	9.75	6.88	8.27	7.59	8.75	4.11	5.69	7.34	0.49	1.64
3	14.47	12.76	14.00	14.33	14.47	11.35	11.72	10.68	13.44	9.50	9.47	12.38	0.58	1.94
4	10.90	11.11	10.55	10.67	9.86	10.15	10.96	10.03	11.36	8.60	9.80	10.36	0.24	0.78
5	10.79	11.11	12.89	12.33	11.40	12.48	9.30	12.24	12.05	11.29	13.48	11.76	0.35	1.15
6	11.28	11.56	12.29	12.88	13.41	13.50	11.81	12.40	14.40	12.90	14.15	12.78	0.31	1.03
7	11.18	11.98	12.02	11.58	12.98	13.46	12.14	12.12	13.35	14.54	14.48	12.71	0.34	1.13
8	11.24	12.68	11.55	12.45	11.87	13.80	12.20	12.04	13.40	13.85	14.85	12.72	0.34	1.12
9	10.19	10.60	11.43	10.20	10.85	12.82	11.32	11.66	11.69	12.95	14.69	11.67	0.41	1.36
10	9.59	9.88	10.40	9.80	10.57	10.90	11.48	10.78	10.37	11.74	11.08	10.60	0.21	0.69
11	8.86	8.97	9.12	9.04	8.74	9.47	9.08	9.59	7.87	9.75	9.94	9.13	0.17	0.57
12	8.21	7.71	8.09	8.41	7.49	8.15	7.59	8.92	7.39	8.59	9.15	8.16	0.18	0.58
13	7.59	7.35	8.50	7.90	7.25	7.81	7.30	8.57	7.36	8.17	8.40	7.84	0.15	0.51
14	7.44	6.88	7.29	6.91	6.35	6.00	7.00	7.00	6.27	6.44	6.97	6.78	0.14	0.45
15	7.16	6.36	5.07	5.73	5.03		6.19	6.57				6.01	0.30	0.79

Table A20: Leaf length-to-width ratio in Oh43E plants

Leaf no.	Plant no. 1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	st. dev.
1	2.89	2.50	2.12	Plant	2.33	1.67	2.53	2.00	2.59	2.63	1.71	2.30	0.13	0.41
2	9.11	9.00	7.75	died	8.50	6.47	7.94	7.31	9.13	9.00	5.74	8.00	0.38	1.19
3	12.65	12.52	11.79		12.35	11.11	12.75	11.78	11.95	12.82	10.17	11.99	0.26	0.84
4	8.89	9.92	10.77		9.86	9.11	10.31	8.83	9.53	10.13	8.96	9.63	0.21	0.67
5	8.44	10.94	10.91		10.38	11.46	9.46	11.24	10.69	9.79	10.08	10.34	0.29	0.92
6	8.94	11.53	12.49		10.79	12.62	10.68	12.23	11.24	10.62	10.69	11.18	0.35	1.11
7	8.97	10.51	11.34		9.64	10.94	10.12	10.93	12.07	9.90	10.43	10.49	0.28	0.89
8	7.96	9.14	10.10		7.56	9.41	8.83	8.86	8.31	8.70	9.60	8.85	0.24	0.77
9	7.46	8.34	9.40		7.23	9.15	7.94	8.84	7.91	8.08	8.11	8.25	0.22	0.70
10	7.88	8.38	10.24		7.77	9.42	8.56	9.06	9.17	7.04	8.73	8.63	0.29	0.92
11	8.19		9.78			9.76	7.26	8.44	8.59	7.60	7.67	8.41	0.34	0.95
12						7.50		8.24			7.47	7.74	0.25	0.43

Table A21: Phase-specific traits of plants used for leaf form measurements

Trait	Plant1	2	3	4	5	6	7	8	9	10	11	mean	s.e.m.	St. dev.
A632														
PR	7	7	6	7	6	7	6	7	6	6	6	6.45	0.16	0.52
GL	6	7	6	7	6	7	6	7	6	7	6	6.45	0.16	0.52
HR	5	4	4	4	4	5	5	5	5	4	4	4.45	0.16	0.52
WX	11	11	11	10	10	11	11	11	11	10	10	10.6	0.15	0.5
EAR	12	12	12	12	12	12	12	12	12	12	12	12	0	0
TLN	18	18	18	18	17	17	17	18	17	17	17	17.5	0.16	0.52
A632E														
PR	6	5	5	5	6	6	6	6	6	6	6	5.73	0.14	0.47
GL	6	6	6	6	6	6	6	6	7	6	6	6.09	0.09	0.3
HR	5	5	4	5	4	5	4	5	4	5	4	4.55	0.16	0.52
WX	10	10	9	10	10	11	10	11	10	10	11	10.2	0.18	0.6
EAR	8	9	7	8	9	9	9	8	9	8	9	8.45	0.21	0.69
TLN	14	16	11	13	13	15	15	13	14	12	15	13.7	0.45	1.49
Oh43														
PR	7	6	6	7	6	6	6	6	5	6	6	6.09	0.16	0.54
GL	6	7	6	7	6	6	6	7	7	7	7	6.55	0.16	0.52
HR	4	4	5	5	4	5	4	4	4	5	5	4.45	0.16	0.52
WX	10	9	9	9	9	10	10	10	9	10	10	9.55	0.16	0.52
EAR	10	9	9	10	10	9	10	10	9	10	9	9.55	0.16	0.52
TLN	15	15	15	15	15	14	15	15	14	14	14	14.6	0.15	0.5
Oh43E														
PR	5	4	5		4	5	5	5	4	5	5	4.7	0.15	0.48
GL	5	5	5		5	5	5	5	5	5	6	5.1	0.1	0.32
HR	3	4	3		3	4	3	4	4	3	4	3.5	0.17	0.53
WX	8	8	8		8	8	8	8	8	8	9	8.1	0.1	0.32
EAR	6	6	6		6	7	7	6	7	6	7	6.4	0.16	0.52
TLN	11	10	11		10	12	11	12	11	11	12	11.1	0.23	0.74

Abbreviations:
PR: Last node with prop roots GL: First leaf with glossy patches HR: First leaf with hairs WX: Last partially glaucous leaf
EAR: Ear placement node TLN: Total leaf number

Table A22: Phase-specific traits in three A632 F2 populations (in position given in leaf number (PR, GL, etc) and in relative position given in percent of TLN (%PR, %GL, etc).

Trait	PR	GL	HR	WX	EAR	TLN	%PR	%GL	%HR	%WX	%EAR
Family H664											
plant no.1	8	6	4	8	11	16	50.0	37.5	25.0	50.0	68.8
2	9	7	5	9	11	16	56.3	43.8	31.3	56.3	68.8
3	8	7	5	8	11	17	47.1	41.2	29.4	47.1	64.7
4	8	7	5	9	11	17	47.1	41.2	29.4	52.9	64.7
5	9	7	5	9	11	17	52.9	41.2	29.4	52.9	64.7
6	9	7	5	9	11	17	52.9	41.2	29.4	52.9	64.7
7	8	7	5	9	11	17	47.1	41.2	29.4	52.9	64.7
8	8	7	5	9	12	17	47.1	41.2	29.4	52.9	70.6
9	7	7	4	9	12	17	41.2	41.2	23.5	52.9	70.6
10	8	6	4	8	12	17	47.1	35.3	23.5	47.1	70.6
11	9	7	5	10	12	17	52.9	41.2	29.4	58.8	70.6
12	8	7	6	9	12	17	47.1	41.2	35.3	52.9	70.6
13	9	7	5	9	12	17	52.9	41.2	29.4	52.9	70.6
14	9	7	5	10	13	17	52.9	41.2	29.4	58.8	76.5
15	8	7	5	9	12	18	44.4	38.9	27.8	50.0	66.7
16	9	7	4	9	12	18	50.0	38.9	22.2	50.0	66.7
17	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
18	9	7	6	10	12	18	50.0	38.9	33.3	55.6	66.7
19	8	7	5	9	12	18	44.4	38.9	27.8	50.0	66.7
20	8	7	5	9	12	18	44.4	38.9	27.8	50.0	66.7
21	8	6	5	9	12	18	44.4	33.3	27.8	50.0	66.7
22	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
23	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
24	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
25	10	7	5	10	12	18	55.6	38.9	27.8	55.6	66.7
26	9	7	4	9	12	18	50.0	38.9	22.2	50.0	66.7
27	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
28	9	7	4	9	12	18	50.0	38.9	22.2	50.0	66.7
29	9	7	4	9	12	18	50.0	38.9	22.2	50.0	66.7
30	9	7	4	9	12	18	50.0	38.9	22.2	50.0	66.7
31	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
32	8	7	5	9	12	18	44.4	38.9	27.8	50.0	66.7
33	10	7	5	10	13	18	55.6	38.9	27.8	55.6	72.2
34	9	7	5	9	13	18	50.0	38.9	27.8	50.0	72.2
35	10	7	4	9	13	18	55.6	38.9	22.2	50.0	72.2
36	9	7	5	10	13	18	50.0	38.9	27.8	55.6	72.2
37	10	7	4	10	13	18	55.6	38.9	22.2	55.6	72.2
38	10	7	5	9	13	18	55.6	38.9	27.8	50.0	72.2
39	9	7	5	10	13	18	50.0	38.9	27.8	55.6	72.2
40	10	7	6	9	13	18	55.6	38.9	33.3	50.0	72.2
41	9	7	5	9	13	18	50.0	38.9	27.8	50.0	72.2
42	10	7	5	9	12	19	52.6	36.8	26.3	47.4	63.2
43	8	7	4	10	12	19	42.1	36.8	21.1	52.6	63.2
44	10	7	5	9	13	19	52.6	36.8	26.3	47.4	68.4
45	9	6	5	9	13	19	47.4	31.6	26.3	47.4	68.4
46	9	7	5	10	13	19	47.4	36.8	26.3	52.6	68.4
47	10	7	5	10	13	19	52.6	36.8	26.3	52.6	68.4
48	9	8	5	10	13	19	47.4	42.1	26.3	52.6	68.4
49	9	7	5	9	13	19	47.4	36.8	26.3	47.4	68.4

continued..

..continued

Trait	PR	GL	HR	WX	EAR	TLN	%PR	%GL	%HR	%WX	%EAR
50	8	7	5	9	13	19	42.1	36.8	26.3	47.4	68.4
51	9	7	5	9	13	19	47.4	36.8	26.3	47.4	68.4
52	9	7	5	9	13	19	47.4	36.8	26.3	47.4	68.4
53	9	7	4	9	13	19	47.4	36.8	21.1	47.4	68.4
54	10	7	5	9	13	19	52.6	36.8	26.3	47.4	68.4
55	9	7	5	9	14	19	47.4	36.8	26.3	47.4	73.7
56	9	7	4	9	14	19	47.4	36.8	21.1	47.4	73.7
57	10	7	5	9	13	20	50.0	35.0	25.0	45.0	65.0
58	10	9	6	10	13	20	50.0	45.0	30.0	50.0	65.0
59	9	7	5	9	13	20	45.0	35.0	25.0	45.0	65.0
60	10	7	6	9	13	20	50.0	35.0	30.0	45.0	65.0
61	10	7	5	9	14	20	50.0	35.0	25.0	45.0	70.0
62	10	7	5	10	14	20	50.0	35.0	25.0	50.0	70.0
63	9	6	6	9	14	20	45.0	30.0	30.0	45.0	70.0
64	10	7	5	9	15	22	45.5	31.8	22.7	40.9	68.2
65	12	7	5	10	15	23	52.2	30.4	21.7	43.5	65.2
Trait	PR	GL	HR	WX	EAR	TLN	%PR	%GL	%HR	%WX	%EAR
Family H665											
Plant no. 1	5	7	5	9	9	15	33.3	46.7	33.3	60.0	60.0
2	8	7	4	8	10	15	53.3	46.7	26.7	53.3	66.7
3	8	7	4	9	11	16	50.0	43.8	25.0	56.3	68.8
4	8	7	5	9	11	16	50.0	43.8	31.3	56.3	68.8
5	7	7	5	9	11	16	43.8	43.8	31.3	56.3	68.8
6	7	7	4	9	11	17	41.2	41.2	23.5	52.9	64.7
7	8	7	5	8	11	17	47.1	41.2	29.4	47.1	64.7
8	7	7	4	9	11	17	41.2	41.2	23.5	52.9	64.7
9	7	7	5	9	11	17	41.2	41.2	29.4	52.9	64.7
10	8	7	4	9	11	17	47.1	41.2	23.5	52.9	64.7
11	9	7	6	9	11	17	52.9	41.2	35.3	52.9	64.7
12	8	7	4	9	11	17	47.1	41.2	23.5	52.9	64.7
13	8	6	5	9	11	17	47.1	35.3	29.4	52.9	64.7
14	8	7	4	9	11	17	47.1	41.2	23.5	52.9	64.7
15	8	7	5	9	11	17	47.1	41.2	29.4	52.9	64.7
16	9	7	4	9	12	17	52.9	41.2	23.5	52.9	70.6
17	9	6	5	9	12	17	52.9	35.3	29.4	52.9	70.6
18	9	6	5	9	12	17	52.9	35.3	29.4	52.9	70.6
19	8	6	5	9	12	17	47.1	35.3	29.4	52.9	70.6
20	9	7	5	9	12	17	52.9	41.2	29.4	52.9	70.6
21	8	6	4	8	12	17	47.1	35.3	23.5	47.1	70.6
22	9	7	5	9	12	17	52.9	41.2	29.4	52.9	70.6
23	10	7	4	9	12	17	58.8	41.2	23.5	52.9	70.6
24	8	7	5	8	12	17	47.1	41.2	29.4	47.1	70.6
25	8	6	5	9	12	17	47.1	35.3	29.4	52.9	70.6
26	8	6	4	9	12	17	47.1	35.3	23.5	52.9	70.6
27	10	7	5	9	12	17	58.8	41.2	29.4	52.9	70.6
28	8	7	4	9	12	17	47.1	41.2	23.5	52.9	70.6
29	9	7	5	8	13	17	52.9	41.2	29.4	47.1	76.5
30	8	7	4	7	11	18	44.4	38.9	22.2	38.9	61.1
31	9	7	4	10	11	18	50.0	38.9	22.2	55.6	61.1
32	10	7	5	9	12	18	55.6	38.9	27.8	50.0	66.7
33	6	8	4	9	12	18	33.3	44.4	22.2	50.0	66.7

continued..

..continued

Trait	PR	GL	HR	WX	EAR	TLN	%PR	%GL	%HR	%WX	%EAR
34	8	7	4	9	12	18	44.4	38.9	22.2	50.0	66.7
35	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
36	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
37	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
38	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
39	8	7	5	9	12	18	44.4	38.9	27.8	50.0	66.7
40	9	8	6	10	12	18	50.0	44.4	33.3	55.6	66.7
41	10	7	5	9	12	18	55.6	38.9	27.8	50.0	66.7
42	8	6	3	10	12	18	44.4	33.3	16.7	55.6	66.7
43	9	6	5	8	12	18	50.0	33.3	27.8	44.4	66.7
44	9	7	4	10	12	18	50.0	38.9	22.2	55.6	66.7
45	10	8	6	10	12	18	55.6	44.4	33.3	55.6	66.7
46	9	7	4	9	13	18	50.0	38.9	22.2	50.0	72.2
47	10	7	5	10	13	18	55.6	38.9	27.8	55.6	72.2
48	10	7	5	8	13	18	55.6	38.9	27.8	44.4	72.2
49	9	7	5	8	13	18	50.0	38.9	27.8	44.4	72.2
50	9	7	5	9	13	18	50.0	38.9	27.8	50.0	72.2
51	9	8	6	10	13	18	50.0	44.4	33.3	55.6	72.2
52	9	7	4	9	13	18	50.0	38.9	22.2	50.0	72.2
53	9	6	5	8	13	18	50.0	33.3	27.8	44.4	72.2
54	10	7	5	9	13	18	55.6	38.9	27.8	50.0	72.2
55	9	6	4	8	13	18	50.0	33.3	22.2	44.4	72.2
56	10	7	5	9	12	19	52.6	36.8	26.3	47.4	63.2
57	9	7	5	9	12	19	47.4	36.8	26.3	47.4	63.2
58	9	7	5	9	12	19	47.4	36.8	26.3	47.4	63.2
59	9	7	5	9	13	19	47.4	36.8	26.3	47.4	68.4
60	9	8	6	11	13	19	47.4	42.1	31.6	57.9	68.4
61	9	7	4	9	13	19	47.4	36.8	21.1	47.4	68.4
62	10	7	6	9	13	19	52.6	36.8	31.6	47.4	68.4
63	9	7	5	9	13	19	47.4	36.8	26.3	47.4	68.4
64	9	7	6	8	13	19	47.4	36.8	31.6	42.1	68.4
65	9	7	4	9	13	19	47.4	36.8	21.1	47.4	68.4
66	9	6	4	8	13	19	47.4	31.6	21.1	42.1	68.4
67	9	7	5	9	13	19	47.4	36.8	26.3	47.4	68.4
68	10	7	6	9	13	19	52.6	36.8	31.6	47.4	68.4
69	9	7	5	9	13	19	47.4	36.8	26.3	47.4	68.4
70	8	7	5	9	13	19	42.1	36.8	26.3	47.4	68.4
71	9	6	4	10	13	19	47.4	31.6	21.1	52.6	68.4
72	10	8	6	10	13	19	52.6	42.1	31.6	52.6	68.4
73	10	7	5	9	13	19	52.6	36.8	26.3	47.4	68.4
74	9	7	4	11	13	19	47.4	36.8	21.1	57.9	68.4
75	10	8	5	10	13	19	52.6	42.1	26.3	52.6	68.4
76	10	7	4	9	13	19	52.6	36.8	21.1	47.4	68.4
77	9	7	5	9	14	19	47.4	36.8	26.3	47.4	73.7
78	9	7	5	10	14	19	47.4	36.8	26.3	52.6	73.7
79	10	7	5	9	14	19	52.6	36.8	26.3	47.4	73.7
80	11	7	5	10	14	20	55.0	35.0	25.0	50.0	70.0
81	9	7	4	9	14	20	45.0	35.0	20.0	45.0	70.0
82	8	7	6	10	14	20	40.0	35.0	30.0	50.0	70.0
83	9	7	5	10	14	20	45.0	35.0	25.0	50.0	70.0
84	10	8	5	10	14	20	50.0	40.0	25.0	50.0	70.0
85	9	7	5	10	14	21	42.9	33.3	23.8	47.6	66.7
86	12		5	9	15	21	57.1	0.0	23.8	42.9	71.4

continued..

..continued

Trait	PR	GL	HR	WX	EAR	TLN	%PR	%GL	%HR	%WX	%EAR
Family H666											
Plant no.1	10	8	6	10	11	16	62.5	50.0	37.5	62.5	68.8
2	10	8	6	10	10	17	58.8	47.1	35.3	58.8	58.8
3	8	7	5	9	11	17	47.1	41.2	29.4	52.9	64.7
4	8	7	5	9	11	17	47.1	41.2	29.4	52.9	64.7
5	9	7	5	9	11	17	52.9	41.2	29.4	52.9	64.7
6	8	7	6	8	11	17	47.1	41.2	35.3	47.1	64.7
7	8	7	5	8	11	17	47.1	41.2	29.4	47.1	64.7
8	10	8	6	9	12	17	58.8	47.1	35.3	52.9	70.6
9	9	7	6	9	12	17	52.9	41.2	35.3	52.9	70.6
10	10	8	6	11	13	17	58.8	47.1	35.3	64.7	76.5
11	8	7	5	9	11	18	44.4	38.9	27.8	50.0	61.1
12	8	6	4	10	11	18	44.4	33.3	22.2	55.6	61.1
13	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
14	7	6	5	9	12	18	38.9	33.3	27.8	50.0	66.7
15	9	7	5	10	12	18	50.0	38.9	27.8	55.6	66.7
16	9	7	5	10	12	18	50.0	38.9	27.8	55.6	66.7
17	10	7	4	10	12	18	55.6	38.9	22.2	55.6	66.7
18	10	7	5	10	12	18	55.6	38.9	27.8	55.6	66.7
19	8	6	5	9	12	18	44.4	33.3	27.8	50.0	66.7
20	9	7	4	9	12	18	50.0	38.9	22.2	50.0	66.7
21	9	7	4	10	12	18	50.0	38.9	22.2	55.6	66.7
22	9	7	5	10	12	18	50.0	38.9	27.8	55.6	66.7
23	9	7	5	10	12	18	50.0	38.9	27.8	55.6	66.7
24	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
25	8	7	5	10	12	18	44.4	38.9	27.8	55.6	66.7
26	10	8	5	10	12	18	55.6	44.4	27.8	55.6	66.7
27	9	7	5	9	12	18	50.0	38.9	27.8	50.0	66.7
28	11	7	4	10	12	18	61.1	38.9	22.2	55.6	66.7
29	8	7	5	10	12	18	44.4	38.9	27.8	55.6	66.7
30	9	7	5	10	13	18	50.0	38.9	27.8	55.6	72.2
31	9	7	5	10	13	18	50.0	38.9	27.8	55.6	72.2
32	10	9	7	11	13	18	55.6	50.0	38.9	61.1	72.2
33	10	7	4	9	13	18	55.6	38.9	22.2	50.0	72.2
34	10	8	8	10	12	19	52.6	42.1	42.1	52.6	63.2
35	10	7	5	9	12	19	52.6	36.8	26.3	47.4	63.2
36	10	7	5	9	13	19	52.6	36.8	26.3	47.4	68.4
37	9	7	6	10	13	19	47.4	36.8	31.6	52.6	68.4
38	9	7	5	9	13	19	47.4	36.8	26.3	47.4	68.4
39	9	8	5	10	13	19	47.4	42.1	26.3	52.6	68.4
40	10	7	6	10	13	19	52.6	36.8	31.6	52.6	68.4
41	10	7	5	10	13	19	52.6	36.8	26.3	52.6	68.4
42	11	8	6	11	13	19	57.9	42.1	31.6	57.9	68.4
43	10	7	5	10	13	19	52.6	36.8	26.3	52.6	68.4
44	10	7	6	9	13	19	52.6	36.8	31.6	47.4	68.4
45	8	7	5	9	13	19	42.1	36.8	26.3	47.4	68.4
46	10	7	5	9	14	19	52.6	36.8	26.3	47.4	73.7
47	10	7	6	10	14	19	52.6	36.8	31.6	52.6	73.7
48	12	8	6	10	14	19	63.2	42.1	31.6	52.6	73.7
49	10	7	5	10	13	20	50.0	35.0	25.0	50.0	65.0
50	10	6	5	9	14	20	50.0	30.0	25.0	45.0	70.0
51	11	8	6	10	14	20	55.0	40.0	30.0	50.0	70.0
52	11	8	6	9	14	20	55.0	40.0	30.0	45.0	70.0

continued..

..continued

Trait	PR	GL	HR	WX	EAR	TLN	%PR	%GL	%HR	%WX	%EAR
53	9	8	5	10	14	20	45.0	40.0	25.0	50.0	70.0
54	10	8	4	9	15	21	47.6	38.1	19.0	42.9	71.4
55	11	7	5	10	15	21	52.4	33.3	23.8	47.6	71.4
All familes: means N=											
6	8.33	7.00	4.83	9.00	11.00	16	52.08	43.75	30.21	56.25	68.75
45	8.44	6.89	4.89	8.93	11.58	17	49.67	40.52	28.76	52.55	68.10
76	9.00	6.99	4.79	9.29	12.25	18	50.00	38.82	26.61	51.61	68.06
54	9.41	7.09	5.09	9.37	13.02	19	49.51	37.33	26.80	49.32	68.52
17	9.76	7.24	5.29	9.53	13.71	20	48.82	36.18	26.47	47.65	68.53
3	10.00	7.33	4.67	9.67	14.67	21	47.62	34.92	22.22	46.03	69.84
s.e.m.'s TLN											
16	0.42	0.26	0.31	0.26	0.00		2.64	1.61	1.92	1.61	0.00
17	0.12	0.07	0.10	0.09	0.10		0.71	0.43	0.57	0.51	0.58
18	0.09	0.05	0.07	0.08	0.06		0.51	0.30	0.41	0.43	0.35
19	0.10	0.06	0.10	0.09	0.07		0.53	0.32	0.53	0.47	0.38
20	0.20	0.18	0.14	0.12	0.11		1.01	0.91	0.71	0.62	0.57
21	0.58	0.33	0.33	0.33	0.33		2.75	1.59	1.59	1.59	1.59

Table A23: Phase-specific traits in four Oh43 F2 populations (in position given in leaf number (PR, GL, etc) and in relative position given in percent of TLN (%PR, %GL, etc).
SHHR: First leaf sheath with hairs
SHWX: Last partially glaucous leaf sheath

Trait	PR	GL	HR	WX	SHWX	SHHR	EAR	TLN	%PR	%GL	%HR	%WX	%SHWX	%SHHR	%EAR
Family A															
plant no.1	4	6	5	8	7	9	6	10	40.00	60.00	50.00	80.00	70.00	90.00	60.00
2	4	6	5	8	7	7	6	11	36.36	54.55	45.45	72.73	63.64	63.64	54.55
3	4	6	5	8	7	7	7	11	36.36	54.55	45.45	72.73	63.64	63.64	63.64
4	4	6	5	9	7	7	7	11	36.36	54.55	45.45	81.82	63.64	63.64	63.64
5	4	6	5	9	7	8	7	11	36.36	54.55	45.45	81.82	63.64	72.73	63.64
6	5	7	6	9	7	8	7	11	45.45	63.64	54.55	81.82	63.64	72.73	63.64
7	4	6	5	9	9	9	7	12	33.33	50.00	41.67	75.00	75.00	75.00	58.33
8	5	7	6	10	8	9	7	12	41.67	58.33	50.00	83.33	66.67	75.00	58.33
9	5	6	5	9	8	8	7	12	41.67	50.00	41.67	75.00	66.67	66.67	58.33
10	6	6	5	8	8	9	7	12	50.00	50.00	41.67	66.67	66.67	75.00	58.33
11	6	6	5	8	8	9	7	12	50.00	50.00	41.67	66.67	66.67	75.00	58.33
12	6	6	6	8	7	8	7	12	50.00	50.00	50.00	66.67	58.33	66.67	58.33
13	6	6	5	9	8	8	7	12	50.00	50.00	41.67	75.00	66.67	66.67	58.33
14	5	6	5	9	8	8	7	12	41.67	50.00	41.67	75.00	66.67	66.67	58.33
15	5	7	5	10	8	8	8	12	41.67	58.33	41.67	83.33	66.67	66.67	66.67
16	6	6	6	9	8	9	8	12	50.00	50.00	50.00	75.00	66.67	75.00	66.67
17	6	6	5	9	8	9	8	12	50.00	50.00	41.67	75.00	66.67	75.00	66.67
18	5	6	5	8	7	8	8	12	41.67	50.00	41.67	66.67	58.33	66.67	66.67
19	5	6	5	9	7	8	8	12	41.67	50.00	41.67	75.00	58.33	66.67	66.67
20	5	6	5	10	8	9	7	13	38.46	46.15	38.46	76.92	61.54	69.23	53.85
21	6	6	5	9	7	8	7	13	46.15	46.15	38.46	69.23	53.85	61.54	53.85
22	6	7	6	10	8	9	8	13	46.15	53.85	46.15	76.92	61.54	69.23	61.54
23	5	6	5	10	8	9	8	13	38.46	46.15	38.46	76.92	61.54	69.23	61.54
24	6	6	6	10	8	8	8	13	46.15	46.15	46.15	76.92	61.54	61.54	61.54
25	5	7	6	10	9	9	8	13	38.46	53.85	46.15	76.92	69.23	69.23	61.54
26	5	7	6	10	9	9	8	13	38.46	53.85	46.15	76.92	69.23	69.23	61.54
27	6	6	5	9	8	9	8	13	46.15	46.15	38.46	69.23	61.54	69.23	61.54

continued..

..continued

Trait	PR	GL	HR	WX	SHWX	SHHR	EAR	TLN	%PR	%GL	%HR	%WX	%SHWX	%SHHR	%EAR
28	5	6	5	9	8	8	8	13	38.46	46.15	38.46	69.23	61.54	61.54	61.54
29	5	6	5	10	8	10	8	13	38.46	46.15	38.46	76.92	61.54	76.92	61.54
30	6	6	5	10	8	10	8	13	46.15	46.15	38.46	76.92	61.54	76.92	61.54
31	6	6	6	9	8	9	8	13	46.15	46.15	46.15	69.23	61.54	69.23	61.54
32	7	7	6	10	8	9	8	13	53.85	53.85	46.15	76.92	61.54	69.23	61.54
33	6	6	6	9	8	9	8	13	46.15	46.15	46.15	69.23	61.54	69.23	61.54
34	5	6	6	10	9	10	9	13	38.46	46.15	46.15	76.92	69.23	76.92	69.23
35	5	6	5	10	8	9	9	13	38.46	46.15	38.46	76.92	61.54	69.23	69.23
36	5	6	5	10	8	10	8	14	35.71	42.86	35.71	71.43	57.14	71.43	57.14
37	6	7	5	10	9	10	9	14	42.86	50.00	35.71	71.43	64.29	71.43	64.29
38	5	6	5	10	8	9	9	14	35.71	42.86	35.71	71.43	57.14	64.29	64.29
39	5	6	6	10	8	9	9	14	35.71	42.86	42.86	71.43	57.14	64.29	64.29
40	5	6	5	10	9	10	9	14	35.71	42.86	35.71	71.43	64.29	71.43	64.29
41	6	6	6	11	9	10	9	14	42.86	42.86	42.86	78.57	64.29	71.43	64.29
42	6	6	5	11	9	11	9	14	42.86	42.86	35.71	78.57	64.29	78.57	64.29
43	5	6	5	11	8	9	9	14	35.71	42.86	35.71	78.57	57.14	64.29	64.29
44	5	6	6	10	9	11	9	14	35.71	42.86	42.86	71.43	64.29	78.57	64.29
45	6	6	5	10	9	11	9	14	42.86	42.86	35.71	71.43	64.29	78.57	64.29
46	7	7	7	10	9	9	9	14	50.00	50.00	50.00	71.43	64.29	64.29	64.29
47	6	6	6	11	9	10	9	14	42.86	42.86	42.86	78.57	64.29	71.43	64.29
48	6	6	5	11	9	10	9	14	42.86	42.86	35.71	78.57	64.29	71.43	64.29
49	5	6	4	10	8	9	9	14	35.71	42.86	28.57	71.43	57.14	64.29	64.29
50	6	6	5	11	8	10	9	15	40.00	40.00	33.33	73.33	53.33	66.67	60.00
51	6	7	5	11	9	10	9	15	40.00	46.67	33.33	73.33	60.00	66.67	60.00
52	7	6	5	11	8	9	9	15	46.67	40.00	33.33	73.33	53.33	60.00	60.00
53	6	7	5	11	8	9	9	15	40.00	46.67	33.33	73.33	53.33	60.00	60.00
54	5	6	5	11	9	11	9	15	33.33	40.00	33.33	73.33	60.00	73.33	60.00
55	6	7	5	11	9	10	9	15	40.00	46.67	33.33	73.33	60.00	66.67	60.00
56	6	6	6	11	9	11	10	15	40.00	40.00	40.00	73.33	60.00	73.33	66.67
57	6	6	5	11	10	11	10	15	40.00	40.00	33.33	73.33	66.67	73.33	66.67
58	7	7	6	11	10	11	10	15	46.67	46.67	40.00	73.33	66.67	73.33	66.67
59	7	7	6	11	10	10	10	15	46.67	46.67	40.00	73.33	66.67	66.67	66.67
60	7	7	5	11	10	10	10	15	46.67	46.67	33.33	73.33	66.67	66.67	66.67

continued..

..continued

Trait	PR	GL	HR	WX	SHWX	SHHR	EAR	TLN	%PR	%GL	%HR	%WX	%SHWX	%SHHR	%EAR
Family B															
Plant no. 1	3	5	4	7	6	7	5	9	33.33	55.56	44.44	77.78	66.67	77.78	55.56
2	4	6	5	8	7	7	6	10	40.00	60.00	50.00	80.00	70.00	70.00	60.00
3	5	6	6	8	7	7	6	10	50.00	60.00	60.00	80.00	70.00	70.00	60.00
4	5	6	5	9	6	8	6	11	45.45	54.55	45.45	81.82	54.55	72.73	54.55
5	4	6	5	9	6	7	7	11	36.36	54.55	45.45	81.82	54.55	63.64	63.64
6	5	6	5	9	7	8	7	11	45.45	54.55	45.45	81.82	63.64	72.73	63.64
7	5	6	5	9	7	8	7	11	45.45	54.55	45.45	81.82	63.64	72.73	63.64
8	6	6	6	9	9	9	7	11	54.55	54.55	54.55	81.82	81.82	81.82	63.64
9	4	6	5	9	7	8	7	11	36.36	54.55	45.45	81.82	63.64	72.73	63.64
10	5	6	5	9	7	8	8	11	45.45	54.55	45.45	81.82	63.64	72.73	72.73
11	6	6	6	9	8	9	7	12	50.00	50.00	50.00	75.00	66.67	75.00	58.33
12	5	6	6	9	8	9	7	12	41.67	50.00	50.00	75.00	66.67	75.00	58.33
13	4	6	5	10	7	8	8	12	33.33	50.00	41.67	83.33	58.33	66.67	66.67
14	5	6	5	9	7	8	8	12	41.67	50.00	41.67	75.00	58.33	66.67	66.67
15	5	6	6	10	8	8	8	13	38.46	46.15	46.15	76.92	61.54	61.54	61.54
16	6	6	6	9	8	9	8	13	46.15	46.15	46.15	69.23	61.54	69.23	61.54
17	6	6	6	9	8	9	8	13	46.15	46.15	46.15	69.23	61.54	69.23	61.54
18	6	7	5	10	8	9	8	13	46.15	53.85	38.46	76.92	61.54	69.23	61.54
19	6	7	6	10	8	9	8	13	46.15	53.85	46.15	76.92	61.54	69.23	61.54
20	5	6	6	10	8	8	8	13	38.46	46.15	46.15	76.92	61.54	61.54	61.54
21	4	6	5	9	7	8	8	13	30.77	46.15	38.46	69.23	53.85	61.54	61.54
22	5	6	5	10	8	9	8	13	38.46	46.15	38.46	76.92	61.54	69.23	61.54
23	6	6	6	10	8	9	8	13	46.15	46.15	46.15	76.92	61.54	69.23	61.54
24	5	6	5	10	8	9	8	13	38.46	46.15	38.46	76.92	61.54	69.23	61.54
25	5	6	5	9	8	9	8	13	38.46	46.15	38.46	69.23	61.54	69.23	61.54
26	5	6	5	10	8	9	8	13	38.46	46.15	38.46	76.92	61.54	69.23	61.54
27	5	6	5	10	8	9	8	13	38.46	46.15	38.46	76.92	61.54	69.23	61.54
28	5	6	6	10	8	9	8	13	38.46	46.15	46.15	76.92	61.54	69.23	61.54
29	5	6	5	8	7	8	8	13	38.46	46.15	38.46	61.54	53.85	61.54	61.54
30	5	6	5	9	8	8	8	13	38.46	46.15	38.46	69.23	61.54	61.54	61.54
31	6	7	6	9	8	9	8	13	46.15	53.85	46.15	69.23	61.54	69.23	61.54
32	5	6	6	10	8	9	9	13	38.46	46.15	46.15	76.92	61.54	69.23	69.23
33	5	6	6	10	8	9	9	13	38.46	46.15	46.15	76.92	61.54	69.23	69.23

continued..

..continued

Trait	PR	GL	HR	WX	SHWX	SHHR	EAR	TLN	%PR	%GL	%HR	%WX	%SHWX	%SHHR	%EAR
34	6	7	5	10	8	10	8	14	42.86	50.00	35.71	71.43	57.14	71.43	57.14
35	7	8	7	12	10	10	8	14	50.00	57.14	50.00	85.71	71.43	71.43	57.14
36	6	7	6	10	9	10	8	14	42.86	50.00	42.86	71.43	64.29	71.43	57.14
37	5	7	5	11	9	11	9	14	35.71	50.00	35.71	78.57	64.29	78.57	64.29
38	5	7	6	11	9	10	9	14	35.71	50.00	42.86	78.57	64.29	71.43	64.29
39	5	6	5	10	8	9	9	14	35.71	42.86	35.71	71.43	57.14	64.29	64.29
40	5	6	5	10	8	9	9	14	35.71	42.86	35.71	71.43	57.14	64.29	64.29
41	5	7	6	10	7	9	9	14	35.71	50.00	42.86	71.43	50.00	64.29	64.29
42	6	6	6	10	8	9	9	14	42.86	42.86	42.86	71.43	57.14	64.29	64.29
43	5	6	6	11	9	11	9	14	35.71	42.86	42.86	78.57	64.29	78.57	64.29
44	5	7	6	10	8	8	9	14	35.71	50.00	42.86	71.43	57.14	57.14	64.29
45	5	7	6	10	8	9	9	14	35.71	50.00	42.86	71.43	57.14	64.29	64.29
46	5	6	5	10	8	9	9	14	35.71	42.86	35.71	71.43	57.14	64.29	64.29
47	5	6	6	10	8	10	9	14	35.71	42.86	42.86	71.43	57.14	71.43	64.29
48	6	7	6	11	9	10	9	15	40.00	46.67	40.00	73.33	60.00	66.67	60.00
49	5	6	6	10	9	10	9	15	33.33	40.00	40.00	66.67	60.00	66.67	60.00
50	6	7	6	11	10	11	9	15	40.00	46.67	40.00	73.33	66.67	73.33	60.00
51	5	6	5	11	9	10	10	15	33.33	40.00	33.33	73.33	60.00	66.67	66.67
Family C															
Plant no. 1	6	6	5	10	9	10	8	13	46.15	46.15	38.46	76.92	69.23	76.92	61.54
2	5	6	5	10	8	9	8	13	38.46	46.15	38.46	76.92	61.54	69.23	61.54
3	5	6	6	10	8	9	8	13	38.46	46.15	46.15	76.92	61.54	69.23	61.54
4	5	6	6	11	9	11	9	14	35.71	42.86	42.86	78.57	64.29	78.57	64.29
5	5	6	6	11	9	11	9	14	35.71	42.86	42.86	78.57	64.29	78.57	64.29
6	5	6	7	10	9	10	9	14	35.71	42.86	50.00	71.43	64.29	71.43	64.29
7	6	6	5	11	10	11	10	15	40.00	40.00	33.33	73.33	66.67	73.33	66.67
8	5	6	6	11	10	11	9	15	33.33	40.00	40.00	73.33	66.67	73.33	60.00
9	5	6	6	11	9	10	10	15	33.33	40.00	40.00	73.33	60.00	66.67	66.67
10	5	6	5	11	9	10	9	15	33.33	40.00	33.33	73.33	60.00	66.67	60.00
11	6	6	5	11	10	11	10	16	37.50	37.50	31.25	68.75	62.50	68.75	62.50

continued..

..continued

Trait	PR	GL	HR	WX	SHWX	SHHR	EAR	TLN	%PR	%GL	%HR	%WX	%SHWX	%SHHR	%EAR
Family D															
Plant no. 1	5	6	5	9	8	9	8	12	41.67	50.00	41.67	75.00	66.67	75.00	66.67
2	5	6	5	10	8	9	8	13	38.46	46.15	38.46	76.92	61.54	69.23	61.54
3	5	6	5	10	8	9	8	13	38.46	46.15	38.46	76.92	61.54	69.23	61.54
4	4	5	5	10	7	9	7	13	30.77	38.46	38.46	76.92	53.85	69.23	53.85
5	5	6	5	9	8	9	7	13	38.46	46.15	38.46	69.23	61.54	69.23	53.85
6	4	6	5	11	8	10	9	14	28.57	42.86	35.71	78.57	57.14	71.43	64.29
7	5	7	6	11	9	10	9	14	35.71	50.00	42.86	78.57	64.29	71.43	64.29
8	6	6	5	10	9	10	9	14	42.86	42.86	35.71	71.43	64.29	71.43	64.29
9	5	6	5	10	8	10	9	14	35.71	42.86	35.71	71.43	57.14	71.43	64.29
10	6	6	5	10	8	10	8	14	42.86	42.86	35.71	71.43	57.14	71.43	57.14
11	5	6	5	10	9	11	9	14	35.71	42.86	35.71	71.43	64.29	78.57	64.29
12	5	7	5	10	10	11	9	14	35.71	50.00	35.71	71.43	71.43	78.57	64.29
13	5	6	6	10	9	10	9	14	35.71	42.86	42.86	71.43	64.29	71.43	64.29
14	5	6	6	10	9	10	9	14	35.71	42.86	42.86	71.43	64.29	71.43	64.29
Family E															
Plant no. 1	5	6	5	10	8	9	8	12	41.67	50.00	41.67	83.33	66.67	75.00	66.67
2	5	7	5	8	7	8	6	12	41.67	58.33	41.67	66.67	58.33	66.67	50.00
3	5	6	5	9	8	9	8	13	38.46	46.15	38.46	69.23	61.54	69.23	61.54
4	5	6	5	10	8	9	9	13	38.46	46.15	38.46	76.92	61.54	69.23	69.23
5	5	6	5	10	8	10	8	13	38.46	46.15	38.46	76.92	61.54	76.92	61.54
6	6	6	5	10	8	9	8	14	42.86	42.86	35.71	71.43	57.14	64.29	57.14
7	5	6	5	10	8	9	9	14	35.71	42.86	35.71	71.43	57.14	64.29	64.29
8	6	6	5	10	8	10	9	14	42.86	42.86	35.71	71.43	57.14	71.43	64.29
9	5	6	5	10	8	9	9	14	35.71	42.86	35.71	71.43	57.14	64.29	64.29
10	6	6	5	10	8	10	9	14	42.86	42.86	35.71	71.43	57.14	71.43	64.29
11	6	6	5	11	9	10	9	15	40.00	40.00	33.33	73.33	60.00	66.67	60.00
12	5	6	6	10	9	10	10	15	33.33	40.00	40.00	66.67	60.00	66.67	66.67
13	5	7	6	12	9	11	10	16	31.25	43.75	37.50	75.00	56.25	68.75	62.50

continued..

..continued

Trait	PR	GL	HR	WX	SHWX	SHHR	EAR	TLN	%PR	%GL	%HR	%WX	%SHWX	%SHHR	%EAR
All families: means N=															
3	4.33	6.00	5.33	8.00	7.00	7.67	6.00	10	43.33	60.00	53.33	80.00	70.00	76.67	60.00
12	4.58	6.08	5.17	8.83	7.00	7.75	6.92	11	41.67	55.30	46.97	80.30	63.64	70.45	62.88
20	5.25	6.15	5.25	8.95	7.75	8.50	7.40	12	43.75	51.25	43.75	74.58	64.58	70.83	61.67
50	5.31	6.13	5.42	9.67	8.00	8.93	8.02	13	40.85	47.18	41.71	74.36	61.54	68.72	61.71
45	5.38	6.29	5.49	10.31	8.53	9.84	8.87	14	38.41	44.92	39.21	73.65	60.95	70.32	63.33
21	5.86	6.38	5.43	10.90	9.19	10.24	9.43	15	39.05	42.54	36.19	72.70	61.27	68.25	62.86
2	5.50	6.50	5.50	11.50	9.50	11.00	10.00	16	34.38	40.63	34.38	71.88	59.38	68.75	62.50
s.e.m.'s TLN=															
10	0.33	0.00	0.33	0.00	0.00	0.67	0.00		3.33	0.00	3.33	0.00	0.00	6.67	0.00
11	0.19	0.08	0.11	0.11	0.21	0.18	0.15		1.75	0.76	1.02	1.02	1.94	1.63	1.35
12	0.14	0.08	0.10	0.15	0.12	0.11	0.13		1.19	0.68	0.83	1.28	1.03	0.96	1.11
13	0.08	0.06	0.07	0.07	0.06	0.08	0.06		0.65	0.44	0.54	0.57	0.46	0.59	0.49
14	0.09	0.08	0.10	0.08	0.09	0.11	0.05		0.65	0.54	0.70	0.55	0.67	0.82	0.37
15	0.16	0.11	0.11	0.07	0.15	0.14	0.11		1.06	0.72	0.74	0.44	0.99	0.91	0.74
16	0.50	0.50	0.50	0.50	0.50	0.00	0.00		3.13	3.13	3.13	3.13	3.13	0.00	0.00

Table A24: Shape factor of leaf epidermal cells in A632 plants

	Plant 1	2	3	Plants 1 to 3
Leaf 3	0.92	0.88	0.91	
	0.92	0.91	0.93	
	0.95	0.93	0.92	
	0.93	0.93	0.89	
	0.95	0.9	0.91	
	0.96	0.91	0.91	
	0.94	0.93	0.91	
	0.96	0.93	0.9	
	0.93	0.9	0.9	
	0.93	0.84	0.95	
	0.93	0.89	0.87	
	0.9	0.88	0.92	
	0.94	0.87	0.91	
	0.94	0.93	0.91	
	0.91	0.92		
	0.94			
mean	0.934	0.903	0.91	0.916
s.e.m.	0.001	0.002	0.001	0.001
st.dev.	0.017	0.027	0.018	0.025
Leaf 4	0.94	0.91	0.95	
	0.93	0.95	0.9	
	0.92	0.91	0.92	
	0.88	0.91	0.92	
	0.94	0.91	0.92	
	0.95	0.89	0.93	
	0.95	0.91	0.93	
	0.9	0.93	0.87	
	0.93	0.94	0.93	
	0.91	0.9	0.96	
	0.9	0.89	0.95	
	0.9	0.86	0.95	
	0.91	0.93	0.93	
	0.93		0.91	
	0.89		0.93	
	0.92			
mean	0.919	0.911	0.927	0.919
s.e.m.	0.001	0.002	0.002	0.001
st.dev.	0.021	0.024	0.023	0.023

continued..

..continued

	Plant 1	2	3	Plants 1 to 3
Leaf 5	0.88	0.93	0.92	
	0.93	0.91	0.89	
	0.91	0.94	0.93	
	0.92	0.94	0.89	
	0.91	0.91	0.93	
	0.9	0.9	0.93	
	0.86	0.92	0.88	
	0.89	0.93	0.89	
	0.89	0.9	0.92	
	0.92	0.92	0.91	
	0.89	0.93	0.89	
	0.89	0.93	0.94	
	0.92		0.93	
	0.92		0.87	
	0.94			
	0.91			
	0.88			
mean	0.904	0.922	0.909	0.910
s.e.m.	0.001	0.001	0.002	0.001
st.dev.	0.021	0.014	0.023	0.021
Leaf 6	0.9	0.93	0.87	
	0.9	0.9	0.85	
	0.93	0.92	0.82	
	0.92	0.86	0.83	
	0.93	0.86	0.8	
	0.85	0.86	0.82	
	0.92	0.91	0.69	
	0.91	0.9	0.79	
	0.93	0.85	0.77	
	0.91	0.92	0.79	
	0.91	0.92	0.85	
	0.88	0.84	0.84	
	0.88	0.91	0.76	
		0.92		
		0.81		
mean	0.905	0.887	0.806	0.867
s.e.m.	0.002	0.002	0.004	0.001
st.dev.	0.024	0.037	0.048	0.056

continued..

..continued

	Plant 1	2	3	Plants 1 to 3
Leaf 7	0.76	0.86	0.81	
	0.86	0.87	0.9	
	0.76	0.75	0.76	
	0.71	0.81	0.77	
	0.9	0.84	0.82	
	0.66	0.85	0.65	
	0.89	0.75	0.68	
	0.93	0.63	0.8	
	0.71	0.61	0.74	
	0.8	0.57	0.8	
	0.91	0.7	0.7	
	0.89		0.82	
	0.89			
mean	0.821	0.749	0.771	0.782
s.e.m.	0.007	0.01	0.006	0.003
st.dev.	0.091	0.108	0.07	0.093
Leaf 8	0.86	0.58	0.82	
	0.83	0.78	0.7	
	0.89	0.83	0.68	
	0.82	0.53	0.79	
	0.87	0.62	0.81	
	0.68	0.86	0.69	
	0.82	0.72	0.81	
	0.77	0.82	0.76	
	0.72	0.69	0.74	
	0.81			
	0.73			
	0.88			
	0.84			
mean	0.809	0.714	0.756	0.766
s.e.m.	0.005	0.013	0.006	0.003
st.dev.	0.066	0.118	0.055	0.089
Leaf 9	0.73	0.81	0.77	
	0.85	0.75	0.78	
	0.77	0.76	0.68	
	0.83	0.75	0.78	
	0.75	0.76	0.73	
	0.72	0.78	0.71	
	0.83	0.78	0.71	
	0.84	0.72	0.82	
	0.84	0.8	0.79	
	0.8	0.75	0.73	
	0.83		0.76	
	0.77		0.86	
			0.77	
			0.77	
mean	0.797	0.766	0.761	0.774
s.e.m.	0.004	0.003	0.003	0.001
st.dev.	0.047	0.027	0.047	0.044

continued..

..continued

	Plant 1	2	3	Plants 1 to 3
Leaf 10	0.73	0.75	0.81	
	0.71	0.76	0.75	
	0.64	0.82	0.71	
	0.64	0.78	0.71	
	0.78	0.77	0.71	
	0.7	0.83	0.71	
	0.82	0.76	0.78	
	0.72	0.68	0.72	
	0.71	0.82	0.68	
	0.82	0.78	0.74	
	0.79	0.8	0.71	
	0.66	0.78		
mean	0.727	0.778	0.73	0.745
s.e.m.	0.005	0.003	0.003	0.002
st.dev.	0.064	0.04	0.037	0.053
Leaf 11	0.69	0.67	0.82	
	0.81	0.67	0.77	
	0.8	0.72	0.74	
	0.77	0.61	0.61	
	0.66	0.67	0.67	
	0.76	0.66	0.71	
	0.74	0.6	0.63	
	0.73	0.71	0.73	
	0.67	0.54	0.78	
	0.68	0.59	0.77	
	0.72	0.55	0.79	
	0.76	0.75	0.78	
	0.82	0.6	0.81	
mean	0.739	0.642	0.739	0.707
s.e.m.	0.004	0.005	0.005	0.002
st.dev.	0.054	0.065	0.067	0.076
Leaf 12	0.81	0.8	0.91	
	0.81	0.81	0.77	
	0.77	0.91	0.87	
	0.8	0.71	0.85	
	0.85	0.68	0.64	
	0.84	0.69	0.7	
	0.76	0.65	0.82	
	0.8	0.59	0.86	
	0.74	0.72	0.79	
	0.83	0.85	0.84	
	0.84	0.82	0.75	
	0.81	0.81	0.78	
	0.8	0.66	0.79	
	0.81			
mean	0.805	0.746	0.798	0.784
s.e.m.	0.002	0.007	0.006	0.002
st.dev.	0.031	0.093	0.073	0.073

Table A25: Shape factor of leaf epidermal cells in A632E plants

	Plant 1	2	3	Plants 1 to3
Leaf 3	0.88	0.88	0.89	
	0.93	0.93	0.94	
	0.9	0.9	0.9	
	0.91	0.9	0.92	
	0.92	0.92	0.93	
	0.92	0.89	0.93	
	0.9	0.91	0.92	
	0.94	0.89	0.91	
	0.83	0.93	0.9	
	0.86	0.92	0.92	
	0.91	0.91	0.93	
	0.92	0.9	0.91	
	0.93	0.92	0.92	
	0.93	0.93	0.93	
	0.93	0.92	0.9	
	0.92	0.91		
	0.83	0.91		
		0.92		
		0.93		
		0.91		
		0.87		
mean	0.904	0.91	0.917	0.91
s.e.m.	0.008	0.004	0.004	0.003
st. dev.	0.034	0.017	0.014	0.023
Leaf 4	0.87	0.94	0.91	
	0.94	0.89	0.87	
	0.89	0.93	0.93	
	0.88	0.91	0.87	
	0.93	0.93	0.91	
	0.92	0.91	0.91	
	0.86	0.93	0.91	
	0.92	0.9	0.88	
	0.93	0.94	0.92	
	0.88	0.94	0.93	
	0.93	0.94	0.93	
	0.9	0.95	0.91	
	0.86	0.92		
	0.88	0.94		
	0.86	0.93		
		0.93		
mean	0.897	0.927	0.907	0.911
s.e.m.	0.008	0.004	0.006	0.004
st. dev.	0.029	0.017	0.022	0.026

continued..

..continued

	Plant 1	2	3	Plants 1 to3
Leaf 5	0.91	0.93	0.92	
	0.92	0.91	0.92	
	0.92	0.86	0.88	
	0.92	0.93	0.92	
	0.92	0.9	0.95	
	0.9	0.92	0.91	
	0.9	0.94	0.93	
	0.94	0.9	0.92	
	0.92	0.94	0.89	
	0.91	0.93	0.91	
	0.94	0.89	0.94	
	0.95	0.95	0.89	
	0.94		0.91	
	0.91		0.93	
	0.94		0.9	
	0.9		0.93	
	0.93		0.94	
	0.9			
	0.89			
mean	0.919	0.917	0.917	0.918
s.e.m.	0.004	0.007	0.005	0.003
st. dev.	0.017	0.026	0.019	0.02
Leaf 6	0.9	0.91	0.95	
	0.87	0.91	0.93	
	0.88	0.91	0.94	
	0.88	0.88	0.91	
	0.9	0.9	0.88	
	0.87	0.91	0.87	
	0.9	0.9	0.87	
	0.83	0.84	0.86	
	0.9	0.94	0.9	
	0.94	0.88	0.87	
	0.88		0.84	
	0.91		0.89	
	0.9		0.9	
	0.88		0.92	
			0.91	
mean	0.889	0.898	0.896	0.894
s.e.m.	0.007	0.008	0.008	0.004
st. dev.	0.025	0.027	0.031	0.028

continued..

..continued

	Plant 1	2	3	Plants 1 to3
Leaf 7	0.69	0.78	0.76	
	0.8	0.8	0.89	
	0.85	0.78	0.85	
	0.76	0.74	0.64	
	0.75	0.67	0.71	
	0.72	0.77	0.78	
	0.74	0.77	0.73	
	0.9	0.8	0.67	
	0.77	0.76	0.65	
	0.85	0.79	0.73	
	0.81	0.74	0.74	
	0.81		0.76	
	0.87		0.8	
	0.81		0.8	
	0.75			
	0.88			
	0.76			
	0.89			
	0.91			
mean	0.806	0.764	0.751	0.778
s.e.m.	0.015	0.011	0.019	0.01
st. dev.	0.066	0.037	0.072	0.066
Leaf 8	0.84	0.85	0.83	
	0.82	0.76	0.77	
	0.69	0.72	0.85	
	0.64	0.82	0.74	
	0.77	0.79	0.88	
	0.8	0.77	0.71	
	0.77	0.66	0.73	
	0.76	0.73	0.81	
	0.67	0.72	0.71	
	0.66	0.76	0.68	
	0.74	0.65	0.72	
	0.8	0.76	0.72	
	0.82	0.74	0.71	
		0.74	0.65	
			0.66	
mean	0.752	0.748	0.745	0.748
s.e.m.	0.019	0.014	0.018	0.01
st. dev.	0.067	0.054	0.069	0.062

continued..

..continued

	Plant 1	2	3	Plants 1 to3
Leaf 9	0.8	0.78	0.78	
	0.75	0.69	0.82	
	0.69	0.82	0.74	
	0.79	0.75	0.73	
	0.69	0.77	0.85	
	0.75	0.75	0.73	
	0.68	0.88	0.75	
	0.66	0.79	0.83	
	0.71	0.74	0.73	
	0.71	0.73	0.83	
	0.76	0.82	0.81	
	0.73	0.71	0.8	
			0.85	
mean	0.727	0.769	0.788	0.762
s.e.m.	0.013	0.015	0.013	0.009
st. dev.	0.044	0.053	0.047	0.054
Leaf 10	0.79	0.72	0.83	
	0.82	0.83	0.7	
	0.88	0.79	0.74	
	0.84	0.71	0.67	
	0.8	0.81	0.83	
	0.8	0.79	0.76	
	0.9	0.66	0.69	
	0.86	0.86	0.85	
	0.87	0.77	0.66	
	0.79	0.8	0.77	
	0.79	0.85		
	0.79	0.87		
	0.79	0.72		
	0.79	0.76		
	0.78	0.73		
	0.8			
mean	0.818	0.778	0.75	0.787
s.e.m.	0.01	0.016	0.022	0.01
st. dev.	0.039	0.061	0.07	0.061

continued..

..continued

	Plant 1	2	3	Plants 1 to3
Leaf 11	Plant	0.83	0.63	
	had	0.82	0.75	
	10	0.78	0.68	
	leaves	0.71	0.73	
	.	0.8	0.75	
		0.87	0.67	
		0.73	0.73	
		0.83	0.84	
		0.84	0.65	
		0.89	0.81	
		0.78	0.74	
		0.78	0.65	
		0.77		
		0.77		
		0.85		
mean		0.803	0.719	0.777
s.e.m.		0.013	0.02	0.014
st. dev.		0.05	0.065	0.071
Leaf 12		Plant	0.79	
		had	0.81	
		11	0.77	
		leaves	0.75	
			0.79	
			0.8	
			0.78	
			0.71	
			0.87	
			0.75	
			0.74	
			0.67	
			0.65	
			0.77	
			0.78	
mean			0.756	
s.e.m.			0.016	
st. dev.			0.057	

Table A26: Shape factor of leaf epidermal cells in Oh43 plants

	Plant 1	2	3	Plants 1 to3
Leaf 3	0.91	0.91	0.92	
	0.93	0.8	0.91	
	0.89	0.9	0.9	
	0.92	0.88	0.93	
	0.94	0.9	0.9	
	0.89	0.86	0.9	
	0.9	0.88	0.9	
	0.87	0.9	0.93	
	0.9	0.9	0.95	
	0.95	0.91	0.93	
	0.88	0.86	0.89	
	0.94	0.85	0.96	
	0.91	0.89	0.92	
		0.86	0.94	
			0.83	
			0.91	
			0.95	
mean	0.91	0.879	0.916	0.902
s.e.m.	0.007	0.008	0.007	0.005
st. dev.	0.025	0.03	0.03	0.033
Leaf 4	0.86	0.83	0.93	
	0.87	0.88	0.94	
	0.92	0.86	0.9	
	0.87	0.86	0.87	
	0.91	0.8	0.95	
	0.91	0.86	0.89	
	0.91	0.85	0.9	
	0.92	0.87	0.83	
	0.89	0.82	0.95	
	0.93	0.84	0.88	
	0.9	0.82	0.93	
	0.89	0.85	0.94	
	0.9	0.86	0.91	
	0.92	0.9	0.92	
		0.82	0.92	
		0.82		
mean	0.9	0.846	0.911	0.884
s.e.m.	0.006	0.007	0.009	0.006
st. dev.	0.021	0.027	0.033	0.04

continued..

..continued

	Plant 1	2	3	Plants 1 to3
Leaf 5	0.92	0.86	0.85	
	0.84	0.9	0.88	
	0.89	0.83	0.88	
	0.87	0.84	0.87	
	0.91	0.83	0.88	
	0.93	0.85	0.86	
	0.88	0.85	0.88	
	0.87	0.89	0.86	
	0.88	0.86	0.94	
	0.91	0.87	0.91	
	0.89	0.8	0.87	
	0.89	0.88	0.88	
	0.82	0.9	0.94	
		0.91	0.93	
mean	0.885	0.862	0.888	0.878
s.e.m.	0.009	0.008	0.008	0.005
st. dev.	0.031	0.032	0.03	0.032
Leaf 6	0.91	0.89	0.76	
	0.89	0.81	0.84	
	0.9	0.9	0.91	
	0.89	0.9	0.88	
	0.81	0.81	0.82	
	0.86	0.85	0.79	
	0.83	0.86	0.9	
	0.84	0.86	0.81	
	0.84	0.86	0.93	
	0.79	0.89	0.75	
	0.92	0.87	0.87	
	0.88	0.86		
	0.86			
mean	0.863	0.863	0.842	0.857
s.e.m.	0.011	0.009	0.018	0.007
st. dev.	0.04	0.03	0.061	0.045
Leaf 7	0.72	0.65	0.87	
	0.72	0.64	0.85	
	0.74	0.68	0.91	
	0.69	0.6	0.84	
	0.72	0.69	0.85	
	0.8	0.7	0.82	
	0.68	0.65	0.85	
	0.75	0.69	0.84	
	0.74	0.64	0.94	
	0.76	0.61	0.85	
	0.81	0.72	0.84	
	0.88	0.72	0.89	
	0.79	0.78	0.79	
	0.81	0.68		
mean	0.758	0.675	0.857	0.761
s.e.m.	0.015	0.013	0.011	0.014
st. dev.	0.055	0.048	0.039	0.088

continued..

..continued

	Plant 1	2	3	Plants 1 to3
Leaf 8	0.66	0.75	0.68	
	0.66	0.8	0.64	
	0.58	0.68	0.75	
	0.69	0.77	0.65	
	0.76	0.73	0.86	
	0.69	0.7	0.66	
	0.53	0.65	0.67	
	0.77	0.71	0.88	
	0.62	0.69	0.75	
	0.64	0.81	0.62	
	0.69	0.71	0.86	
	0.69	0.68	0.83	
			0.77	
			0.73	
mean	0.665	0.723	0.739	0.711
s.e.m.	0.02	0.014	0.024	0.013
st. dev.	0.068	0.05	0.09	0.077
Leaf 9	0.89	0.64	0.71	
	0.67	0.74	0.78	
	0.72	0.77	0.88	
	0.74	0.69	0.83	
	0.65	0.71	0.81	
	0.79	0.57	0.8	
	0.83	0.64	0.85	
	0.66	0.76	0.74	
	0.79	0.7	0.77	
	0.51	0.72	0.75	
	0.67	0.81	0.59	
	0.69	0.81	0.72	
		0.59		
mean	0.718	0.704	0.769	0.729
s.e.m.	0.029	0.021	0.022	0.014
st. dev.	0.1	0.077	0.077	0.087
Leaf 10	0.82	0.73	0.74	
	0.8	0.75	0.8	
	0.72	0.58	0.7	
	0.71	0.72	0.76	
	0.75	0.66	0.78	
	0.87	0.75	0.63	
	0.69	0.67	0.76	
	0.77	0.76	0.71	
	0.74	0.6	0.81	
	0.81	0.69	0.77	
		0.74	0.87	
		0.78	0.81	
		0.79		
		0.75		
mean	0.768	0.712	0.762	0.744
s.e.m.	0.018	0.017	0.018	0.011
st. dev.	0.057	0.064	0.062	0.065

continued..

..continued

	Plant 1	2	3	Plants 1 to3
Leaf 11	0.63	0.77	0.85	
	0.73	0.75	0.78	
	0.67	0.85	0.81	
	0.59	0.72	0.82	
	0.74	0.73	0.67	
	0.79	0.78	0.65	
	0.67	0.8	0.73	
	0.9	0.74	0.8	
	0.68	0.74	0.86	
	0.65	0.8	0.79	
	0.64	0.76	0.75	
	0.59	0.75	0.88	
		0.77		
		0.76		
mean	0.69	0.766	0.783	0.747
s.e.m.	0.026	0.009	0.021	0.012
st. dev.	0.089	0.034	0.072	0.077
Leaf 12	0.66	0.68	0.78	
	0.59	0.67	0.76	
	0.55	0.7	0.78	
	0.6	0.7	0.83	
	0.62	0.69	0.84	
	0.61	0.7	0.74	
	0.79	0.69	0.8	
	0.66	0.68	0.82	
	0.57	0.8	0.75	
	0.55	0.72	0.82	
	0.62	0.58	0.72	
	0.56	0.71	0.87	
	0.7	0.71	0.82	
	0.67	0.66		
		0.57		
mean	0.625	0.684	0.795	0.699
s.e.m.	0.018	0.014	0.012	0.014
st. dev.	0.067	0.054	0.044	0.089

Table A27: Shape factor of leaf epidermal cells in Oh43E plants

	Plant 1	2	3	Plants 1 to3
Leaf 3	0.81	0.89	0.86	
	0.89	0.9	0.93	
	0.82	0.91	0.87	
	0.86	0.89	0.84	
	0.86	0.91	0.88	
	0.89	0.91	0.85	
	0.86	0.86	0.93	
	0.76	0.9	0.91	
	0.85	0.91	0.86	
	0.85	0.84	0.91	
	0.86	0.84	0.93	
	0.87	0.78	0.91	
		0.84		
		0.86		
		0.88		
		0.86		
		0.9		
mean	0.848	0.875	0.89	0.872
s.e.m.	0.011	0.009	0.01	0.006
st. dev.	0.036	0.036	0.034	0.038
Leaf 4	0.88	0.83	0.92	
	0.85	0.82	0.82	
	0.82	0.88	0.97	
	0.83	0.86	0.9	
	0.87	0.91	0.79	
	0.89	0.89	0.9	
	0.8	0.91	0.85	
	0.77	0.87	0.87	
	0.87	0.88	0.91	
	0.81	0.79	0.87	
	0.87	0.78	0.89	
	0.87	0.77	0.92	
			0.93	
			0.87	
mean	0.844	0.849	0.886	0.861
s.e.m.	0.011	0.014	0.012	0.008
st. dev.	0.038	0.05	0.046	0.048
Leaf 5	0.81	0.83	0.79	
	0.73	0.87	0.85	
	0.89	0.77	0.86	
	0.78	0.76	0.75	
	0.76	0.7	0.68	
	0.72	0.81	0.71	
	0.81	0.93	0.85	
	0.87	0.65	0.86	
	0.75	0.93	0.67	
	0.8	0.86	0.81	
	0.87	0.91		
	0.82	0.9		
	0.87			
mean	0.806	0.827	0.783	0.807
s.e.m.	0.016	0.026	0.024	0.013
st. dev.	0.057	0.091	0.076	0.075

continued..

..continued

	Plant 1	2	3	Plants 1 to3
Leaf 6	0.65	0.59	0.75	
	0.6	0.66	0.61	
	0.8	0.61	0.66	
	0.71	0.6	0.77	
	0.68	0.66	0.84	
	0.7	0.7	0.73	
	0.71	0.67	0.72	
	0.64	0.62	0.69	
	0.76	0.69	0.72	
	0.71	0.71	0.76	
	0.62	0.78	0.87	
	0.7	0.64	0.7	
	0.8		0.68	
			0.75	
			0.83	
mean	0.698	0.661	0.739	0.702
s.e.m.	0.017	0.016	0.018	0.011
st. dev.	0.062	0.054	0.07	0.069
Leaf 7	0.6	0.6	0.8	
	0.68	0.67	0.72	
	0.69	0.59	0.63	
	0.65	0.67	0.69	
	0.69	0.65	0.69	
	0.8	0.66	0.55	
	0.69	0.59	0.62	
	0.76	0.54	0.69	
	0.65	0.62	0.6	
	0.73	0.64	0.75	
	0.62	0.62	0.71	
	0.74	0.66	0.79	
	0.71	0.62		
		0.67		
mean	0.693	0.629	0.687	0.668
s.e.m.	0.016	0.01	0.022	0.01
st. dev.	0.056	0.039	0.076	0.064
Leaf 8	0.66	0.73	0.63	
	0.72	0.8	0.64	
	0.75	0.72	0.69	
	0.64	0.76	0.61	
	0.65	0.8	0.78	
	0.62	0.8	0.76	
	0.63	0.69	0.61	
	0.73	0.81	0.59	
	0.74	0.77	0.69	
	0.64	0.71	0.59	
	0.53	0.66	0.58	
	0.71	0.72	0.6	
	0.62	0.67	0.67	
	0.69	0.67		
	0.68	0.66		
mean	0.667	0.731	0.649	0.684
s.e.m.	0.015	0.014	0.018	0.01
st. dev.	0.058	0.055	0.065	0.068

Table A28: Phase-specific traits in A632 plants used for sectioning

Trait	PR	GL	HR	WX	EAR	TLN
plant no.1	7	7	5	11	11	17
2	7	7	6	11	12	18
3	7	7	5	11	12	18
4	7	7	5	11	12	18
5	7	7	5	10	12	18
mean	7	7	5.2	10.8	11.8	17.8
s.e.m.	0	0	0.2	0.2	0.2	0.2
st. dev.	0	0	0.447	0.447	0.447	0.447

Table A29: Phase-specific traits in A632E plants used for sectioning

Trait	PR	GL	HR	WX	EAR	TLN
plant no.1	6	6	6	9	8	10
2	7	6	5	11	8	11
3	7	6	6	10	8	12
4	6	6	5	11	8	11
5	7	6	6	10	8	12
mean	6.6	6	5.6	10.2	8	11.2
s.e.m.	0.245	0	0.245	0.374	0	0.374
st. dev.	0.548	0	0.548	0.837	0	0.837

Table A30: Phase-specific traits in Oh43 plants used for sectioning

Trait	PR	GL	HR	WX	EAR	TLN
Plant no. 1	6	6	6	10	9	14
2	6	6	5	11	9	14
3	6	6	6	11	10	15
4	6	7	5	11	10	15
5	6	6	6	11	10	15
mean	6	6.2	5.6	10.8	9.6	14.6
s.e.m.	0	0.2	0.245	0.2	0.245	0.245
st. dev.	0	0.447	0.548	0.447	0.548	0.548

Table A31: Phase-specific traits in Oh43E plants used for sectioning

Trait	PR	GL	HR	WX	EAR	TLN
Plant no. 1	4	5	5	7	6	9
2	5	5	5	7	6	8
3	5	5	5	7	6	9
4	5	5	5	7	6	9
5	5	5	5	7	6	9
mean	4.8	5	5	7	6	8.8
s.e.m.	0.2	0	0	0	0	0.2
st. dev.	0.447	0	0	0	0	0.447

Table A32: Phase-specific traits in A632 plants used for leaf staining

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	7	7	4	11	14	21
2	7	7	5	13	14	21
3	7	8	5	12	14	21
4	7	7	5	12	14	21
5	6	7	5	12	14	21
6	7	7	5	10	13	19
7	8	7	5	10	13	20
8	7	7	5	12	14	21
9	7	7	4	11	14	21
mean	7.00	7.11	4.78	11.44	13.78	20.67
s.e.m.	0.17	0.11	0.15	0.34	0.15	0.24
st. dev.	0.50	0.33	0.44	1.01	0.44	0.71

Table A33: Phase-specific traits in A632E plants used for leaf staining

Trait	PR	GL	HR	WX	EAR	TLN
plant no.1	7	7	5	13	11	17
2	6	7	5	12	11	17
3	6	7	5	13	11	17
4	7	7	5	13	11	17
5	6	7	6	13	10	17
6	6	7	5	12	10	16
7	6	7	5	12	10	16
8	6	7	5	12	10	16
9	6	7	5	12	10	16
10	6	7	5	12		17
mean	6.20	7.00	5.10	12.40	10.44	16.60
s.e.m.	0.13	0.00	0.10	0.16	0.18	0.16
st. dev.	0.42	0.00	0.32	0.52	0.53	0.52

Table A34: Phase-specific traits in Oh43 plants used for leaf staining

Trait	PR	GL	HR	WX	EAR	TLN
plant no.1	6	7	5	11	10	16
2	6	6	4	12	11	17
3	6	7		11	10	15
4	6	7	5	12	11	16
5	6	7	5	11	11	16
mean	6.00	6.80	4.75	11.40	10.60	16.00
s.e.m.	0.00	0.20	0.25	0.24	0.24	0.32
st. dev.	0.00	0.45	0.50	0.55	0.55	0.71

Table A35: Phase-specific traits in Oh43E plants used for leaf staining

Trait	PR	GL	HR	WX	EAR	TLN
plant no.1	4	6	4	8	6	10
2	4	5	4	8	6	10
3	4	6	4	9	7	13
4	5	6	4	10	7	12
5	5	6	4	10	7	12
mean	4.40	5.80	4.00	9.00	6.60	11.40
s.e.m.	0.24	0.20	0.00	0.45	0.24	0.60
st. dev.	0.55	0.45	0.00	1.00	0.55	1.34

Table A36: Phase-specific traits and expression of *Rg1* in A632 plants

Trait	First ragged leaf	WX	EAR	TLN
plant no.1	6	8	14	19
2	6	8	13	18
3	6	8	13	18
4	6	8	14	19
5	6	8	14	19
6	5	8	12	17
7	5	7	13	18
8	5	7	14	19
9	6	8	16	22
10	6	9	14	20
11	6	9	14	20
12	6	8	13	18
13	5	8	11	16
14	5	8	12	17
15	6	8	13	18
16	6	8	14	19
17	6	8	12	18
mean	5.71	8.00	13.29	18.53
s.e.m.	0.11	0.12	0.28	0.33
st. dev.	0.47	0.50	1.16	1.37

Table A37: Phase-specific traits and expression of *Rg1* in A632E plants

Trait	First ragged leaf	WX	EAR	TLN
plant no.1	5	8	10	15
2	5	8	10	16
3	6	8	9	14
4	6	8	10	13
5	6	8	10	14
6	6	8	10	14
7	6	8	9	14
8	6	8	10	15
9	5	8	11	15
mean	5.67	8.00	9.89	14.44
s.e.m.	0.17	0.00	0.20	0.29
st. dev.	0.50	0.00	0.60	0.88

Table A38: Phase-specific traits and expression of *Rg1* in Oh43 plants

Trait	First ragged leaf	WX	EAR	TLN
plant no.1	4	7	10	17
2	5	7	12	17
3	5	7	14	19
4	5	7	13	18
5	6	8	13	19
6	5	7	11	17
7	5	8	12	17
8	5	7	13	17
9	5	8	10	16
10	5	8	10	16
11	4	7	12	17
12	5	8	11	17
13	5	7	12	18
14	4	7	13	17
15	4	7	11	17
16	4	7	14	20
17	5	7	11	16
18	4	7	12	17
19	4	6	13	17
20	4	7	11	17
21	3	6	11	16
22	4	8	11	17
mean	4.55	7.18	11.82	17.23
s.e.m.	0.29	0.25	0.52	0.44
st. dev.	0.67	0.59	1.22	1.02

Table A39: Phase-specific traits and expression of *Rg1* in Oh43E plants

Trait	First ragged leaf	WX	EAR	TLN
plant no.1	5	7	8	12
2	4	8	8	13
3	5	7	8	13
4	5	7	8	12
5	5	6	7	12
6	4	7	7	12
7	4	6	10	15
8	5	7	8	12
9	5	6	6	11
10	4	7	8	13
11	4	6	7	11
12	2	5	6	11
13	5	6	8	12
14	5	8	7	12
15	4	6	8	13
16	4	7	7	12
17	5	7	9	14
18	5	7	7	12
19	5	6	8	13
mean	4.47	6.63	7.63	12.37
s.e.m.	0.18	0.17	0.22	0.23
st. dev.	0.77	0.76	0.96	1.01

Table A40: Phase-specific traits in A619

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	7	7	4	11	11	16
2	8	8	3	11	11	17
3	7	7	4	11	11	16
4	10	7	4	11	11	17
5	8	7	3	11	11	16
6	8	7	4	12	11	17
7	8	7	4	11	11	16
8	9	8	4	12	11	17
9	9	7	5	11	11	16
10	9	7	4	11	10	15
11	9	7	5	11	11	16
12	9	7	5	11	11	17
mean	8.42	7.17	4.08	11.2	10.9	16.3
s.e.m.	0.26	0.11	0.19	0.11	0.08	0.19
st. dev.	0.9	0.39	0.67	0.39	0.29	0.65

Table A 41: Phase-specific traits in A619E

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	7	6	3	9	9	14
2	7	6	3	9	9	13
3	8	7	3	9	9	14
4	8	7	4	9	10	15
5	8	7	4	9	10	14
6	8	7	4	9	9	14
mean	7.67	6.67	3.5	9	9.33	14
s.e.m.	0.21	0.21	0.224	0	0.21	0.2582
st. dev.	0.52	0.52	0.548	0	0.52	0.6325

Table A 42: Phase-specific traits in A635

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	9	8	5	13	15	20
2	10	9	4	12	16	21
3	10	9	5	12	16	21
4	9	9	5	12	15	22
5	9	8	5	11	15	21
6	10	8	5	10	15	20
7	10	8	5	10	14	19
8	10	8	6	10	14	19
9	9	8	5	10	13	19
10	9	8	5	10	14	19
mean	9.5	8.3	5	11	14.7	20.1
s.e.m.	0.17	0.15	0.15	0.37	0.3	0.35
st. dev.	0.53	0.48	0.47	1.15	0.95	1.1

Table A43: Phase-specific traits in A635E

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	10	7	5	10	14	20
2	10	8	5	12	13	19
3	10	7	4	10	12	19
4	8	7	4	10	11	18
5	8	7	5	8	11	16
6	8	7	5	9	11	16
7	9	7	4	9	11	16
mean	9	7.1	4.57	9.71	11.9	17.7
s.e.m.	0.38	0.1	0.2	0.47	0.46	0.64
st. dev.	1	0.4	0.53	1.25	1.21	1.7

Table A44: Phase-specific traits in B73

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	12	7	7	7	16	21
2	11	6	6	7	16	21
3	12	7	7	7	16	21
4	11	7	7	8	15	21
5	12	6	6	7	15	20
6	11	6	6	7	16	20
mean	11.5	6.5	6.5	7.17	15.7	20.7
s.e.m.	0.22	0.22	0.22	0.17	0.21	0.21
st. dev.	0.55	0.55	0.55	0.41	0.52	0.52

Table A45: Phase-specific traits in B73G

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	9	5	6	6	12	17
2	10	6	6	6	12	17
3	10	6	5	6	12	17
4	10	6	5	7	12	17
5	10	6	6	7	13	18
6	9	6	5	6	12	17
7	11	6	6	7	12	17
mean	9.86	5.86	5.57	6.43	12.1	17.1
s.e.m.	0.26	0.14	0.2	0.2	0.14	0.14
st. dev.	0.69	0.38	0.53	0.53	0.38	0.38

Table A46: Phase-specific traits in C123

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	8	5	5	6	12	16
2	7	5	4	6	11	16
3	8	5	4	6	12	16
4	7	5	4	6	12	16
5	7	5	4	6	11	15
6	7	5	4	6	12	16
7	7	5	5	6	11	16
8	7	5	4	6	12	16
9	7	5	5	6	11	15
10	7	5	5	6	12	16
11	7	5	4	6	12	16
12	7	5	5	6	11	15
13	8	5	5	6	12	16
14	8	5	5	6	11	15
15	7	5	4	6	11	15
mean	7.3	5	4.5	6	11.5	15.7
s.e.m.	0.1	0	0.1	0	0.13	0.13
st. dev.	0.5	0	0.5	0	0.52	0.49

Table A47: Phase-specific traits in C123E

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	7	5	4	6	10	14
2	7	5	4	6	10	14
3	7	5	3	7	10	15
4	8	5	5	6	11	15
5	7	5	4	6	10	14
6	7	5	4	6	10	14
7	7	5	4	6	10	14
8	7	5	4	6	10	14
9	7	5	4	6	10	14
10	7	5	4	6	10	14
mean	7.1	5	4	6.1	10.1	14.2
s.e.m.	0.1	0	0.3	0.2	0.2	0.27
st. dev.	0.3	0	0.5	0.3	0.32	0.42

Table A48: Phase-specific traits in H100

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	11	7	6	9	16	23
2	10	7	6	9	15	23
3	11	7	6	9	15	23
4	12	7	6	9	15	23
5	12	7	6	9	15	23
6	12	7	6	10	16	24
7	11	7	7	9	16	23
8	12	7	6	9	16	24
9	12	7	6	9	15	20
10	13	7	7	8	14	20
11	10	7	7	9	14	19
12	11	7	7	9	14	20
13	10	7	7	9	15	20
mean	11.3	7	6.38	9	15.1	21.9
s.e.m.	0.26	0	0.14	0.11	0.21	0.5
st. dev.	0.95	0	0.51	0.41	0.76	1.8

Table A49: Phase-specific traits in H100E

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	10	7	6	8	14	19
2	11	7	7	9	14	20
3	11	7	6	9	14	19
mean	10.7	7	6.33	8.67	14	19.3
s.e.m.	0.33	0	0.33	0.33	0	0.33
st. dev.	0.58	0	0.58	0.58	0	0.58

Table A50: Phase-specific traits in Mo17

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	5	5	5	7	10	15
2	6	5	5	7	10	15
3	5	5	5	7	10	15
4	5	5	5	7	10	15
5	5	5	5	7	11	15
mean	5.20	5.00	5.00	7.00	10.20	15.00
s.e.m.	0.20	0.00	0.00	0.00	0.20	0.00
st. dev.	0.45	0.00	0.00	0.00	0.45	0.00

Table A51: Phase-specific traits in Mo17E

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	6	5	5	7	9	14
2	6	5	5	7	8	14
3	6	5	5	7	9	14
4	6	5	5	7	9	14
5	6	5	5	7	9	13
mean	6.00	5.00	5.00	7.00	8.80	13.80
s.e.m.	0.00	0.00	0.00	0.00	0.20	0.20
st. dev.	0.00	0.00	0.00	0.00	0.45	0.45

Table A52: Phase-specific traits in N28

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	14	7	5	8	16	22
2	14	7	5	8	17	23
3	14	7	5	8	17	22
4	14	7	5	8	16	22
5	14	7	5	8	17	23
6	13	6	5	7	16	22
7	13	6	6	7	16	21
mean	13.7	6.71	5.14	7.71	16.4	22.1
s.e.m.	0.18	0.18	0.14	0.18	0.2	0.26
st. dev.	0.49	0.49	0.38	0.49	0.53	0.69

Table A53: Phase-specific traits in N28E

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	12	7	5	8	14	20
2	12	7	5	8	14	20
3	11	7	5	7	14	19
4	12	7	6	8	14	20
5	11	7	5	8	13	19
6	12	6	5	7	13	19
7	11	7	6	7	14	19
mean	11.6	6.9	5.29	7.57	13.7	19.4
s.e.m.	0.2	0.1	0.18	0.2	0.18	0.2
st. dev.	0.53	0.4	0.49	0.53	0.49	0.53

Table A54: Phase-specific traits in Sc76

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	10	7	3	9	18	24
2	9	7	3	9	17	23
3	10	8	4	9	17	22
4	9	7	3	9	18	23
5	10	7	3	9	17	23
6	10	7	3	9	18	23
7	10	7	3	9	18	24
8	10	7	4	8	19	24
9	11	7	4	10	19	25
10	11	7	4	9	18	23
mean	10.00	7.10	3.40	9.00	17.90	23.40
s.e.m.	0.21	0.10	0.16	0.15	0.23	0.27
st. dev.	0.67	0.32	0.52	0.47	0.74	0.84

Table A55: Phase-specific traits in Nc264

Trait	PR	GL	HR	WX	EAR	TLN
Plant no.1	9	6	3	8	14	19
2	9	7	3	7	14	18
3	10	7	4	9	14	18
4	9	7	4	8	13	17
5	9	7	3	8	14	19
6	8	6	3	7	13	18
7	9	7	3	8	13	18
8	9	7	4	8	14	20
9	9	7	4	8	14	20
10	9	7	5	9	14	18
11	9	7	3	8	13	18
12	9	6	4	8	13	18
13	10	6	3	8	14	18
14	9	6	4	7	13	18
mean	9.07	6.64	3.57	7.93	13.57	18.36
s.e.m.	0.13	0.13	0.17	0.16	0.14	0.23
st. dev.	0.47	0.50	0.65	0.62	0.51	0.84

Table A56: Effect of SD on TLN and ear placement node in A632 and A632E

	LD			SD (8-38 days after planting)						
				Visible ligules	Visible leaves	Visible ligules	Visible leaves			
Trait	WX	EAR	TLN	before SD		after SD		WX	EAR	TLN
A632										
Plant no. 1	10	12	19	0	2	5	7	10	11	16
2	10	12	18	0	2	4	7	10	10	15
3	11	12	18	0	1	5	7	9	11	16
4	11	12	18	0	2	4	7	11	10	17
5	10	12	18	0	2	4	7	11	11	15
6	11	12	18	0	1	4	7	11	10	15
7	11	13	19	0	2	4	7	11	10	15
8	10	13	19	0	2	4	7	11	10	15
9	11	12	18	0	2	5	7	11	10	14
mean	10.56	12.22	18.33	0.00	1.80	4.40	7.00	10.56	10.33	15.33
s.e.m.	0.18	0.15	0.17	0.00	0.13	0.16	0.00	0.24	0.17	0.29
st. dev.	0.53	0.44	0.50	0.00	0.42	0.52	0.00	0.73	0.50	0.87
A632E										
Plant no. 1	9	9	14	0	1	5	8	10	8	12
2	10	9	14	1	2	4	8	9	8	12
3	10	8	14	0	1	4	8	9	7	12
4	10	9	15	0	2	4	8	9	7	12
5	9	8	14	1	2	4	8	9	7	12
6	9	8	14	1	2	5	8	9	8	13
7	9	9	14	1	2	5	8	9	7	12
8	9	8	13	0	2	5	8	10	7	12
9	9	8	15							
mean	9.33	8.44	14.11	0.50	1.80	4.50	8.00	9.25	7.38	12.13
s.e.m.	0.17	0.18	0.20	0.16	0.13	0.17	0.00	0.16	0.18	0.13
st. dev.	0.50	0.53	0.60	0.46	0.42	0.53	0.00	0.46	0.52	0.35

Table A57: Effect of SD on TLN and ear placement node in Oh43 and Oh43E

	LD			SD (8-38 days after planting)						
	WX	EAR	TLN	Visible ligules before SD	Visible leaves	Visible ligules after SD	Visible leaves	WX	EAR	TLN
Oh43										
Plant no. 1		9	14	0	2	4	6	9	8	12
2	10	10	15	0	2	4	6		7	11
3	10	9	14	0	2	4	6	9	7	11
4	9	9	14	0	2	4	7	9	8	12
5	10	9	14	0	2	4	7	9	8	12
6		9	14	0	2	4	6	9	7	12
7	10	9	13	0	2	4	6	9	7	11
8	10	10	14	0	2	4	7	10	8	12
9				0	2	4	7	9	8	12
10				0	2	4	6	9	7	12
mean	9.83	9.25	14.00	0.00	2.00	4.00	6.40	9.11	7.50	11.70
s.e.m.	0.17	0.17	0.20	0.00	0.00	0.00	0.16	0.11	0.17	0.15
st. dev.	0.41	0.46	0.53	0.00	0.00	0.00	0.52	0.33	0.53	0.48
Oh43E										
Plant no. 1	7	5	9	0	2	4	6	7	5	9
2	7	5	9	0	1	3	6	7	5	8
3	7	5	9	0	1	3	6	7	5	9
4	8	6	10	0	1	4	6	7	6	9
5	8	7	12	0	2	4	6	7	5	9
6	7	5	9	0	2	3	6	6	5	8
7	8	6	10	0	2	4	6	7	5	9
8	8	6	10	0	1	4	6	7	5	9
9				0	1	4	6	7	5	9
10				0	1	4	6	7	5	8
mean	7.50	5.63	9.75	0.00	1.40	3.70	6.00	6.90	5.10	8.70
s.e.m.	0.19	0.26	0.37	0.00	0.16	0.15	0.00	0.10	0.10	0.15
st. dev.	0.53	0.74	1.04	0.00	0.52	0.48	0.00	0.32	0.32	0.48

Table A58: TLN in wild type and total number of nodes Tp1 plants in A632, A632/A632E and A632E.

Genotype	wild type			*Tp1/+*		
	A632	A632/A632E	A632E	A632	A632/A632E	A632E
Plant no. 1	21	19	17	25	21	21
2	21	18	18	23	20	23
3	21	18	18	23	20	22
4	21	18	17	25	22	22
5	19	18	17	25	19	18
6	20	19	19	22	21	20
7	21	19	19	23	21	
8	21	19	19	23	21	
9		20	17	23		
10		18	17	26		
11		19	16	23		
12			17			
13			20			
14			18			
mean	20.63	18.64	17.79	23.73	20.63	21.00
s.e.m.	0.26	0.24	0.40	0.45	0.32	0.63
st dev	0.74	0.67	1.12	1.27	0.92	1.79

Table A59: TLN in wild type and total number of nodes Tp1 plants in Oh43, Oh43/Oh43E and Oh43E.

Genotype	wild type			*Tp1/+*		
	Oh43	Oh43/Oh43E	Oh43E	Oh43	Oh43/Oh43E	Oh43E
Plant no. 1	14	14	10	15	15	13
2	14	14	11	15	15	13
3	14	14	11	16	16	13
4	14	14	11	16	16	13
5	15	15	12	16	16	13
6	15	15	12	16	16	14
7	15		12	16	16	14
8	15		12	16	16	14
9	15			16	16	14
10	15			16	17	14
11	15			16	17	15
12	16			16	17	15
13	16			16	18	
14				17		
15				17		
16				17		
17				17		
18				17		
19				17		
20				17		
21				17		
22				17		
23				17		
24				17		
25				17		
26				17		
27				17		
28				17		
29				18		
30				18		
31				18		
32				18		
33				18		
34				18		
35				18		
36				18		
37				18		
38				18		
39				18		
40				18		
41				18		
42				18		
43				19		
44				19		

Table A60: TLN in wild type and total number of nodes Tp1 plants in F2 families segregating the early flowering trait in A632.

Genotype	wild type		*Tp1/+*	
A632	F2 (1)	F2 (2)	F2 (1)	F2 (2)
Plant no. 1	16	16	19	17
2	16	17	19	17
3	17	17	19	18
4	17	17	19	18
5	17	17	19	18
6	18	17	20	18
7	18	17	20	18
8	18	17	20	19
9	18	17	20	19
10	18	18	20	19
11	18	18	20	19
12	18	18	20	19
13	18	18	20	20
14	18	18	21	20
15	18	18	21	20
16	19	18	21	20
17	19	18	21	20
18	19	18	21	20
19	19	18	21	20
20	19	18	21	20
21	19	18	21	20
22	19	18	22	20
23	19	18	22	20
24	19	19	22	20
25	20	19	22	20
26	20	19	22	20
27	20	19	22	21
28	20	19	22	21
29	20	19	22	21
30	20	19	22	21
31	20	19	22	21
32	20	19	22	21
33	20	19	22	21
34	20	19	22	21
35	20	19	22	21
36	20	19	22	21
37	20	19	22	21
38	20	19	22	21
39	20	20	22	21
40	20	20	22	22
41	20	20	23	22
42	20	20	23	22
43	20		23	22
44	20		24	22
45	20		24	22
46	20		24	22
47	20		24	22
48	20		24	23
49	21		24	23
50	21		25	23

continued..

..continued

Genotype	wild type		*Tp1/+*	
A632	F2 (1)	F2 (2)	F2 (1)	F2 (2)
51	21		25	23
52			26	23
53			27	23
54				23
55				23
56				24
57				24
58				24
59				25

Table A61: TLN in wild type and total number of nodes Tp1 plants in F2 families segregating the early flowering trait in Oh43.

Genotype		wild type			*Tp1/+*	
Oh43	F2 (1)	F2 (2)	F2 (3)	F2 9!0	F2 (2)	F2 (3)
Plant no.1	10	9	12	12	10	13
2	11	10	12	12	11	14
3	11	10	12	12	11	14
4	11	11	13	12	12	14
5	11	11	13	12	12	14
6	11	11	13	13	12	14
7	12	11	13	13	12	14
8	12	11	13	13	12	14
9	12	11	13	13	12	15
10	12	11	13	13	13	15
11	12	12	13	14	13	15
12	12	12	13	14	13	15
13	12	12	13	14	13	15
14	12	12	14	14	13	15
15	12	13	14	14	13	15
16	12	13	14	14	14	15
17	12	13	14	14	14	15
18	12	13	14	14	14	15
19	12	13	14	14	14	15
20	13	13	14	14	14	15
21	13	13	14	14	14	15
22	13	13	14	14	14	15
23	13	13	14	15	14	15
24	13	13	14	15	14	16
25	13	13	14	15	14	16
26	13	13	14	15	14	16
27	13	13	14	15	14	16
28	13	13	14	15	14	16
29	13	13	14	15	14	16
30	13	13	14	15	14	16
31	13	13	15	15	14	16
32	13	13	15	15	14	16
33	13	13	15	15	15	16
34	13	14	15	15	15	16
35	13	14	15	15	15	16
36	14	14	15	15	15	16
37	14	14	16	15	15	16
38	14	14	16	16	15	16
39	14	14		16	15	16
40	14	14		16	15	16
41	14	14		16	15	16
42	14	14		16	15	17
43	14	14		16	15	17
44	14	14		16	15	17
45	14	14		16	15	17
46	14	14		16	15	17
47	14	14		16	15	17
48	14	15		16	15	17
49	14	15		17	15	17
50	15	15		17	15	17
51	15	15		17	15	17

continued..

.continued

Genotype		wild type			*Tp1/+*	
Oh43	F2 (1)	F2 (2)	F2 (3)	F2 9!0	F2 (2)	F2 (3)
52	15			17	15	17
53	15			17	16	17
54	15			17	16	17
55	15			17	16	17
56	15			17	16	17
57	15			17	16	18
58	15			17	16	18
59	15			17	16	18
60	15			17	16	18
61				17	16	19
62				17	16	19
63				18	16	20
64				18	16	
65				18	16	
66					16	
67					16	
68					16	
69					16	
70					16	
71					16	
72					16	
73					16	
74					16	
75					16	
76					16	
77					16	
78					16	
79					17	
80					17	
81					17	
82					17	
83					17	
84					17	
85					18	
86					18	
87					18	

Table A62: TLN in wild type and total number of nodes Tp2-E2 plants in A632, A632/A632E and A632E.

Genotype	wild type			*Tp2-E2/+*		
	A632	A632/A632E	A632E	A632	A632/A632E	A632E
Plant no. 1	19	17	16	21	19	17
2	20	18	16	21	20	18
3	20	18	16	21	20	18
4	20	18	17	22	20	19
5	20	18		22	20	
6	20	18		23	20	
7	21	18		23	21	
8		18		23		
9		18		23		
10		18				
11		19				

Table A63: TLN in wild type and total number of nodes Tp2 plants in Oh43, Oh43/Oh43E and Oh43E.

Genotype	wild type			*Tp2/+*		
	Oh43	Oh43/Oh43E	Oh43E	Oh43	Oh43/Oh43E	Oh43E
Plant no. 1	17	13	10	18	18	14
2	17	14	11	22	20	15
3	17	14	11	22	20	16
4	17	15	11	23	21	17
5	18	15	11	23	22	17
6	18	15	11	23	22	17
7	18	15	12	23	22	18
8	18	15	12	23	22	
9	18	15	12			
10	18		12			
11	18		12			
12	19		12			
13	19		12			
14			13			
15			13			
16			13			
17			13			
18			13			
19			14			

Table A64: TLN in wild type and total number of nodes and tassel score in Tp2 plants in F2 families segregating the early flowering trait.

Genotype	A632			Oh43		
	wild type	*Tp2-E2/+*		wild type	*Tp2/+*	
Trait	TLN	No. of nodes	Tassel score	TLN	No. of nodes	Tassel score
Plant no.1	15	17	4	10	13	7
2	15	17	3	11	13	7
3	16	17	3	11	13	7
4	16	18	3	11	13	7
5	16	18	4	12	14	7
6	17	18	4	12	14	7
7	17	19	3	12	14	7
8	17	19	3	12	14	7
9	17	19	3	12	14	7
10	17	19	4	12	14	7
11	17	19	3	12	15	7
12	17	19	3	12	15	3
13	17	19	4	12	16	7
14	17	19	5	12	17	7
15	17	19	3	12	17	7
16	17	19	3	12	17	7
17	17	19	2	12	18	5
18	17	19	3	12	18	7.5
19	17	19	4	13	18	6
20	18	19	3	13	18	7.5
21	18	20	3	13	18	8
22	18	20	4	13	18	8
23	18	20	4	13	18	8
24	18	20	3	13	18	8
25	18	20	3	13	18	5
26	18	20	4	13	19	7.5
27	18	20	3	13	19	6
28	18	20	3	13	19	8
29	18	20	3	13	19	8
30	18	20	3	13	19	7
31	18	20	3	13	19	7.5
32	18	20	3	13	19	7.5
33	18	20	4	13	19	8
34	18	20	3	13	19	8
35	18	21	3	13	19	8
36	18	21	3	13	19	8
37	18	21	3	13	19	8
38	18	21	4	13	19	6
39	19	21	3	13	19	5
40	19	21	3	13	19	8
41	19	21	4	13	19	8
42	19	21	4	13	19	7
43	20	21	4	14	19	8
44	20	21	4	14	19	7
45	20	22	3	14	19	8
46	20	22	3	14	20	8
47	21	22	3	14	20	7

continued..

..continued

Genotype		A632			Oh43	
	wild type	*Tp2-E2/+*		wild type	*Tp2/+*	
Trait	TLN	No. of nodes	Tassel score	TLN	No. of nodes	Tassel score
48		23	3	14	20	5
49		23	4	14	20	6
50				14	20	8
51				14	20	5
52				14	20	5
53				14	20	7.5
54				14	20	7
55				14	20	7
56				15	20	8
57				15	20	6
58				15	20	8
59				15	20	8
60				15	21	8
61				15	21	7
62				15	21	3
63				15	21	8
64				15	21	8
65				15	21	8
66				15	21	5
67				16	21	5
68				16	21	8
69				16	21	7.5
70				16	21	8
71				16	22	7
72				17	22	7
73				17	22	5
74				17	23	8
75					23	7
76					23	8

65

Table A The effect of SD conditions on tassel initiation and ear placement in A632 and A632E plants. (N is the number of plants scored. All errors are given as ± 2 s.e.m.)

	A632					A632E				
SD Treatment (DAP)	No. of emerged ligules	No. of emerged leaves	Ear placement node	Total leaf number	N	No. of emerged ligules	No. of emerged leaves	Ear placement node	Total leaf number	N
None			12.2±0.3	18.3±0.3	9			8.4±0.4	14.1±0.4	9
8-38	0-5	2-7	**10.3±0.3**^{c,3}	**15.3±0.6**^{c,3}	10	0-5	2-8	**7.4±0.4**^{c,2}	**12.1±0.3**^{c,3}	8
18-23	2-3	4-5	12.0±0.0	18.0±0.4	8	2-3	4-5	8.0±0.5	**13.6±0.3**^{a}	10
23-28	3-4	5-6	11.9±0.2	**17.8±0.3**^{a}	10	3-4	5-6	**8.1±0.2**^{a}	13.8±0.3	10
28-33	3-4	6-7	**11.5±0.3**^{b,1}	**16.7±0.4**^{c,3}	10	3-4	6-7	8.4±0.3	13.9±0.4	10
33-38	4-5	7-8	12.0±0.0	**17.0±0.5**^{c,2}	9	4-5	7-8	8.1±0.4	14.0±0.3	10

a, 1: significantly smaller than LD control at p≤0.05 (a: 1-tailed *t* test, 1: 1-tailed *U* test)
b, 2: significantly smaller than LD control at p≤0.01 (b: 1-tailed *t* test, 2: 1-tailed *U* test)
c, 3: significantly smaller than LD control at p≤0.001 (c: 1-tailed *t* test, 3: 1-tailed *U* test)
a, b, c refer to results from *t* test; 1, 2, 3 refer to results from *U* test

66

Table A The effect of SD conditions on tassel initiation and ear placement in Oh43 and Oh43E plants (N is the number of plants scored. All errors are given as ± 2 s.e.m.).

	Oh43					Oh43E				
SD Treatment (DAP)	No. of emerged ligules	No. of emerged leaves	Ear placement node	Total leaf number	N	No. of emerged ligules	No. of emerged leaves	Ear placement node	Total leaf number	N
None			9.3±0.3	14.0±0.4	8			5.6±0.5	9.8±0.7	8
8-38	0-4	2-6	**7.5±0.3**[c,3]	**11.7±0.3**[c,3]	10	0-4	1.5-6	**5.1±0.2**[a]	**8.7±0.3**[b,1]	10
8-13	0-1	2-3	9.0±0.0	14.0±0.0	9	0-1	1-3	**5.0±0.4**[a]	**8.2±0.4**[c,2]	10
13-18	1-2	3-4	9.0±0.0	14.0±0.0	8	1-2	3-4	5.2±0.4	9.1±0.5	10
18-23	2	4-4.5	9.0±0.0	14.0±0.5	10	2	4-4.5	5.6±0.3	9.1±0.5	10
23-28	2.5-3	4.5-5.5	**8.7±0.3**[a,1]	**12.8±0.3**[c,3]	9					
28-33	3-4	5.5-6.5	**8.9±0.3**[a]	**13.4±0.4**[a,3]	8					
33-38	4-5	6.5-7.5	9.1±0.2	13.8±0.3	9					

a, 1: significantly smaller than LD control at p≤0.05 (a: 1-tailed *t* test, 1: 1-tailed *U* test)
b, 2: significantly smaller than LD control at p≤0.01 (b: 1-tailed *t* test, 2: 1-tailed *U* test)
c, 3: significantly smaller than LD control at p≤0.001 (c: 1-tailed *t* test, 3: 1-tailed *U* test)

Table A67: Effective SD treatments in A632 and A632E

SD treatment	None (LD control)		8-38 DAP (SD control)		18-23 DAP		23-28 DAP		28-33 DAP		33-38 DAP	
Trait	EAR	TLN	EAR	TLN	EAR	TLN	EAR	TLN	EAR	TLN	EAR	TLN
A632	12	19	11	16	12	18	12	18	12	17	12	17
Plant no.1	12	18	10	15	12	18	12	17	12	16	12	18
2	12	18	11	16	12	18	12	18	11	16	12	17
3	12	18	10	17	12	19	12	18	12	17	12	17
4	12	18	11	15	12	17	12	18	11	17	12	17
5	12	18	10	15	12	18	12	18	11	16	12	16
6	13	19	10	15	12	18	12	18	12	17	12	17
7	13	19	10	15			12	18	11	16	12	18
8	12	18	10	14			12	18	11	17	12	16
9							11	17	12	18		
mean	12.22	18.33	10.33	15.33	12.00	18.00	11.90	17.80	11.50	16.70	12.00	17.00
s.e.m.	0.15	0.17	0.17	0.29	0.00	0.22	0.10	0.13	0.17	0.21	0.00	0.24
st. dev.	0.44	0.50	0.50	0.87	0.00	0.58	0.32	0.42	0.53	0.67	0.00	0.71
A632E												
Plant no.1	9	14	8	12	8	13	8	14	8	14	9	14
2	9	14	8	12	9	14	8	14	9	15	8	14
3	8	14	7	12	7	14	8	14	9	14	9	14
4	9	15	7	12	9	14	9	14	9	14	8	14
5	8	14	7	12	9	14	8	14	9	14	8	14
6	8	14	8	13	8	13	8	14	8	13	8	14
7	9	14	7	12	7	14	8	13	8	14	8	14
8	8	13	7	12	8	14	8	14	8	13	8	13
9	8	15			7	13	8	14	8	14	8	14
10					8	13	8	13	8	14	7	15
mean	8.44	14.11	7.38	12.13	8.00	13.60	8.10	13.80	8.40	13.90	8.10	14.00
s.e.m.	0.18	0.20	0.18	0.13	0.26	0.16	0.10	0.13	0.16	0.18	0.18	0.15
st. dev.	0.53	0.60	0.52	0.35	0.82	0.52	0.32	0.42	0.52	0.57	0.57	0.47

Table A68: Effective SD treatments in Oh43 and Oh43E

SD treatment	None (LD control)		8-38 DAP (SD control)		8-13 DAP		13-18 DAP		18-23 DAP		23-28 DAP		28-33 DAP		33-38 DAP	
Trait	EAR	TLN	EAR	TLN	EAR	TLN	EAR	TLN	EAR	TLN	EAR	TLN	EAR	TLN	EAR	TLN
Oh43	9	14	8	12	9	14	9	14	9	13	8	12	8	13	9	14
Plant no.1	10	15	7	11	9	15	9	14	9	13	8	12	9	13	9	14
2	9	14	7	11	9	14	9	14	9	15	8	13	9	14	9	14
3	9	14	8	12	9	14	9	14	9	14	9	13	9	14	9	13
4	9	14	8	12	9	14	9	14	9	14	9	13	9	13	9	14
5	9	14	7	12	9	14	9	15	9	13	9	13	9	13	10	14
6	9	13	7	11	9	14	9	14	9	14	9	13	9	14	9	14
7	10	14	8	12	9	14	9	14	9	15	9	13	9	13	9	13
8			8	12	9	14	9	14	9	14	9	13			9	14
9			7	12					9	15						
mean	9.25	14.00	7.50	11.70	9.00	14.11	9.00	14.11	9.00	14.00	8.67	12.78	8.88	13.38	9.11	13.78
s.e.m.	0.17	0.20	0.17	0.15	0.00	0.11	0.00	0.11	0.00	0.26	0.17	0.15	0.13	0.18	0.11	0.15
st. dev.	0.46	0.53	0.53	0.48	0.00	0.33	0.00	0.33	0.00	0.82	0.50	0.44	0.35	0.52	0.33	0.44
Oh43E																
Plant no.1	5	9	5	9	4	8	4	8	5	7						
2	5	9	5	8	5	8	5	9	5	9						
3	5	9	5	9	5	8	5	9	6	9						
4	6	10	6	9	5	8	6	10	6	9						
5	7	12	5	9	6	9	6	10	5	9						
6	5	9	5	8	4	7	6	10	6	10						
7	6	10	5	9	5	8	5	9	6	10						
8	6	10	5	9	5	8	5	9	6	9						
9			5	9	6	9	5	9	5	9						
10			5	8	5	9	5	8	6	10						
mean	5.63	9.75	5.10	8.70	5.00	8.20	5.20	9.10	5.60	9.10						
s.e.m.	0.26	0.37	0.10	0.15	0.21	0.20	0.20	0.23	0.16	0.28						
st. dev.	0.74	1.04	0.32	0.48	0.67	0.63	0.63	0.74	0.52	0.88						

Table A69: Phase-specific traits in Lfy1 plants

Genotype	wild type				*Lfy1/+*			
Trait	PR	WX	EAR	TLN	PR	WX	EAR	TLN
A632								
Plant no. 1	11	9	14	20	10	11	16	28
2	11	10	15	20	9	10	17	34
3	10	10	14	19	9	10	15	29
4	9	9	14	20	11	10	15	28
5	10	11	14	20	9	10	14	30
6	8	11	13	19	12	10	18	31
7	8	11	14	20	11	11	15	25
8	10	10	14	20	10	10	16	30
9	9	9	14	20	9	10	15	31
10	9	10	14	21				
11	9	10	14	20				
12	10	9	15	21				
13	11	10	15	21				
mean	9.62	9.92	14.15	20.08	10.00	10.22	15.67	29.56
s.e.m.	0.29	0.21	0.15	0.18	0.37	0.15	0.41	0.84
st. dev.	1.04	0.76	0.55	0.64	1.12	0.44	1.22	2.51
A632								
Plant no. 1	10	10	14	20	11	11	16	30
2	10	10	14	20	11	10	16	30
3	11	10	13	19	8	10	15	27
4	10	11	13	19	10	11	16	30
5	9	10	14	20	10	10	15	32
6	8	11	14	20	9	9	16	35
7	10	10	14	20	9	10	16	30
8	9	10	14	20	11	12	18	34
9	10	10	14	20	9	9	17	35
10	9	10	14	20	10	10	16	32
11	9	10	15	20	10	10	15	34
12					9	10	15	31
13					11	10	16	29
14					10	10	18	34
15					10	9	15	27
mean	9.55	10.18	13.91	19.82	9.87	10.07	16.00	31.33
s.e.m.	0.25	0.12	0.16	0.12	0.24	0.21	0.26	0.69
st. dev.	0.82	0.40	0.54	0.40	0.92	0.80	1.00	2.66
A632/A632E								
Plant no. 1	10	10	13	19	9	10	14	30
2	10	10	13	19	11	10	14	26
3	10	10	12	18	10	9	14	27
4	10	9	12	18	10	9	13	27
5	10	9	12	17	9	10	14	26
6	9	9	13	19	9	10	14	27
7	10	9	13	18	10	10	14	31
8	10	9	12	18	9	10	13	26
9	10	9	13	19	10	9	16	32
10					10	9	14	27
11					10	9	15	26
mean	9.89	9.33	12.56	18.33	9.73	9.55	14.09	27.73
s.e.m.	0.11	0.17	0.18	0.24	0.19	0.16	0.25	0.66
st. dev.	0.33	0.50	0.53	0.71	0.65	0.52	0.83	2.20

continued..

..continued

Genotype	wild type				*Lfy1/+*			
Trait	PR	WX	EAR	TLN	PR	WX	EAR	TLN
A632E (3)								
Plant no. 1	10	9	12	17	9	10	13	23
2	10	10	12	17	8	10	12	24
3	10	10	12	17	9	10	12	23
4	9	9	13	18	11	10	14	24
5	9	10	11	16	9	10	13	22
6	9	10	12	17	9	9	13	21
7	9	10	11	16	10	9	12	21
8	9	9	11	16	9	9	12	23
9					9	10	12	21
10					9	10	12	23
11					9	10	12	20
mean	9.38	9.63	11.75	16.75	9.18	9.73	12.45	22.27
s.e.m.	0.18	0.18	0.25	0.25	0.23	0.14	0.21	0.41
st. dev.	0.52	0.52	0.71	0.71	0.75	0.47	0.69	1.35
A632E (3)								
Plant no. 1	9	10	11	17	10	10	13	25
2	9	10	11	17	9	10	13	24
3	9	11	12	16	9	10	12	26
4	9	10	11	17	10	10	12	24
5	8	9	10	16	10	10	12	23
6	9	10	10	16	8	10	12	21
7	9	9	11	17	8	10	12	22
8					9	10	12	23
9					9	10	12	24
10					8	8	10	20
11					10	9	13	24
12					10	9	13	23
mean	8.86	9.86	10.86	16.57	9.17	9.67	12.17	23.25
s.e.m.	0.14	0.26	0.26	0.20	0.24	0.19	0.24	0.48
st. dev.	0.38	0.69	0.69	0.53	0.83	0.65	0.83	1.66
A632E (3)								
Plant no. 1	9	11	11	17	8	10	12	24
2	8	9	11	16	8	10	11	22
3	8	8	9	14	9	10	12	21
4	9	9	10	14	9	9	12	23
5	8	9	10	14	9	10	11	20
6	8	9	10	16	8	10	12	20
7	8	9	10	15	9	9	10	22
8	8	10	10	16				
9	8	10	10	16				
10	8	10	10	15				
11	9	10	11	16				
12	8	9	10	15				
mean	8.25	9.42	10.17	15.33	8.57	9.71	11.43	21.71
s.e.m.	0.13	0.23	0.17	0.28	0.20	0.18	0.30	0.57
st. dev.	0.45	0.79	0.58	0.98	0.53	0.49	0.79	1.50

continued..

..continued

Genotype	wild type				*Lfy1/+*			
Trait	PR	WX	EAR	TLN	PR	WX	EAR	TLN
wild type								
Plant no. 1	9	5	8	14	19			
2	10	5	9	14	20			
3	9	4	8	13	19			
mean	9.33	4.67	8.33	13.67	19.33			
s.e.m.	0.33	0.33	0.33	0.33	0.33			
st. dev.	0.58	0.58	0.58	0.58	0.58			
Tp2/+								
Plant no. 1	21		25	27	22	39	40	50
2	17		22	26	21		40	49
3	19		24	27	26	36	43	52
4	22		24	28				
mean	19.75		23.75	27.00	23.00	37.50	41.00	50.33
s.e.m.	1.11		0.63	0.41	1.53	1.22	1.00	0.88
st. dev.	2.22		1.26	0.82	2.65	2.12	1.73	1.53

Table A70: Phase-specific traits in wild type siblings in a family segregating *id1* (family J579).

Trait	PR	GL	HR	WX	EAR	TLN
Plant no. 1	8	7	7	8	10	15
2	8			7	12	16
3	9	7	6	8	11	16
4	8	7	7	8	12	17
5	8			7	13	17
6	8			7	11	17
7	9	7	7	9	12	17
8	8			7	13	18
9	8			7	12	18
10	8			7	12	18
11	9	7	6	8	12	18
12	9			8	13	18
13	9			8	13	18
14	10	7	7	8	12	18
15	7			7	13	19
16	8			6	13	19
17	8			7	13	19
18	8			7	13	19
19	8			7	12	19
20	8			7	13	19
21	9			8	14	19
22	9			8	14	19
23	9			8	13	19
24	9			7	14	19
25	9			8	13	19
26	10			8	13	19
27	11	8	8	10	13	19
28	9			8	14	20
29	9			8	14	20
30	9			7	14	20
31	9			8	14	20
32	10	6	6	8	14	20
33	10	7	8	8	15	21
mean	8.7273	7	6.8889	7.6364	12.848	18.455
s.e.m.	0.1461	0.1667	0.2606	0.1292	0.1853	0.2307
st. dev.	0.8394	0.5	0.7817	0.7424	1.0642	1.325

Table A71: Phase-specific traits in id1 siblings in a family segregating *id1* (family J579).

Trait	PR	GL	HR	WX	EAR	TLN*
Plant no. 1	13			8	no	28
2	13			10	ears	28
3	24			8	in	28
4	13	7	7	8	any	29
5	17			8	of	29
6	12			8	these	30
7	15			8	plants	30
8	24			8		30
9	25	7	6	8		32
10	21			8		33
11	26			8		34
12	14	7	7	9		36
13	22			8		36
mean	18.385	7	6.6667	8.2308		31
s.e.m.	1.4332	0	0.3333	0.1601		0.7792
st. dev.	5.3625	0	0.5774	0.5991		2.9155

*: These plants had not produced tassels by the time they died in the autumn, so TLN here denoted the last leaf visible in the whorl when the plants dried up.

Table A72: Phase-specific traits in wild type siblings in a M3 family segregating *dlf* (family J580).

Trait	PR	GL	HR	WX	EAR	TLN
Plant no. 1	10	7	5	9	15	20
2	9	7	5	9		19
3	9	7	5	9	16	21
4	9	7	5	10	14	20
5	10	6	4	9	14	20
6	10	7	6		14	20
7	10	6	4	9	14	20
8	9	7	5	9	14	18
9	9	6	4	9	14	20
mean	9.4444	6.6667	4.7778	9.125	14.375	19.778
s.e.m.	0.1757	0.1667	0.2222	0.1179	0.248	0.2778
st. dev.	0.527	0.5	0.6667	0.3536	0.744	0.8333

Table A73: Phase-specific traits in dlf siblings in a M3 family segregating *dlf* (family J580).

Trait	PR	GL	HR	WX	EAR	TLN
Plant no. 1	10	6	5	10	16	24
2	10	6	5	9	16	23
3	11	6	5	9	16	24
4	10	7	4	10	16	23
5	10	6	4	10	17	23
mean	10.2	6.2	4.6	9.6	16.2	23.4
s.e.m.	0.2	0.2	0.2449	0.2449	0.2	0.2449
st. dev.	0.4472	0.4472	0.5477	0.5477	0.4472	0.5477

Table A74: Phase-specific traits in A632 plants (family J576)

Trait	PR	GL	HR	WX	EAR	TLN
Plant no. 1	10	7	5	10	12	18
2	9	7	5	10	14	20
3	9	7	5	10	14	20
4	9	7	5	10	14	20
5	9	7	5	10	14	20
6	9	7	4	10	14	19
7	9	7	5	10	13	19
8	9	7	5	10	14	20
9	9	7	5	9	13	19
10	9	7	4	10	14	20
11	9	7	5	10	14	20
12	9	7	5	9	14	20
13	9	7	5	10	14	19
14	9	7	5	10	14	20
15	9	8	5	10	15	20
16	9	7	5	10	14	20
mean	9.0625	7.0625	4.875	9.875	13.813	19.625
s.e.m.	0.0625	0.0625	0.0854	0.0854	0.1638	0.1548
st. dev.	0.25	0.25	0.3416	0.3416	0.6551	0.6191

Table A75: Phase-specific traits in wild type siblings in a M3 family in A632 segregating *ltf* (family J584).

Trait	PR	GL	HR	WX	EAR	TLN
Plant no. 1	10	7	5	9	14	18
2	9	7	4	10	14	19
3	10	7	5	9	14	19
4	10	7	4	9	14	19
5	10	7	5	9	15	19
6	9	7	5	10	14	20
7	11	7	5	9	15	20
8	10	7	5	9	16	20
9	10	7	5	9	16	20
10	10	7	4	9	14	21
11	10	7	5	10	15	21
12	10	7	5	9	15	21
13	10	7	5	9	15	21
14	10	7	4	10	16	21
15	10	7	5	9	16	21
16	10	7	4	10	16	21
mean	9.9375	7	4.6875	9.3125	14.938	20.063
s.e.m.	0.1106	0	0.1197	0.1197	0.2135	0.2495
st. dev.	0.4425	0	0.4787	0.4787	0.8539	0.9979

Table A76: Phase-specific traits in ltf siblings in a M3 family in A632 segregating *ltf* (family J584).

Trait	PR	GL	HR	WX	EAR	TLN
Plant no. 1	15	7	5	10	21	28
2	14	7	5	9	22	28
3	15	6	4	9	22	28
mean	14.667	6.6667	4.6667	9.3333	21.667	28
s.e.m.	0.3333	0.3333	0.3333	0.3333	0.3333	0
st. dev.	0.5774	0.5774	0.5774	0.5774	0.5774	0