INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

THE ROLE OF THE ABSCISSION ZONE IN DEVELOPMENT OF THE TOBACCO INFLORESCENCE

by

YI ZHANG

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1998

UMI Number: 9830785

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

4/20/98
Date

Jack G Valdovinos
Chairman of Examining Committee
Dr. Jack G. Valdovinos, Lehman College

4/28/98
Date

Richard L. Chappell
Executive Officer
Dr. Richard L. Chappell

Thomas E. Jensen
Dr. Thomas E. Jensen, Lehman College

Dwight Kincaid
Dr. Dwight T. Kincaid, Lehman College

David L Lentz
Dr. David Lentz
New York Botanical Garden

Neil Grant
Dr. Neil Grant
William Patterson College of New Jersey

Supervising Committee

The City University of New York

Abstract

THE ROLE OF THE ABSCISSION ZONE IN DEVELOPMENT OF THE TOBACCO INFLORESCENCE

by

YI ZHANG

Advisor: Professor Jack G. Valdovinos

The abscission zone of the tobacco flower pedicel is a regulatory tissue in the development of the tobacco inflorescence. The abscission zone of each flower pedicel acts as a node (flower node). It is composed of several layers of small cells containing dense cytoplasm in the cortex and the pith on both sides of the groove mark. The proximal side is a potential meristem for flower bud formation and the distal side is the separation layer responsible for the induction of flower abscission. The terminal tobacco inflorescence is a typical compound cymose with many branch types which include monochasium branch types to trichasium branch types at each flower node. Except for the terminal flower most other flowers are initiated from the prior flower's abscission zone. The young inflorescence increases flower number rapidly by developing dichasium and trichasium branch types. The abscission zone is morphologically differentiated when the flower bud is about 1.2 mm in length. Flower bud formation may occur at the base of a flower pedicel even before the abscission zone becomes morphologically differentiated. The flower bud become inducible to abscission when the flower bud reaches about 10 mm in length. The abscission of a flower is prevented by rapid secondary growth of the vascular tissue after anthesis. Some flowers can be broken not at the abscission zone but further out the pedicel before any induction with ethylene. The abscission zone may not be the weakest point because of morphological differentiation but it becomes the weakest point in all inducible flowers after three hours treatment of ethylene. The abscission zone in a thin-layer explant is the most active tissue with respect to flower bud formation and other organogenesis under tissue culture conditions. Flower bud formation and other organogenesis can be induced from the proximal side of the abscission zone rapidly with proper conditions. The combination indole-3-acetic acid and kinetin causes the flower bud formation to develop from two ends of the abscission zone in an explant. However the combination of α-naphthalene-acetic acid and 6-benzylamino-purine causes the flower bud formation to develop from the whole abscission zone in an explant.

ACKNOWLEDGMENTS

I am deeply grateful to Professor Jack G. Valdovinos, who kindly provided the opportunity for me to study at Lehman College, helped me to choose a research project, gave me great freedom in research and advice for my thesis preparation. I thank Professor Thomas E. Jensen the department chairman for the use of his laboratory facilities, for continuous financial support and for his advice for my research. I wish to thank Mr. Mike Baxter who was very helpful in offering technical training on the EM facilities. My thanks are extended to Professor Dwight Kincaid from whom I received my initial computer training and the extremely useful methods for experimental design and analysis. My thanks are also extended to Professor Dominick Basile for his excellent classes; I was interested in the correlation of plant form and function and I learned the thin layer explant tissue culture techniques in his laboratory. I thank Dr. David Lentz and Dr. Neil Grant for their kindly serving on my defense committee and Dr. Michael Nee and Dr. William Corpe for their serving on my second examination committee. Sincere thanks are also extended to Ms. Dolores Vitanza and Dr. Antonio Pappantoniou for encouragement and helpful advice during the time I was a graduate student. I thank Dr. Edythe Jones and Mr. Kat Lee for their patience and their time helping me with English writing and speaking.

I would like to express the deepest appreciation and gratitude to my wife, Jia Yu, who I met and married in New York. Our family life became a strong support for me to

finish my thesis. I also thank my mother-in-law, Yi-Ping Zhang, whose support was an essential factor that allowed me to finish writing. I am deeply grateful to my cousin, Lynn Chang, for her help and support that enabled me to continue graduate school in New York.

Table of Contents

Page

Lists of Tables

Lists of Figures

Lists of Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
ACC	1-aminocyclopropane-1-carboxylic acid
AZ	abscission zone
AZbk	abscission zone break
BAP	benzyl-aminopurine
CH	chloroplast
CTA	∂-(3-chloro-o-tolyl)acetic acid
cv.	cultivar
CW	cell wall
ETH	ethylene
F1	flower 1
F1reg	flower 1 regenerate
FAA	Formaldehyde (5), Acetic acid (5) and Alcohol (90)
GB	Golgi body
GR	granular component
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
IPA	N6-(△2-isopentenyl)adenine
k	kilo, one thousand
kD	kilo Dalton

KIN	kinetin (or 6-furfarylaminopurine)
MB	microbody
MI	mitochondrion
NAA	∂-naphthalene acetic acid
NL	nucleolus
NU	nucleus
OsO4	osmium oxide
Pbk	pedicel break
PM	plasma membrane
RER	rough endoplasmic reticulum
SEM	scanning electron microscope
TEM	transmition electron microscope
VA	vacuole
W1	week 1
W2	week 2
W3	week 3
W3reg	week 3 regenerate

INTRODUCTION

In the tobacco plant development of the inflorescence is a process which starts with the transition from a vegetative phase to a reproductive phase and ends with the production of seeds. Development of an inflorescence passes through a series of changes where the inflorescence becomes a mature one which consists of a huge terminal inflorescence with a great number of tiny seeds. During the development of a tobacco inflorescence flower formation and flower abscission are two of the most important incidents. They regulate the size of inflorescence through increasing or decreasing the number of fruits on the inflorescence and finally determine the quantity of seeds produced by the plant.

The terminal inflorescence of tobacco plant

A description of the tobacco inflorescence lacks clarity. This can be seen from the description of the tobacco inflorescence in the literature. When Carolus Linnaeus named *Nicotiana tabacum* L. (Linnaeus, 1753) the terminal inflorescence of tobacco plants was described as "*Flores in racemos pancos, longos*". In most current plant classification books the tobacco inflorescence has been called a "terminal panicle" or a "racemose" (Robinson and Fernald, 1908; Bailey, 1949; Gleason and Cronquist, 1963 and 1991; Wiggins and Porter, 1971). However in some recent research papers (Trull and Malmberg, 1994; Kelly et al., 1995) the inflorescence of tobacco is called a "determinate" without considering the description from the plant classification manuals.

Inflorescences are traditionally classified as "determinate" (cymose) and "indeterminate" (racemose) inflorescence types (Richett, 1944). They are distinguished by their developmental patterns. In an indeterminate inflorescence, the inflorescence blooms from the base upwards so that theoretically it could continue to elongate indefinitely (Cronquist, 1988). In a determinate inflorescence the apical meristem differentiated to a single terminal flower first.

Study of the inflorescence pattern is a developmental question which further relates to the regulation of the inflorescence development. For an indeterminate inflorescence the continued development of the inflorescence is contributed to by the apical meristem which is called an inflorescence meristem (Weigel et al., 1992; Trull and Malmberg, 1994). Since the apical meristem is differentiated to a single flower in the determinate inflorescence there must be sub-apical tissue to continue the inflorescence development. Otherwise the plant would not form an inflorescence.

Tobacco plants have been used as one of the most popular experimental materials (Tso, 1972). But development of a tobacco inflorescence is not a well studied area. The development of a tobacco inflorescence has been described briefly as that when the inflorescence of the tobacco plant is formed the terminal bud develops into a single flower; at the basis of its flower stalk, three branches emerge from the main stem (Croes et al., 1985). This description clearly shows that the tobacco inflorescence is a determinate inflorescence according to the definition (Cronquist, 1988) in that a determinate inflorescence has its terminal flower blooming first. Trull and Malmberg (1994) state that the tobacco has a determinate inflorescence. They further describe that wild-type tobacco plants have a cymose inflorescence in which the terminal flower develops first followed by the production of inflorescence branches by axillary buds. The most recent description of tobacco inflorescence development (Kelly et al., 1995) states that the shoot apex of tobacco is consumed in the production of a terminal flower that is subtended by cymose inflorescence branches. However none of these papers cite any works about tobacco inflorescence development. A poster presentation was given based on this study in 1995 (Zhang and Valdovinos, 1995).

The genetic regulation of the conversion between an inflorescence meristem and a flower meristem is demonstrated in *Antirrhinum* and *Arabidopsis* both of which have an indeterminate inflorescence (Trull and Malmber, 1994). In *Antirrhinum*, a *flo* gene (FLORICUALA) regulates the formation of the flower meristem but this gene is normally inhibited by a *cen* gene (CENTRORADIALIS) which is expressed in the inflorescence meristem. In the *cen* mutant, the terminal meristem expressed with a *flo* gene forms a terminal flower (Bradley et al.,1996a and 1996b). In *Arabidopsis*, a *tfl* gene (TERMINAL FLOWER) is a homolog of the *cen* gene of *Antirrhinum* (Shannon and MEEKS-Wagner, 1993). A mutant of the *tfl* gene also causes terminal flowering (Alvarez et al., 1992). A *lfy* gene (LEAFY) is a homolog of the *flo* gene of *Antirrhinum*. A *lfy* gene mutant shows a certain degree of inflorescence characteristics (Weigel et al.,1992). These results did not show a continued development following formation of the terminal flower. There are issues of timing and branching control that are not addressed by either *cen* or *tfl* genes and these mutants are certainly not switching the wild-type inflorescence from the racemose to the cymose form (Battey, 1996).

In the determinate inflorescence (e.g. in the tobacco plant) the apical meristem differentiates into a terminal flower and other subapical tissue is re-initiated to a flower meristem, which is usually at the base of the terminal flower. A *nfl* gene (NFL) is a tobacco homolog gene as the *flo* gene in *Antirrhinum* and *the lfy* gene in *Arabidopsis.* The *nfl* gene is not sufficient for the initiation of floral meristem development in tobacco plants (Kelly et al.,1995) because the *nfl* gene is expressed in the flower apex as well as in a vegetative apex. NFL transcripts accumulate in cells that have been displaced a given distance from the central region of the meristem. Tthe resulting pattern can be described as a compartment of NFL-expressing cells that maintains a constant radius from the central axis and one which overlaps the sites of organ initiation. NFL is likely to be under negative regulation in the central of the meristem so that indeterminacy is maintained until the meristem is committed to reproductive development.

Studying inflorescence development is not an easy task. Greyson (1994) pointed out that inflorescence terminology was accumulated to fill taxonomic and morphological objectives and developmental insights were frequently shallow or missing. This situation can be easily sensed from those famous inflorescence works (Rickett, 1944; Weberling, 1965 and 1989; Stebbens, 1974). Distinctions between patterns of flower initiation, patterns of flower-opening on the inflorescence and the mature flower display are not always maintained. Therefore studies on inflorescence classification (Rickett 1944; Weberling, 1965) suggest that a clear-cut distinction between determinate and indeterminate inflorescences may not exist.

Recently there is increasing interest in studying inflorescences. The key word 'inflorescence' can lead to at least 120 references within the recent 10 years in several databases (CUNY+ DPER, Medline, Carl Uncover). More studies have been done in relation to genes which regulate inflorescence structure and development (Chen et al.,1997; Bonhomme et al.,1997; Bradley et al., 1997). However there is not a general method in studying inflorescence development. The following is a summary about the origin of ideas for developing the methods used in this investigation to study tobacco inflorescence development.

The most widely used method in studying an inflorescence is the generation of line drawings through the use of a dissection microscope in the study of herbarium specimens. In fact the line drawings of major cymose and racemose inflorescence types can be found in most plant classification manuals. Some of the fine line drawings can be found in books (Cronquist, 1988; Bell, 1991; Weberling, 1989). Bell (1991) emphasized the branching patterns and branch sequence in his chapter on reproductive morphology. In this study of tobacco the inflorescence is rich in a variation of branch types. A detailed recording of branch types provides data not only on organization but also on development of the tobacco inflorescence. For the tobacco plant there are some published line drawings (Tran Thanh Van, 1973a and Croes et al., 1985) and published photographs (Tso, 1972); however they are not designed to show the inflorescence pattern.

For plant taxonomists specimens of most wild *Nicotiana spp.* grown in wild are tall ones up to 2 to 3 meter with huge inflorescences at a very mature stage. Most specimens do not have the terminal flower, the patterns of flower opening on the inflorescence are missing from the specimens on the herbarium sheets, and that the inflorescence has been described as a “panicle” (Dr. Nee, personal communication). However the description of the tobacco inflorescence as a panicle was ignored by researchers who grew tobacco in greenhouses (Trull and Malmberg 1994; Kelly et al., 1995).

Collecting specimens periodically throughout the inflorescence developmental period increases the accuracy in describing inflorescence development. Kual (1995) collected buds and inflorescences of a *Populus* at weekly intervals from March through September and biweekly collections from October through February. That resulted a detailed description of the development and organogenesis of a poplar inflorescence. The various stages of inflorescence development were chosen according to the plant studied. In the tobacco plant inflorescence stages were described by groups who collected thin layer explants from tobacco plants. Croes et al. (1985) published a schematic representation of part of the inflorescence of *Nicotiana tabacum*. They designed the inflorescence stage according to the flowering sequence of the flower on the subbranches from the terminal node. The stages are indicated by Roman numerals. The numeral refers to the position of the flower bud at or one day before anthesis. When flower bud “1" is at the stage of anthesis the inflorescence is in “stage I”, At “stage 0" the terminal bud of the main stem is at or near anthesis. That method failed to describe the whole developmental process because it only describes the development of a subbranch. It cannot be assumed that all the subbranches are in the same stage. In Yager’s dissertation (1957), he chose the opening of the terminal flower as a reference stage to determine a certain developmental status. This stage is similar to the “stage 0" from Croes et al. (1985). It is a highly distinguishable stage.

Inflorescence size can be expressed in seed number, seed dry weight, flower number or fruit number. Campbell (1988) studied the seed production and flower number of several populations of *Ipomopsis aggregata* in the mountainous area of Washington and northern Idaho. The data of seed production and flower number were used to analyze the effects of pollination. A similar study was applied to *Zigadenus paniculatus* (Emms et al., 1997). Both of the studies used inflorescence size to evaluate the male fitness. However inflorescence size is a valuable characteristic not only for male fitness but also for a number of physiological regulations. Best of all inflorescence sizes are collected as quantitative data which provide a mean for inflorescence comparison.

In studying inflorescence development one needs to take into account that the distribution of various developmental stages of flowers of an inflorescence will result in a different size of inflorescence. There are several studies of tobacco flower development where flowers have been grouped according to size (Goldberg, 1988; Reinold et al.,1993; Hill et al.,1987). These workers studied the 1-aminocyclopropanecarboxylic acid (ACC) accumulation (Hill et al., 1987) or genes expression (Goldberg, 1988; Reinold et al., 1993) in relation to flower sizes. Goldberg (1988) designated 12 groups of flowers with group 1 of an average length of 0.8 cm and group 12 of an average length of 4.6 cm. However, since fruiting stage and smaller flower buds (under 0.8 cm) were not included, Goldberg’s 12 groups of tobacco flowers are not a complete set for studying tobacco inflorescence development.

The abscission and abscission zone of the tobacco flower

The abscission of tobacco flower have been studied for more than a century (Von Mohl, 1860; Kendall, 1918; Yager, 1957; Valdovinos et al., 1972; Valdovinos et al., 1985). The abscission of tobacco flowers is a physiologically regulated phenomenon in that abscission occurs in a defined location of the plant referred to as the separation layer portion of the abscission zone. Abscission is responsive to various stimuli including pollination and presence of auxin and ethylene.

If abscission occurs the cellular changes of separation will take place within the abscission zone (Addicott, 1982). The abscission zone is usually located at the base of the discrete organ. In flowers or fruits, abscission zones may located at the base, top, or middle of the pedicel (Kendall, 1918) and this region is usually morphologically and anatomically recognizable. The distinctions between the abscission zones and its neighboring cells in morphology and anatomy indicate that the abscission zone is comprised of a localized special tissue. Biochemical characteristics of the abscission zone suggest further that the abscission zone is a specifically differentiated tissue to support the abscission function.

In the tobacco plant the abscission zone is located at the base of the pedicel of the flower or the fruit. An indentation or groove marks the proximal portion of the abscission zone (Kendall, 1918). The groove is not present in very young floral buds (shorter than 2 to 3 mm long) but appears in flower buds which reach 2 to 3 mm in length (Kendall, 1918; Yager, 1957). The groove appears first on the abaxial side of the pedicel and gradually forms a ring around the abscission zone (Kendall, 1918). Cell separation does not occur throughout the entire abscission zone but is typically confined to a one-to-five-cell wide separation layer at the distal side of the abscission zone (toward the flower). For ‘Little Turkish’ tobacco plants the separation layer is confined to one to two cell layers distal to the most invaginated groove marker point (Yager, 1957).

Von Mohl (1860) described the abscission zone as being composed of cells that are smaller and more meristematic than the cells of the adjacent stem and petiole. In tobacco the abscission zone is composed of several tiers of small cells including a complete layer across the organ to be shed (Jensen and Valdovinos, 1967). The smaller cells found in the cortical region of abscission zones are often isodiametric, more densely protoplasmic, have smaller intercellular spaces, and highly branched plasmodesmata (Kendall, 1918). The groove (average width 0.2 um) is delineated by an indentation of the epidermal tissue, extending for some distance into the pedicel (Jensen and Valdovinos, 1967). Within the abscission zone the development of vascular tissue has been observed. The primary xylem is differentiated with less secondary cell wall material. The secondary vascular tissue does not develop until after fertilization. However the secondary vascular tissue develops rapidly with the growth of the fruit after fertilization (Yager, 1957).

The ultrastructure of the abscission zone of the pedicel of the tobacco plant was first studied by Jensen and Valdovinos (1967). The groove of the abscission zone was found to extend some distance into the pedicel with branchings off the main groove. Branched plasmodesmata were observed in the middle lamellae of the abscission zone cells. Microbodies with crystalloid cores appeared with frequency in cells of the abscission zone. Chloroplasts was found to contain a granular component which is membrane-enclosed. The inner membrane of the chloroplasts is highly invaginated.

A morphometric study describes the relative cell volume of the abscission zone cell components (Lieberman et al., 1983). Abscission zone cells have large vacuoles of 75.8% and small nucleus of 3.2% (total cell volume). In comparison with tobacco apical meristem cells (Kanchanapoom and Thomas, 1987 a and b) the vacuoles and nuclei of the apical cells take up 8.6% and 44.1% respectively. The volume of chloroplasts in the abscision zone cells is 3.9% and in the apical meristem cells is 3.7%. Mitochondria takes up 2.1% in the abscision zone cells and 3.3% in the apical meristem cells. Chloroplasts and mitochondria take up comparable spaces in both types of tissues in tobacco plant. These data may indicate a similar need for energy by abscission zone and apical cells. However, cells of the abscission zone appear to be more differentiated with regard to the presence of microbodies with crystalloid cores and vacuoles of 75.8% total cell volume than cells of the apical meristem.

During the abscission processes caused by the preventing of pollination (Valdovinos and Jensen, 1968, Jensen and Valdovinos, 1968) the ultrastructure of the abscission zone cells was changed in several ways. One of the most significant changes in cell structure observed was the increase in the surface area of the rough endoplasmic reticulum (RER). This was also found in the case where inflorescence of tobacco plants were treated with ethylene (Valdovinos et al., 1972). Ethylene increased the surface area of RER as well as accelerated abscission. Morphometric studies by Lieberman et al. (1983) confirmed this effect of ethylene. By applying inhibitors of translation and transcription to tobacco flowers the results indicate that new mRNA production is apparently not necessary for abscission (Valdovinos et al., 1985). However, mRNA apparently must be translated in order for abscission to occur . The inhibitor of translation (cycloheximide C15H23NO4) can inhibit the ethylene-induced RER formation and cause the significant delay of flower abscission normally induced by ethylene. In contrast to the translation inhibitor, the transcription inhibitor (Actinomycin D) did not inhibit the ethylene-induced RER formation and did not cause the delay of ethylene-induced flower abscission. These changes also confirmed that cell wall break down resulted in a weakening of the separation layer. The cell wall break-down was thought to be due to cellular activities of the abscision zone cells including the synthesis of enzymes and the secretion of enzymes into the cell wall.

Since abscission is a localized phenomenon usually involving 2 or 3 cell layers specific cell wall degrading enzymes and mRNA should also be localized according to Abeles (1968). Two major groups of cell-wall-degrading enzymes studied in bean abscission zones were pectinases and cellulases. Pectic enzymes have been found at a high activity level in the tobacco flower abscission zone (Yager, 1960). The activity could be affected by treating plants with Indole-3-acedic acid (IAA) and methionine. Some of the available commercial pectic enzymes (Pectinase from Nutritional Biochemicals Corporation) caused the separation of cells of tissue slices from the abscission zone (Yager, 1960). An increase in soluble pectins of the middle lamella in abscising tissue has been reported (Morre, 1968). One of the pectinases, polygalacturonase, has been found in the abscission zone of bean leaves (Berger and Reid, 1979). The activity of the enzyme in abscission zones is substantially high and rather constant. However, no correlation between level of polygalacturonase activity and the rate of abscission in bean was observed. In ripening fruit of tomato, polygalacturonase may responsible for initiating wall disruption by attacking the middle lamella region between cellule layers (Crookes and Grierson, 1983).

Cellulase [Endo-(1,4)-B-D-glucanase] is one of the cell wall degrading enzymes which has been a focal point for study of leaf abscission in bean plants (Tucker et al.,1991). An increase in cellulase during abscission in bean leaves was first demonstrated by Lewis and Varner (1970). Several cellulases could be isolated from the bean abscission zones (del Compillo et al., 1988) but only a 51 kD protein with an isoelectic point of pH 9.5 appeared to increase as abscission occurred (Reid et al., 1990). Antibodies raised against pure "9.5 cellulase" have been shown to be specific for this enzyme. They lack cross-reactivity with other cellulases from the been abscission zone. A cDNA clone from bean has been identified as coding for the 9.5 cellulase (Tucker et al., 1988; Tucker et al.,1991). Results of immunolocalization on tissue prints (Reid et al., 1990) and *in situ* hybridization (Tucker et al., 1991) indicate that both 9.5 cellulase and its coding mRNA are localized in a same restricted pattern in the bean leaf abscission zone and its surrounding vascular tissue.

The antibody for bean 9.5 cellulase did not react in *Coleus* abscission zone. However, cellulase activity was detected and localized primarily in the cell wall, middle lamella and paramural bodies of the abscission zone cells of *Coleus* (Baird and Reid, 1992). Thus, the cellulases could be organismic-specific.

In tobacco plants, only exocellulase appears to be associated with the abscission process (Lieberman, 1984). The cellulase activity is also localized in the middle lamella region of the cell wall during advanced stages of abscission of tobacco flowers.

The 9.5 cellulase is also found in organs of the bean flower where cells are undergoing major wall disruption (del Compillo and Lewis, 1992). It was suggested that 9.5 cellulase exists in situations where cell wall dissolution occurs during development. Therefore 9.5 cellulase may not be a specific abscission zone-specific-protein marker.

According to Hannig (1913) and Loewi (1907), abscission maybe induced in two different ways. First by abnormal external conditions ("spontaneous" or premature abscission) and second by normal internal conditions at a normal time ("automatic" or normal abscission). However most studies considered only abscission induced under abnormal external conditions (Kendall, 1918). In tobacco plants, the fruits stay firmly on the inflorescence even though abscission may occur in the cortex; this is evidenced by the presence of a white powdery substance at the base of the pedicel (Kendall, 1918). Abscission of tobacco flowers is different from the leaf abscission of most other plants in that flowers may not always abscise. Studies of tobacco flower abscission usually involve the prevention of pollination of flowers or other treatments. However, the abscission of flowers where the inflorescence appears "over-loaded" with flower has not been studied.

Prevention of pollination is a technique used most often to induce abscission of tobacco flowers (Kendall, 1918; Yager, 1957; Valdovinos and Jensen, 1968). One way to prevent pollination is that the stigma is cut away in addition to removing the anthers before anthesis (Kendall, 1918). That resulted in tobacco flowers abscising in about 5 days. However, that method might also involved tissue damage which might lead to abscission. A aluminum cap foil was used to cover the stigma to prevent pollination by Yager (1957) and Valdovinos and Jensen (1968). Again tissue damage may occur since the aluminum foil cap was placed over the stigma before the corollas open. The degradation processes leading to cell separation in pedicel abscission tissue of unpollinated tobacco flowers appear to commence between 3-4 days or longer after anthesis. Flowers without pollination are obviously of no value for seed production of the inflorescence.

Rapid induction of flower abscission was achieved by treatment of the inflorescence with illuminating gas (Kendall, 1918). A small shoot of the plant placed in water and was placed under a bell jar containing 1.5% illuminating gas. Flower abscission was induced in 8 hours. Ethylene was found to be the active ingredient of the illuminating gas. Ethylene has since been identified as a natural regulator of abscission (Jackson and Osborne, 1970; Lieberman, 1979). Tobacco inflorescences treated with 5 ppm ethylene causes the break strength to decrease in 3 to 5 hours (Valdovinos et al., 1972). That ethylene can induce flower abscission in five hours suggests that this is a good system for research.

Addicott (1982) in his book on abscission suggests that abscission is a correlation phenomenon. Development of early formed flowers can lead to the abortion and abscission of later developing flowers. If for any reason the early flowers do not develop later flowers can set and the fruits develop normally. The role of abscission of flowers appears to be for developing and maintaining a desirable balance in the developmental and physiological activities of the inflorescence. The processes of abscission are sensitive to a variety of internal and external conditions. Therefore plants appear to have found many ways to utilize abscission for benefit and survival of the species and the individual. One example is where premature leaf abscission is induced as a defense against gall aphid attack. (William and Whitham, 1986). They found that the abscission of leaves infected by aphids can reduce the aphid population by 28 % in one poplar species and 50% in another poplar species.

Interpretation of the function of abscission appears to be based very often on speculation rather than on scientific evidence. If tobacco flower abscission is to benefit the tobacco plant obviously it will not benefit the flower discarded but rather the remainder of the plant body or the remainder of the inflorescence. Thus the abscission zone where abscission occurs provides a function for the benefit of the remainder of the inflorescence. Perhaps it is fair to say that the abscission zones are important components of the inflorescence. The benefits of flower abscission are appearently for the removal of excess flowers and to allow fewer fruits for the development of more mature seeds.

Flower bud formation from tissue culture

In the case of a determinate inflorescence the tobacco apical meristem differentiates to a terminal flower. The continued development of the inflorescence requires secondary meristems for flower bud formation (Kelly et al., 1995). In the studies described here, the positional association of the subsequent flower bud formation and the abscission zone appears obvious on the intact inflorescence. Whether or not the abscission zone serves as a source for flower bud formation could be demonstrated by the well established tissue culture system.

Flower bud formations from tissue culture have been conducted using explants from stem segments of plants (Aghion-Prat, 1965). Formation of flower buds has almost always been observed after callus formation. It is generally impossible to compare the explants rigorously.

The tobacco "thin-layer explant" organogenesis system has been developed by Tran Thanh Van and co-workers (Tran Thanh Van, 1973a; Tran Thanh Van, 1973b; Tran Thanh Van et al., 1974). In this system the small explants are composed of 3 to 6 layers of epidermal and cortex cells excised from floral branches of *Nicotiana tabacum*. They are capable of *de novo* flower organogenesis with no intermediate callus or leaf formation. The same kind of explants can be induced to form floral buds, vegetative buds, roots or callus under appropriate conditions (Tran Thanh Van et al., 1974). The best floral bud formation was obtained when explants were taken from plants where the terminal bud was in the green-fruit stage and the explants were cultured on a medium with IAA and Kinetin both at 1μM and with 3% sucrose in the light. For the differentiation of vegetative buds explants taken from the flower branch of a green fruit stage inflorescence were cultured on a medium with 1μM IAA and 10 μM kinetin (with 3% sucrose concentration). Optimal conditions for root formation were where explants were taken from plants where the terminal bud carried a mature fruit. For root formation the culture medium contained 0.1 μM kinetin, 10 μM IAA and 1% sucrose, and the tissue was cultured in darkness. The optimal medium for callus formation contained 5μM 2,4-D and 0.1 μM kinetin and 3%

sucrose.

The thin layer explant organogenesis system for flower bud formation is both similar and different in several ways from the development of tobacco inflorescences. The explants must be from the “source plant” of a flowering plant or from a flowering determinate plant. Explants from most stem surfaces (except the basal part of stem) are able to induce a flower bud. However explants vary in potential to produce the flower bud depending on where the explant is excised. Explants can vary in the number of flower buds they form even though they are taken from a similar position on the stem.

For inducing floral buds explants must be excised from a flowering plant. When the system was introduced by Tran Thanh Van (1973a) they reported that best floral-bud formation was obtained when explants were taken from plants where the terminal bud was in the green-fruit stage. Most studies were followed using only explants from the flowering branch. The main apex becomes florally determined prior to the morphological appearance of the terminal flower in the apical meristem (Singer and McDaniel, 1986 and 1987; McDaniel et al., 1989). For flower bud formation the “source plants” must be determined to flower (Altamura and Captitani, 1992). Explants from young non-flowering plants were unable to form any floral buds (Kaur-Sawhney et al., 1990). Thin layer explants taken from vegetative plant parts are only capable of regenerating vegetative buds (van den Ende et al., 1984). Rajeevan and Lang (1993) argues that since the “source plants” were all in a flowering stage, and that no flower bud formation can be obtained in explants from strictly vegetative plants it would appear that flower formation in the explants is not identical with *de novo* flower formation in a hitherto vegetative plant.

Explants vary in the potential to form floral buds. Not all explants can be induced to form flower buds. Tran Thanh Van (1973a) studied the plant in five parts as a flower branch, a flower zone of the stem, a sub-flower zone of the stem, a medium zone of the stem and a basal zone of the stem. Explants taken from a basal zone of the stem cannot be induced to form flower buds and can form only vegetative buds. Seventy-five percent of the explants taken from a medium zone of the stem formed vegetative buds and 25 percent of the explants formed flower bud. Sixty percent of the explants taken from the a sub-flower zone of the stem formed vegetative buds and 40 percent of the explants formed flower buds. Thirty-eight percent of the explants taken from a floral zone of the stem formed vegetative buds and 62 percent of the explants formed flower buds. One hundred percent of the explants taken from flower branches formed flower buds. Heylen and Vendrig(1988) found that in the same kind of explants taken from flower branches flower bud formation depends on which auxin and cytokinin combinations are used. Among the five auxins (IAA, IBA, NAA, CTA, 2,4-D) and the four cytokinins (BAP, Kinetin, Zeatin and IPA) tested IAA/BAP, IAA/Kinetin, IBA/BAP and NAA/BAP combinations in the media produce 100 percent explants with flower bud formation. 2,4-D with Kinetin, Zeatin or IPA and CTA with IPA, NAA with IPA in the culture media result in poor flower bud formation (lower than 60 percent). All other combinations of auxin and cytokinin caused 85 to 96 percent of the explants to form flower buds.

The capacity to form flower buds from a thin layer explant in culture is also affected by which tobacco cultivar or species is used (Rajeeven and Lang, 1993). For some *Nicotiana* species only 72% of explants from the pedicel of the flower produced flower buds. Two day-neutral tobacco plant cultivars -- cv. Wisconsin 38 and cv. Samsun-- are the most popular materials used by most groups and 100 percent of their explants from pedicels of flowers produced flower buds. The capacity to form flower buds does not depend on which *Nicotiana* biotypes are used. For example explants from long-day, short day or day-neutral tobacco plants all form flower buds as long as the explants are collected from the flowering branches.

Croes et al. (1985) has concluded that a floral gradient exists along the axis of the inflorescence branch. The floral gradient is expressed in terms of the number of floral buds formed on the explants. The gradient exists in both the flower pedicel and in the inflorescence internode tissues in that the explant taken from the apical part of the branch forms more flower buds than the explant taken from the basal portion of the branch forms. The capacity to generate these buds is largely determined by tissue age at the time of excision. Consequently the gradient moves along the axis during the outgrowth of the inflorescence. Tiburcio et al. (1988) also found that the explants from various parts of the inflorescence are different with regard to floral bud formation. Explants with the highest potential for flower bud formation are from the pedicel of green fruit. A variation in flower bud formation can be obtained by applying different auxins and cytokinins in the culture media. IAA and kinetin was originally reported as the best hormone combination for flower bud formation (Tran Thran Van, 1973 a and b). However using NAA instead of IAA resulted in the formation of more flower buds on the explants (Tiburcio et al., 1988). In the presence of synthetic auxins (NAA, CTA) more flower buds were generated than in cultures containing the natural auxins (IAA, IBA). The most active cytokinin was BAP

(Heylen and Vendrig, 1988).

Polyamines, peroxidase isoenzymes and pectic cell wall fragments are several other factors which appear to be related to flower bud formation in the thin layer explant organogenesis system. Several polyamines increase when the vegetative or floral bud is formed from tissue cultures derived from the thin layer explant of the tobacco inflorescence. Large increases of spermidine are especially associated with floral bud differentiation (Tiburcio et al., 1988). Inhibition of spermidine synthesis by cyclohexylamine (C6H13N) prevents the rise in the spermidine titer and floral bud initiation is also inhibited. Exogenously applied spermidine causes floral initiation in cultures which would otherwise form vegetative buds (Kaur-Shawney et al., 1988). Spermidine is required from the start of cultures for about three weeks but it can not replace kinetin in cultures at the time of flower bud formation. When kinetin is present spermidine increases the number of floral buds that develop into normal flowers. No condition can induce thin layer explants from young non-flowering plants to form floral buds (Kaur-Shawney et al., 1990). Among 47 identified peroxidase isoenzymes 25 have been correlated to specific developmental events during the thin layer explants floral organogenesis (Kay and Basile, 1987). Thus the specific isoperoxidases play roles in different flower developmental processes. Pectic cell wall fragments from suspension-cultured sycamore cells or tobacco cells contain major oligosaccharins and these increase floral bud formation when added to the transition medium. The flower-inducing activity of the fragments was stable to heat treatment and proteolytic digestion (Eberhard et al., 1989).

As concluded by Rajeevan and Lang (1993) flower formation in the explants is not identical with *de novo* flower formation in a hitherto vegetative plant. It is rather the expression of a floral state already established in the plant. Culture conditions that permit flower bud formation in an explant are conditions which maintain the floral state and encourage its expression. Conditions under which no flower buds are formed reduce this state and /or prevent its expression. In general explants from either a floral branch or a floral stalk on the inflorescence with full green fruit are preferred and are used in most experiments.

Objectives of this investigation

Research on inflorescence development, flower abscission and flower bud formation has traditionally been separate endeavors. However these three processes now appear to be correlated. The objectives of this investigation are to:

1. Devise a method to describe developmental form of the tobacco inflorescence.
2. Use the method to follow the development of the inflorescence.
3. Study the relationship between abscission and flower bud formation.
4. Study using tissue culture the formation of flower buds from explants.

MATERIALS AND METHODS

1. Plant material

Tobacco plants (Nicotiana tabacum L. cv, "Little Turkish") were grown in the greenhouses and in large environmental chambers to flowering stages under the following conditions: An approximate 14-hour photoperiod at minimum temperatures of 25°C during the day and 20°C at night was observed in the greenhouses. In environmental chambers, the plants were exposed to 14 hours per day of light from cool-white fluorescent and incandescent lamps at an intensity of 10,000 Lux. and temperatures of 27±1°C during the day cycle and 20±1°C during the night cycle (Valdovinos et al., 1972).

2. Flower development stages

Flower development was divided into 12 stages according to floral size which was measured in length from the abscission zone to the tip of the corolla or the calyx. The 12 groups were assigned by the following formula: Group n is within the length from $((n-1)\times 0.7)^2$ mm to $(n \times 0.7)^2$ mm. The "n" is the group number from 1 to 12 (Table 1). Representatives of the 12 groups are shown in a photograph (Fig. 1). For the fruit group (group 12), if the corolla had abscised, 30 mm was arbitrarily added to the length of the fruit.

3. Inflorescence development stages

Four stages of inflorescence development was studied. The first stage is when the first flower (always the terminal flower) is blooming (corolla opens). This is called the F1 stage (Fig. 8). F1 stage inflorescence may last 1 to 2 days. Based on the F1 stage, W1 stage is one week after F1 stage, W2 stage is two weeks after F1 stage, and W3 stage is three weeks after F1 stage. Thus F1, W1, W2, and W3 are the four stages of inflorescence development in this study. Based my data, the F1 stage and W1 stage are flowering stages, and the W2 stage and W3 stage are fruiting stages.

4. Some reproductive characteristics of "Little Turkish" tobacco

The following measurements were taken from the experimental plant material. At the F1 stage, the height of plant was measured from soil level to the top of the terminal flower, and the number of nodes on the main stem was counted from the cotyledon node to the terminal flower node. At the W3 stage, the numbers of fruits (group 12) were counted. Mature dry fruits were collected before their dehiscence. After the fruits had dried for several months in the laboratory seeds of each fruit were collected. The average weight of a seed was calculated based on ten independent weights (10 seeds were weighed each time) on an analytical balance (Mettler H20T, variation: 0.01 mg). Seeds within an entire fruit from randomly selected dry fruits were collected and weighed. The total seeds of a fruit and the total seeds of a plant were calculated based on the average seed weight,

weight of seeds in a whole fruit, and number of fruits of a plant.

5. Development of the tobacco inflorescence

Development of tobacco inflorescences of tobacco plants was studied over a three year period on plants grown in greenhouses. The inflorescences from F1, W1, W2, and W3 stages were studied with regard to the composition of flowers and flower arrangement on the inflorescences. Recording was done by using the specifically designed inflorescence formula. F1 and W1 stages were recorded in detail by the flowering stage formula (Fig. 2), and W2 and W3 stages were recorded by the simplified fruiting stage formula (Fig. 3). With each of the stages, at least six inflorescences had been recorded and analyzed statistically.

Branch types were studied through observation. The abscission zone at the base of each flower was used as the major reference to indicate the flower node. Number of subbranch(es) was identified. If more than one subbranch was observed, the relative sizes of the subbranches were evaluated. At some point in the inflorescence where the abscission zone did not exist, a bract was used to indicate the node. The flower developmental sequence in the inflorescence was observed on a daily basis starting with the appearance of a flower bud progressing to the W1 stage inflorescence. Major branch types were illustrated in line-drawings by using the CorelDRAW.

6. Abscission pattern of flowers

Terminal 25 to 30 cm portions of the plant at W1 stage were removed and placed with the basal ends in a beaker containing water in an environmental chamber at low light intensity and a temperature of 25+/-1 °C. A continuous flow of air (60 l / min) with or without ethylene (5 ul / min) was provided to the chamber (Valdovinos et al., 1972). With ethylene the induction of abscission lasted for 6 hours. The inflorescence was then pushed with the basal end against a VORTEX-GENIE mixer at the maximum speed (setting 10) for 1 minute to remove loosely attached flowers. The flowers (both the abscised and not abscised) were then collected. The sizes (lengths) of the flowers were measured from the abscission zone to the tip of the corolla of the flower. The percentage of flowers abscising in each flower grouping of the inflorescence was calculated. Data for the abscission pattern are the averages from six independent inflorescences.

Without ethylene the induction of flower abscission was approximately 48 hours. The flowers (abscised and not abscised) were collected respectively. The sizes (lengths) of the flowers were measured from the abscission zone to the top of the flower. The percentage of flowers abscised from each flower group of each inflorescence was calculated. Data for the abscission pattern are the averages from six independent inflorescences.

7. Break strengths for flower abscission

Break strengths were measured by using an Accu Force Cadet digital force gage (Ametex) which can automatically record the maximum force from 0 to ~700 grams with a sensitivity of 0.1 gram. The whole inflorescence was fastened to the force gage (Fig. 4), then the flower to be tested was pulled down by holding the flower in the hand until the flower broke or the force gage went out of range. An out-of-range measurement was recorded as 700 grams. After breaking, the breaking point of the flower was checked to see if it matched the groove of the abscission zone. The breaking point at the groove was a break at the abscission zone (AZbk) and the breaking point on the pedicel of the flower is a "pedicel zone break"(Pbk). The size of each flower was then measured. The final recordings include flower size, break strength, AZbk or Pbk. With 5 ppm of ethylene treatment, the measurements were done every hour from 0 to 6 hours.

Without ethylene, the measurements were done from 0 to 72 hours at 12 hours intervals. For each reading, several inflorescences were used. Starting at 36 hours at each time of measurement there were some flowers which had abscised without applying any forces. These flowers with breaking points all at their abscission zones were collected and their sizes were measured.

8. Preparation for light and electron microscopes

Free hand sections were used for rapid light microscope observation. The materials were either held on one hand between thumb and index fingers or pressed against a micro slide class. A single-edged razor blade wetted with water was used to cut sections continually. All the sections (after 20 were cut) were put into water in a petri dish, only the even and thin (near transparency) sections was selected and mounted in water on slides for observation. No staining was applied; the cells of most sections contained chloroplasts which provided substantial contrast. Microscopic measurements were done by using a micro ruler installed in the eye piece of a Beck Krassis microscope. The micro ruler was calibrated by using a stage micro ruler. The calibration chart is shown in (Table 2). Good sections were about 20 to 30 μm thick.

The tissue culture materials were fixed in FAA solution (5% Acetic acid, 5% Chloroform in 70% Ethanol), dehydrated in an ethanol series, followed by toluene infiltration to paraffin. Sections were cut at 10 μm on an A/O rotary microtome with metal knives. They were stained with Safranin O and Fast Green and mounted the Permount medium.

For fine light microscopic observations, the pedicel tissue (1 to 2 mm) containing the abscission zone was fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 1 to several hours at 4°C and then rinsed for several hours in the buffer with 5 to 6 changes. The material was then dehydrated through an ethanol series, propylene oxide, and embedded in Epon (Luft, 1961). 1 μm thick sections were cut with a Leica microtome by a triangle glass knife with a small water reservoir. The sections were collected with a hair loop and placed on microscope slides. These were dried on a hot plate. The sections were stained in either methylene blue or basic fuchsin, observed under microscope and photographed with an Olympus microscope.

For scanning electron microscope observations, pedicels from various sizes of flowers containing the abscission zones were cut into two halves and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 2 hours. The materials were rinsed in the buffer, and dehydrated in a fine ethanol serial, then critical-point dried. Both cutting and epidermic surfaces were glued on a holder and coated with gold. The material was observed and photographed on a JSM-U3 scanning electron microscope.

For observing the ultrastructure of the abscission zone cells, the pedicel tissue (1 to 2 mm) containing the abscission zone was fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 1 to several hours at 4°C, and rinsed several hours in the buffer with 5-6 changes. The tissue was post fixed in 1% OsO4 in 0.1 M phosphate buffer for 1 hour at 4°C, dehydrated through ethanol series, propylene oxide, and Epon (812) (Luft, 1961), and embedded in Epon. The block were trimmed into a trapezoid less than 0.5 X 0.5 mm on any side with the abscission zone cells. Ultrathin sections at 60 nm were cut with a diamond knife on a Leica microtome and collected on 300 mess copper grid. The sections were stained in both uranyl acetate and lead citrate. The section was observed with a Hitatch-7000 TEM.

9. Floral bud formation conditions for "Little Turkish" tobacco

The culture media were modified from Tran Thanh Van (1973 b). A basal medium composed of macro- and micro- elements according to Murashige and Skoog (1962) was purchased from Sigma. To the basal medium, the following substances were added: 3% sucrose. 100mg/l myo-inositol, 0.1mg/l thiamine.HCl, auxin (IAA, or NAA) 1μM, cytokinin (KIN, or BAP) 1μM. The pH value was adjusted to 5.1 before autoclaving.

The thin layer explants were taken from the pedicels of flowers or fruits when the inflorescences were at the "green fruit stage" (about W2 stage inflorescence). The whole pedicel was cut off and first put into 70% ethyl alcohol for 30 seconds then into 7% commercial Clorox bleach for 10 minutes then rinsed three times in sterilized water and put into a wet petri dish. In the petri dish the two ends of the pedicel were cut off, the explants were peeled from the middle part of the pedicel, and transferred to "baby food" jars containing testing media. The "baby food" jars were each covered with three layers of aluminum foil and sealed with Parafilm. The culture temperature was 25±1 °C under continuous light.

Explants containing the abscission zone were sliced off of the pedicel and the internode of a sub-branch described as the "F portion" shown in (Figure 5). The "F portion" was cut off from the inflorescence, first put into 70% ethyl alcohol for 30 seconds and then put into 7% commercial Clorox bleach for 10 minutes, then rinsed three times in sterilized water and put into a wet petri dish. In the petri dish, the two ends of the "F portion" were cut off, leaving about 3 to 4 mm of tissue on each side of the abscission zone. The adaxial side was trimmed off evenly, so that the "F portion" could lay down with the abaxial side face up. The final explants with abscission zones were then sliced off parallel through the abaxial side. The explants were usually thick at both ends and thin at the abscission zone.

Photographs were taken daily from day zero to day 15, these explants were then fixed in FAA solution at 4°C. They were embedded in paraffin and sectioned at 10 μm, stained with Safranin O and fast green, and mounted with the Permount medium.

The explants containing the abscission zone were also cultured in the four media which were reported to be the best conditions for promoting vegetative bud formation, floral bud formation, root formation, or callus formation (Tran Thanh Van et al., 1974). The major differences of the four organogenesis conditions are shown in (Table 3). The time required for organogenesis were in reference to the flower bud formation conditions so that the dark condition remained until the time of photographing.

10. The effects of plant hormones on intact inflorescences

Indole-3-acetic acid (IAA), kinetin, and combinations of IAA and kinetin were each sprayed onto F1 stage inflorescences at concentrations of 1 μm for IAA, 1 μm for kinetin, and 1 μm/ 1 μm for IAA and kinetin. The plants were returned to the greenhouse and continued to grow in the greenhouse. At the W3 stage, the inflorescences were recorded by using the fruiting stage formula (Fig. 3). At least six inflorescences for each treatment were observed and the data were compared with the data of untreated groups.

For ethylene treatment, the six F1 stage inflorescences were covered by plastic bags sealed with parafilm. 0.2 ml of pure ethylene was injected into the plastic bag. The plastic bags remained on the inflorescences for six hours. Then the plastic bags were removed, the inflorescences were shaken by hand to remove all of the loosely attached flowers. The plants were put back into the greenhouse and continued to grow in the greenhouse. The abscised flowers after six hours of ethylene treatment were collected and the sizes of those flowers were measured. For these plants (since all terminal flowers were removed) the date of the next blooming flower was called the F1reg stage. Three weeks after the F1reg stage was the W3reg stage. The W3reg stage inflorescences were recorded by using the fruiting stage formula (Fig. 3). The number of scars left on the inflorescence by abscised flowers were carefully counted in the W3reg stage (Only four plants reached the W3reg stage).

RESULTS

Part I. The plant material and reproductive characteristics

Under greenhouse conditions, the "Little Turkish" tobacco plant *(Nicotiana tabacum* L. cv. "Little Turkish") usually takes 100 to 120 days to develop from seed germination to anthesis. It takes 12 to 14 days from the first sign of a visible floral bud to the first blooming flower. The inflorescence at the date of the first flower blooming is named "F1 stage inflorescence". This first blooming flower usually takes 5 days to have its fruit develop to a regular fruit size, and this fruit may remain green for about 2-3 weeks before turning yellow.

"Little Turkish" tobacco plants develop to an average height 117 cm at F1 stage (Table 4). There are 42 internodes on the main stem at anthesis. At a W3 stage, each plant can produce about 68 mature fruits and each fruit contains an average of 2,188 seeds with an average weight of 62 μg per seed. Therefore each plant on the average can produce about 9.2 gram of seeds or 150,000 seeds.

The wide ranges of all the six traits observed (number of internodes at F1 stage, height of plant at F1 stage, number of fruit set at W3 stage, weight of a tobacco seed, number of seeds per fruit, number of seeds per plant) were based on six randomly selected plants. The tobacco plant is a large annual plant and produces very tiny seeds in a huge terminal inflorescence. The range of the number of internodes at anthesis, the range of height of plant at F1 stage and the range of the weight of a tobacco seed are relatively narrow (narrower than 30 percent). These characteristics may be less dependent on environmental conditions. The range of fruit set at W3 stage and the range of the number of seeds per fruit are relative higher. The relative range of the number of seeds per fruit is 105.1 percent of the average number of seeds per fruit which is much higher than the relative range of the number of fruit set at W3 stage which is 68.8 percent of the average number of fruit set per plant. Although the fruit number has been used to describe the inflorescence size, the number of seeds per fruit appears to be more important in determining the final seed production of a plant. The "Little Turkish" tobacco plant has been cultured in the greenhouses for years, and it is basically a self pollinated species. The cause for the range of fruit set at W3 stage and the range of the number of seeds per fruit is likely a response to external environment rather than various genetic backgrounds.

The abscission of tobacco flowers have been studied for more than a century (Mohl, 1860). Flower abscission can be induced by external ethylene within 5 hours (Valdovinos et al., 1972). In this study, many flowers and flower buds were removed by ethylene treatment within six hours (Fig. 6). In contrast with the abscission of flowers leaves of the tobacco plant do not abscise from the plant upon to ethylene induction or when the shoot was removed from the root or even after the plant becomes yellow and dry. When vegetative parts of the plants were treated with 5ppm ethylene for six hours, there was no sign of abscission to the leaves. After these plants were placed back to the greenhouse, they transited to flowering stage at the same time as the control group did and they developed the size of inflorescence within the same range of the control group. Dry leaves hang on the plant as long as no mechanical damage occurs (Fig. 7).

Part II. Organization of the tobacco terminal inflorescence

The tobacco terminal inflorescence is a dynamic compound cymose which arises from the four terminal nodes on the main stem (Fig. 8). The first node (node 'a') develops a terminal flower (Fig. 8, 54) and three sub-branches (Fig. 8, A1, A2, A3). This node is a typical equal trichasium branch type (Fig. 12, C). The second node ("b"), third node ("c") and fourth node ("d") each develops a single sub-branch (Fig. 8, B, C, D).

The F1 stage inflorescence is the reference stage studied in this investigation (Fig. 9). Based on six randomly selected plants, the average flower number at F1 stage inflorescence is 135.5. The distribution of flowers according to the 12 flower groups is shown in the F1 stage inflorescence statistics table (Table 5). The arrangement of flowers, developing sequence of flowers and major branch types are shown in the F1 inflorescence formula (Fig. 2). Flowering sequence of flowers is recorded as the sizes of flowers. The smaller the flowers are, the later they develop. The position of the terminal flower (54) and developmental sequence of flowers are the essential characteristics to confirm that the tobacco inflorescence is a determinate type.

The most interesting structure of the tobacco inflorescence is the abscission zone at the base of the pedicel of each flower. The photographs (Fig. 10) show the abscission zone is recognizable by naked eye on large (group 11, 12) and small (group 8, 5) flowers (buds). The abscission zone may be a potential meristem identified by a region of small cells (Fig. 11) which extends completely across the pedicel and half way across the derived subbranch. The abscission zone of a tobacco flower is positionally predictable and morphologically distinguishable at the base of the pedicel of flower in tobacco plant (details in Part III).

Branch types found in tobacco inflorescences

A tobacco inflorescence becomes complex due to the most complex branching processes. Nine major branch types in tobacco inflorescences are illustrated by line drawings (Fig. 12). They are named as *monochasium*, *equal dichasium*, *equal trichasium*, *unequal dichasium*, *bract-monochasium*, *bract unequal dichasium*, *unequal trichasium*, *bract unequal trichasium* and *two-AZ trichasium*. Definitions of these terms are included in a glossary in the appendix A.

Among these branch types, some are found only at specific locations. The equal trichasium is always found at the terminal node of the main stem on the inflorescence or the terminal node of a lateral inflorescence. The equal dichasium occasionally appears on the lateral inflorescence from the fifth terminal node (node "e") on the main stem. It is a transition type between a sub-branch and a lateral inflorescence (Fig. 22).

A monochasium branch type may have a fixed location and never change to unequal dichasium. It is usually found at the second or the third node of a subbranch (Fig. 14, C, the third node). In most cases monochasia are temporary forms and they will further develop to unequal dichasium and even unequal trichasium types. The unequal dichasium type is more common than the unequal trichasium type under greenhouse conditions.

The bract involved branch types only roughly represent the extremely diverse branch types. The bract can be clearly visible or reduced. The relationship between the bract and the abscission zone is uncertain. When the bract is from an abscission zone of a flower, it may represent the reduced leaf of the node (Fig. 12, F, H, I). However a bract may be some distance from any abscission zone; it appears that the bract inserts a node on the internode of two abscission zones (Fig. 12, E, the second node).

The two-AZ trichasium type (Fig. 12, I; Fig. 14, D) is an extreme type of all branching processes. It usually occurs at the upper portion of a subbranch where space is easily available. The two flowers share the same growth point for a common subbranch development but have independent abscission zones for each flower. More subbranches may develop from the abscision zones with or without bracts.

In the tobacco inflorescence the maximum number of subbranches at any node is three. Most nodes having less than three subbranches tend to develop up to three subbranches. There are two basic routines for subbranch development (Fig. 13). One of the routines (routine I) begins with monochasium type (Fig. 13, I-A) which first develops to an unequal dichasium type (Fig. 13, I-B) and then to an unequal trichasium type (Fig. 13, I-C). Under the best growth conditions the unequal trichasium may close up to an equal trichasium (Fig. 13, I-D). The equal trichasium can be considered as the most effective branch type of tobacco plants for producing a maximum number of flowers within a minimum distance from the first terminal node on the main stem.

The second routine (Fig. 13, routine II) is to insert a node which is marked by a bract. The insertion may occur to the internode (Fig. 13, II-B). But bracts may also develop from the abscission zone of a former flower (Fig. 13, II-C, II-D).

Development of a lateral flower

A lateral flower contrasts with the terminal flower. It develops mostly from the base of a pre-existing flower or occasionally from an axillary of a bract. Only the lateral flowers originating from flower bases have been studied in detail.

The flower primordia may develop from the base of a pedicel of a former flower at a very early stage. In one of the free-hand sections (Fig. 15, A) two flower primordia (micro buds) are adjacent to one another, the large one is about 0.33 mm and the small one is about 0.11 mm in length. They develop from the proximal side (toward the main body of the plant) of an abscission zone of a small bud (group 5). The smaller primordium develops from the base of the pedicel of the larger one which has a clear differentiation of floral body and pedicel but has no morphologically recognizable abscission zone. In another free-hand section (Fig. 15, B) two other tiny flower buds develop from the base of a small bud (group 5), the larger one is about 0.80 mm (group 2), and smaller one is about 0.17 mm (group 1) in length. The abscission zone of the 0.80 mm long flower bud is not morphologically recognizable. The smaller bud clearly develops from the base of the pedicel of the larger one. These observations indicate that the morphological differentiation of the abscission zone is not required for a flower bud to develop from the base of the pedicel of a flower.

Pedicel length and pedicel diameter are somewhat correlatively developed in relation to flower body development (Fig. 1). From group 5 to group 12, the flower length increases about 6 fold from 9.96 mm to 62.70 mm (Table 6). The pedicel length increases about 10 times from 1.28 mm to 12.65 mm. The pedicel diameter increases about 2 times from 1076.0 μm to 2046.7 μm. The flower length appears linearly correlated to the flower groups (R square = 0.984). The pedicel length (R square = 0.848) and pedicel diameter (R square = 0.943) are less correlated to the flower groups than flower length is. A histograph (Fig. 16) shows that pedicel length and pedicel diameter develop in a similar pattern in that they develop slowly from group 5 to group 10 and develop rapidly from group 10 to group 12 (in comparison to the flower length). The pedicel length and pedicel diameter could represent the capacity of nutrient translocation and the support for spacing. So that nutrition requirement and space requirement may increase at group 10 and reach maximum at group 12.

Cross section of the pedicel of a tobacco flower shows that the pedicel has a similar structure to the tobacco stem in that the vascular tissue appears as a closed cycle (Fig. 17, 18). However a gap appears in the cross section of the pedicel at the abscission zone (Fig. 19). Parenchyma tissue of the gap connect the parenchyma tissues in cortex and in pith. The cortex has about 13 layers of parenchyma cells across the pedicel. The diameter of pedicel increases about 2 times from 1076.0 μm in group 5 to 2046.7 μm in group 12. Most of the increase of pedicel diameter is from the expansion of cortical parenchyma cells from the group 5 to group 11 (1823.3 μm). Secondary growth of the vascular tissue occurs clearly in group 5 and larger flowers --the secondary xylem vessels (dark) are 2 to 3 layers in group 5 bud, 3 to 5 layers in group 11 flower and 8-10 layers in the group 12 fruit. The rapid secondary growth of the vascular tissues in group 11 flower to group 12 fruit contributes mostly to the final increase of the pedicel diameter.

Development of inflorescence

During the reproductive phase of the tobacco plant the four terminal nodes contribute to the terminal inflorescence. Under greenhouse conditions it requires about 12 days from the appearance of a flower bud to the F1 stage inflorescence. The internodes on the terminal main stem are not well elongated (Fig. 20) and the subbranches are crowded and not very distinguishable until the F1 stage (Fig. 9). A terminal floral bud and three subbranches develop from the first terminal node (node "a"). The terminal flower is the largest flower of the inflorescence and the three subbranches are the largest subbranches. Other subbranches (3) develop from terminal nodes "b", "c", "d" correspondently (Fig. 8). They develop in a very crowd space, and are smaller than those from node "a". However they have almost the same number of detectable flower buds as do the node "a" subbranches (Table 12).

At F1 stage the terminal flower is the only one blooming. This stage can last for 1 to 2 days before other flowers from the node "a" subbranches start blooming. In the late F1 stage, some of the largest flower buds in the subbranches from node "a" are very close to blooming and their sizes may be longer than 49 mm. Therefore they may be grouped into flower group (group 11). The average number of flowers for group 11 is 2.7 (Table 5). The internodes on the main stem of the F1 stage inflorescence are clearly elongated (Fig. 9) and the four terminal nodes and the six subbranches are distinguished easily (Fig. 8). The "D" subbranch is much smaller than other subbranches (Fig. 9), its largest flower bud is only about 14 mm in length (group 6) (Fig. 2). However the number of nodes on the "D" subbranch is compatible to all the other subbranches at F1 stage (Table 12).

The flowering period extending from the F1 stage to the W1 stage leads to formation of the development of the tobacco inflorescence. Flower number increases from an average of 135.5 per inflorescence at the F1 stage to an average of 212.8 per inflorescence at the W1 stage (Table 7). The inflorescence becomes mature in that flowers from groups 7 to group12 increase from 9.9 percent of total flowers at F1 stage to 21.6 percent of total flowers at W1 stage (Table 8). Mature fruits (group 12) reach an average of 9.5 per inflorescence on the W1 stage (Table 9).

The W1 stage to the W2 stage is a transition period during the development of the tobacco inflorescence because flower abscission occurs during this period (Fig. 21). The total number of flowers of an inflorescence may continue increasing to about 400. Mature fruits (group 12) also increase from an average of 9.5 per inflorescence at the W1 stage to an average of 23 per inflorescence at the W2 stage (Table 9). Therefore the inflorescence at the W2 stage is more mature than it is at the W1 stage. Abscission of flowers could be a signal to indicate that inflorescence at the W2 stage becomes "over-loaded".

The period extending from W2 stage to the W3 stage is the major phase for fruit formation during the overall development of the tobacco inflorescence. Fruit set (group 7 to group 12) increases from an average of 66.6 per inflorescence at the W2 stage to an average of 96.6 per inflorescence at the W3 stage (Table 10). Formation of new flowers during the period W2 stage to W3 stage is slow and some of the very young flower buds (group 3 to group 5) wither on the inflorescence. The number of flowers from group 7 to group 11 at the W3 stage is even less than the number of the flowers at the W2 stage (Table 9). Developed fruits increased from 23 per inflorescence at the W2 stage to 66.6

per inflorescence at the W3 stage. Some fruits which developed at an earlier time start turning yellow. Abscission of flower buds between group 5 to group 10 commonly occurs.

The dynamic change of branch type is a powerful source to increase inflorescence size in terms of flower number. However the final fruit set is more dependent upon the monochasium-originated flowers. At flowering stages the proportion of the monochasium-originated flowers is less than the non-monochasium-originated flowers (Table 11). The proportion of the monochasium-originated flowers is 40.7 percent of the total flowers at F1 stage and 35.0 percent of the total flowers at the W1 stage. At fruiting stages the proportion of the monochasium-originated flowers is more than the proportion of non-monochasium-originated flowers (Table 11). The proportion of the monochasium-originated flowers is 68.0 percent of the total flowers at the W2 stage and 59.8 percent of the total flowers at the W3 stage. The flower number of monochasium-originated flower at F1 stage is 55.2 per inflorescence. This is very close to the number (57.8 per inflorescence) of fruits of monochasium-originated fruits at the W3 stage . Therefore most early monochasium-originated flower buds developed into fruits. Non-monochasium-originated fruits at the W3 stage is only 38.8 out of the total fruit set (96.6). This is much less than the non-monochasium-originated flowers at F1 stage which is 80.3 out of the total flowers (135.5). The non-monochasium-originated flower buds increase the total flower number, but less important for fruit set.

The tobacco plant apparently is not programmed to develop a long subbranch on its determinate inflorescence. At the F1 stage (the beginning of flowering) the length of subbranches, in terms of number of nodes, is already close to the length to carry all of the fruits at the W3 stage (Table 12). The average subbranch lengths at F1 stage (group 2 to group 11) is 9.2 nodes; at W1 stage (group 2 to group 12) is 12.4 nodes; at W2 stage (group 7 to group 12) is 7.6 nodes and at W3 stage (group 7 to group 12) is 9.6 nodes. There is a significant increase in the number of nodes on subbranches at the W1 stage in comparison with the number of nodes on subbranches at F1 stage. However most flower buds at the increased number nodes on subbranches at the W1 stage never develop past a group 7 flower stage and they often abscise.

Note----Forming lateral inflorescence may be an important alternative source for increasing the seed production of the tobacco plant. On a healthy plant, 4 to 5 more terminal nodes following node "d" may develop their lateral inflorescences. These lateral inflorescences have a new secondary terminal node in the equal-trichasium branch type and several other nodes with secondary subbranches. The largest flower of the lateral inflorescence is the terminal flower on the secondary terminal node. There are two to many well developed leaves at the basal end of the lateral inflorescence (Fig. 22).

Part III. Morphology of the tobacco abscission zone

Anatomy of tobacco flower abscission zone

The abscission zone of a tobacco flower is marked by a groove which is clearly visible at the base of the pedicels of tobacco flowers and fruits (Fig. 23). Many gland trichomes are around the groove area (Fig. 24). A groove may appear as a sharp angle with dense trichomes (Fig. 25) or as a smooth curve with few trichomes (Fig. 26). The SEM photograph also shows that the abscission zone is composed of several layers of small cells in which the nuclei are the dominate cell content. However, the nuclei are not dominate in other parts of the cortex cells of the pedicel (Fig. 25).

The abscission zone is a part of the shoot system of the plant and has the same tissue types as those of the regular stem or the pedicel. In the cross section of the pedicel of a group-5-flower (Fig. 27) these tissues can be identified as epidermis, cortex, vascular tissues and pith. The vascular tissue appears to be a closed cylinder in the section or a closed cylinder in the shoot system.

In longitudinal sections of tissues containing the abscission zone a groove delineates the zones (seen on both sides of the section) (Fig. 28, A). Adjacent to the groove area, there are several layers of small cells. The small cells are parenchyma cells which comprise the cortex and pith. At regions the adjunction between the pedicel and its subbranch (Fig. 28, B) small cells extend to the proximal side of the groove and continue to the subbranch. The number of the cortical cells of the abscission zone is about the same number as the number of cortex cells of the pedicel on distal side of the abscission zone.

The formation of the groove may be because the cortical cells of the abscission zone do not expand as the diameter of the pedicel increases. The pith cells of the abscission zone are smaller and more numerous than the pith cells on distal side of the abscission zone. The pith cells of the abscission zone appear to occupy a little more space than the pith cells occupy on distal side of the abscission zone. The cylinder of vascular tissue appears larger in diameter in the abscission zone than it appears in the pedicel.

The vascular tissue is not passing through the abscission zone area as continuous as they are in the other part of the pedicel. The vascular tissue is discontinuous at the abscission zone area; a gap filled with small parenchyma cells connects the pith and cortex regions at the abscission zone (Fig. 29). Xylem vessels appears irregular at the abscission zone. They are not neatly arranged along the axial but are mixed with irregularly distributed parenchyma ray cells. It appears that during development the vessel cells are rearranged before and after they pass the abscission zone (Fig. 30).

The gap in vascular tissue and the potential for developing subbranches are characteristics of a node. However the abscission zone is different from a regular node as defined in a vegetative shoot. The abscission zone apparently develops subtending a terminal bud which differentiated into a flower. The abscission zone retains small cells which make up the whole cortex and pith of the abscission zone. The abscission zone appears to respond to the stimuli for flower abscission. The abscission zone also appears to have potential for developing a trichasium branch type. Usually a subbranch develops from the proximal side of the abscission zone and which continues to become larger than the pedicel of the flower (Fig. 31, 32). The abscission zone appears to have some aspects of a flower node.

The differentiation and development of the abscission zone

Morphological differentiation of the abscission zone is not observed until the flower bud develops to about 1.2 mm in length. None of the micro buds (group 1, from 0.1 mm to 0.4 mm) have a distinguishable abscission zone (Fig. 33, A). Flowers in group 1 are flower primordia and flower buds with only the distinction between a flower body and a pedicel. The abscission zone is differentiated within the tiny buds (group 2, from 0.5 mm to 1.9 mm). At the group 2 stage a 0.6 mm long tiny bud has no distinguishable abscission zone (Fig. 33, B). Details of a 0.95 mm long tiny bud shows that the cells at the base of the pedicel are nearly the same sizes as the cell at the middle of the pedicel (Fig. 34, A), and none groove can be distinguished. A groove appears in the 1.2 mm long tiny bud (Fig. 33, C) and becomes much clearer in the 1.8 mm long tiny bud (Fig. 33, D). In a 2.0 mm long minute bud (group 3) the groove and the small cells are clearly differentiated (Fig. 34, B). The cells of the pedicel are elongated and those of the abscission zone are not elongated. The abscission zone cells appear smaller in the radial dimension as well. The small cells of the abscission zone appear not to be expanded in contrast to neighboring cells of either side of the abscission zone.

During the development of the tobacco flower the pedicel increases in both length and diameter (Table 6). The cortical cells of the abscission zone have only a limited amount of cell expansion with no increase in cell number. They become compressed axially at flower stage (group 11) resulting in a radial length of the cells averaging 22.1

μm and an axial length averaging 17.1 μm (Fig. 35, B). The groove becomes deeper as the cells of the abscission zone retain their size and a 200 μm deep groove is observed in flowers of group 11 (Fig. 35, A). The pith cells of the abscission zone remain small. However there are many more cells in the pith region of the abscission zone than the number of cells in the pith region of the pedicel. Of course they were a result of cell division.

Ultrastructure of the abscission zone

In the cortical cells of the abscission zone the vacuole occupies about two thirds of the cell volume (Leiberman et al., 1983). The vacuole is much smaller than the vacuole of cortical cells of the pedicel (Fig. 30 B). Micrographs of the ultrastructure (Fig. 36, 37, 38, 39) show that the plasma membrane lies firmly adjacent to the cell wall. Degraded cytoplasmic material is of low frequency in the vacuole of the cortical cells. The cortical cells have interphase nuclei with one or more nucleoli and appears have a typical staining pattern. Chloroplasts have a membrane-enclosed granular body as well as well organized thylakoids. The inner membrane of chloroplasts contains many invaginations. These invaginations often form small vesicles of light electron density. There are also one to several starch granules in the chloroplasts. Chloroplast division is seen frequently . The microbodies are usually found with a crystalloid core (Fig. 36, 37). The mitochondria are rich and very dense in content (Fig. 37, 38, 39). There is frequent ER (Fig. 37). The Golgi bodies present appear to be very compact with limited surrounding vesicles (Fig. 38).

Cells of abscission zones of inflorescences exposed to a 6 hour ethylene treatment show significant changes in the ultrastructure (Figs. 40, 41, 42, 43). The plasma membrane is separate from the cell wall (Figs. 40, 41, 42). Many Golgi bodies with well developed vesicles appear around cell membranes (Figs. 40, 42) and near the vacuole membranes (Fig. 43). RER is observed with increasing frequency (Fig. 41). Degraded cytoplasmic material is observed in the vacuoles at a higher frequency than in vacuoles of abscission cells of inflorescence not treated with ethylene. Mitochondria are less frequency and appear in a certain degree of degradation (Figs. 41, 42).

The Golgi bodies may be involved in both cell wall thickening and cytoplasm degradation in the vessel cells (Fig. 44). The annular-type of vessels with the secondary cell wall formation are undergoing cytoplasm degradation. The nucleus is elongated to fit the elongated vessel cell. Golgi bodies are the most abundant organelles in the developing vessel cell. But the functions of the Golgi bodies in the vessel cell is different from the Golgi bodies in the cortical cells of the abscission zone of inflorescence treated with ethylene.

Part IV. Abscission of the tobacco flowers

Flower abscission pattern

Not all flowers can be induced to abscise by ethylene treatment for six hours or by air treatment for 48 hours. Abscission of tobacco flowers was studied according to the 12 groups of flowers on the W1 stage inflorescences. The fruits (group 12), group 1 and group 2 flowers (buds) are not abscising (Table 13). More than 90 percent of the flowers between group 6 to group 10 were induced to abscise. Flowers from group 3 and group 4 have a very low abscission rate with about 11 percent to 17 percent in group 4 and 0.3 percent to 5 percent in group 3. Flowers from group 5 have a much higher rate of abscission (from 60 percent to 83 percent). Flowers (group 11) can be induced at a very low rate (7 percent for the air treatment) to a relatively high rate of 49 percent in the case of ethylene treatment.

The flower abscission pattern induced by ethylene is different from the flower abscission pattern induced by air (Fig. 45). For both conditions, the fruits (group 12) and flowers from group 1 and group 2 cannot be induced to abscise. 100 percent of flowers from group 8 and group 9 are induced to abscise. The abscission induced by ethylene treatment is higher in group 11 than that induced by air. In group 11 ethylene induces nearly half of the flowers abscise but air treatment induces only 7 percent of the flowers abscise. In contrast ethylene induced abscission is lower in the flower group 3, 4, 5, 6, and 7 than the abscission induced by a 48 hour air treatment. The rapid induction of abscission by ethylene treatment for six hours appears to cause only the currently inducible flowers to abscise.

Flower break strength

With ethylene treatment of up to 2 hours the break strength of a flower is correlated with its flower length (Fig. 46). The top three lines (0 hour, 1 hour and 2 hours) show the break strengths for flower groups 5 to 11. Smaller flower buds are weaker than larger flower buds. In general a flower of group 5 can be broken with about 150 gram of pulling force while a flower of group 11 is difficult to break requiring about 600 gram of pulling force. After three hours treatment of ethylene the break strength at the abscission zone of a flower pedicel decreases substantially. The correlation of break strength to flower groups is obvious from group 8 to group 11, and the correlation of break strength with flower group is less obvious from group 5 to group 8.

With up to 2 hours ethylene treatment the break strength of a flower was not decreased. The decrease in the break strength of a flower as induced by ethylene appears three hours after ethylene treatment for all the seven groups of flowers (Fig. 47). After five hours to six hours treatment of ethylene the break strength dropped to under 100 gram in pulling force except in the flowers (group 11). The break strength of flowers in group 11 are much higher than all the other flower groups.

The break strength of flowers in group 11 could be induced to decrease by ethylene treatment with the break strength decreasing to about 150 gram of pulling force after six hours of ethylene treatment. However some group 11 flowers shown a weaker point in their pedicels other than at the abscission zone. The break strengths of the pedicel break zones did not show any decrease of the break strength for six hours of ethylene treatment (Fig. 48). The break strengths for flowers (group 11) can not be significantly induced by air induction for 72 hours either at the abscission zone or at the pedicel break zone (Fig. 49).

Selective abscission

Breaks in the pedicels are common to certain groups of flowers and at some induction periods. With ethylene treatment of six hours the pedicel break is found to occur in group 11 flowers at all induction times (Table 14), apparently some group 11 flowers developed which were not inducible to abscission. In the ethylene treatment of one hour and two hours the pedicel break occurred in all flower groups (Table 14). However no decrease of break strength was observed (Fig. 47). In the air treatment of 48 hours pedicel breaks are found in flower group 10 and 11 of all induction times (Table 15).

Many flowers abscised without any pulling force during the air induction period between 36 hours to 72 hours (Table 16). These flowers are from flower groups 5 to 10. In reference to the W1 population, flowers from group 5 and group 10 may only partially abscise and flowers from groups 6 to 9 may abscised by 100 percent in this none-force manner.

Part V. Floral bud formation from thin layer explants of "Little Turkish" tobacco

Conditions for flower bud formation

The medium required for flower bud formation for the "Little Turkish" tobacco plant is essentially the same as for other tobacco cultivars (Rajeevan and Lang, 1993). The medium contains MS salts, 30 g/L sucrose, 100 mg/L myoinositol, 0.4 mg/L thiamine.HCl. Plant growth regulators (IAA 0.175 mg/L and KIN 0.216 mg/L or NAA 0.186 mg/L and BAP 0.225 mg/L), 10 g/L agar, pH 5.7. It was cultured at 25 °C with continuous lighting. With these conditions about 10 percent of thin-layer explants from the green-fruit stage inflorescence developed flower buds in 25 to 35 days. Usually a few leaves differentiated in advance (Fig. 50). Ten percent of explants taken from flower pedicels, fruit pedicels, branch internodes between flowers, and branch internodes between fruits developed flower buds. However the combination of plant growth regulators (IAA/ KIN or NAA/BAP) affected the development of the explants. NAA / BAP promoted more callus formation and more buds developed. IAA / KIN induced less callus and fewer buds. In addition to the plant growth regulators mentioned he two organic compounds -- myo-inosital and thiamine hydrocloride are sufficient for flower bud formation as well as five organic compounds (100 mg / L myoinosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl).

Flower bud formation from the abscission zone

One hundred percent of explants containing abscission zones developed flower buds. The buds developed from the proximal side of the abscission zone (Fig. 51, 52). The proximal and distal ends of the explant were distinguished in that the proximal end is usually larger and has some callus developed (Fig. 52). The flower buds were clearly distinguishable by the naked eye within ten days and some of the flower buds reached anthesis in about 20 to 25 days after the beginning of the of tissue culture (Fig. 53).

In the medium for flower bud formation which contained the growth regulators NAA/BAP, each explant developed several flower buds crowded along the abscission zone (Fig. 54, B). In addition to the formation of flower buds callus was also formed from the lower side of the explants. In contrast explants in the same medium, but with the growth regulators IAA/KIN, only developed flower buds from two points each located at one end of the abscission zone of the explants. There was very limited callus formation and explants were remained small (Fig. 54, A).

In comparing the development of explants with or without abscission zone tissue paired explants from the same flower were cultured in the same container. The explants with abscission zone tissue always developed flower buds (Fig. 55, 56). The explant from upper part of a flower pedicel (without abscission zone tissue) had no flower bud formation during the cultural period up to 40 days. In the flower bud formation medium with growth regulators NAA/BAP both explants with or without abscission zone tissue developed callus from the lower side of the explants (Fig. 55, B,C; Fig. 56, C, D). However explants in the flower bud formation medium with plant hormones IAA/KIN only developed a limited amount of callus from the proximal end of the explants (Fig. 55, a; Fig. 56, A, B).

Differentiation potential of the abscission zone

According to Tran Thanh Van et al. (1974) thin-layer explants are capable of developing directly flower buds, vegetative buds, roots or callus at certain cultural conditions. These conditions are also true for "Little Turkish" tobacco explants which include abscission zone tissue. In vegetative bud formation conditions (MA salts with 30 g / L sucrose, 100 mg / L myoinosital, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA. 2.16 mg / L KIN and 10 g / L agar, pH at 5.7 and constant lighting) 100 percent of the explants cultured developed vegetative buds (Fig. 57, A). In flower bud formation conditions (MA salts with 30 g / L sucrose, 100 mg / L myoinosital, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA. 0.216 mg / L KIN and 10 g / L agar, pH at 5.7 and constant lighting), 100 percent of the explants cultured developed flower buds (Fig. 57, B). In the callus promoting medium (MA salts with 30 g / L sucrose, 100 mg / L myoinosital, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA. 0.022 mg / L KIN and 10 g / L agar, pH at 5.7 and constant lighting), 4 percent of the explants developed callus from abscission zone (Fig. 57, C); the rest (96 percent) of the explants did not change significantly (Fig. 57, D). In the root promoting conditions (MA salts with 30 g / L sucrose, 100 mg / L myoinosital, 0.1 mg / L thiamine.HCl, 1.75 mg / L IAA. 0.022 mg / L KIN and 10 g / L agar, pH at 5.7 and darkness), only 19 percent of the explants developed roots from the abscission zone (Fig. 57, E). Nine percent of the explants developed flower buds from the abscission zone (Fig. 57, F) and the other 72 percent of the explants shown callus which was not developed from the epidermal abscission zone but rather from the lower side of the explant (Fig. 57, G). The 9 percent of the explants with flower bud formation under root formation conditions is an interesting result. It indicates that the abscission zone is a potential tissue for flower bud formation and the flower bud formation does not completely require a lighting condition.

The organogenesis results from explants cultures in glass baby food jars and in plastic petri dishes are quite different. In both containers containing flower bud formation medium (MA salts with 30 g / L sucrose, 100 mg / L myoinosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA. 0.216 mg / L KIN and 10 g / L agar, pH at 5.7) explants on media in the baby food jars developed flower buds (Fig. 51). Explants in the plastic petri dishes developed callus from the abscission zone (Fig. 58).

Flower bud formation from the abscission zone during the first 15 days

Flower bud formation from thin layer explants including abscission zone tissue was observed for 15 days on a daily basis. During the first three days the tissue culture containing explants with abscission zone tissue underwent graduate changes. The explants expanded slightly (Fig. 59, A, B, C, D) and the cells of the abscission zone became enlarged and loosely in contact with each other (Fig. 61, A, B). Flower primordia appeared after about 4 to 5 days of onset of the culture and became visible at 6 to 7 days of the beginning of the culture (Fig. 59, E, F, G, H). The primordia developed first from one end of the abscission zone on the explant and from the proximal side of the abscission zone. An early differentiated stage of a flower primordium is seen from the section of an explant after six days of culture (Fig. 61, C). From day 8 to day 11 both ends of the abscission zone tissue of the explants developed flower buds (Fig. 60, A, B, C, D). The flower bud is a group 2 flower bud with the distinction of a "flower body" and a "pedicel" on the section of an explant cultured for eight days (Fig. 61, D). From day 12 to day 15 more flower buds developed from the abscission zones (Fig. 60, E, F, G, H). They are usually from the two ends of the abscission zone on the explants. In some explants several flower buds were arranged as a subbranch of an inflorescence.

Part VI. Plant hormones' effects on the development of tobacco inflorescence

Ethylene's effects on the inflorescence development:

Ethylene treatment of F1 stage inflorescences can remove most flowers (buds) from group 5 to group 11. An average of 22 flowers and buds including the terminal flower were removed from the F1 stage inflorescence after 6 hours ethylene treatment (Table 17). Flowers and buds from group 7 to group 11 of the F1 stage inflorescences were completely removed by shaking the plants after 6 hours ethylene treatment at F1 stage(Table 18), flower buds from group 5 and group 6 were partially removed by shaking the plants after 6 hours ethylene treatment at F1 stage. None flower buds from group 4 of smaller were removed. This result is similar to the result of the abscission pattern of W1 stage inflorescences in that flower buds from group 1 to group 4 were not inducible to abscission (Fig. 49). However at W3reg stage inflorescence the number of scars of the abscised flowers increased to an average of 34 (Table 17). Therefore more flower buds had been induced into the abscission process but they were not removed by shaking the plants after 6 hours ethylene treatment at F1 stage.

The average number of fruits set on the W3reg stage inflorescences (treated with ethylene at F1 stage) is 124 which is somewhat higher than that of the control inflorescences at W3 stage which is 96.6. That ethylene treatment increases fruit set is an surprising. It may be a result of the longer time the F1 stage to be reached in the regenerated inflorescence (as compare to time for the development of the F1 stage in the normal inflorescence). Another reason may be that the removal of the earlier flowers and buds results in more space for flowers to develop. The treatment with ethylene at the F1 stage inflorescence results not only in the W3reg inflorescence being larger but also more mature in that only 1.8 percent of the fruits set from groups 7 to 11 (Table 19). However effect of ethylene on the F1 stage inflorescence is likely to only accelerate the abscission of the inducible flowers and buds. It probably does not affect the nutritional (resource) status of the plant.

The effect of auxin and cytokinin on inflorescence development

Auxins and cytokinins are plant growth promoting hormones. When they are applied to the F1 inflorescence they stimulate the elongation of the flower pedicels, the corolla tubes and the internodes of the subbranches of the inflorescence. However none of these treatments increase significantly the size of inflorescences (as measured by number of fruit set in Table 19). IAA may promote both fruit set and restrain flower abscission; therefore more flowers from groups 7 to 11 were on the W3 stage inflorescences of the IAA-treated group than on inflorescences not treated with IAA (Table 19). Kinetin may promote fruit set and may not restrain flower abscission so that fewer flowers from groups 7 to 11 were on the W3 stage inflorescence of the kinetin-treated group. Combined IAA and kinetin treatment did not increase the inflorescence size. The combined treatment apparently helped the inflorescence to become more mature at the W3 stage: only 2 percent of total fruits set from groups 7 to 11 were on the W3 stage inflorescences treated with IAA and kinetin.

These results appear to suggest that nutritional status or resource limitation is not changed by applying plant hormones to the inflorescence. In the other words the reproductive loading capacity is not determined by plant hormones.

DISCUSSION

Methods for studying tobacco inflorescence development

The post-flowering development of the plant reproductive structure "inflorescence" is rarely studied. The development of an inflorescence is the result of a large number of parallel reaction sequences that are coordinated in time and space to produce numerous reproductive structures in a supposedly harmonious fashion. There are many developmental responses that the plant has to make during the formation of the inflorescence. Earlier investigators of inflorescences have not attempted to describe developmental processes but rather the diversity of inflorescences among plant species (Weberling, 1989). In this investigation tobacco has been used as a model plant to study inflorescence development. In order to study this "new subject" many methods were established to study the development of the inflorescence. The data collected using these methods are valuable for describing the development of the tobacco inflorescence. These methods will also be useful in the areas of morphology, physiology and ecology to further address tobacco reproductive biology.

Tobacco flowers grouped by size has been used in previous studies (Goldberg, 1988). The purpose of using flower groups in this study is to describe the development of the inflorescence. For the first time flowers of all sized have been studied. A method of dividing tobacco flowers into twelve groups is carried out in this study. This method reflects the accelerated elongation of the flower length during the developmental period. Random sampling of flower length from group 5 to group 12 is nearly linearly correlated

(Fig. 16). Using this method the fruits and flowers (with open corollas) are two independent groups along with other smaller flower buds which are divided into ten groups mathematically. The data from flower group 1 is impossible to collect in its entirety since flower group 1 includes all flower buds larger than zero mm to 0.49 mm in length. Flower buds larger than 0.5 mm in length can be counted accurately.

It is more difficult to distinguish among developmental stages of the tobacco inflorescences than it is to simply divide flowers into groups. An inflorescence is more dynamic and more variable in its development than a flower is. A method was used by Croes et al. (1985) and Tiburcio et al. (1988) to name the inflorescence developmental stages for the purpose of identifying where thin layer explants were collected. Yager (1957) used the stage of inflorescence where the terminal flower had just "opened" as a reference stage for experimentation because inflorescences earlier in development than this stage lack obvious descriptive characteristics. In this study the stage of inflorescence where the terminal flower is open is called the F1 stage. After this stage the inflorescence is on its way toward maturity and it takes approximately three weeks to have its first fruit turning a pale yellow in color. This is not only a marker of maturity but is also a marker of senescence of the inflorescence. Collecting data from the F1 stage to the W3 stage results in a manageable size of data. Of course it requires three to four months for the tobacco plant in the greenhouses to grow to the F1 inflorescence stage.

The use of tobacco instead of *Arabidopsis* allows questions to addressed concerning inflorescence development which cannot be properly addressed by using *Arabidopsis*. The source of tissue contributed for the flower bud formation is different between tobacco and *Arabidopsis*. There is no flower abscission to reduce the size of a inflorescence in *Arabidopsis*. In addition the recording methods are not suitable for *Arabidopsis* because as is the case in many mustard weeds there is the development of many axillary inflorescences. These may appear in the same time period in a similar if not even larger size than the size of the real terminal inflorescence.

Recording data concerning the inflorescence was done by dissecting the inflorescence in a top-to-down order. The subbranches, flowers and their arrangement were recorded by drawing a draft of the inflorescence formula (Fig. 2, Fig. 3). To record data concerning the F1 stage and the W1 stage inflorescence in detail is both essential and manageable. To record data concerning the W2 stage and W3 stage inflorescence in detail may also help to obtain more data for analysis. But due to the extremely large number of flowers and the complications flower abscission, the detail recording of the W2 stage and W3 stage inflorescence is not attempted in this research.

The data collected using the inflorescence formula were analyzed in order to obtain the following characteristics: Flowers of monochasium-and-non-monochasium-original, the number of nodes on the subbranches, degree of maturity of inflorescences and size of inflorescences. These characteristics provide a numerical comparison of inflorescences and enable the description of inflorescence development precisely.

The method for determining the abscission pattern was heavily dependent on the flower group assignment and the inflorescence developmental stages. They constitute the comparison basis for inflorescences. For the first time when doing break strength analysis the size of the flower was considered. It was found that break strength of the abscission zone is correlated with the flower length when the break strength has not reduced during 0 to 2 hours ethylene treatment (Fig 46, 47).

The new instrument (Fig. 4) used to measure break strength in this investigation also provides more useful information in that the abscission zone may not be the weakest point in the pedicel of the flower. It is impossible to find this with a top-load balance which can only measures the bending break strength (Valdovinos et al., 1972).

Application of plant hormones to the intact inflorescence is a difficult experiment. Generally it is difficult to have a simple effect which can be traced during the development of the inflorescence. Using the methods developed in this study the numerical comparisons among inflorescences solved this problem. The effects of plant hormones on the development of the whole inflorescence can be studied. In this respect these methods have wide usage. Most defined environmental factors can be tested by this method to see how they affect the development of an inflorescence.

Tobacco inflorescence is a dynamic compound cymose

An inflorescence is the part of shoot system for reproduction. Instead of producing leaves and branches it produces bracts and flowers. Plants are characterized by an "open system of growth" (Foster and Gifford, 1974). Theoretically plants can grow to an unlimited size. The body plan continues to be elaborated through the entire post-embryonic phase of the life span. The tobacco inflorescence is a dynamic compound cymose. Although the cymose is a type of determinate inflorescence, but as the branch of shoot, it only represents the branch type of the inflorescence following the sympodial manner, that every meristem differentiates to a single flower. Then the sub-terminal tissue continues to form new flowers.

The compound cymose develops on a solitary stem and is a restricted determinate inflorescence rather than a mixed determinate and indeterminate as explained by Cronquist (1988). The terminal flower always blooms first. On the other branches the flowering sequence is following a restricted-determinate-manner in that flowers are developed from the abscission zone of a pre-existing flower. Development of the inflorescence appears to be under precise regulation and the key structure to perform such a regulation is the abscission zone of each flower.

The potential for dynamic changes in the inflorescence is an important characteristic of the tobacco inflorescence. It helps the tobacco plant to adjust its inflorescence size and to adapt better to environmental variations. For example, a certain sequence in the flowering pattern may or may not be repeated during the development of the inflorescence. Nevertheless there appears to be a well regulated system operating in tobacco for inflorescence development. Any node develops from monochasium to dichasium and to trichasium branch types. It appears that it is not necessary to follow a restricted trichasium branch type. The advantage is obvious in that it is a logical design to allow the plant to use the available space which gradually becomes available with the elongation of internodes and pedicels.

The abscission zone is a flower node

The abscission zone is actually a flower node which originates from a terminal meristem. However the terminal meristem differentiates into a single flower and the apical dominance is then released by the terminal flower. Thus the abscission zone becomes active for developing subbranches. The "node and internode" architecture is a basic-and-essential pattern of a shoot system in plants. A flower node is the analog of a node in the vegetative shoot system. For the tobacco plant the flower node is different from the vegetative node in that the flower node is the uppermost node and its apical meristem differentiates to a flower. There is no longer apical dominance by the respective flower node and this situation will encourage further flower bud development from the node. The flower node tends to develop one to three subbranches instead of only one branch developing from a vegetative node in the tobacco plant.

The abscission zone of a tobacco flower is morphologically recognized by the ring or groove. Small cells are across all the cortex and the pith of the abscission zone. The vascular tissue is broken by one to three gaps also filled with small cells. The vascular tissue irregularly passes by the abscission zone due to the reassociation with the vascular tissue of subbranch(es). Unlike the vegetative node with either a developed or dormant bud the flower node usually develops one subbranch and has the potential for developing one or two more subbranches. Tissue culture studies show that the flower bud formation is not restricted to any particular point on the abscission zone but a regulatory mechanism restricts the maximum number of subbranches to three on the intact inflorescence.

In the development of a flower the position of the abscission zone can be predicted at quite an early time. When the flower is at a primordium stage, the next smaller flower primordium may already appear underneath. When the flower body and pedicel are recognizable the position of the abscission zone can predicted precisely. However the morphologically recognizable abscission zone appears when the flower bud is about 1.2 mm in length and the pedicel starts to elongate and to increase in girth. The cells of the abscission zone remain as small cells without significant increase of cell size in any direction. The pedicel continues to develop in length with a significant increase of girth. When the flower is about 10 mm in length the secondary vascular tissue can be easily identified in a cross section of the pedicel. However the secondary vascular tissue develops slowly until anthesis and then rapid growth appears. According to Muir (1942) growth hormones are transported to the pedicel from the ovary after pollination. According to Dalessandro and Roberts (1971), a high auxin-to-cytokinin ratio (IAA 5.0 mg/liter, Zeatin 0.1 mg/liter) can induce xylogenosis in the pith explants of *Lactuca*. Thus the rapid growth of the vascular tissue may be due to the growth hormones transported from the ovary following pollination.

In reference to the groove the small-cell-area of the abscission zone can be divided into a proximal side and a distal side. The significance of this differentiation is that flower abscission occurs at the distal side and the flower bud formation is from the proximal side. Flower bud formation and flower abscission are two opposite processes in the development of an inflorescence; both processes occur in the well differentiated local tissues. The two local tissues are closely associated and logically fit their functions quite well in that the distal side separation layer only removes the flower when abscission occurs but the remaining part still has the tissue for flower bud formation.

Abscission is a prepared regulatory mechanism

Abscission is a localized phenomenon (Sexton, 1983). It occurs only at the abscission zone of the discrete organ. Abscission of the tobacco flower happens only at the abscission zone at the base of the pedicel and more precisely it only happens on the distal side of the abscission zone. However the abscission zone may not be the weakest point in comparison to the pedicel of the same flower but it becomes the weakest point with the induction of abscission. Therefore abscission at the abscission zone is not only morphologically determined but is also physiological determined.

In these studies the abscission zone is morphologically differentiated when the flower length is only about 1.2 mm. [Yager (1957) and Kendall (1918) reported a value of 2 to 3 mm in length]. The flower buds inducible to abscission must be about 8 mm in length. Flowers longer than 8 mm in length are easily induced by ethylene treatment for six hours except for the fruits. For fruits the abscission process may be prevented by growth hormones transported from the ovary and the rapid secondary growth of vascular tissue further stabilizes the fruits on the inflorescence.

Ethylene treatment induces the abscission of flower in only few hours. In a comparison of the ultrastructure of control and six-hour ethylene treated cortical cells of the abscission zone, several significant differences are the contact of plasma membrane to cell wall, the Golgi bodies and the degraded cytoplasmic material in the vacuole. However the most obvious organelles such as nucleus, chloroplasts, mitochondria and microbodies are of no observable difference. A significant increase of RER was reported (Valdovinos et al., 1972; Lieberman et al., 1983; Valdovinos et al., 1985). Obviously the effect of ethylene treatment is rather specific to initialize the abscission process which requires the synthesis and secretion of cell wall degradation enzymes. The degraded cytoplasmic materials in the vacuole may be a response to the rapid increase of the cellular secretion processes. The normal cellular organization in cytoplasm is not disrupted as in comparison to the vessel element cell where the cytoplasm is eventually completely degraded. However, mRNA production may involved due to ethylene treatment (Abeles, 1968). In a recent study on the abscission of tomato flower, the Egase mRNA accumulation increases with ethylene treatment in two days and with air in 4 days (Lashbrook et al. 1994). Whether or not mRNAs are involves in the ethylene-induced abscission of tobacco flower is not clear.

The break strength is correlated to flower length. Larger flowers require more force to break. But this situation is changed after a three hour ethylene treatment. The break strength starts to decrease after a three-hour treatment of ethylene. This result is similar to that published earlier (Valdovinos et al., 1972). Rapid decreases of break strength are observed in flowers from group 6 to group 10 after three-hour ethylene treatment.

The abscission of tobacco flowers is prepared prior to the abscission process. The differentiation of the abscission zone is morphological evidence. Physiologically ethylene can trigger the abscission process in very short time and ethylene only induces the abscission at the abscission zone. Valdovinos et al. (1985) tested the effect of ethylene on the abscission process; their results suggest that only translation is required for the abscission induced by ethylene treatment. Thus the mRNA for the cell wall degradation enzymes should exist in the cells of the abscission zone. With a six-hour treatment of ethylene the increase of Golgi body activity and the separation of plasma membrane from the cell wall further suggest that the cell wall degradation is related to rapid cellular secretion. If any mRNA for a cell-wall-degradating enzyme existed before any induction was applied then this mRNA must be the tissue specific and it should not exist in other tissue.

In the abscission of the leaf of bean, cellulase and its mRNA existed in the same pattern around the abscision zone (Tucker et al., 1991). Unfortunately they did not test the existence of cellulase mRNA in the tissue where there was no induction of abscission.

Flower bud formation is from the abscission zone

Flower bud formation originates from the proximal side of the abscission zone in both the intact inflorescence and the explant under tissue culture. It was suggested earlier that the abscission zone is actually a flower node which is right beneath a determinate apex. The abscission zone can potentially develop up to three subbranches on the intact inflorescence. The most obvious trichasium branch type is the one from the terminal node under the terminal flower. A simple description of this branch type was also reported by Croes et al. (1985).

The origination of flower buds (except the terminal bud develops from the vegetative apical meristem) is mostly from the proximal sides of the abscission zones of a pre-existing flower. This is a regular function of a node as in the vegetative shoot. However the abscission zone is different from a vegetative node in that flower primordia are not always initiated on the abscission zone; in most cases one flower primordium is initiated and developed but the entire abscission zone retains the potential to develop one to two more flower bud(s). This is in contrast to a vegetative node where one axillary bud is always present.

Under the tissue culture conditions with IAA and KIN flower buds are formed from the two extreme ends of the abscission zone on the explant. This phenomenon indicates that the branch characteristics exists in the abscission zone even though they are removed from the intact plant. However the growth regulators NAA and BAP can easily break the branch characteristics in that many flower buds are formed from the entire abscission zone on the explant. Thus the hormones'effect on the organogenesis is not simply to initiate the organogenesis process but may also be involved in the patterning of branch during the organogenesis.

Plant hormones

Plant hormones play important roles in the regulation of growth and development in plants. Development of the tobacco inflorescence involves many complicated processes which interact with the signals from both internal and external sources. Verification of the roles of the various plant hormones in the development of the tobacco inflorescence would be of great interest. The following discussion will focus on the involvement of plant hormones in flower bud formation, flower abscission and inflorescence development.

Flower bud formation under tissue culture is a well established model since the work of Tran Thanh Van (1973a). The explant must be obtained from the plant which is florally determined (Altamura and Capitani, 1992; Rajeevan and Lang 1993). The 1:1 ratio of auxin and cytokinin at 1μM concentration of each hormone is required in the media. Different cytokinins and auxins in the culture media affect organogenesis (Heylen and Vendrig, 1988). Media with IAA/KIN or NAA/BAP result in different numbers of flower buds and in a different pattern of the arrangement of flower buds. However the thin layer tissue culture model may not be the same for flower bud regulation in the intact inflorescence. Most flower buds from tissue culture remain poorly developed and some times are irregular. A continuous development of inflorescence pattern was not studied in the tissue cultures. Therefore the complicated regulatory system in the intact inflorescence is not clear.

There are no solid data about the concentrations of hormones in the abscission zone of the intact plant. Muir (1942) reported that the growth hormones (auxin) increased in the ovary after about two days of pollination and these hormones were transported to the pedicel. A peak in growth hormones in the pedicel occurred about three days after pollination. According to tissue culture results (Tran Thanh Van et al., 1974), higher auxin levels promotes root differentiation. Therefore the auxin in the pedicel from the ovary may not be of the desirable level to promote flower bud formation at the abscision zone on the intact inflorescence. In fact flower bud formation on the intact inflorescence developing from an abscission zone may occur much earlier than flower anthesis. The external applications of hormones of IAA, KIN and the combination of IAA and KIN provide no clue as to whether or not they affect flower bud formation from the abscission zone on the intact inflorescence. The uptake and metabolism of NAA and BAP in explants of tobacco tissue not containing the abscission zone were studied by Barendse et al. (1987). The uptake of both NAA and BAP by the explants was shown to be proportional to the concentrations supplied in the media. Both growth regulators were rapidly metabolized by the explants. This information indicates that plant hormones at the abscission zone may be from other sources and they may or may not concentrate at the abscission zone.

The role of auxin as the main internal factor inhibiting abscission was quantitatively well established for *Coleus* leaves (Jacobs, 1968). In the studies of Muir (1942) unpollinated flowers had very low levels of growth hormones in either ovary and pedicel and the flowers whithered and abscission occurred within three to four days after the flowers reached full development. These data suggest that low auxin levels in the pedicel may be the cause for abscission. However auxin may not only prevent the abscission process but in addition it may also promote the rapid development of the secondary vascular tissue in the pedicel. In such a case it is the secondary vascular tissue that prevents the fruit from abscising. Kendall (1918) reported that abscission process occurs in the cortex of fruits which had remained firmly on the inflorescence. A similar phenomenon was observed in the fruits on the inflorescence of tobacco which had been placed in the laboratory for days with the basal end in water. The auxin transported to the pedicel may help induce secondary growth of the vascular tissue which in turn prevents the abscission.

Ethylene is the plant hormone which induces abscission rapidly (only three hours). Electron microscopic studies show that ethylene treatment can induce the RER increase

(Valdovinos et al., 1972; Lieberman et al., 1983; Valdovinos et al., 1985). Ethylene treatment for six hours also increases the Golgi bodies in the cortical cells of the abscission zone. These observations suggest that ethylene may rapidly induce proteins synthesis and/or secretion.

Whether or not ethylene is involved in the natural abscission of flowers is not clear. According to Yang and Hoffman (1984), ethylene is produced in most tissues of the plant. Hill et al. (1987) reported that pollen grains contain high ACC (~2700nmol/g) --the ethylene precursor and pollination caused the release of ethylene in the tobacco flower. At least ethylene causes the abscission of corollae. Therefore accumulation of the released ethylene in air may also cause flower abscission particularly the natural abscission occurring between W1 and W2 stages when pollination is at the peak period. However the young inflorescences (F1 stage) do not appear to be affected by the surrounding older inflorescences. Reid et al. (1984) reported that the senescence of carnation petals may not be caused directly from the ethylene released from the gynoecium but rather by the ethylene released from petals where ACC was transported from the gynoecium. In contrast whether or not the abscission zone of a tobacco flower produces ethylene itself could be important to understanding natural abscission.

In the overall development of inflorescences how plant hormones are involved is a challenging question. This involves the internal regulation of production, transportation and metabolism of plant hormones. The treatment of inflorescences externally by hormones promotes the elongation of pedicels and internodes. This may result in more space for flowers to develop. However the size of the inflorescence does not increase significantly. KIN treatment may cause the inflorescence to be more mature because KIN can generally attract nutrients and enhance the development of the fruits. Treatment by IAA caused a few fruits to develop and more flowers to be delayed developing into fruit . These results may be similar to IAA effects in Jacobs' (1968) studies.

Treatment with ethylene removes most inducible flowers at the F1 stage inflorescence. However it appears that ethylene does not inhibit the development of the flowers which are not inducible to abscission. Inflorescences treated with ethylene at F1 stage can continue to develop and replace the flowers which abscised. The inflorescence may become larger at the W3reg stage than a regular inflorescence one which had not been treated with ethylene at the W3 stage.

Plant hormones are not nutrient resources for plants as they are effective at very low concentrations and would not act as a carbon source. The interesting questions are: can resources affect the concentrations of hormones at the abscission zone? How do hormones affect the resources? These are very interesting questions in plant physiology.

Reproductive strategy

A reproductive strategy is an evolved complex of traits (Lovett Doust, 1989). More broadly plant reproductive strategy can be characterized by patterns of biomass allocation, mineral nutrient partitioning and the commitment of shoot meristem to particular developmental fates. The ecological concern of a reproductive strategy is to interpret the result of the performance of the reproductive strategy. Reproductive strategy may reflect how plants respond to certain environmental changes. The reproductive strategy may involve the genetic capability in that each plant has its own ways to respond to stimuli. Ideally the genetic capability is encoded in the genome and then is expressed biochemically (and /or morphologically). The plant parts carrying the positional differentiation information can perform specific functions. By triggering these functions appropriately the regulatory mechanism is executed.

It have been reported the plants pose some reproductive strategy which provides the opportunities to ensure minimal seed set and to optimize the reproductive yield in response to environmental variation. This is called the "reproductive insurance hypotheses" (Le Corff, 1993). In this hypothesis the first important event is that the plant should only flower when conditions can ensure a minimum seed set. Certain vegetative development is required for a tobacco plant to flower. This is likely a genetically-determined characteristic because the seeds of tobacco plant are very tiny (a seed averaging weights 62 μg). It is impossible to apply the transitional conditions (from vegetative to flowering) for a seedling to flower. Most studies about transition always involve a well developed vegetative plant. Floral determination has been described and characterized in *Nicotiana* (McDaniel et al., 1992). The shoots (either adventitious shoots or produced by a seedling meristem) can produce the same number of nodes before flowering (Rivers and Marcotrigiano, 1994). The tobacco "Wisconsin" 38 produces between 30 to 35 vegetative nodes, or 32 to 38 from regenerated shoots. In "Little Turkish", the shoot from the seedling produces 34 to 47 nodes. In tissue culture only the explant from flower-determined plants can be induced to form flower buds. Since the tobacco seed is so small the storage of nutrients will definitely not be able to support much initial growth. Due to final size of the plant and its potential to produce so many seeds (150 k per plant) the plant may genetically require certain basic vegetative growth achievement before the transition from vegetative growth to flowering can be possible.

For optimizing seed production the most challenging decision is about the balance between the quantity and quality of seeds because only the survivable seeds have value and the immature seeds only waste resources. During the reproductive processes--which start with the transition from vegetative to reproductive phases and end with the production of seed--plants are unable to avoid environmental changes physically due to their nutritional manner. To optimize the reproductive yield the tobacco plant may use an over-flowering strategy to provide maximum seed production, selectively aborting some of the developing flowers to allocate resources to the best candidates and thus to ensure the quality of seeds. The more interesting questions are how do plants detect the resource status and how do plants adjust their activities to the current resource status.

The abscission zone (flower node) with functions for both flower bud formation and flower abscission may represent a concrete reproductive strategy. Flower bud formation and flower abscission are the two opposite processes which contribute to the regulation of tobacco inflorescence development. The integral coordination of these two processes is an interesting phenomenon. Obviously the abscission zone could be a specialized structure for regulating the development of the tobacco inflorescence. The abscission zone is recognized morphologically and it posses a potential which does not exist in other tissue. Beyond the morphologically differentiated abscission zone there must be a whole set of complex system or positional information which allows the abscission zone to respond to both internal and external factors and to perform the defined processes. The ecological studies define the relationship between resources and the quantity of product (Lovvett Doust, 1989). Results of this study indicate that plants are prepared to perform their reproductive strategy.

Models of the regulatory mechanisms

Based on these results and the literature, some models of tobacco inflorescence development are proposed. These models may help to clarify characteristics of development of the tobacco inflorescence.

Flowering determination

A certain degree of vegetative growth is essential for the plant to be able to flower. Plants flower due to photoperiod induction or after reaching to certain number of vegetative nodes. "Flower-determined" plants has a stable commitment of a vegetative meristem to become a flower meristem. There must be some internal measurement which indicates when the degree of vegetative development has been reached for flowering to begin.

Segmentation in an "open-system of growth"

The development of a tobacco inflorescence follows two basic rules of the development of a plant shoot system. 1. It develops in a node-internode alternation fashion which is resembles the segmentation in animals. 2. It develops in a "open-system of growth"in that it can continue to add new nodes. Segmentation is a way to allow plant tissues carry the positional information.

A node of the tobacco flower

The flower node of a tobacco flower is specialized in comparison to a node on the vegetative stem. The flower node is the uppermost node of a branch where its terminal meristem differentiates into a flower. Thus unlike a regular node on the tobacco vegetative stem apical dominance no longer exists at the flower node. Another difference between a flowering node and a vegetative node is that the former tends to develop flower buds in the pattern of a reproductive branching manner. It tends to develop one to three flower buds whereas the tobacco vegetative node develops one vegetative bud. The node of a tobacco flower is also the abscission zone of the flower.

Signals affecting the action of the abscission zone

A combination of signals may either promote more flower bud formation or restrain flower bud formation. Conditions for more flower bud formation will allow the inflorescence to repeat flower initiation, abscission zone differentiation and floral bud formation to increase the size of the inflorescence. Otherwise flower abscission occurs.

Acting manner of the abscission zone

The abscission zone appears to be an integral structure for development of the tobacco inflorescence in that it serves both on increase and decrease in the size of the inflorescence. The abscission zone appears to have dual functions for flower bud formation and flower abscission. Although these two functions are opposite processes they appear to cooperate in an integral manner without interrupting each other.

The tissues responding to the flower bud formation and flower abscission are located in what appear to be two isolated sides of the abscission zone. The proximal side of the abscission zone is associated with flower bud formation and the distal side of the abscission zone is associated with flower abscission.

Conditions for flower bud formation and for flower abscission may be different and they may not be coincident for both processes. In the whole inflorescence flowers abscise while at the same time new flower buds form.

SUMMARY

The present studies involve methods for recording and describing the development of a tobacco inflorescence. The tobacco terminal inflorescence is a compound cymose with many dynamic branch types. The abscission zone of each flower is identified as the most important structure during the tobacco inflorescence development; it regulates the size of the inflorescence by having the functions of flower bud formation and flower abscission. The regulatory mechanism for size of the inflorescence is further facilitated with a fine differentiation of the abscission zone in that the distal side of the abscission zone responds for flower abscission and the proximal side for flower bud formation. The coordination of the growth conditions with flower bud formation and flower abscission at each abscission zone leads to the formation of the fully developed inflorescence.

These studies provide knowledge about the development of tobacco inflorescence in a number of ways. Morphologically the abscission zone at the base of the pedicel of a tobacco flower is recognized as a positionally differentiated flower node of the tobacco reproductive structure--the terminal inflorescence. The complete determinate compound-cymose of the tobacco inflorescence is described following the developmental process. Although the tobacco inflorescence is a determinate type it also "obeys" the rule of the general growth pattern of a shoot system described as "an open-system of growth". Theoretically the inflorescence can develop new flowers indefinitely. The compound cymose is similar to the shoot branch type sympodial found in plants other than tobacco where a terminal bud may abort and one, two or more sub-terminal buds emerge for

indefinite development. The differentiation of node and internode of a shoot system is also displayed on the inflorescence and the positional information is implemented into the segmentation of "an open-system of growth".

The abscission zone of a tobacco flower is a special flower node on the inflorescence. The apical meristem differentiates into a flower resulting in the loss of apical dominance, and one to three flower buds may now develop from this node. The abscission zone is further differentiated into two parts: the distal part responds in flower abscission and the proximal part is for flower bud formation.

Abscission can only be induced in flowers which are at stages group 5 to group 11. Flowers smaller than group 5 (shorter than about 8 mm in length) are not usually inducible to abscission by a six-hour ethylene treatment. Fruits do not abscise from the inflorescence because of the rapid increase of secondary vascular tissue in the pedicel. The break strength is correlated with size of flowers where abscission was not ninduced by ethylene. With ethylene treatment 3 hours and longer break strengths of flowers in groups 5 to 11 decrease rapidly.

As stated above flower buds develop from the proximal side of the abscision zone. On the intact inflorescence flower primordia usually develop from the proximal side of the abscission zone of a pre-existing flower at an very early stage. Under tissue culture flower buds can develop from any point on the proximal side of the abscission zone, but usually from the two extreme ends of the abscission zone of the explants receiving IAA an KIN in the medium.

Flower bud formation and flower abscission are the two opposite processes localized on the two sides of the abscission zone. From a coordination of integral functions the abscission zone adjusts the development of the tobacco inflorescence.

The present studies describe a complicated system in tobacco plants which apparently has a fine tuned regulatory mechanism in response to the growth conditions. The morphological characteristics are correlated with their functions. The tobacco inflorescence system could be useful in several further studies. 1. inflorescence development, 2. physiological and ecological studies of plant reproduction. 3. molecular genetic studies about the differentiation of the abscission zone.

Table 1. Assignment of the flower groups

Flower Groups		Flower length		
No.	Name	Measure (mm)**	Represent (mm)**	Square root**
1	Micro bud	0.1 - 0.4	0.00 - 0.49	0.0 - 0.7
2	Tiny bud	0.5 - 1.9	0.49 - 1.96	0.7 - 1.4
3	Minute bud	2.0 - 4.4	1.96 - 4.41	1.4 - 2.1
4	Small bud	4.5 - 7.8	4.41 -7.84	2.1 - 2.8
5	Medium bud	7.9 - 12.2	7.84 -12.25	2.8 - 3.5
6	Large bud	12.3 - 17.6	12.25 - 17.64	3.5 - 4.2
7	Short tube	17.7 - 24.0	17.64 - 24.01	4.2 - 4.9
8	Medium tube	24.1 - 31.3	24.01 - 31.36	4.9 - 5.6
9	Long tube	31.4 - 39.6	31.36 - 39.69	5.6 - 6.3
10	Pre-flower	39.7 - 49.0	39.69 - 49.00	6.3 - 7.0
11	Flower	49.1 - 59.2	49.00 - 59.29	7.0 - 7.7
12	Fruit*	59.3 - 70.5	59.29 - 70.56	7.7 - 8.4

* For group 12 fruit 30 mm is added to the length of a fruit without a corolla tube

** "Square root" is a mathematic approach of assignment of the flower groups; each step increases by 0.7. "Represent" is the square values of those in "Square root" and "Measure" is the truncated values of those in "Represent".

Table 2. Microscope calibration

Microscopes	Objective lens	Calibration
Beck Kassel-K106 (In Room 037)	5X	1/10=0.4 mm, 1/100=40μm
	10X	1/10=0.15mm, 1/100=15μm
	43X	1/10=37μm, 1/100=3.7μm
Olympus with camera (In Room 026)	4X	21 X on 135 negative
	10X	52 X on 135 negative
	20X	103 X on 135 negative
	40X	207 X on 135 negative
	100X	517 X on 135 negative

Table 3. Major differencies of four organogenesis conditions

	IAA*	Kinetin	Sucrose	Lighting
Vegetative bud formation	1μM	10μM	3%	Light
Flower bud formation	1μM	1μM	3%	Light
Callus formation	1μM	0.1μM	3%	Light
Root formation	10μM	0.1μM	1%	Dark

*IAA = Indole-3-acetic acid

Table 4. Some reproductive characteristics of "Little Turkish" tobacco

Trait	Average	Range	% of Range*
Number of internodes at F1 stage	42	34 -- 47	16.7%
Height of plant at F1 stage	117 cm	100 -- 130 cm	25.6%
Number of fruit set at W3 stage	68	44 -- 88	68.8%
Weight of a tobacco seed	62 μg	55 μg -- 68 μg	21.0%
Number of seeds per fruit	2188	900 -- 3200	105.1%
Weight of seeds per plant	9.2 g	2.2g -- 19.1 g	183.7%
Number of seeds per plant	150 k	40 k --282 k	161.3%

* The percent of range is the percent of the difference of range divided by the Average.

Table 5. Statistics* of the F1 stage inflorescence

Flower group		F1 inflorescence (n=6)			
No.	Name	Mean	Std E	%	Range
1	micro bud	data incomplete			
2	tiny bud	42.7	2.29	31.5	37-49
3	minute bud	33.7	4.57	24.8	21-49
4	small bud	21.3	1.33	15.7	17-26
5	medium bud	14.7	1.67	10.8	11-22
6	large bud	9.8	1.05	7.3	6-13
7	short tube	3.3	0.42	2.5	2-5
8	medium tube	3.2	0.40	2.3	2-5
9	long tube	1.3	0.42	1.0	0-3
10	pre-flower	2.8	0.48	2.1	1-4
11	flower	2.7	0.67	2.0	1-5
12	fruit	0	0	0	0
total		135.5	8.74	100	113-168

* Mean: the average number of flowers of six inflorescences. Std E: standard error. %: the percent of "Mean" divided by the total "Mean". Range: minimum and maxmum numbers of flowers of an inflorescence observed in the group.

Table 6. Correlation among of flower length, pedicel length and pedicel diameter

Flower group		Ct*	Flower length		Pedicel length		Pedicel diameter	
No	Name		mm	Std E	mm	Std E	μm	Std E
5	medium bud	25	9.96	0.31	1.28	0.09	1076.0	19.5
6	large bud	27	14.41	0.25	2.48	0.10	1269.6	18.1
7	short tube	13	19.92	0.57	3.39	0.18	1369.2	20.3
8	medium tube	15	27.33	0.43	4.07	0.18	1454.7	27.7
9	long tube	11	34.73	0.54	4.36	0.20	1494.5	22.1
10	pre flower	14	45.07	0.77	5.21	0.19	1575.7	22.3
11	flower	24	56.08	0.52	9.13	0.45	1823.3	46.5
12	fruit**	23	62.70	0.41	12.65	0.36	2046.7	37.9

*Ct: number of flowers of the group examined. Std E: standard error.

** 30 mm was added to the length of fruit if corolla tube abscised.

Table 7. Statistics* of the flowering stage inflorescences

Flower group		F1 inflorescence (n=6)				W1 inflorescence (n=6)			
No	Name	Mean	Std E	%	Range	Mean	Std E	%	Range
1	micro bud	data incomplete							
2	tiny bud	42.7	2.29	31.5	37-49	55.3	4.29	26.0	41-70
3	minute bud	33.7	4.57	24.8	21-49	47	4.51	22.1	37-62
4	small bud	21.3	1.33	15.7	17-26	23.7	2.39	11.1	16-31
5	medium bud	14.7	1.67	10.8	11-22	23.3	3.44	11.0	14-36
6	large bud	9.8	1.05	7.3	6-13	17.5	1.41	8.2	12-21
7	short tube	3.3	0.42	2.5	2-5	9.3	1.23	4.4	7-15
8	medium tube	3.2	0.40	2.3	2-5	4.5	0.43	2.1	3-6
9	long tube	1.3	0.42	1.0	0-3	3.5	1	1.6	0-7
10	pre-flower	2.8	0.48	2.1	1-4	4.8	1.01	2.3	2-9
11	flower	2.7	0.67	2.0	1-5	14.3	1.52	6.7	10-20
12	fruit	0	0	0	0	9.5	0.63	4.5	8-12
total		135.5	8.74	100	113-168	212.8	13	100	175-257

* Mean: the average number of flowers of six inflorescences. Std E: standard error. %: the percent of "Mean" divided by the total "Mean". Range: minimum and maxmum numbers of flowers of an inflorescence observed in the group.

Table 8. Flowering tendency of the F1 and W1 stage inflorescences

	F1 stage inflorescence	W1 stage inflorescence
Total (group 2 - 12)	135.5	212.8
Flower bud (2 - 6)	122.2 (90.1%)	166.9 (78.4%)
Flower+fruit(7 - 12)	13.3 (9.9%)	45.9 (21.6%)

Table 9. Fruiting tendency of the F1, W1, W2 and W3 stage inflorescences

	F1 stage	W1 stage	W2 stage	W3 stage
Total (7-12)	13.3	45.9	66.6	96.6
Flower (7-11)	13.3 (100%)	36.4 (79.3%)	43.6 (65.5%)	28.2 (29.2%)
Fruit (12)	0 (0%)	9.5 (20.7%)	23 (34.5%)	68.4 (70.8%)

Table 10. Statistics* of the fruiting stage inflorescences

	W2 inflorescence (n=6)				W3 inflorescence (n=6)			
	Mean	Std E	%	Range	Mean	Std E	%	Range
Flowers(7-11)**	43.6	3.5	65.5	28-54	28.2	2.3	29.2	18-38
Fruit (12)	23	1.6	34.5	18-30	68.4	4.6	70.8	44-88
Total	66.6	4.7	100	49-84	96.6	4.7	100	80-123

*Mean: the average number of flowers of six inflorescences. Std E: standard error. %: the percent of "Mean" divided by the total "Mean". Range: minimum and maxmum numbers of flowers of an inflorescence observed in the group.

**Flowers include all from groups 7 to 11.

Table 11. Number and percent of the monochasium and non-monochasium originated flowers

Infl. Stage*	Total	Monochasium	non-monochasium
F1(2-12)	135.5	55.2 (40.7%)	80.3 (59.3%)
W1(2-12)	212.8	74.5 (35.0%)	138.3 (65.0%)
W2(7-12)	66.6	45.3 (68.0%)	21.3 (32.0%)
W3(7-12)	96.6	57.8 (59.8%)	38.8 (40.2%)

* Inflorescence stages. For F1 and W1 stages number of flowers was counted from flower groups 2 to 12 and for the W2 and W3 stages the number of flowers was counted from flower groups 7 to 12.

Table 12. Number of nodes on subbranches*

Infl. Stage**	A1	A2	A3	B	C	D	Average
F1(2-12)	9.2	9.7	9.3	9.2	9.0	8.8	9.2
W1(2-12)	12.8	12.3	12.2	12.3	12.0	12.8	12.4
W2(7-12)	8.0	7.5	8.0	7.2	7.3	7.3	7.6
W3(7-12)	10.2	9.8	9.7	9.2	10.3	8.7	9.6

* A1, A2, A3, B, C, D are the six subbranches of the tobacco terminal inflorescence.

** Inflorescence stages. For the F1 and W1 stages number of nodes was counted from flower groups 2 to12 and for the W2 and W3 stages number of nodes was counted from flower groups 7 to 12.

Table 13. Abscission pattern of the W1* stage inflorescences

Flower group		Air (48 hours)		Ethylene (6 hours)	
No.	Name	%	Std E	%	Std E
1	micro bud	0.000	0.000	0.000	0.000
2	tiny bud	0.000	0.000	0.000	0.000
3	minute bud	4.847	3.678	0.303	0.303
4	small bud	17.396	5.842	10.795	4.394
5	medium bud	83.432	5.341	59.557	7.783
6	large bud	100.000	0.000	97.145	1.288
7	short tube	100.000	0.000	98.333	1.667
8	medium tube	100.000	0.000	100.000	0.000
9	long tube	100.000	0.000	100.000	0.000
10	pre-flower	94.722	4.092	100.000	0.000
11	flower	6.577	3.004	48.667	4.107
12	fruit	0.000	0.000	0.000	0.000

* For both air and ethylene treatments six W1 stage inflorescences were studied
%: the average percent of abscised flowers in the flower group of six inflorescences.
Std E: standard error.

Table 14. Number of flowers studied in ethylene induced break strength analysis

Flower group		5	6	7	8	9	10	11
0h	Total	2	2	10	9	8	2	21
	AZbk*	1	2	10	9	8	1	17
	Pbk**	1	0	0	0	0	1	4
1h	Total	15	23	13	9	2	6	20
	AZbk	11	13	5	4	1	2	18
	Pbk	4	10	8	5	1	4	2
2h	Total	11	20	8	10	5	5	14
	AZbk	4	13	2	4	2	3	7
	Pbk	7	7	6	6	3	2	7
3h	Total	9	17	11	5	5	4	11
	AZbk	9	13	11	5	5	4	5
	Pbk	0	4	0	0	0	0	6
4h	Total	14	26	18	6	9	6	24
	AZbk	9	26	18	6	9	6	19
	Pbk	5	0	0	0	0	0	5
5h	Total	19	32	22	5	7	8	27
	AZbk	19	32	22	5	7	8	26
	Pbk	0	0	0	0	0	0	1
6h	Total	25	28	17	11	4	11	31
	AZbk	25	28	17	11	4	11	30
	Pbk	0	0	0	0	0	0	1

*AZbk: break at the abscission zone of the flower.

**Pbk: break on the pedicel of the flower.

Table 15. Number of flowers studied in air induced break strength analysis

Flower group		5	6	7	8	9	10	11
0h	Total	2	2	10	9	8	2	21
	AZbk*	1	2	10	9	8	1	17
	Pbk**	1	0	0	0	0	1	4
12h	Total	0	2	4	4	4	6	5
	AZbk	0	2	4	4	4	5	4
	Pbk	0	0	0	0	0	1	1
24h	Total	1	3	4	0	1	4	7
	AZbk	1	3	4	0	0	1	6
	Pbk	0	0	0	0	1	3	1
36h	Total	0	0	1	0	4	7	16
	AZbk	5	0	1	0	2	1	6
	Pbk	0	0	0	0	2	6	10
48h	Total	0	0	0	0	0	8	8
	AZbk	0	0	0	0	0	3	4
	Pbk	0	0	0	0	0	5	4
60h	Total	0	0	0	0	0	6	11
	AZbk	0	0	0	0	0	4	4
	Pbk	0	0	0	0	0	2	7
72h	Total	0	0	0	0	0	3	11
	AZbk	0	0	0	0	0	1	5
	Pbk	0	0	0	0	0	2	6

* AZbk: break at the abscission zone of the flower.

** Pbk: break on the pedicel of the flower.

Table 16. Distribution* of non-force abscission of flowers during air induction from 36 to 72 hours

Flower group		% of non_force abscission	% of W1 stage population	% of W1 stage popul. adjusted**
3	minute bud	0.9	35.2	--
4	small bud	6.10	17.7	--
5	medium bud	21.8	17.4	34.4
6	large bud	24.9	13.1	25.9
7	short tube	21.8	7.0	13.8
8	medium tube	9.6	3.4	6.7
9	long tube	10.5	2.6	5.2
10	pre-flower	4.4	3.6	7.6
total		229	133.6	67.6

* The percent distribution of flowers in the flower groups 3 to 10 is calculated according to the total flower number from group 3 to 10. Flowers of total collection of non-force abscission are 229. In W1 stage inflorescence this flowers from flower groups 3 to 10 are 133.6.

** The percent distribution of W1 stage population adjusted is based on the assumption that all flowers between flower group 5 to10 are abscised, but the flowers of group 3 and group 4 are partially abscised.Thus the possible non-abscised flowers of group 3 and group 4 are deducted from the total. The adjusted total flowers are 67.6, all of them from flower group 3 to 10, and all might abscise as that of the non-force abscission.

Table 17. Effect of ethylene on inflorescence size

Exp. plant*	No. of flowers abscised at F1 stage	No. of scars at W3reg stage	No. of fruits set at W3reg stage
A	12	35	146
B	14	19	120
C	34	42	113
D	29	40	117
Average	22	34	124

* Four plants were tested, they are labeled A, B, C, D. Ethylene treatment of six hours was applied to these plants when at F1 stage. Flowers abscised were collected after the ethylene treatment. After the ethylene treatment when the next flower blooming was recorded as F1reg stage, and three weeks after F1reg stage is W3reg stage when the inflorescences were recorded by using the fruiting stage formula with special attention to the scars of abscised flowers.

Table 18. Ethylene* induced abscission on the intact F1 stage inflorescence

Flower group		Percent of abscission
12	fruit	N/A
11	flower	100%
10	pre-flower	100%
9	long tube	100%
8	medium tube	100%
7	short tube	100%
6	large bud	56%
5	medium bud	20%
4 and smaller	small bud and smaller	0%

* Ethylene was injected to a plastic bag which covered the inflorescence. The plastic bag was removed after six hours and abscised flowers were collected and measured. The remaining flowers on the inflorescence were evaluated (group 6 and group 5). No flower from group 4 and smaller abscised.

Table 19. Effect of plant hormones* on inflorescence development

	Total fruit set (group 7 -12)	% of fruits (group 12)	% of flowers (group 7-11)
Control (n=6)	96.6	70.8%	29.2%
IAA (n=6)	111.8	66.6%	33.5%
Kinetin (n=6)	92.9	84.2%	15.8%
IAA + Kinetin (n=6)	109.8	98.0%	2.0%
Ethylene (n=4)	124	98.2%	1.8%

* IAA (1μM), Kinetin (1μM), or IAA (1μM) + Kinetin (1μM) was sprayed on the F1 stage inflorescences. Ethylene was injected to plastic bags which wrapped the F1 inflorescences for six hours. The effects of IAA treatment, Kinetin treatment and IAA + Kinetin treatment were determined at the W3 stage. The effects of the ethylene treatment were determined at the W3reg stage.

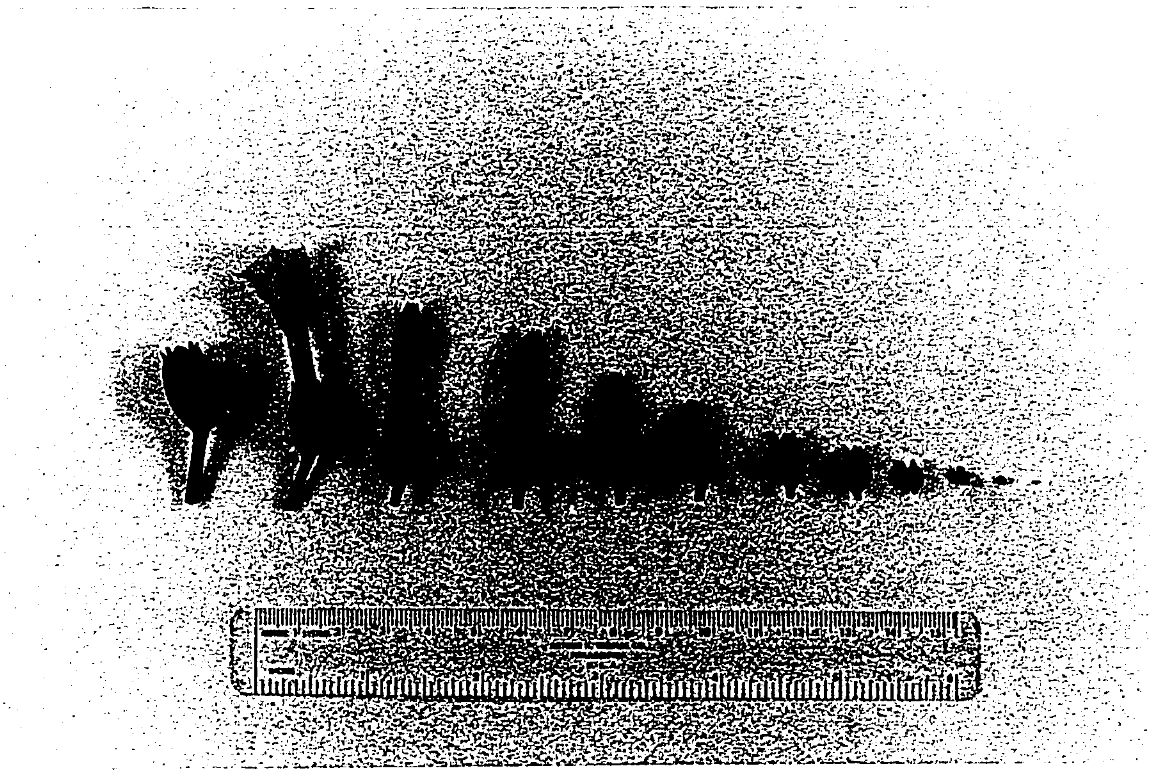


Fig. 1. Representatives of the 12 groups of tobacco flowers (See Table 1).

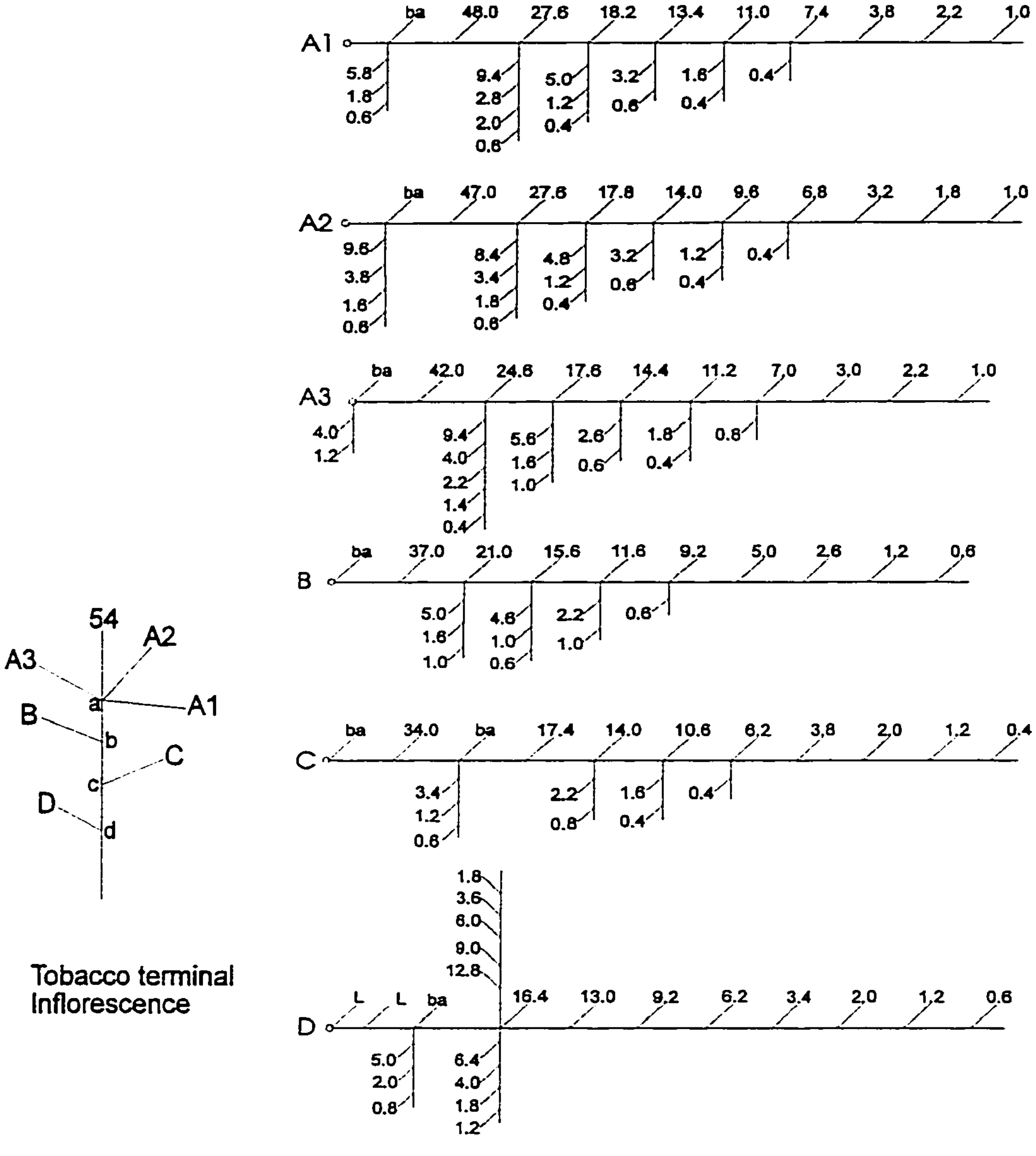


Fig. 2. The flowering inflorescence formula is used for recording the detail of F1 and W1 stages of tobacco inflorescences. This figure shows a typical F1 stage inflorescence. The left part shows the inflorescence coming from the four terminal nodes which are named "a", "b", "c", and "d". On the "a" node, there is a terminal flower which measured 54 mm in length and there are three sub-branches called "A1", "A2", "A3". "B", "C", "D" are three sub-branches from "b", "c", "d" nodes respectively. The right part shows the detail of the six sub-branches including the flower population and flower arrangement. Numbers are the size of each individual flower or floral bud in mm. ba: bract, L: leaf.

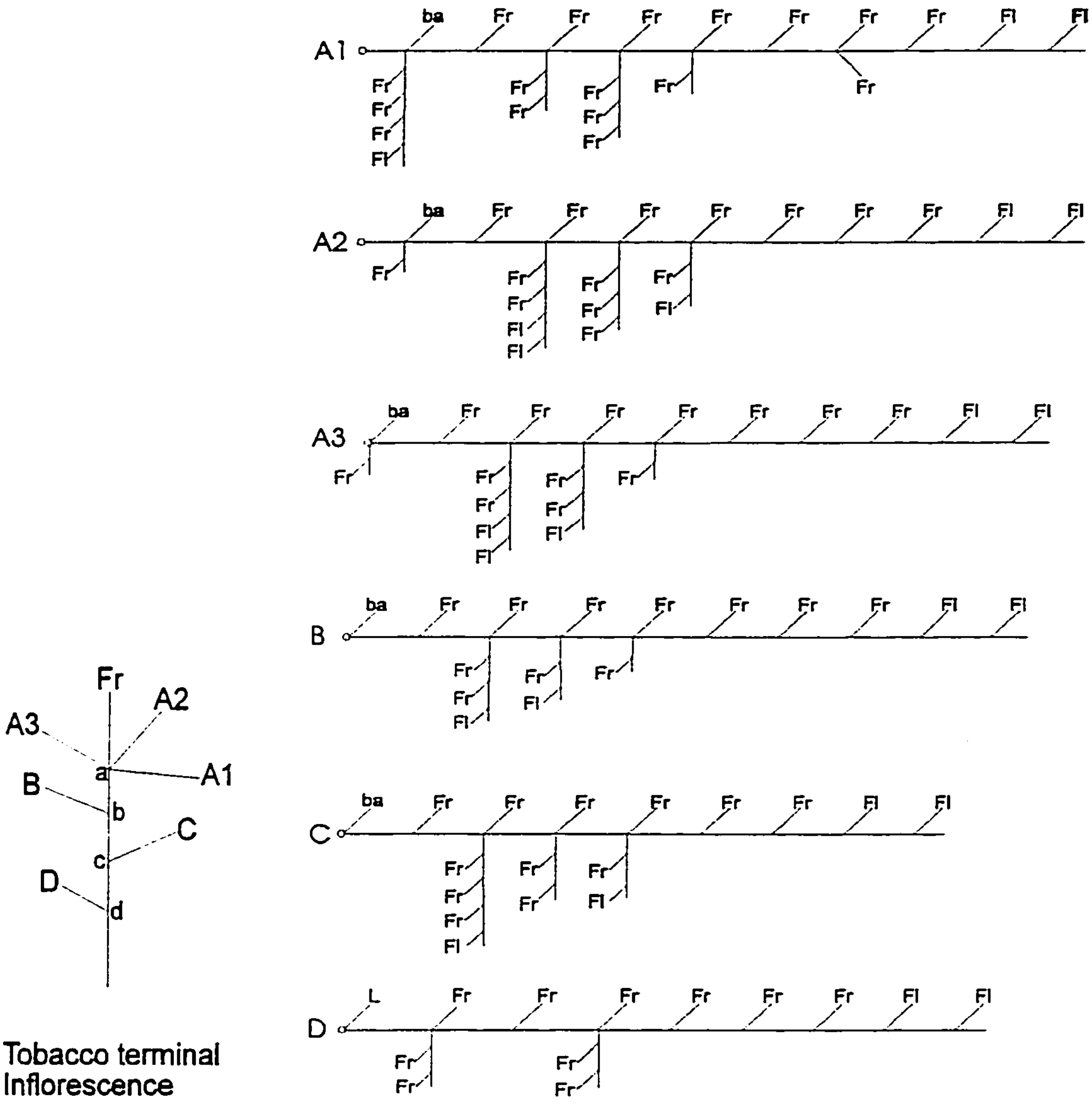


Fig. 3. The fruiting inflorescence formula is used for recording W2 and W3 stages of tobacco inflorescence. This figure shows a typical W3 stage inflorescence. The left part shows that only the terminal four nodes (a, b, c, d) are used. Fr is the terminal fruit. A1, A2, A3, B, C, D are the six subbranches from the four nodes. The right part shows the detail of the six subbranches including the fruit and flower population and arrangement. The Fr is stand for fruit (group 12); the Fl is stand for flower (including group 7 to group 11). Ba: bract, L: leaf.

]

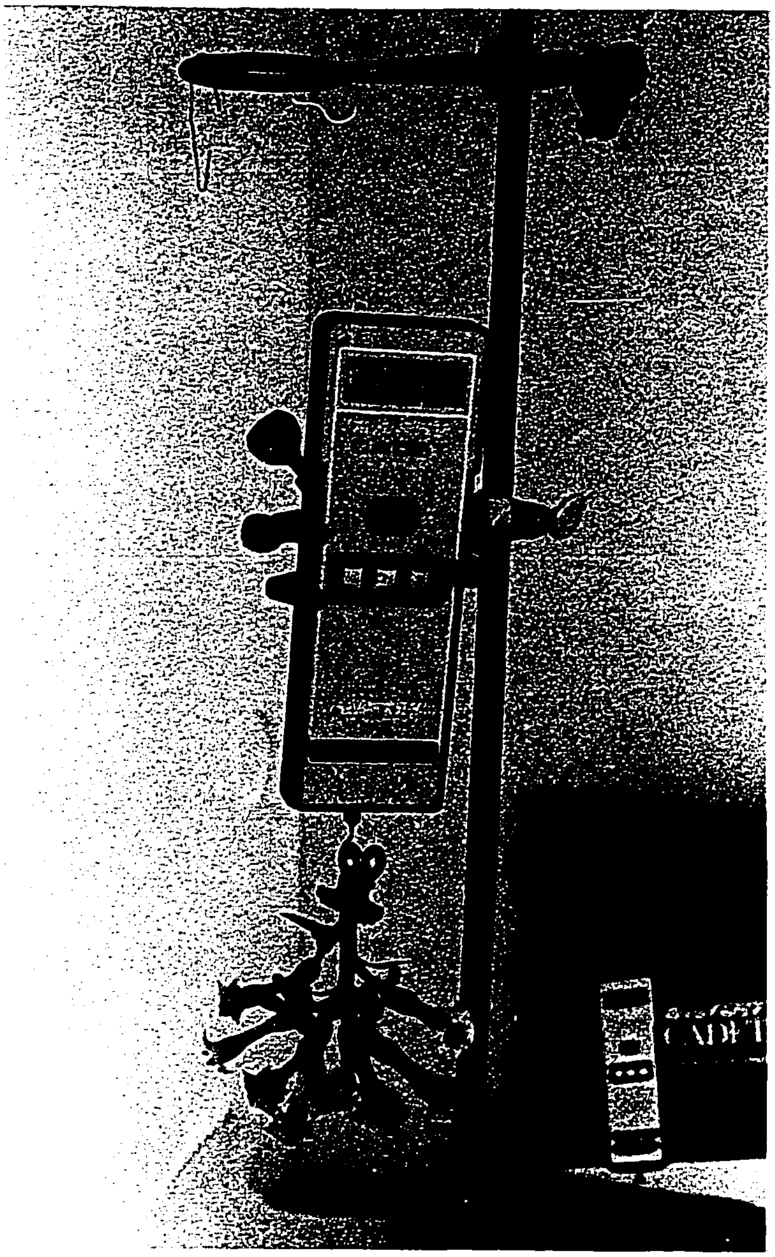


Fig. 4. An Accu Force Cadet digital force gage (Ametex) was used to measure the break strength during the induction of tobacco flower abscission. The photograph shows the force gage is fastened on a support stand. The tobacco inflorescence is fastened to the force gage by a clip. The break strength is then ready to be measured for each individual flower. The peak reading is automatically recorded (resolution of 0.1 gram). The “zero” key is pushed to reset the gage.

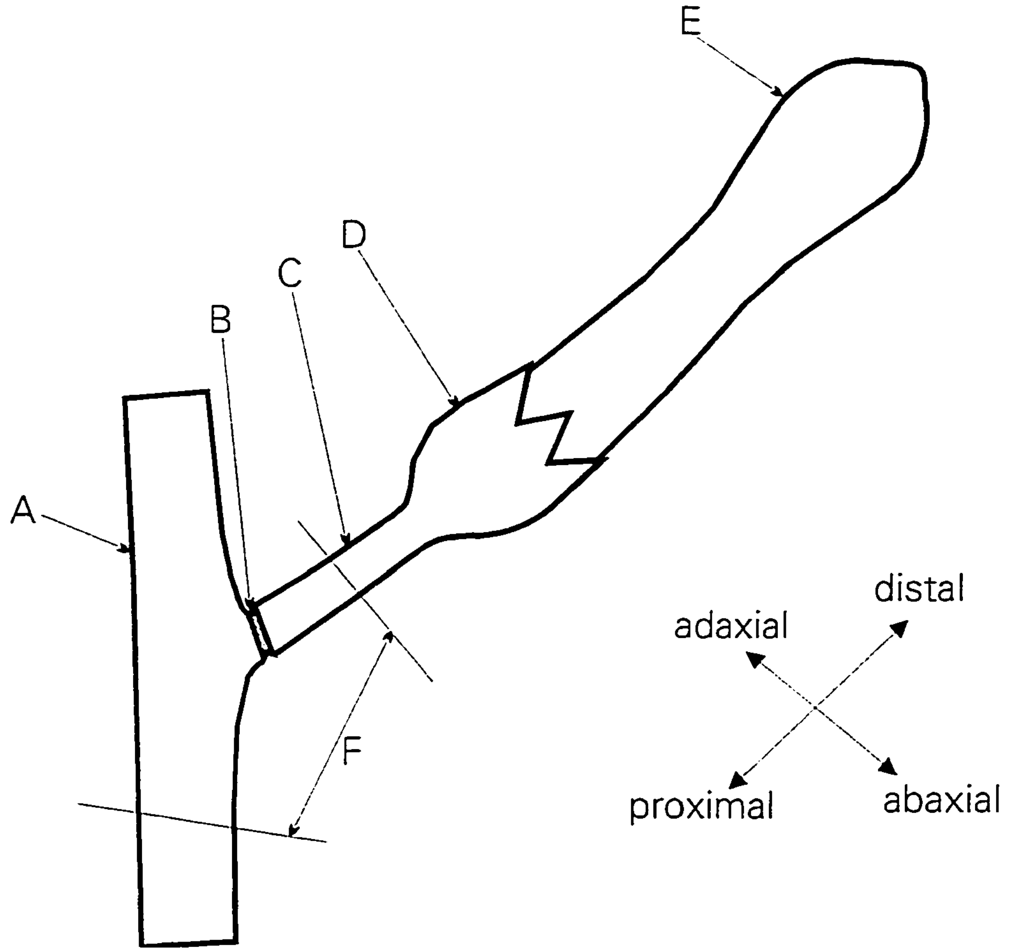


Fig. 5. A tobacco flower and terms for orientations. The orientations (adaxial, abaxial, distal, proximal) are in reference to the abscission zone (B) which is marked by the groove at the base of the pedicel of a flower. A: subbranch, B: abscission zone, C: pedicel, D: calyx, E: corolla tube, F: The "F-portion" which includes the abscission zone, part of the pedicel, and part of the internode of subbranch. A thin layer explant containing abscission zone was sliced off of the abaxial side of this "F-portion".

Fig. 6. The terminal inflorescence after ethylene treatment for six hours. The photograph shows that only fruits and very small flower buds stay on the inflorescence. Scars of abscised flowers and flower buds are seen on the nodes between the fruits and the small flower buds.

Fig. 7. Basal part of a tobacco plant showing two dry leaves hanging on the stem without abscission.

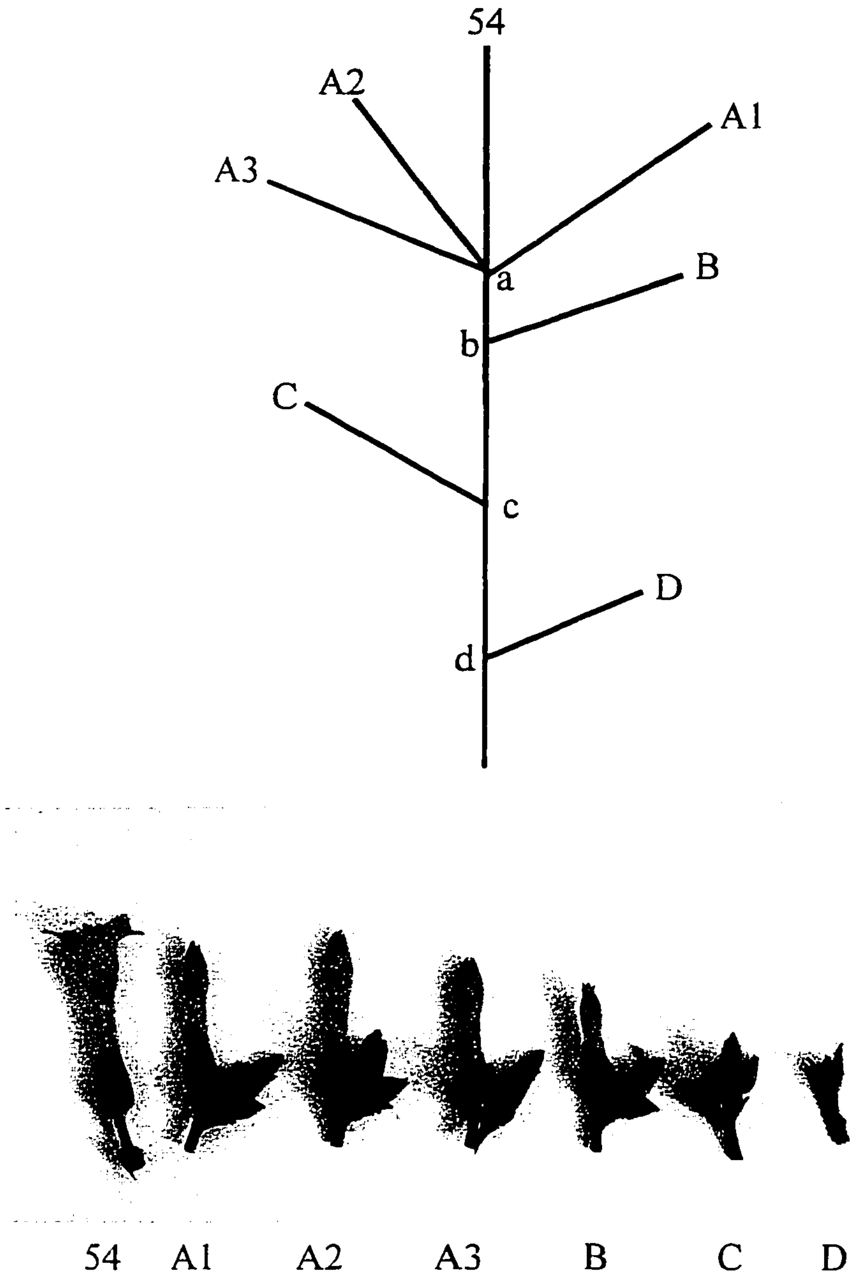


Fig. 8. A tobacco terminal inflorescence and subbranches. The top part illustrates that four terminal nodes (a,b,c,d) were studied. The terminal flower is a group 11 flower in length of 54 mm. The six sub-branches are named after the node from which they developed as A1, A2, A3, B, C, D. The terminal node "a" has three subbranches and one terminal flower, it is a typical trichasium. The photograph at the bottom shows the terminal flower (54) and six subbranches (A1, A2, A3, B, C, D) dissected from an F1 stage inflorescence.

Fig. 9. An F1 stage inflorescence of the tobacco plant (photograph).

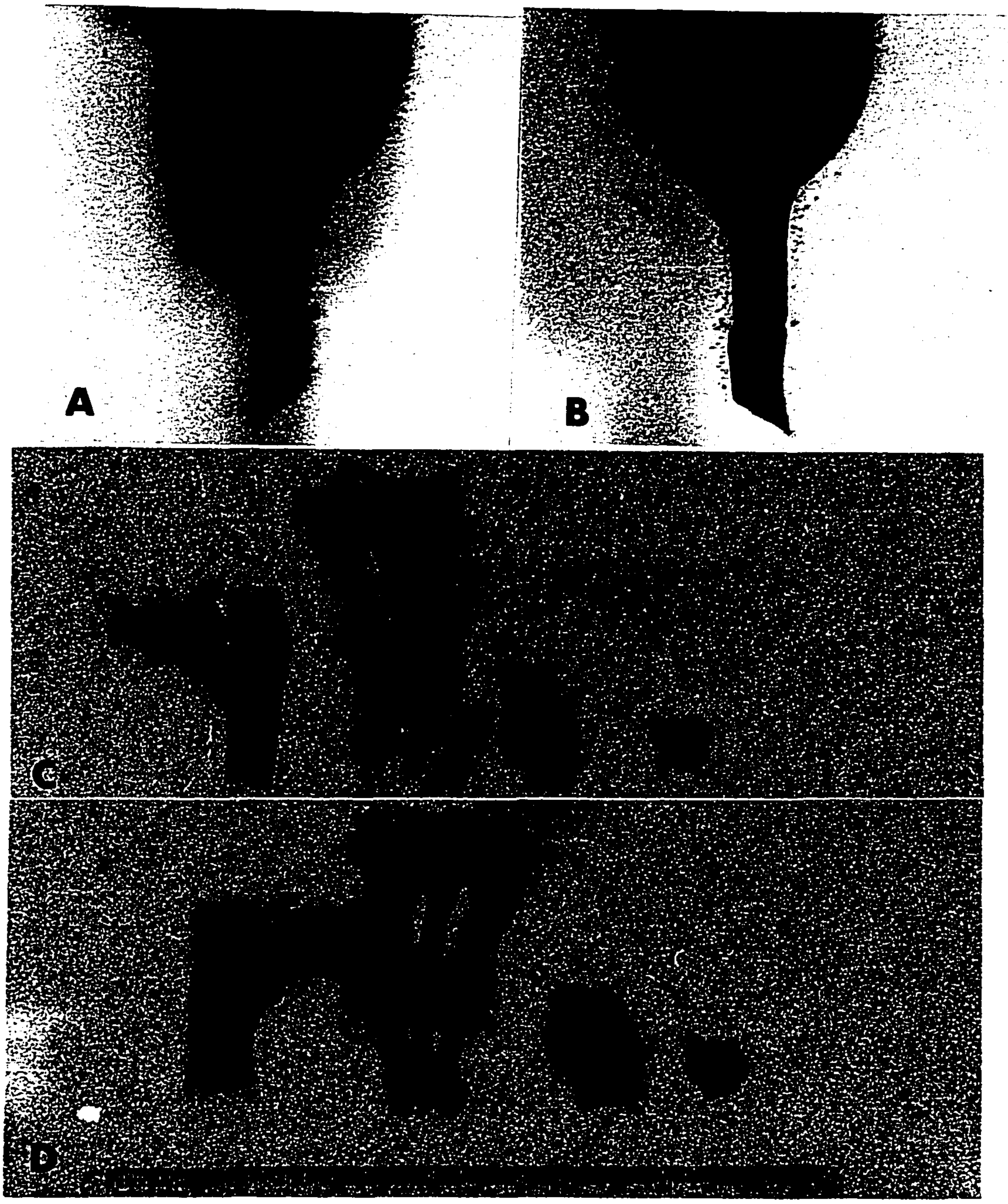


Fig. 10. The groove "marker" of the abscission zone at the base of the pedicels of flowers. A and B are the outside view and the inside view of the abscission zone of a fully developed fruit (group 12, size at 65mm). C and D are the inside view and the outside view of several flowers from different groups (from left to right: group 12, group 11, group 8 and group 5).

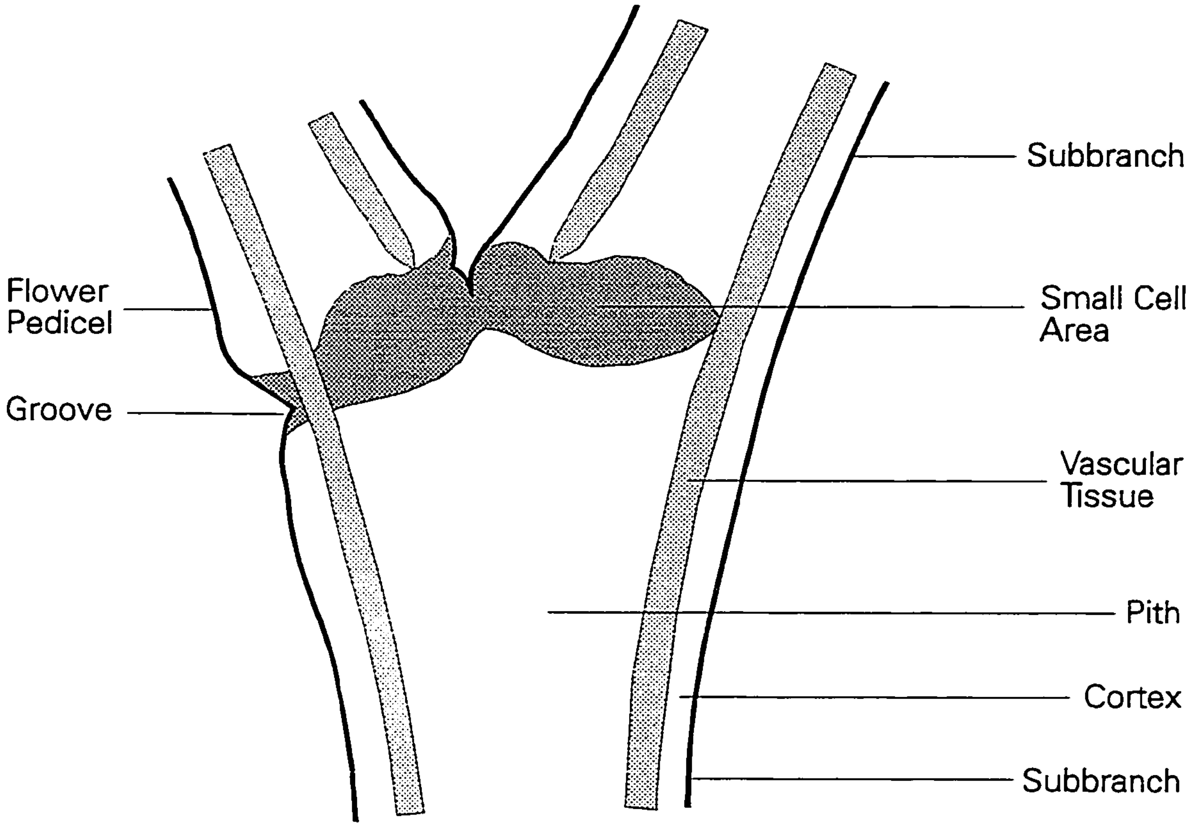


Fig. 11. Small cell area and vascular tissue around the abscission zone. The figure also shows the relationship among the pedicel and its up and down subbranches.

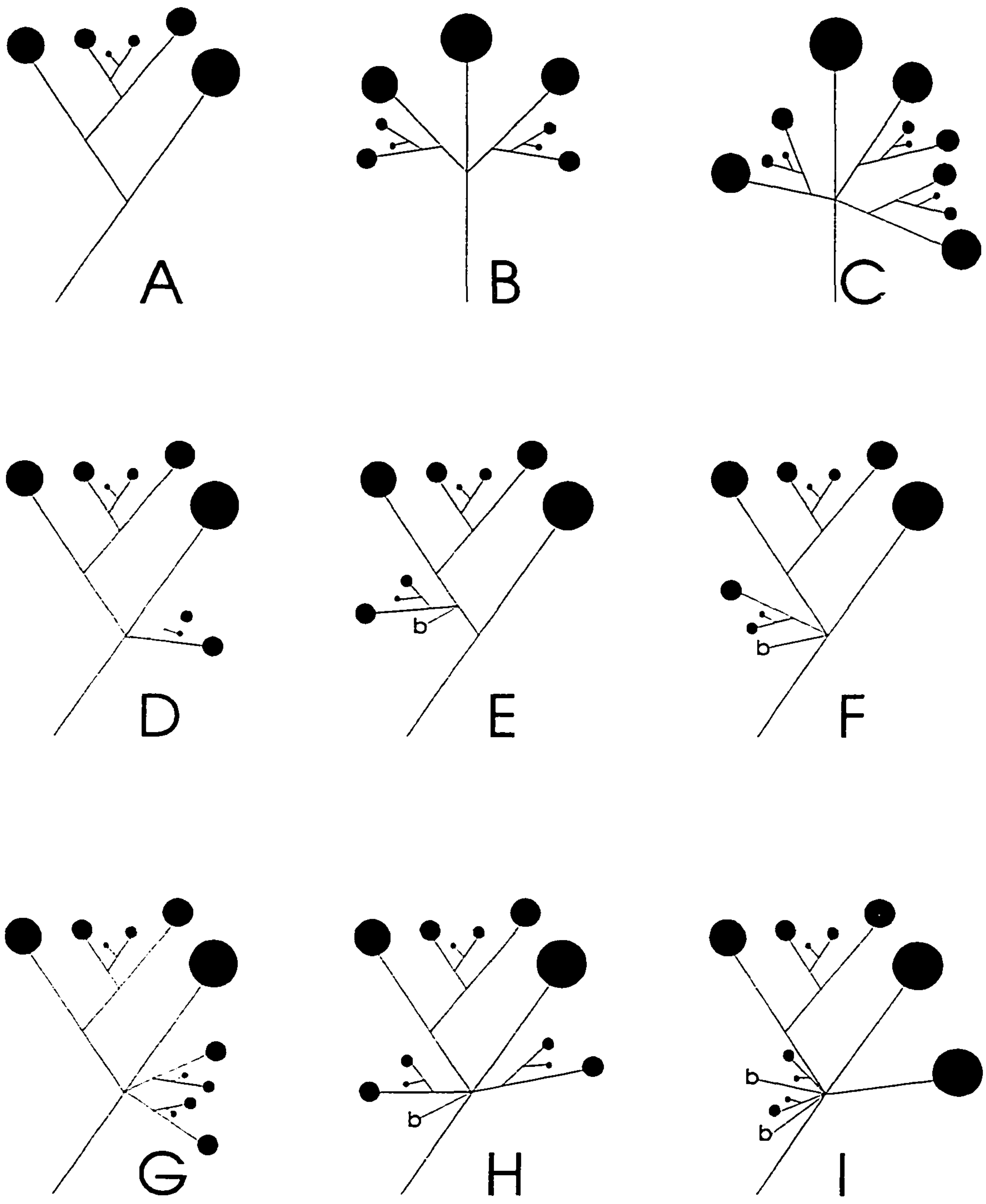
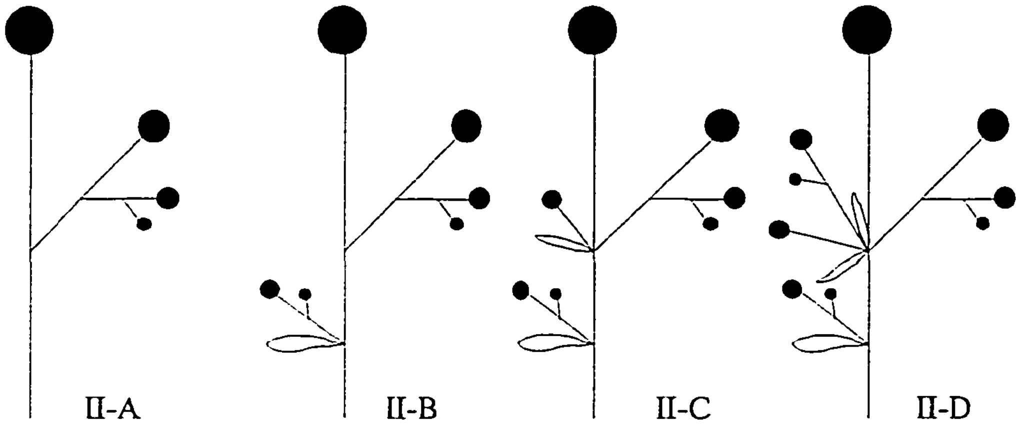
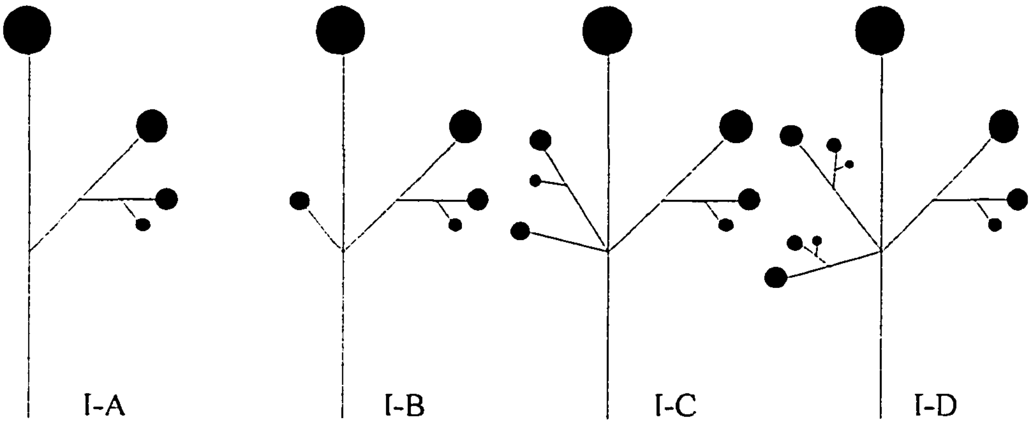


Fig. 12. Major branch types found in the tobacco inflorescence.
A: Monochasium, B: Equal dichasium, C: Equal trichasium, D: Unequal dichasium, E: Bract-monochasium, F: Bract-unequal dichasium, G: Unequal trichasium, H: Bract-unequal trichasium, I: Two-AZ trichasium, b: bract.



Routine II

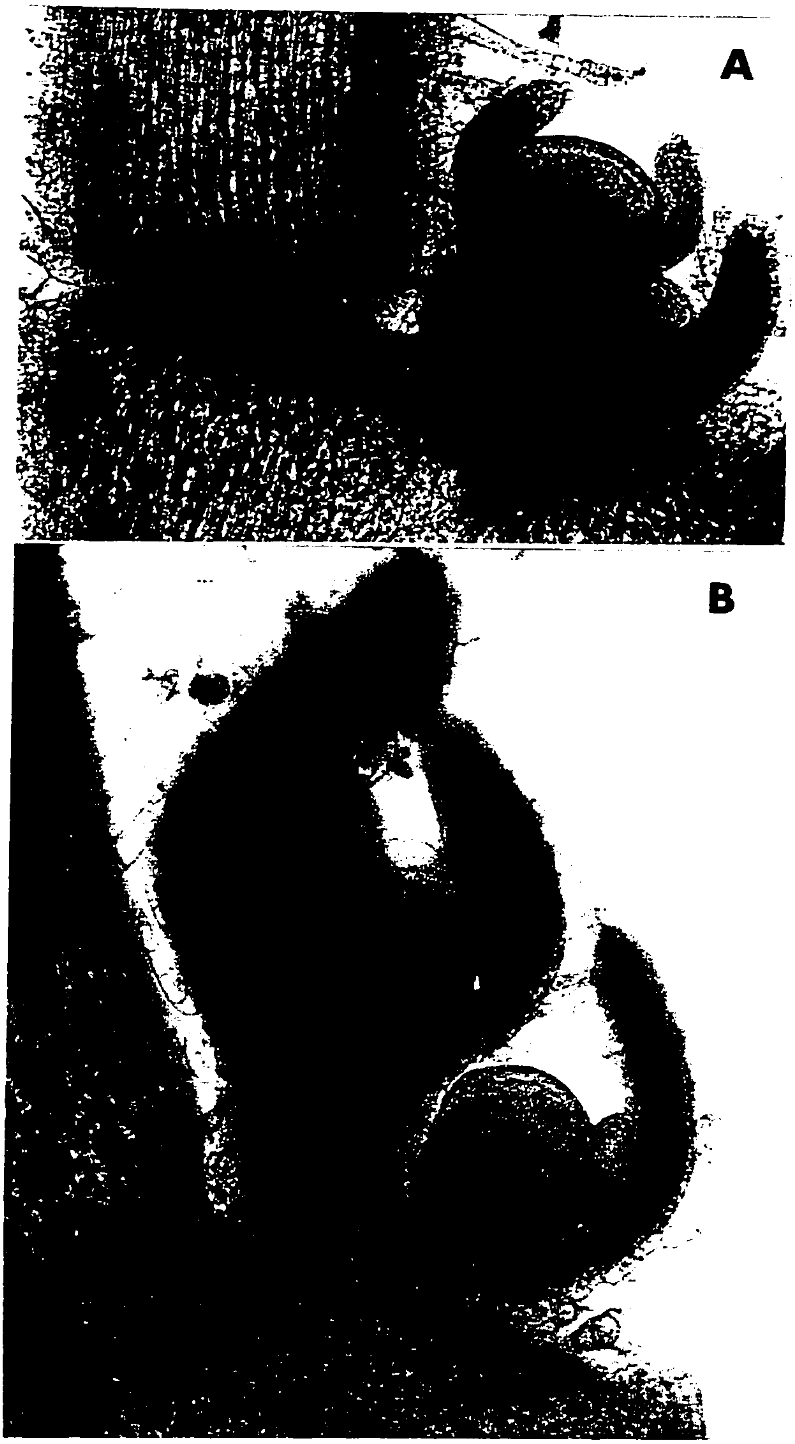


Routine I

Fig. 13. The dynamic changes of branch types in the tobacco inflorescence.
Routine I illustrates the changes of branch types at an abscission zone of a flower, the branch types are intended to develop from a monochasium (I-A) to an unequal dichasium (I-B) and to an unequal trichasium (I-C). It may develop to an equal trichasium (I-D). Routine II illustrates the bract involved in flower bud formation at an abscission zone of a flower (II-C, II-D) or at internode of the subbranch (II-B).

Fig. 14. Some typical branch types of the tobacco inflorescence. A: the equal trichasium type on the terminal node, B: unequal dichasium type, C: a fixed monochasium type at the third node of this subbranch, D: The two-AZ trichasium type from upper portion of many subbranches.

Fig. 15. Photographs of free-hand sections showing "micro bud" and "tiny bud" of flowers. The top one shows longitudinal section of two micro flower buds (group 1). The larger flower bud is about 0.33 mm, has differentiated to flower body and pedicel. The smaller bud is only about 0.11 mm, it is only a seemingly disorganized group of primordial cells. Both of the flower buds have developed from the proximal side of the abscission zone of a medium bud (group 5). X 84, 1cm bar = 119 μm . The bottom photograph shows longitudinal sections of other two flower buds. The large flower bud is about 0.80 mm, belongs to tiny bud (group 2). It has a well differentiated flower body and pedicel. The abscission zone is not identified . The small bud is about 0.17 mm and belongs to micro bud (group 1). It develops from the base of the pedicel of the larger one. Its pedicel is not clear. X 120, 1 cm bar = 83 μm.


A
B

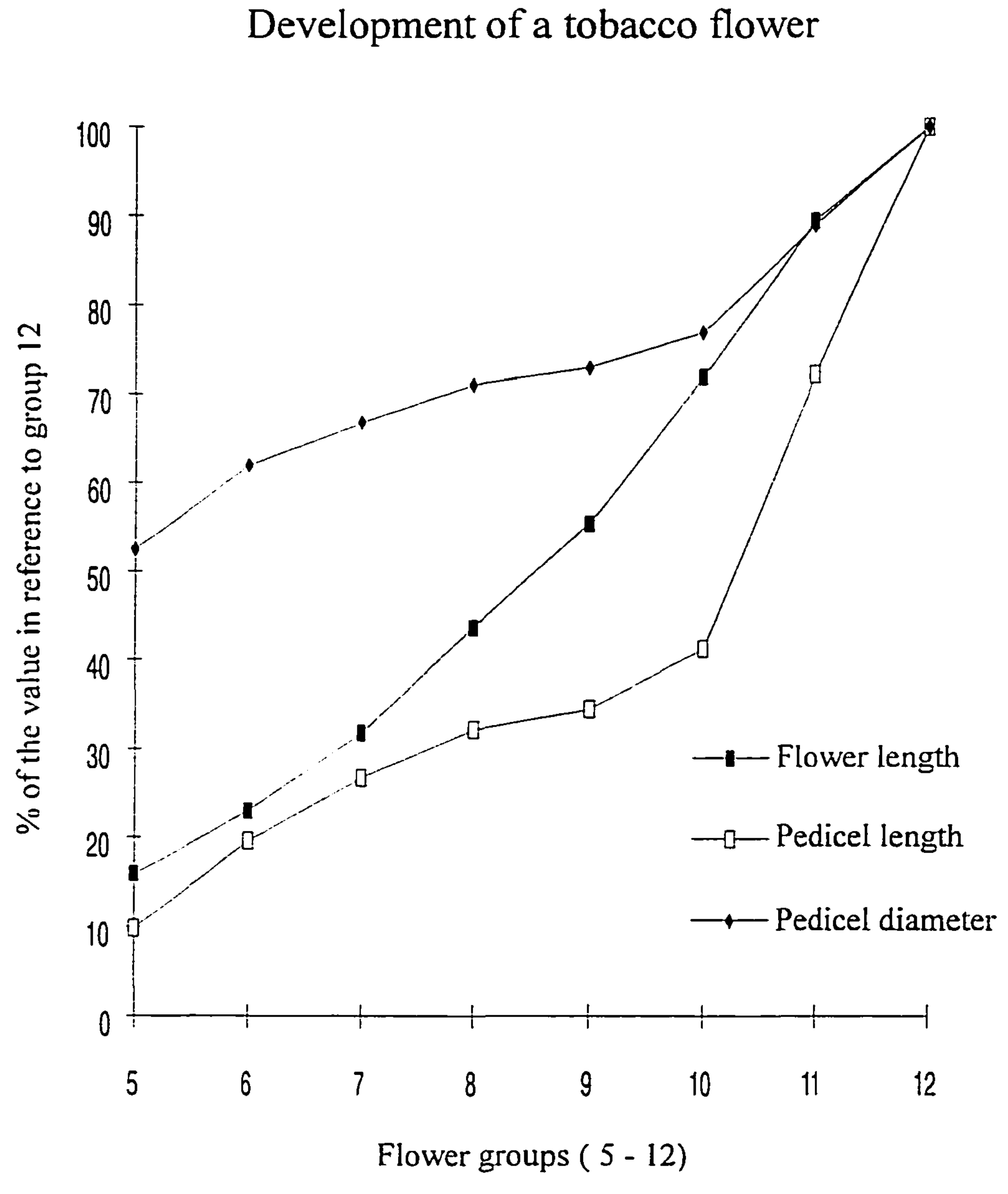


Fig. 16. Correlative development of flower length, pedicel length and pedicel diameter of tobacco flowers from group 5 to 12 (See Table 6). The graph is plotted in a percent scale in reference to the group 12 values which are flower length 62.7mm, pedicel length 12.7 mm and pedicel diameter 2049 μm. The figure shows that the flower length is the most linearly correlated to flower groups and both pedicel diameter and pedicel length increase slowly between group 6-9 and rapidly between group 10-12.

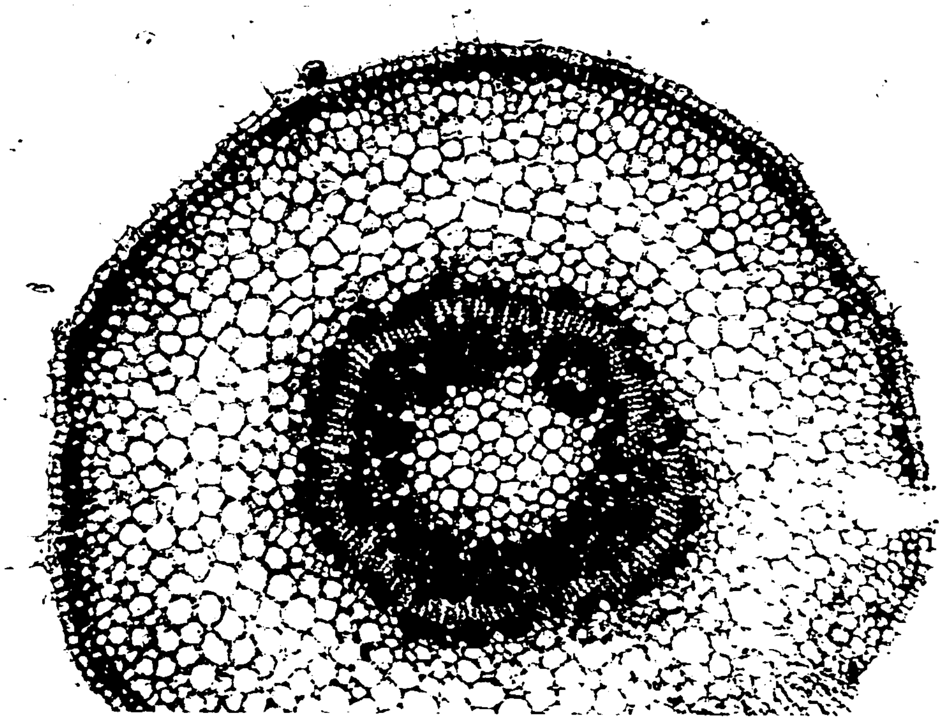


Fig. 17. Photograph of a free-hand section showing the anatomy of the pedicel of a group 5 flower bud. The photograph shows the circled vascular tissue with secondary growth (2-3 layers of secondary xylem vessels); the cortex has about 13 layers of parenchyma cells. X100, 1 cm bar = 100μm.

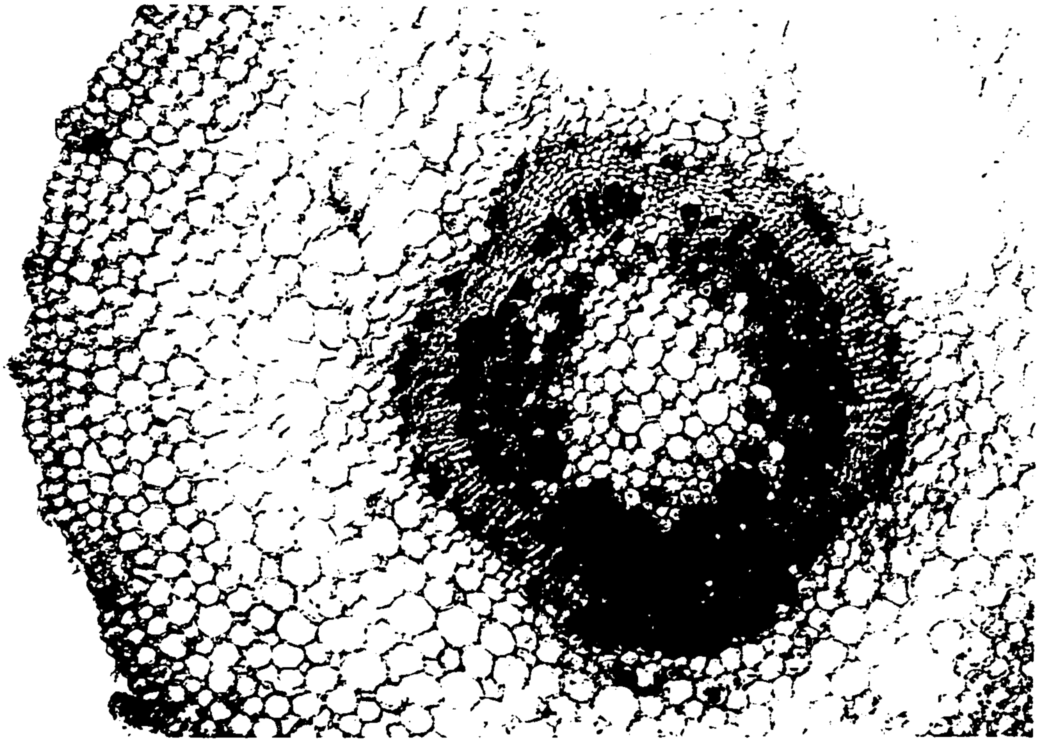


Fig. 18. Photograph of a free-hand section showing the anatomy of the pedicel of a group 11 flower. The photograph shows the circled vascular tissue with secondary growth (3-5 layers of secondary xylem vessels). The cortex has 13 layers of parenchyma cells. X100, 1 cm bar = 100μm.

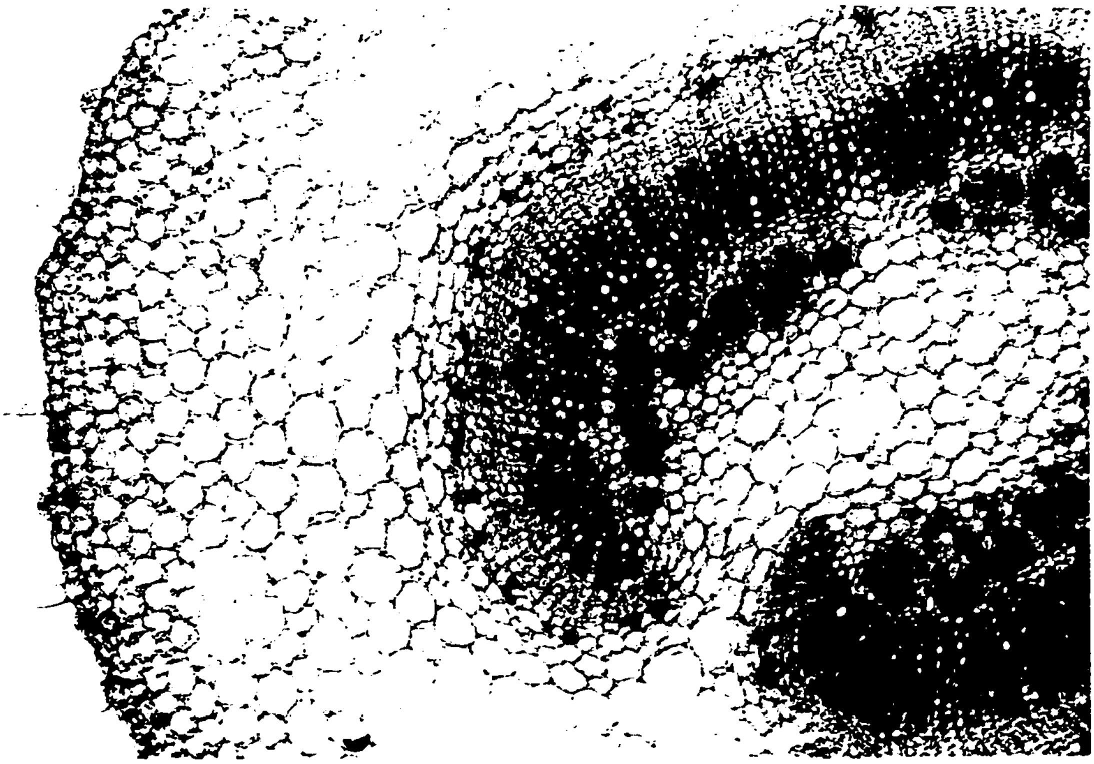


Fig. 19. Photograph of a free-hand section showing the anatomy of the abscission zone of a group 12 fruit. The photograph shows the vascular tissue has a break. The secondary xylem has 8-10 layers of vessels. The cortex has 13 layers of parenchyma cells. X100, 1 cm bar = 100μm.

Fig. 20. Photographs of four terminal inflorescence stages before the F1 stage.
A: 3 days after the appearance of foral bud. B: 7 days after the appearance of floral bud.
C: 9 days after the appearance of floral bud. D: 10 days after the appearance of floral bud.

Fig. 21. Photograph of a W2 stage tobacco inflorescence

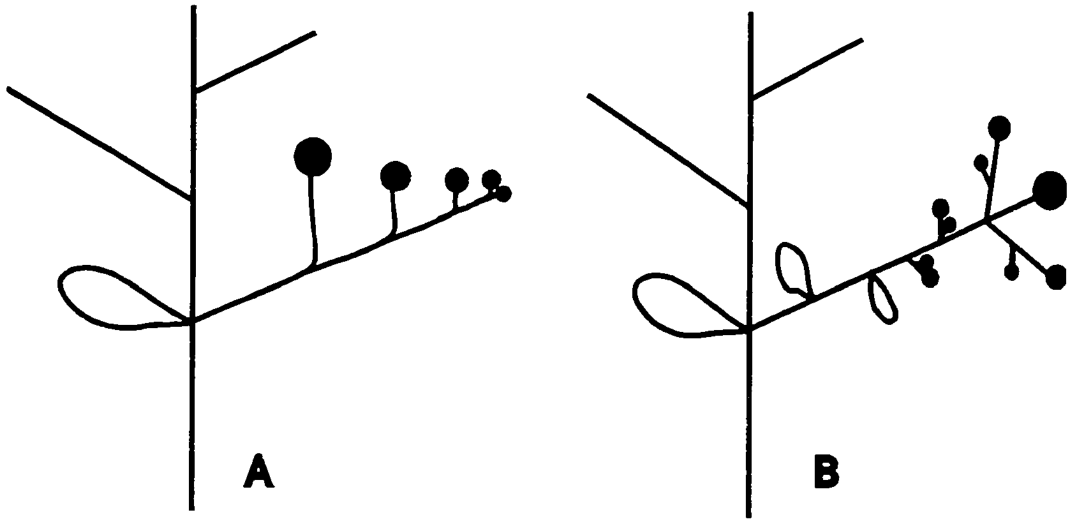


Fig. 22. A subbranch and a lateral inflorescence.
A: subbranch, B: lateral inflorescence

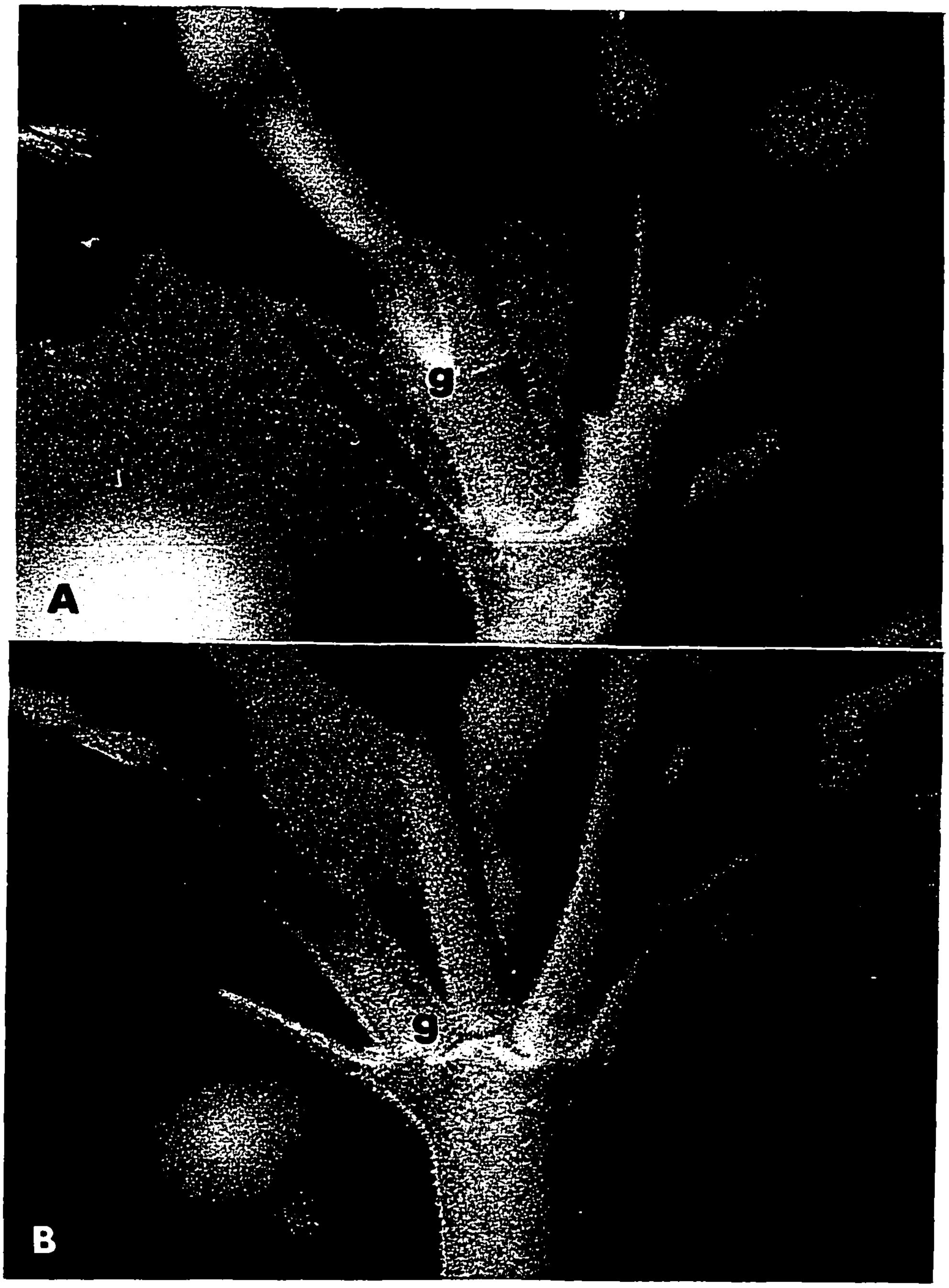


Fig. 23. Photographs of the visible abscission zones of tobacco flowers
A: a groove of a group 11 flower. B: a groove of the terminal fruit (group 12). g: groove

Fig. 24. An SEM photograph showing the overview of an abscission zone. X 400.

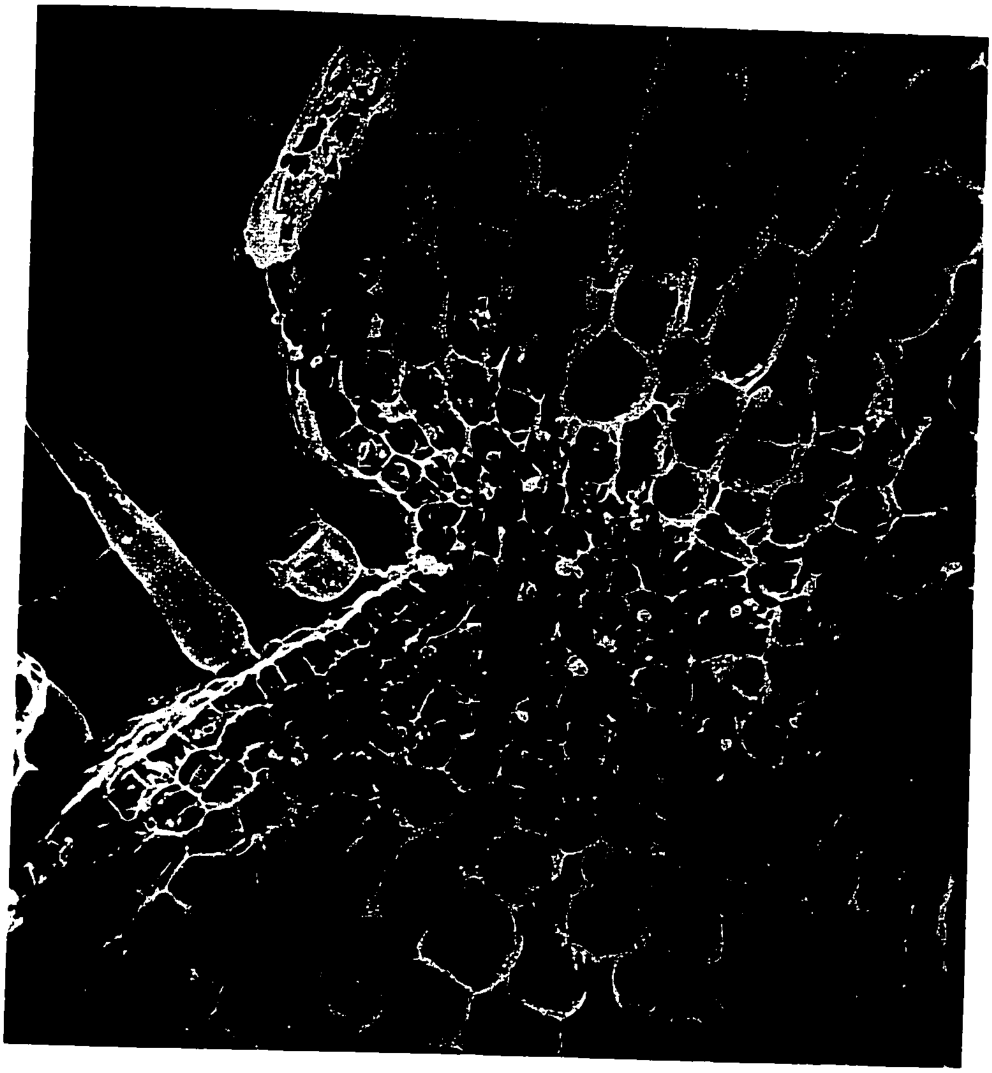


Fig. 25. An SEM photograph showing the cutting surface of an abscission zone X 400.

Fig. 26. An SEM photograph showing an angled-view of an abscission zone X 140.

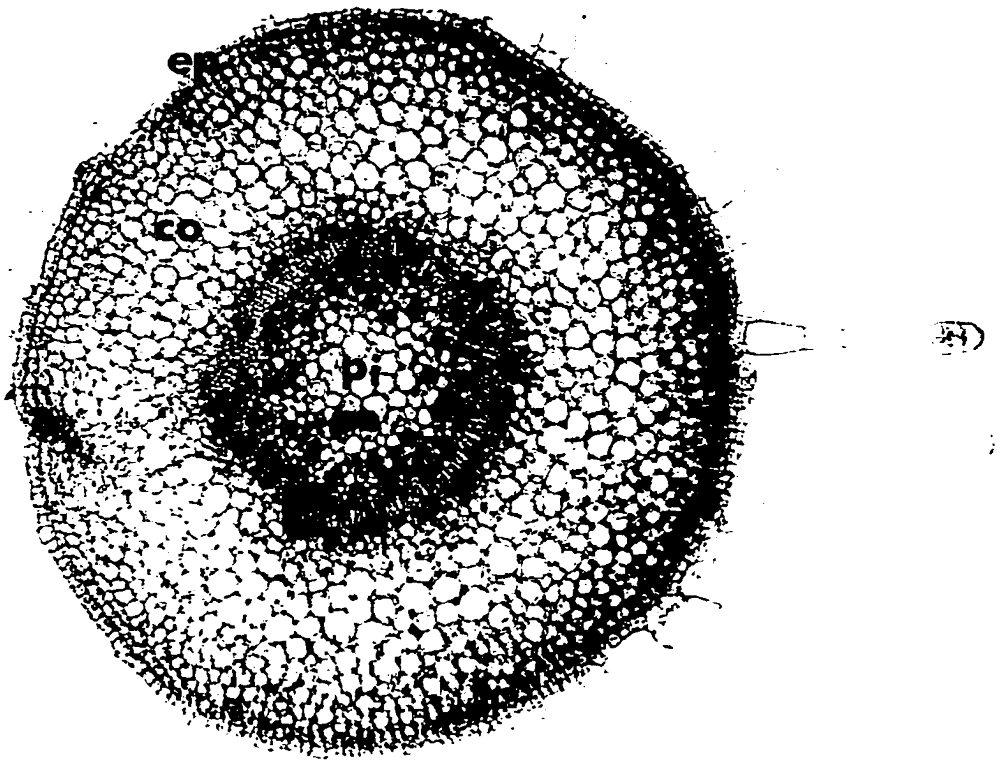


Fig. 27. Photograph of a f ree-hand section showing the anatomy of the pedicel of group 5 bud. ep: epidermis, co: cortex, vt: vascular tissue, pi: pith. X 100, 1 cm bar = 100μm

Fig. 28. Phtographs of semi-thin sections showing the abscission zone of group 9 buds. A: parallel axial section, B: perpendicular axial section. X 80, 1 cm bar = 125μm

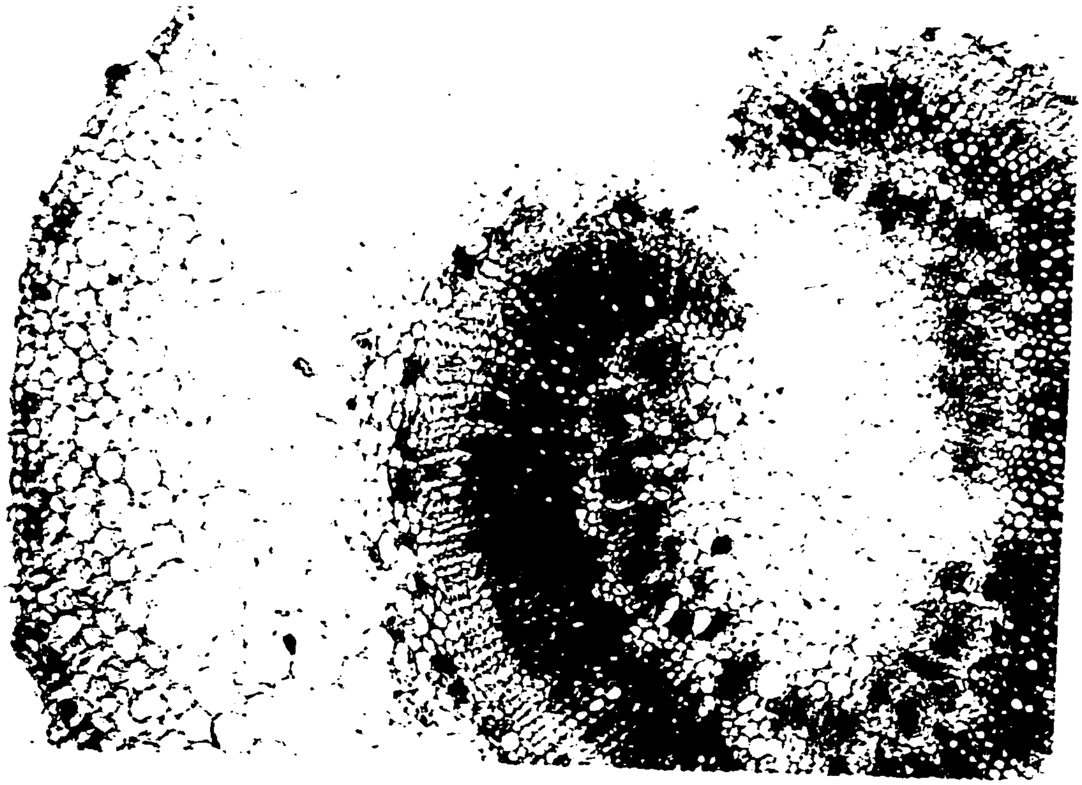


Fig. 29. Photograph of a free-hand section showing the anatomy of the abscission zone of a group 12 fruit. X 100, 1cm bar =100 μm.

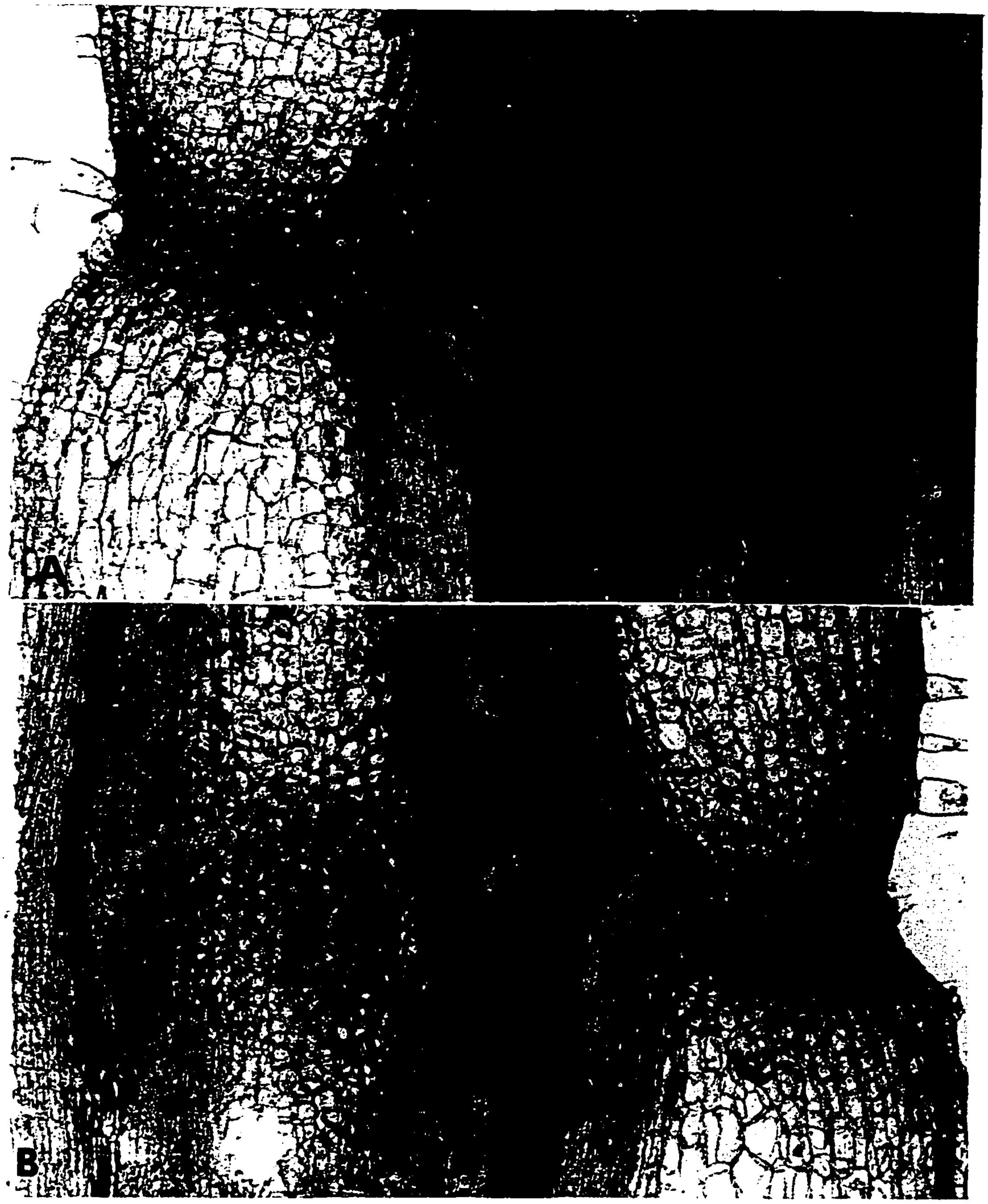


Fig. 30. Photographs of free-hand sections showing the irregular vascular tissue in the abscission zones of group 11 flowers. X 93, 1cm bar = 107 μm.

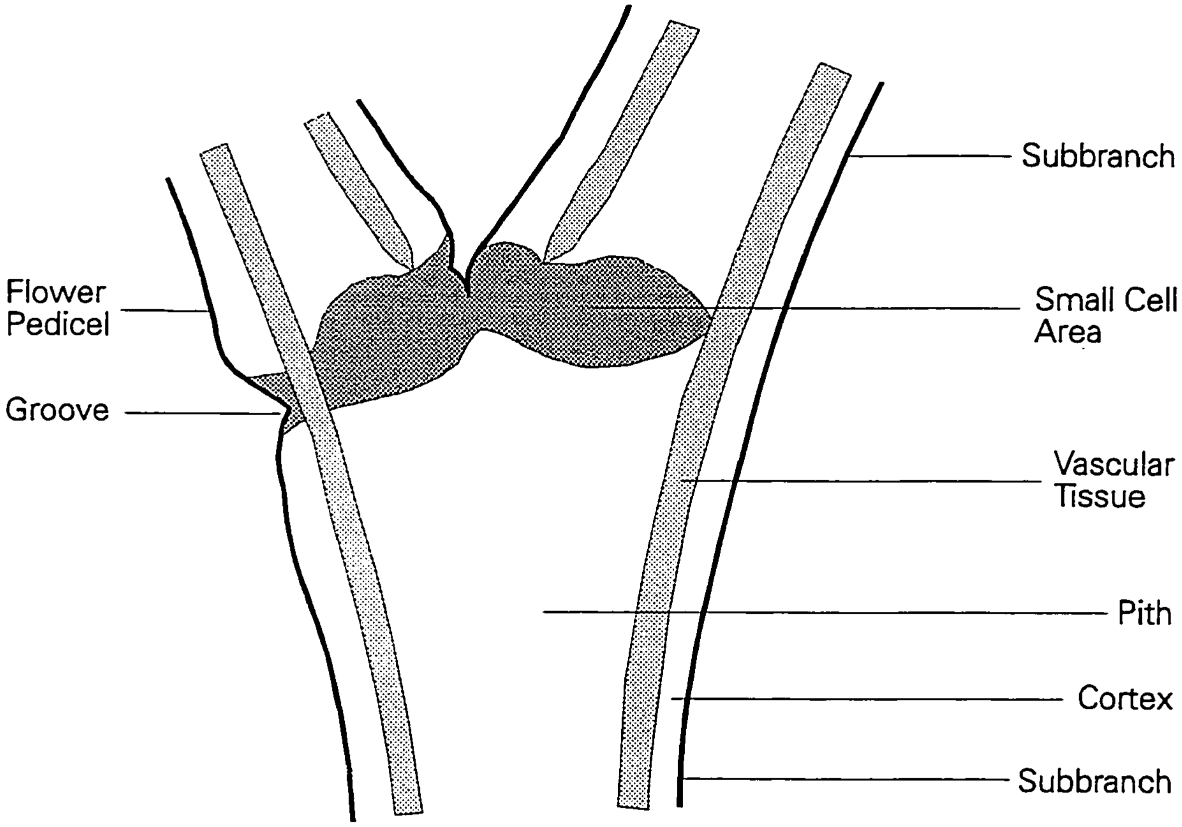


Fig. 31. Small cell area and vascular tissue around the abscission zone

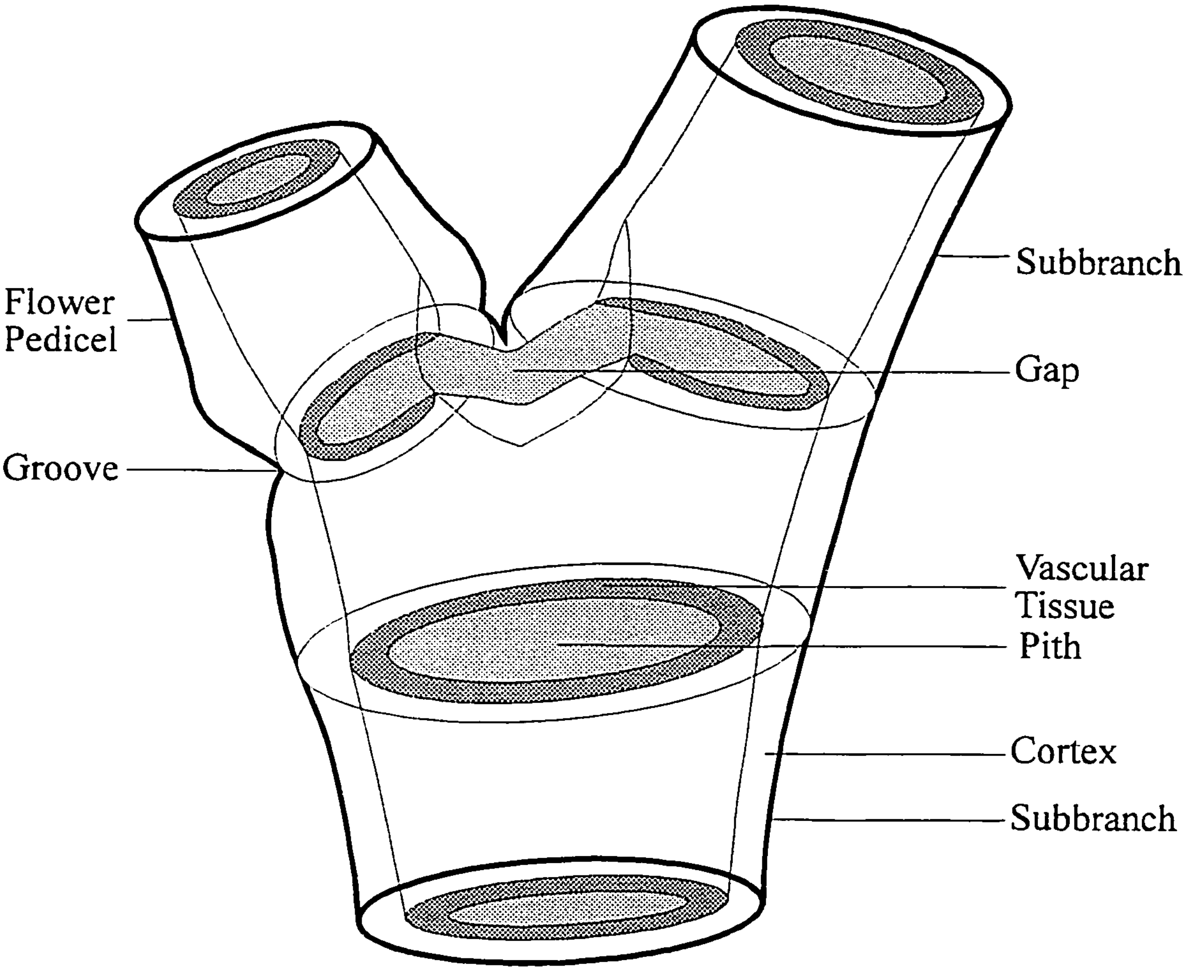
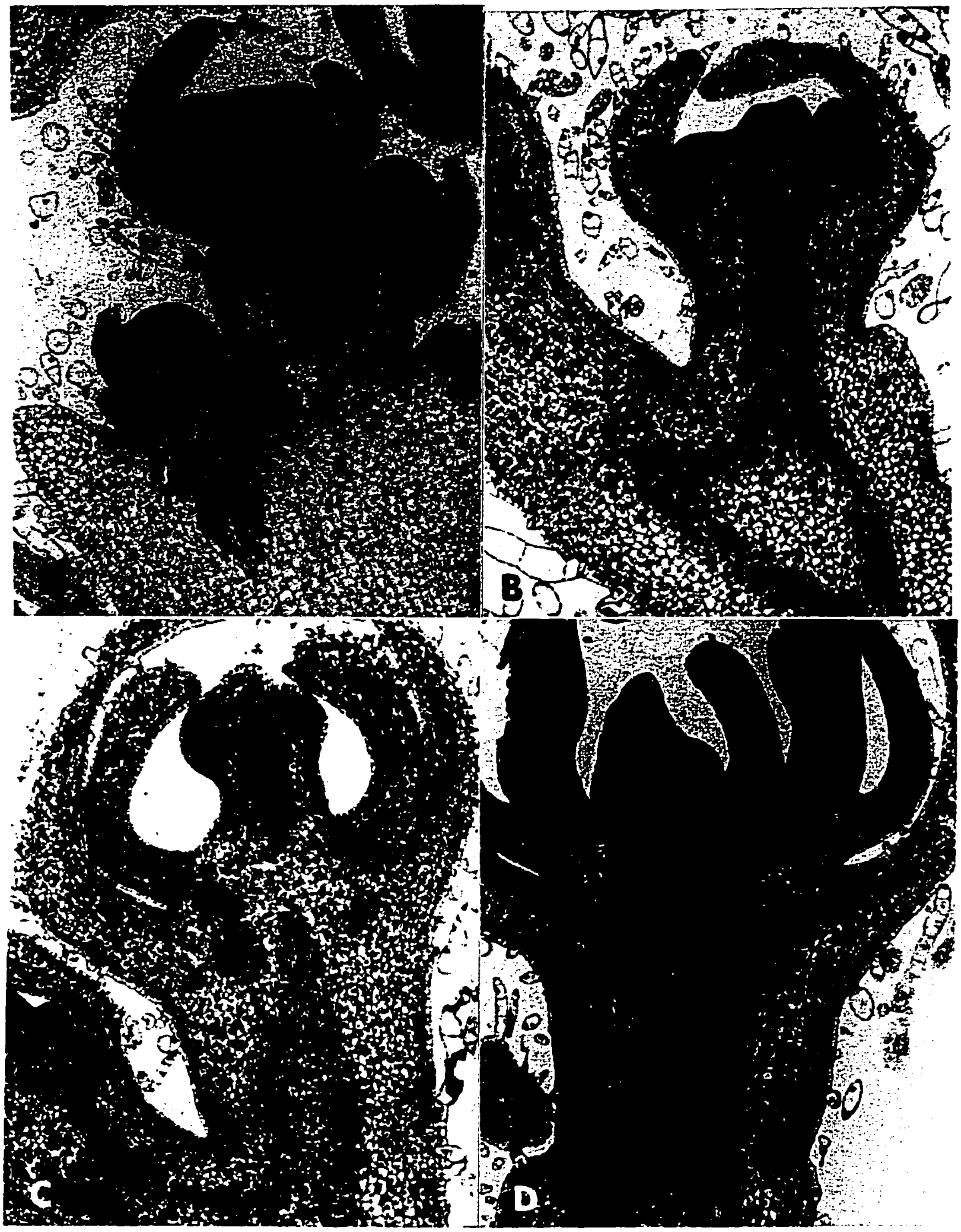


Fig. 32. Vascular system around the abscission zone of a tobacco flower. A gap free of vascular tissue exists between the abscission zone of the flower and the subbranch developed from the abscission zone.

Fig. 33. Photographs of semi-thin sections showing the development of micro buds (group 1) and tiny buds (group 2). A: Four micro buds (0.47mm, 0.22mm, 0.17mm, 0.10mm), none AZ recognizable. B: A 0.6 mm tiny bud, none AZ recognizable. C: A 1.2 mm tiny bud, the AZ is just start to be recognizable. D: A 1.8 mm tiny bud, the Az is clearly identified. X 73, 1cm bar = 138 μm.


B
C
D

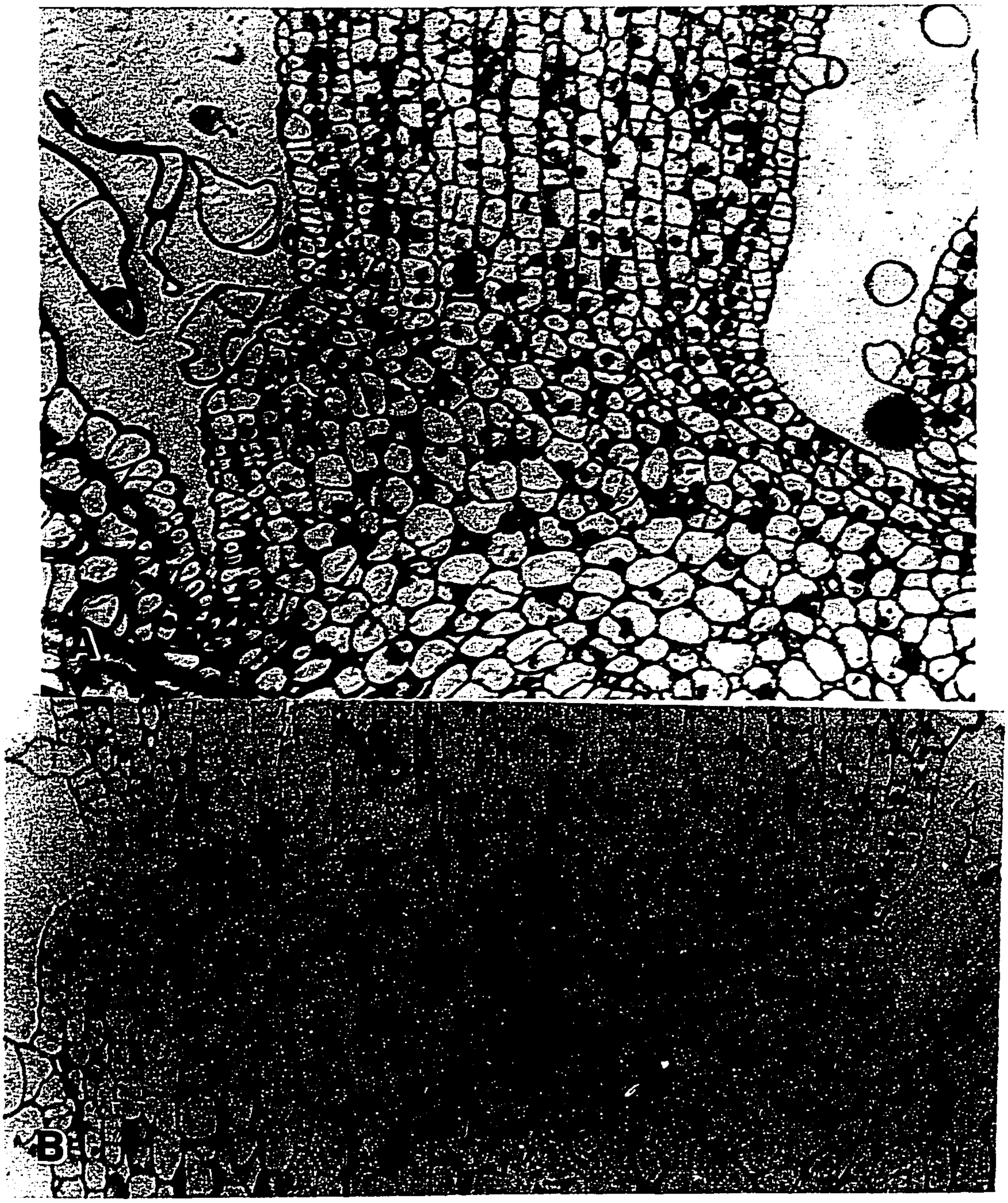


Fig. 34. Photographs of semi-thin sections showing the differentiation of the abscission zone in “tiny” and “minute” buds. A: the 0.95 mm long bud without a clear differentiated abscission zone, X 300, 1 cm bar = 33 μm. B: the 2.0 mm long bud with clear distinguishable groove and small cells area, X 260, 1 cm bar = 38 μm.

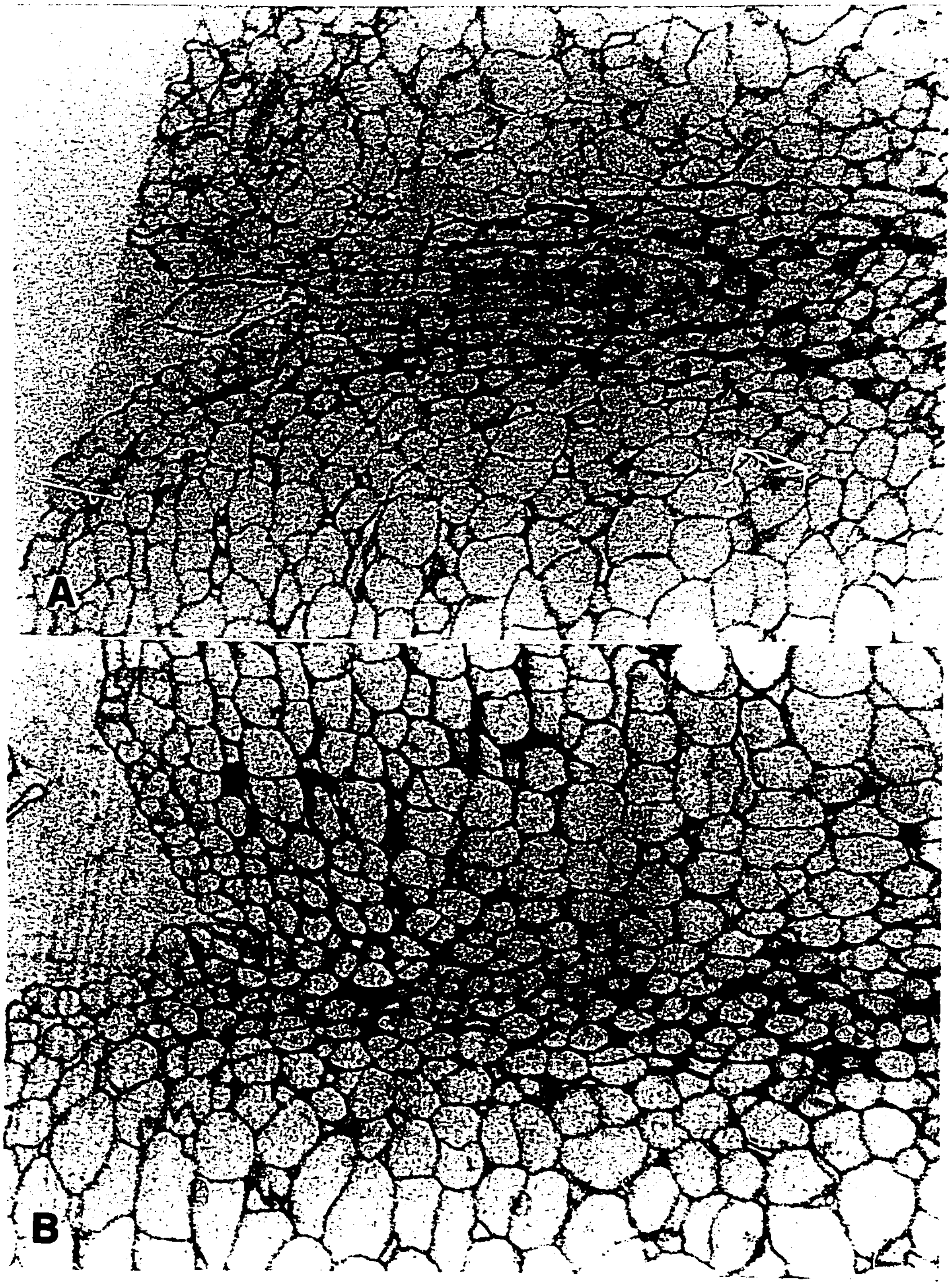


Fig. 35. Photographs of semi-thin sections showing the very differentiated abscission zones of group 11 flowers. A: the groove can extent 200 μm into the pedicel. B: the small cells are at both sides of the groove. Both X 225, 1cm bar = 44 μm.

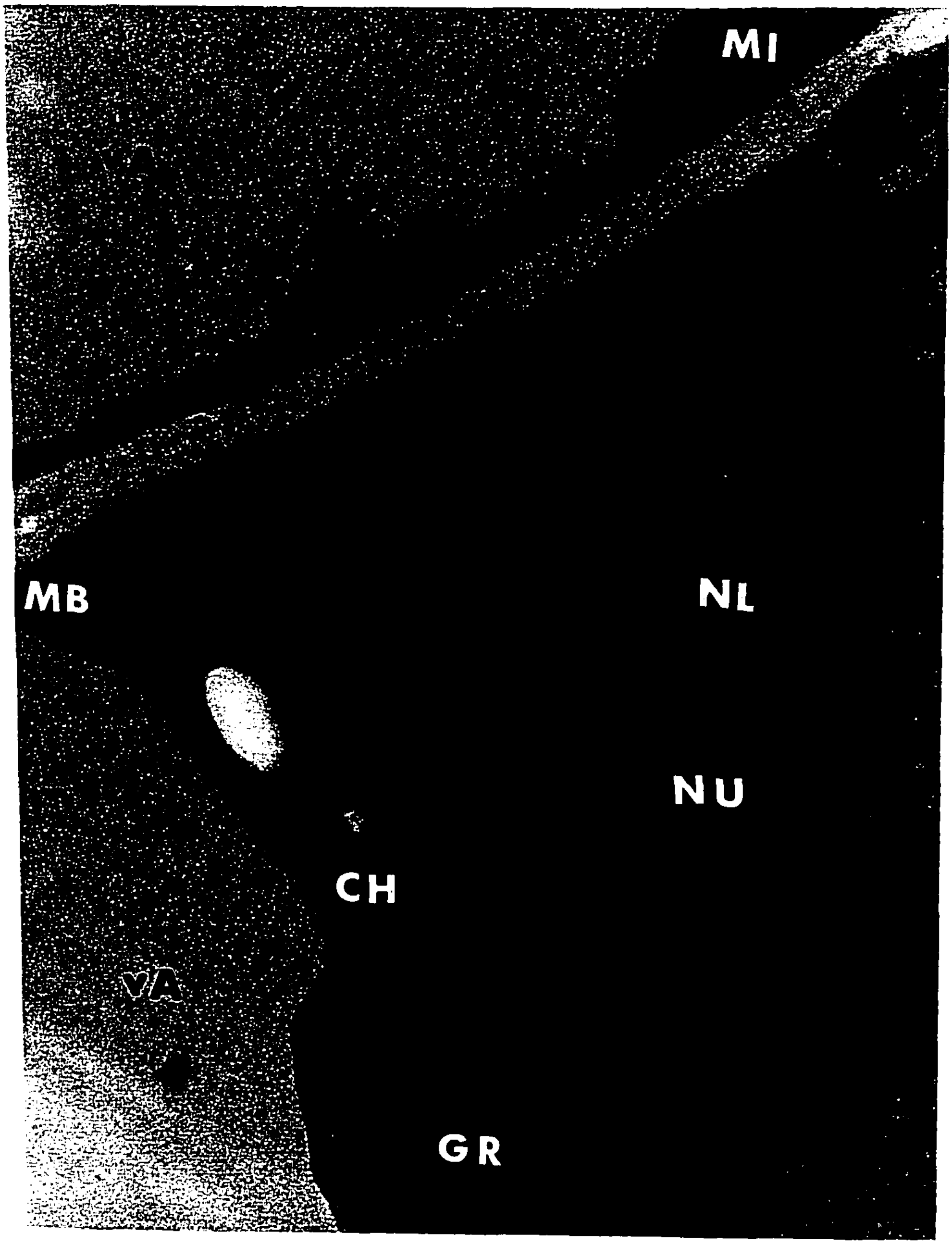


Fig. 36. A TEM photograph of control cortical tissue showing that the interphase nucleus has nucleolus and normal stain, the cell membrane is tightly contact with cell wall, the chloroplasts are normal and microbody has crystalloid core. CH:chloroplast, CW:cell wall, GR:granular component, MB: microbody, MI:mitochondrion, NL:nucleolus , NU:nucleus, VA:vacuole. X20,000

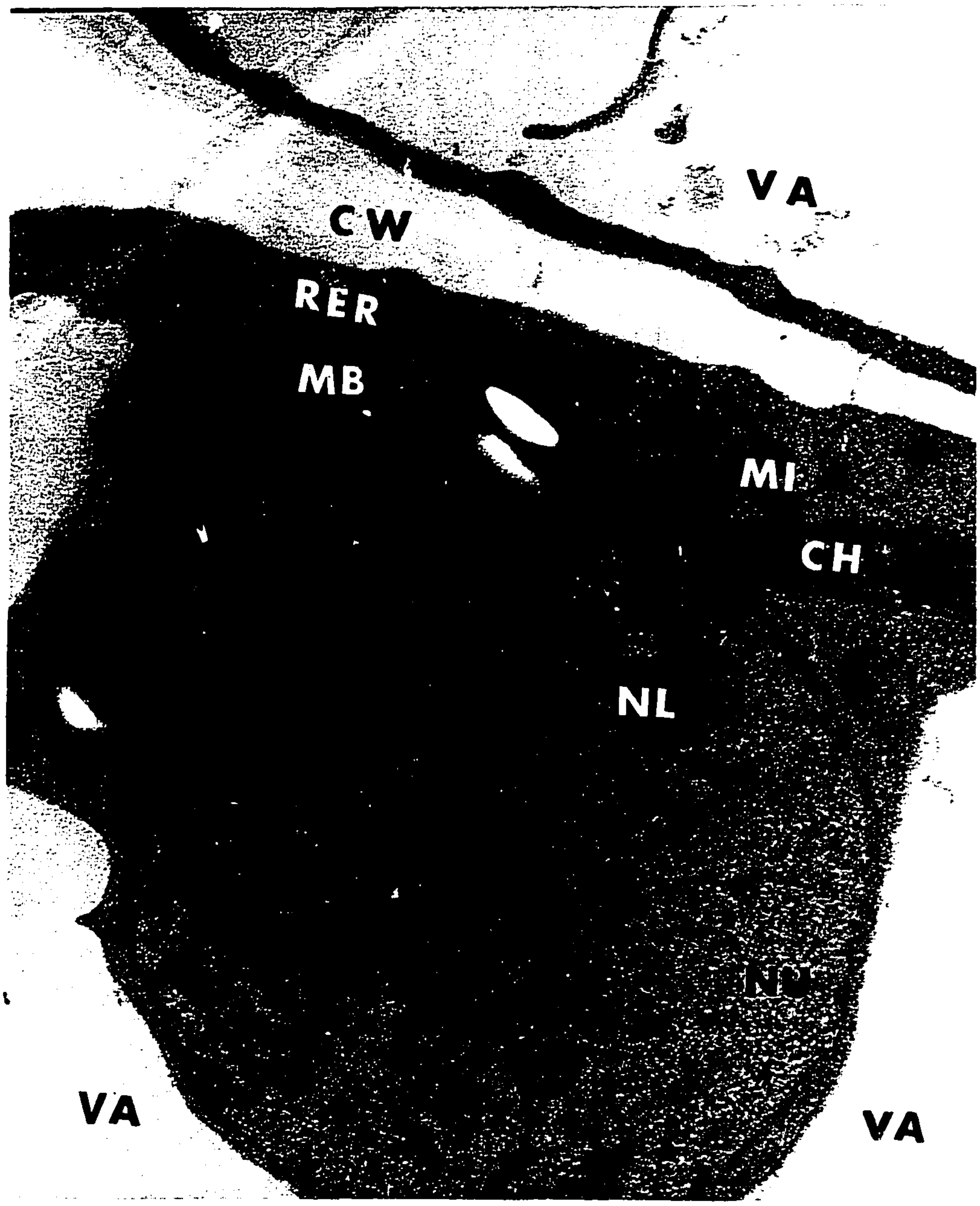


Fig. 37. A TEM photograph of control cortical tissue showing nucleus, chloroplasts, mitochondia, RER and microbody. CH:chloroplast, CW:cell wall, MB:microbody, MI:mitochondrion, NL:nucleolus, NU:nucleus, RER:rough endoplasmic reticulum, VA:vacuole. X16,000

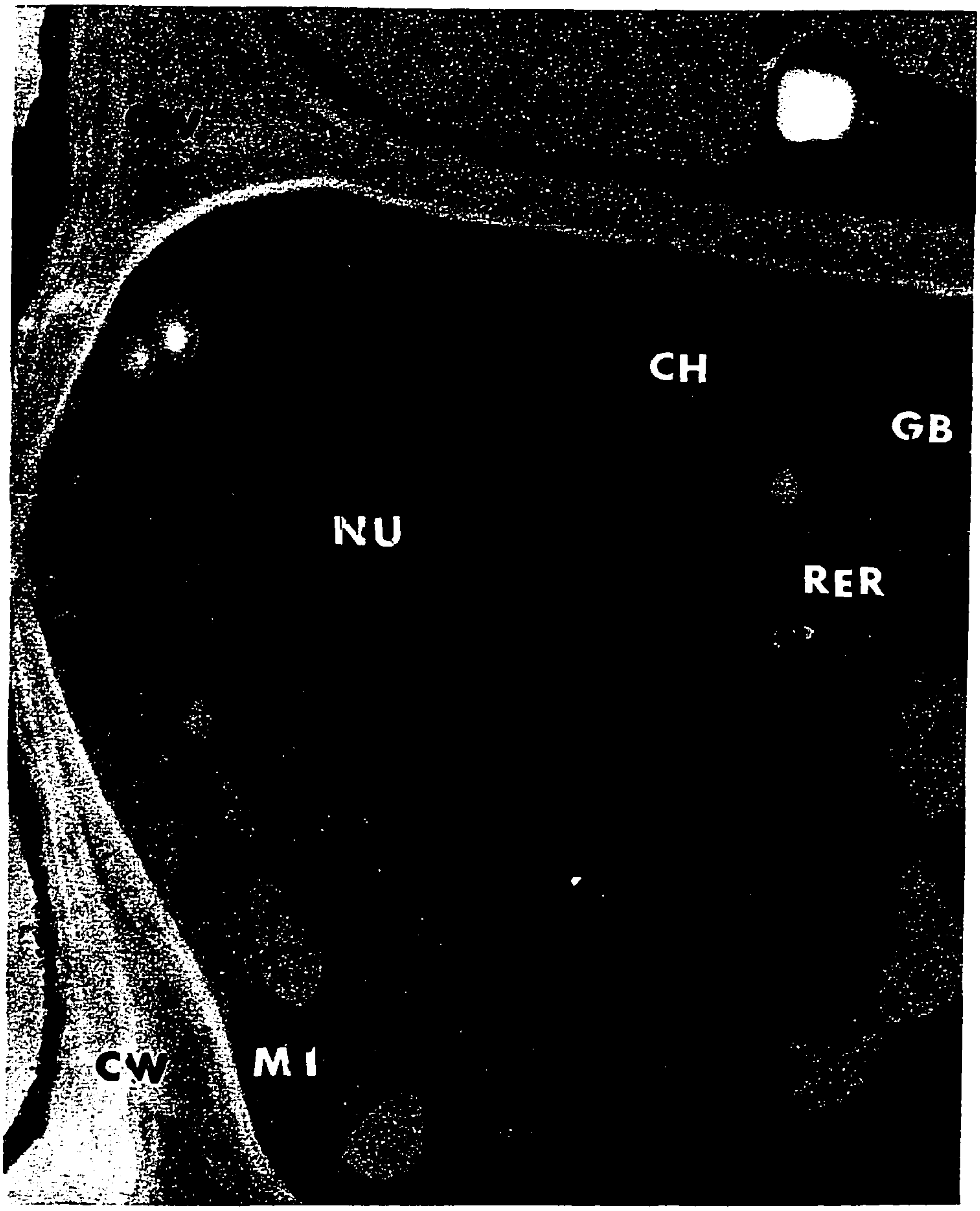


Fig. 38. A TEM photograph of control cortical tissue showing that there are many mitochondia; the nuleus has an unusual invagination. Golgi body is highly impact. CH:chloroplast, CW:cell wall, GB:Golgi body, MI:mitochondrion, NU:nucleus, RER:rough endoplasmic reticulum, VA:vacuole. X14,000

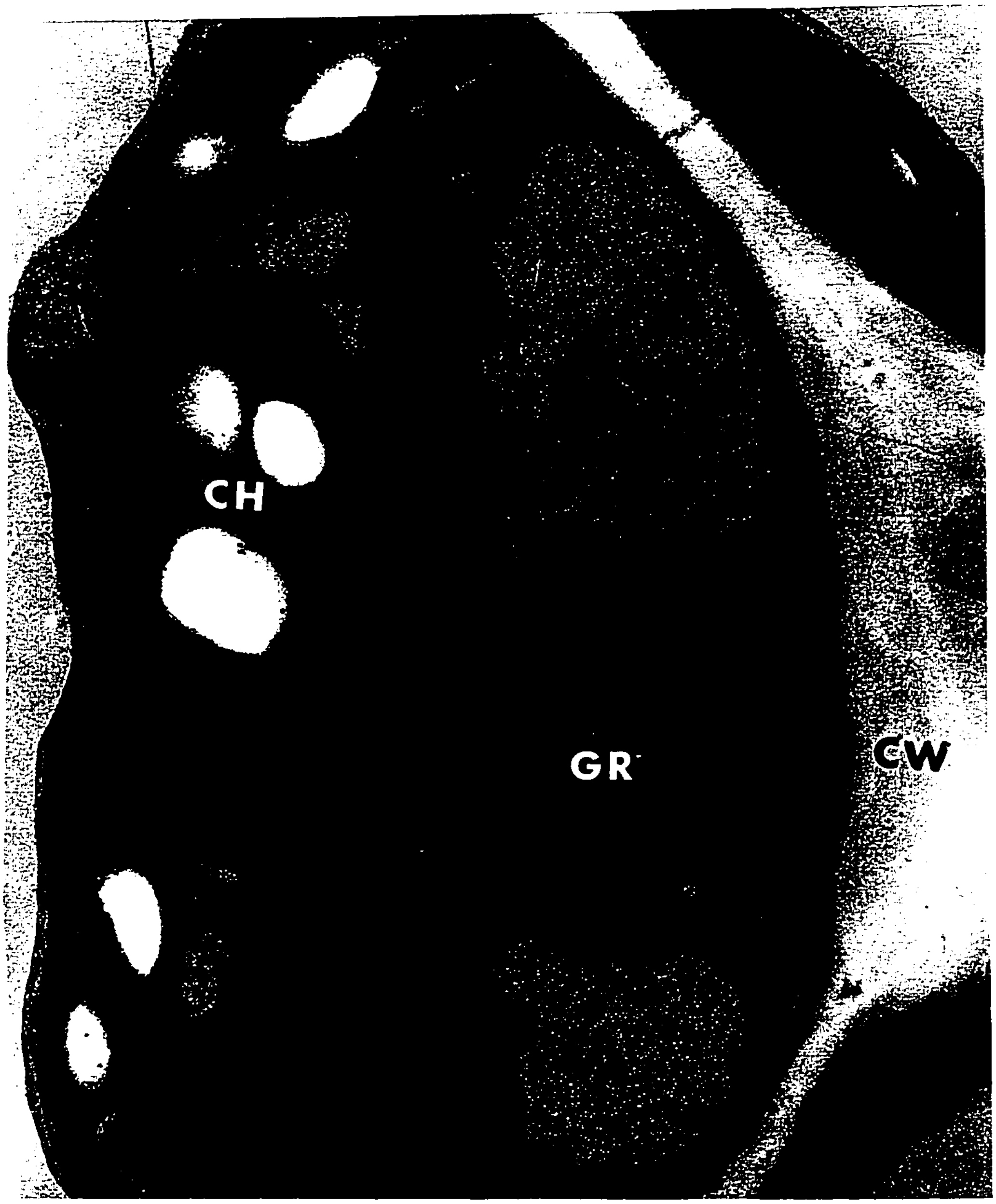


Fig. 39. A TEM photograph of control cortical tissue showing that there are many mitochondia and chloroplasts. CH:chloroplast, CW:cell wall, GR:granular component, MI:mitochondrion, VA:vacuole. X20,000

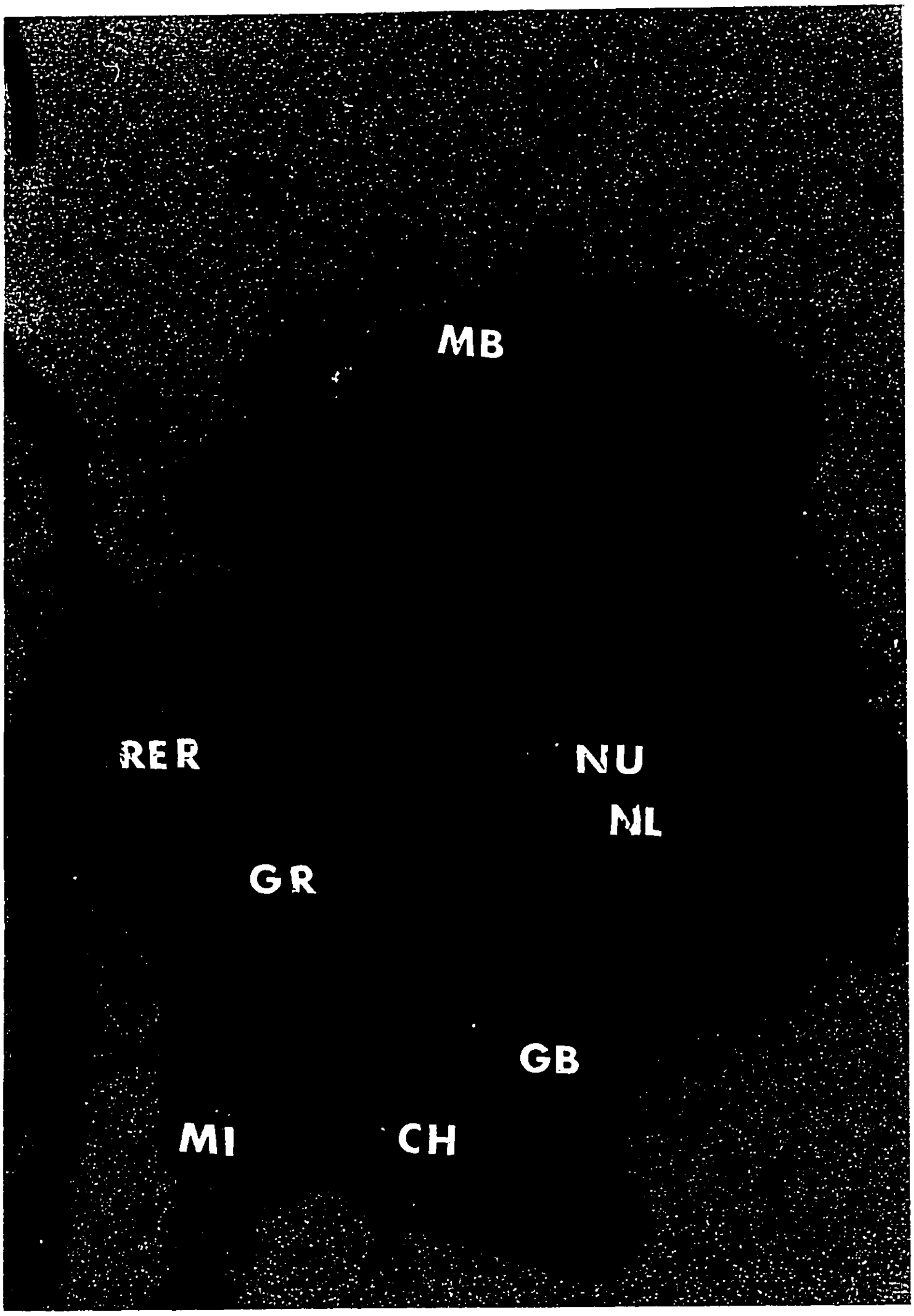


Fig. 40. A TEM photograph of six hour ethylene-treated cortical tissue showing that many Golgi bodied are around the cell membrane and vacoule membrane and the cell membrane is separated from cell wall. CH:chloroplast, CW:cell wall, GB:Golgi body, GR:granular component, MB: microbody, MI:mitochondrion, NL:nucleolus, NU:nucleus, RER:rough endoplasmic reticulum, VA:vacuole. X20,000

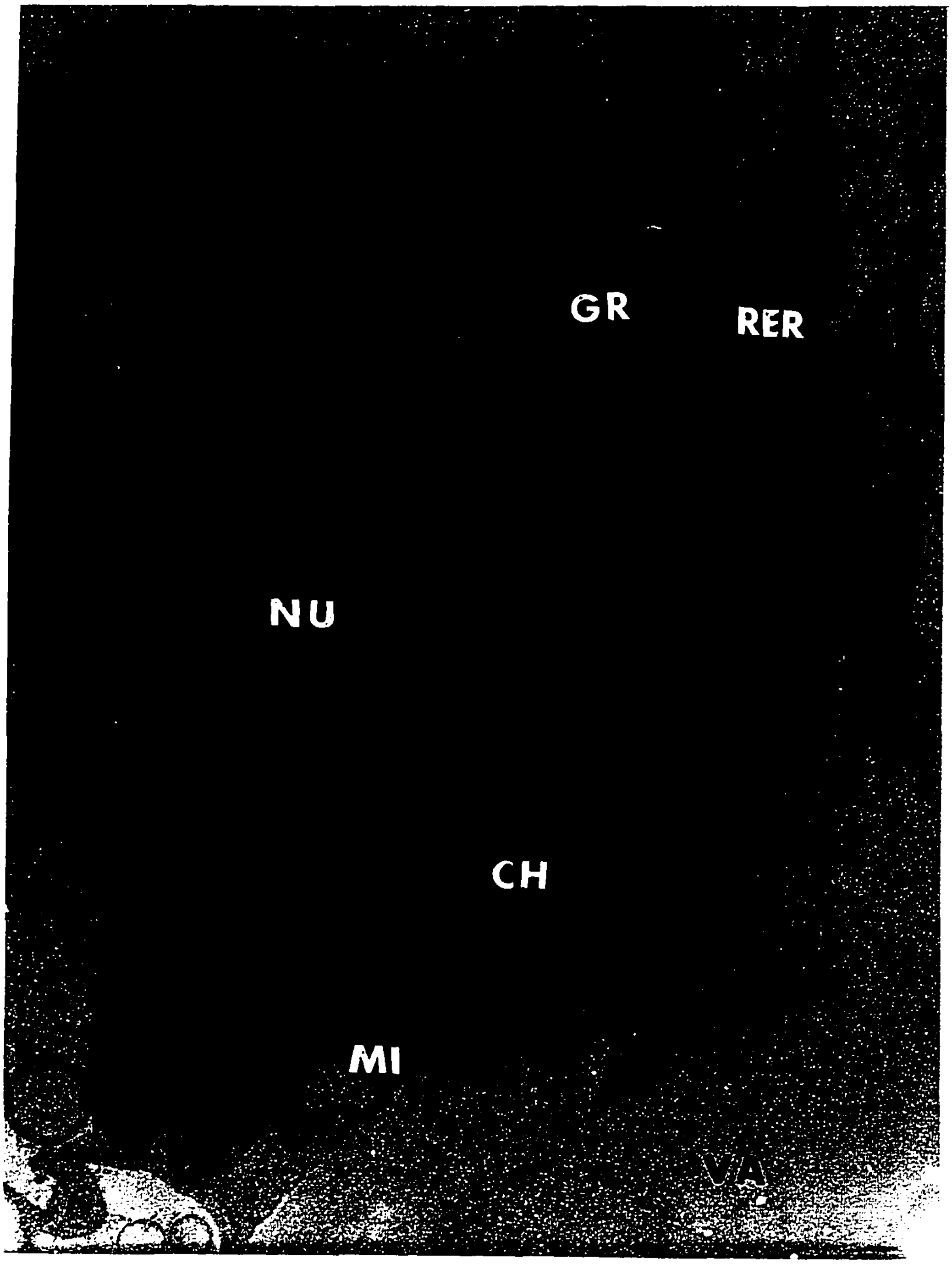


Fig. 41. A TEM photograph of six hour ethylene-treated cortical tissue showing that rich RER is around the plasma membrane which is separated from cell wall. CH:chloroplast, CW:cell wall, GR:granular component, MI:mitochondrion, NU:nucleus, RER:rough endoplasmic reticulum, VA:vacuole. X12,000

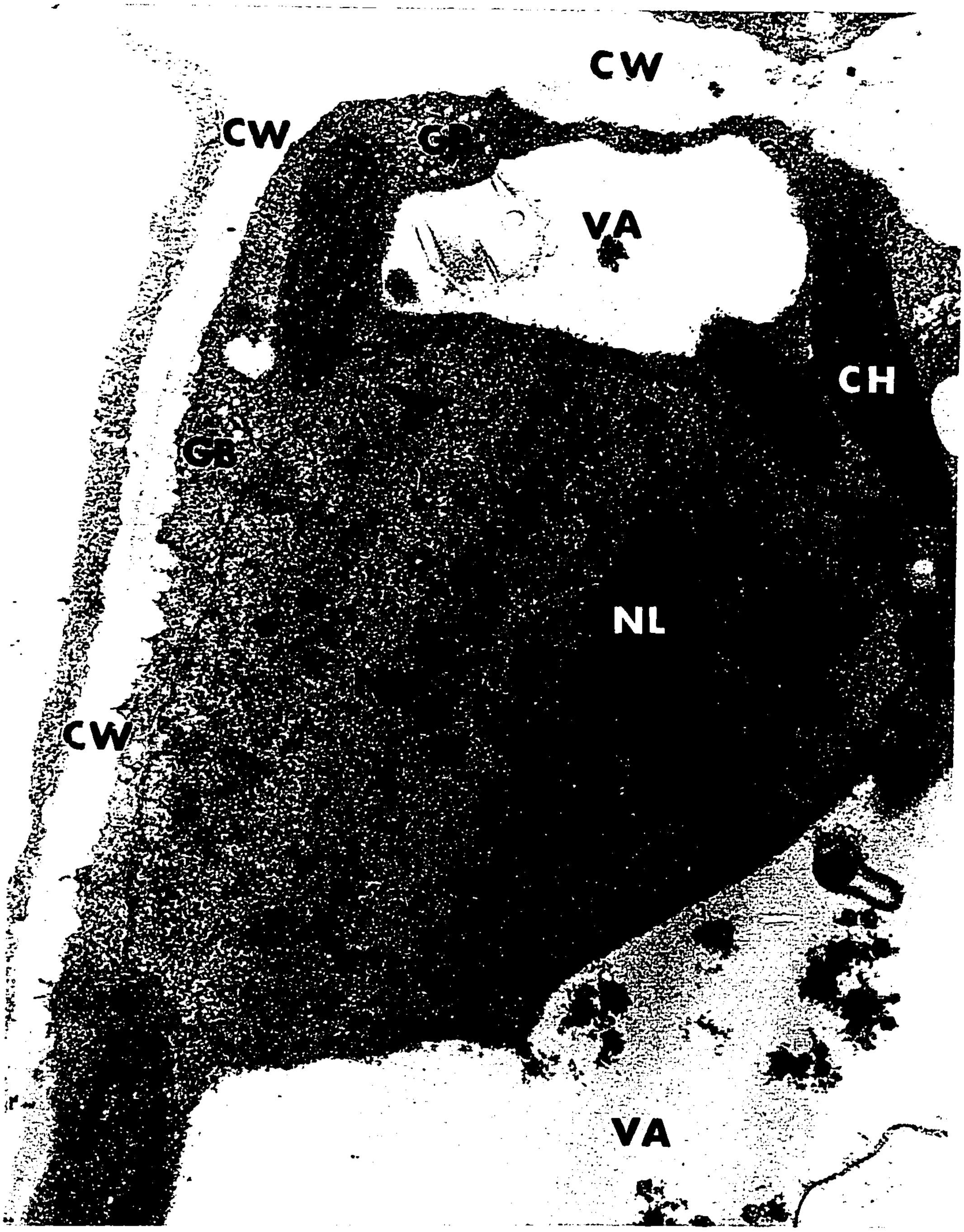


Fig. 42. A TEM photograph of six hour ethylene-treated cortical tissue showing that many Golgi bodies are around the plasma membrane which is separate from cell wall, a lot of degraded cytoplasm material is in vacoule. CH:chloroplast, CW:cell wall, GB:Golgi body, MI:mitochondrion, NL:nucleolus, NU:nucleus, VA:vacuole. X14,000

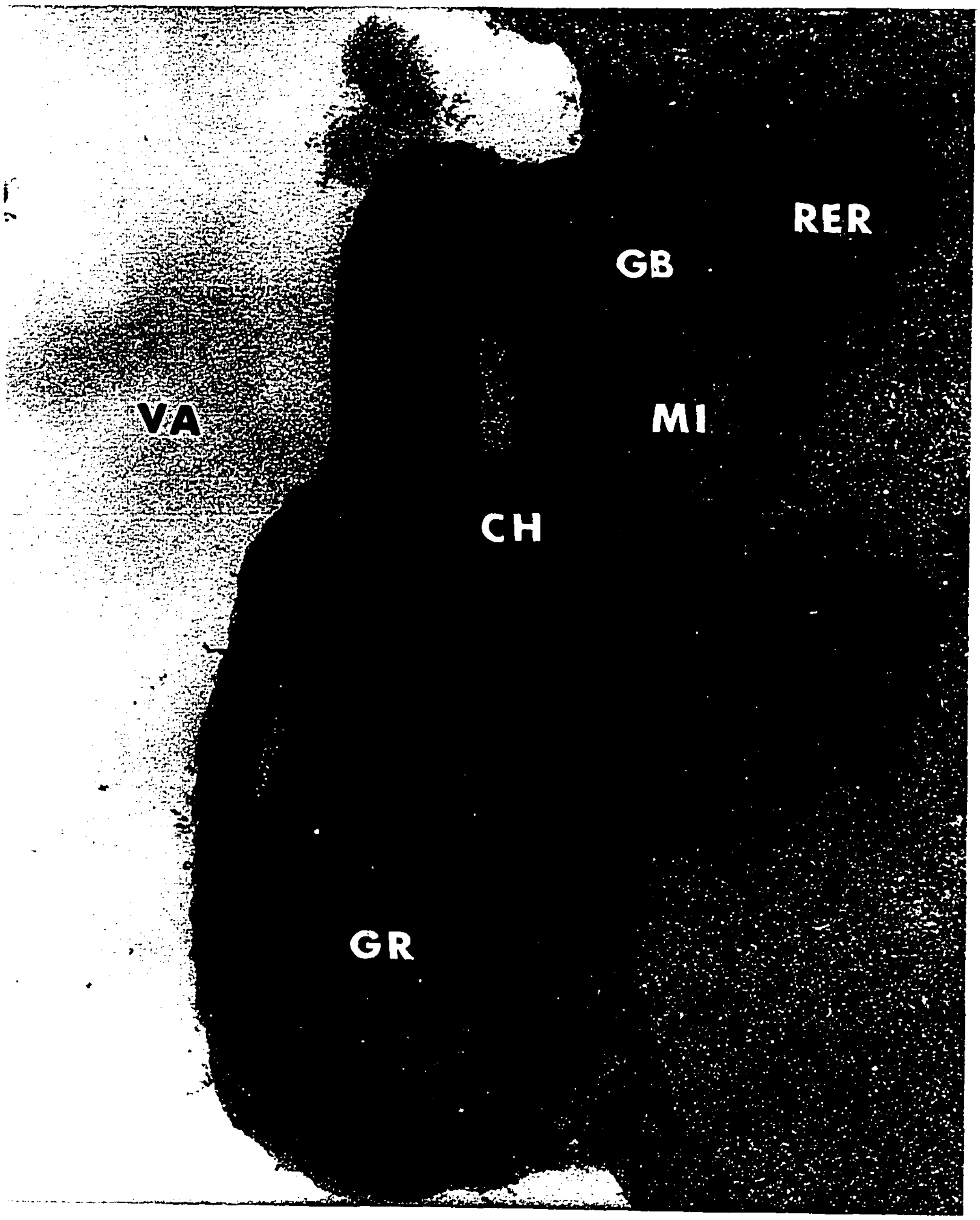


Fig. 43. A TEM photograph of six hour ethylene-treated cortical tissue showing the normal chloroplasts and many Golgi bodies around the vacoule membrane. CH:chloroplast, GB:Golgi body, GR:granular component, MI:mitochondrion, RER:rough endoplasmic reticulum, VA:vacuole. X40,000

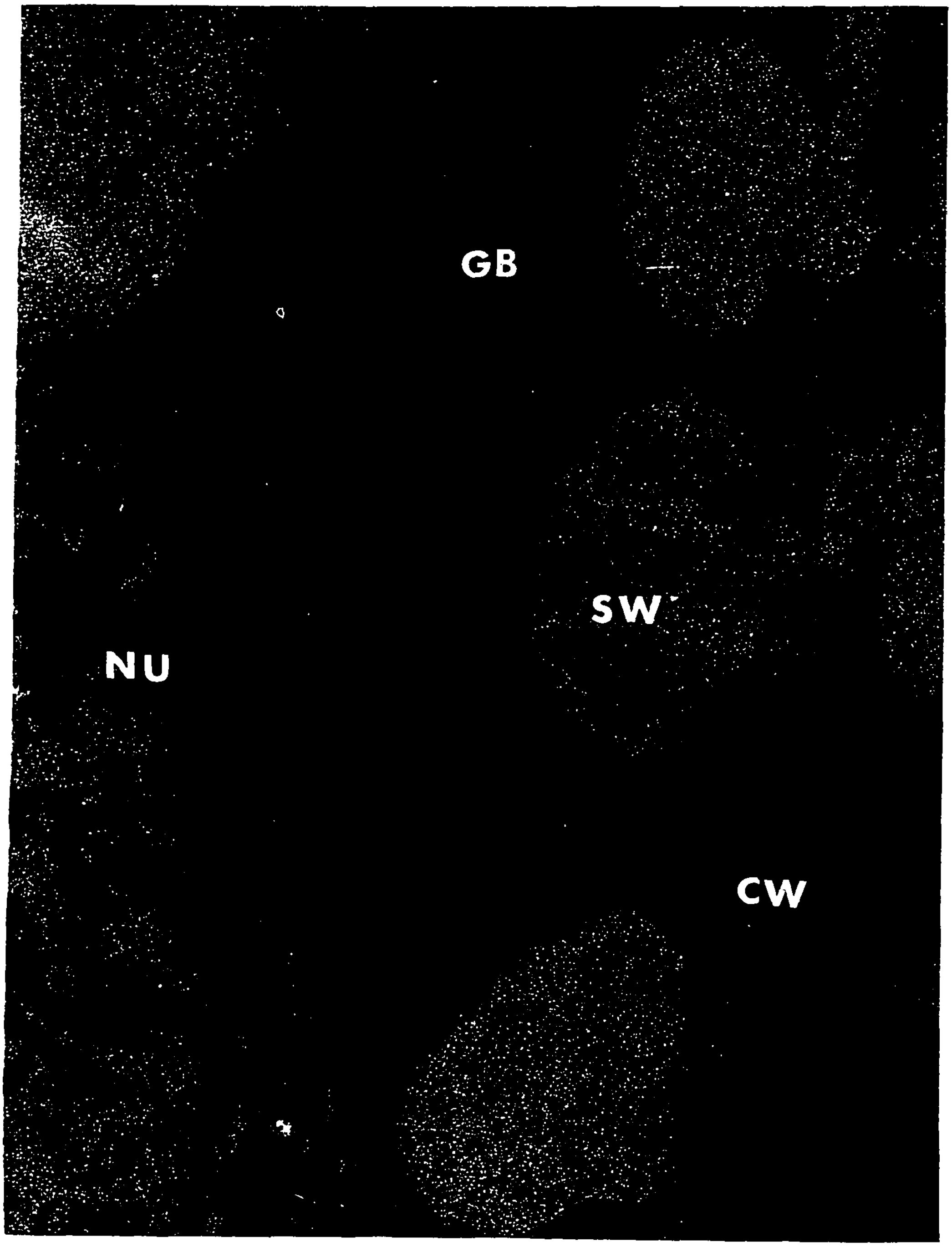


Fig. 44. A TEM photograph of control xylem vessels showing that the Golgi bodies are related to the secondary cell wall development and the cytoplasm degradation. The nucleus becomes elongated with the vessel. CW:cell wall, GB:Golgi body, NU:nucleus, SW:secondary wall, VA:vacuole. X20,000

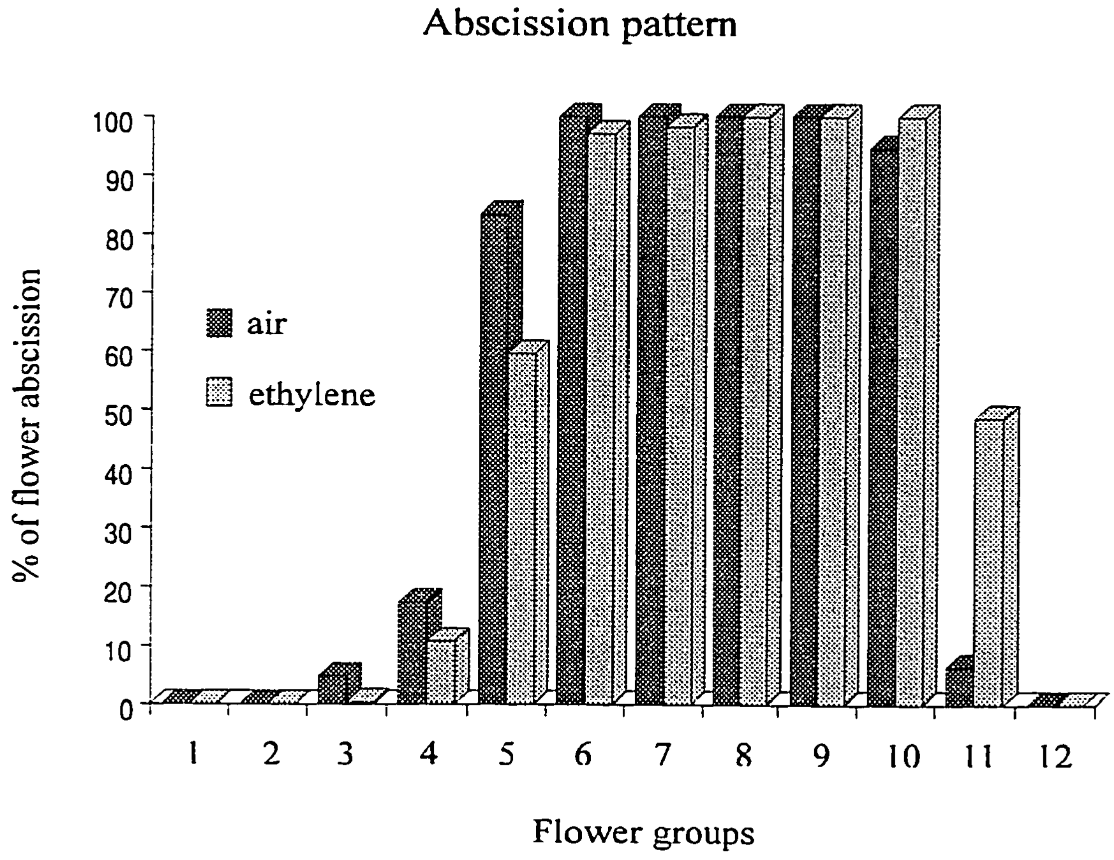


Fig. 45. Abscission pattern of W1 stage inflorescence as induced by ethylene treatment for six hours or as induced by air for 48 hours.

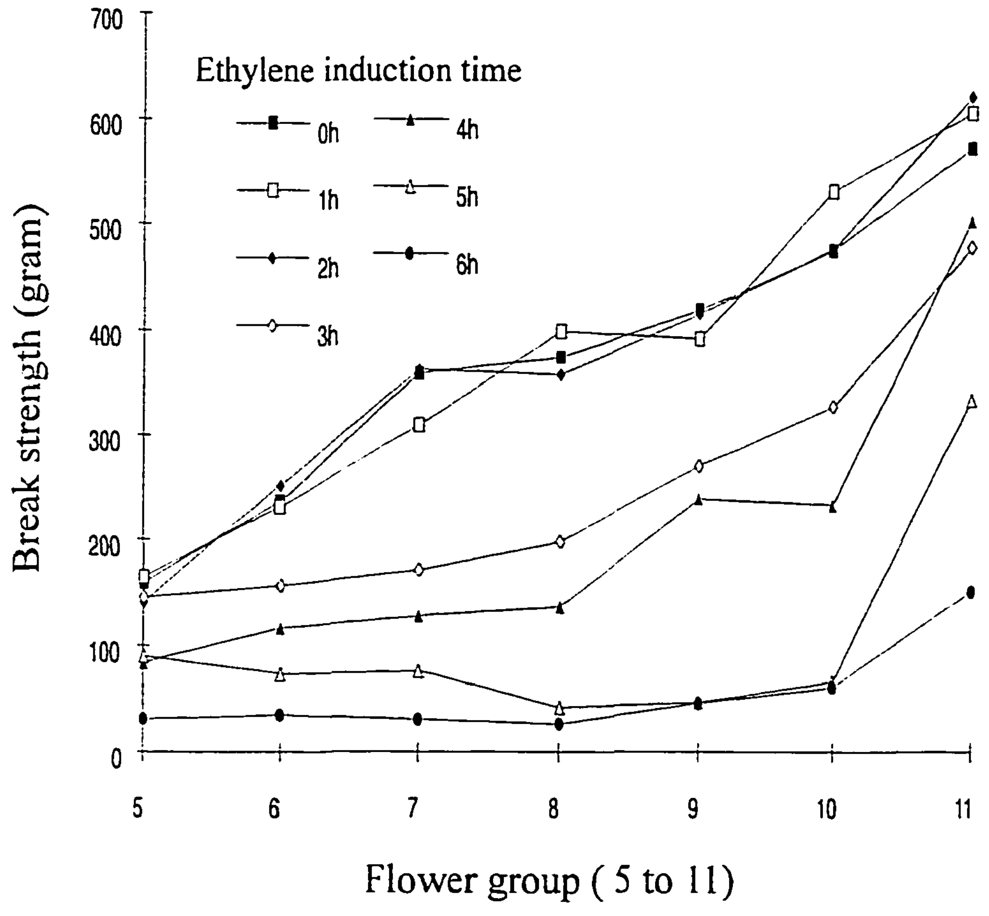


Fig. 46. Break strength changes as related to flower groups. Breaks are at the abscission zone.

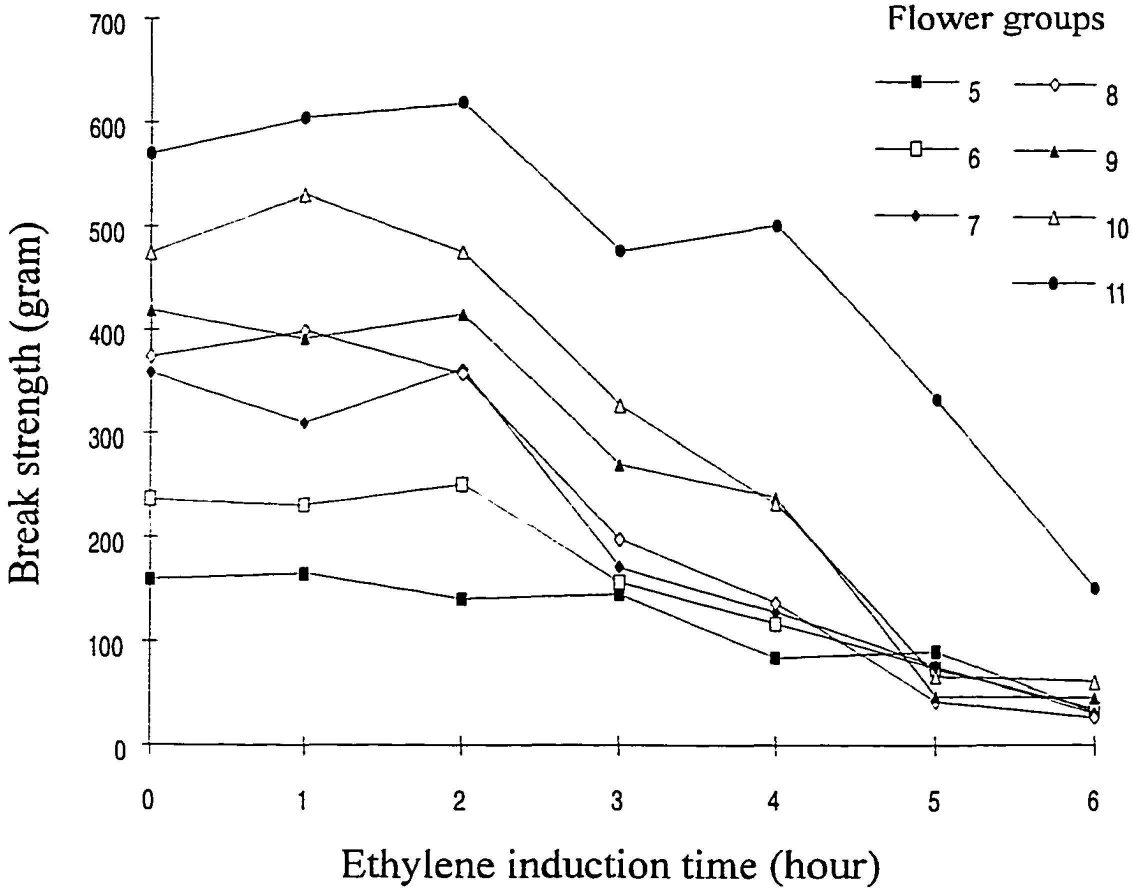


Fig. 47. Break strength changes as related to ethylene induction time. Breaks are at the abscission zone.

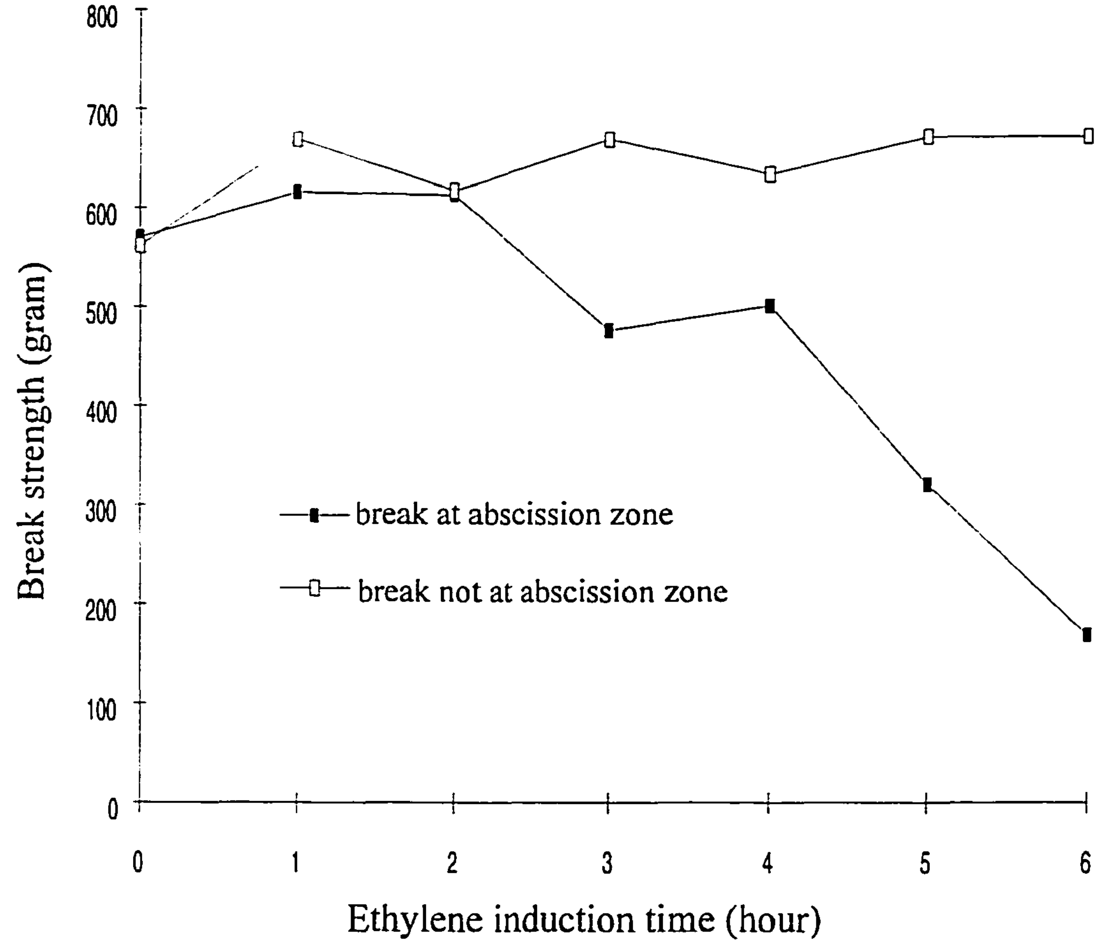


Fig. 48. Break strength changes of the group 11 flowers as related to ethylene induction time. Breaks are at either the abscission zone or "pedicel break zone".

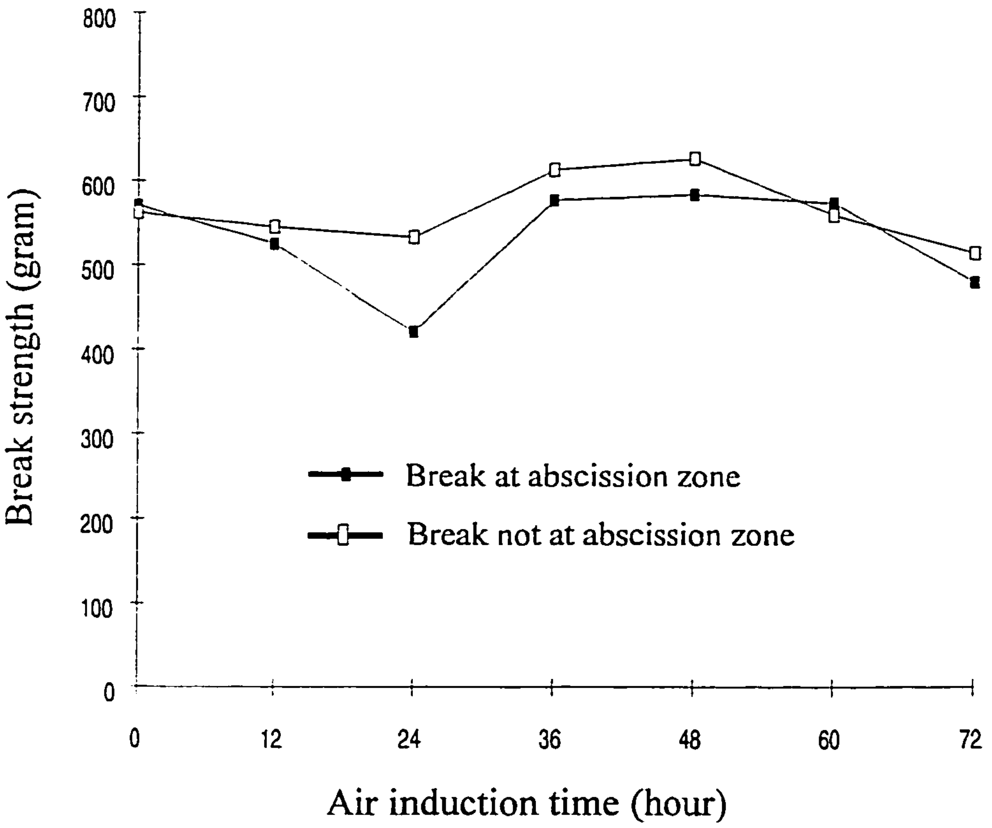


Fig. 49. Break strength changes in the group 11 flowers as related to air induction time. Breaks are at either the abscission zone or "pedicel break zone".

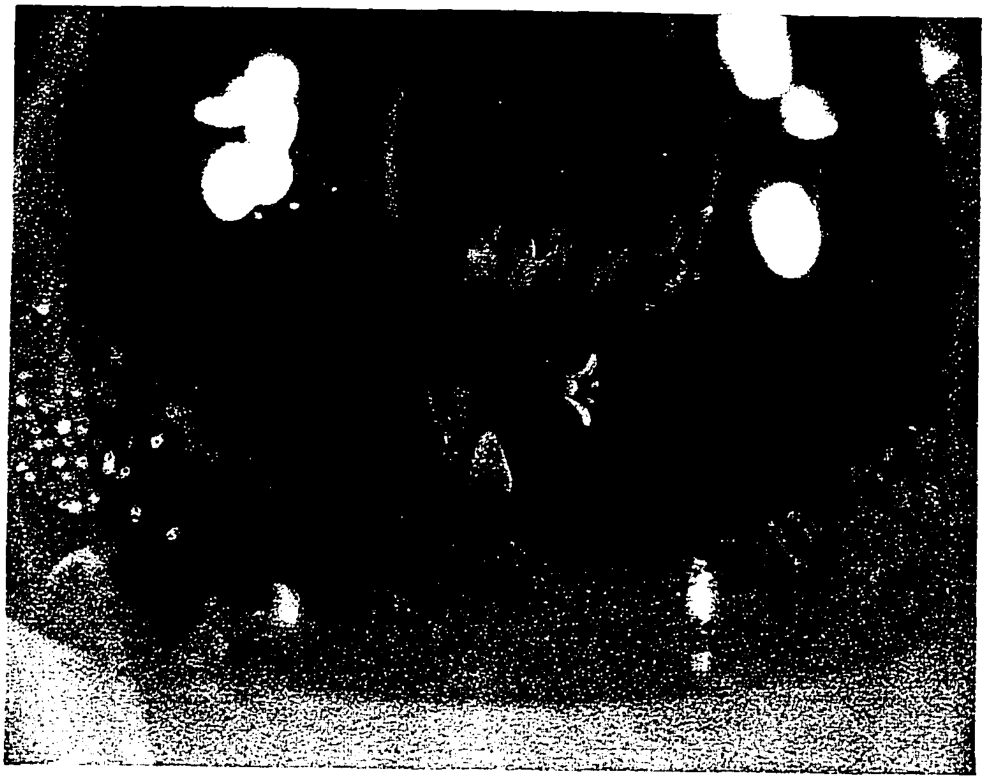


Fig. 50. Flower bud formation from a thin-layer explant under tissue culture.
The explant was obtained from the pedicel of a green fruit. It was cultured in MS salts with 30g / L sucrose, 100mg / L myoinosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L KIN and 10 g / L agar, pH at 5.7. The flower buds first appears at 35 days after the culture and the photo was taken at 50 days of culture.

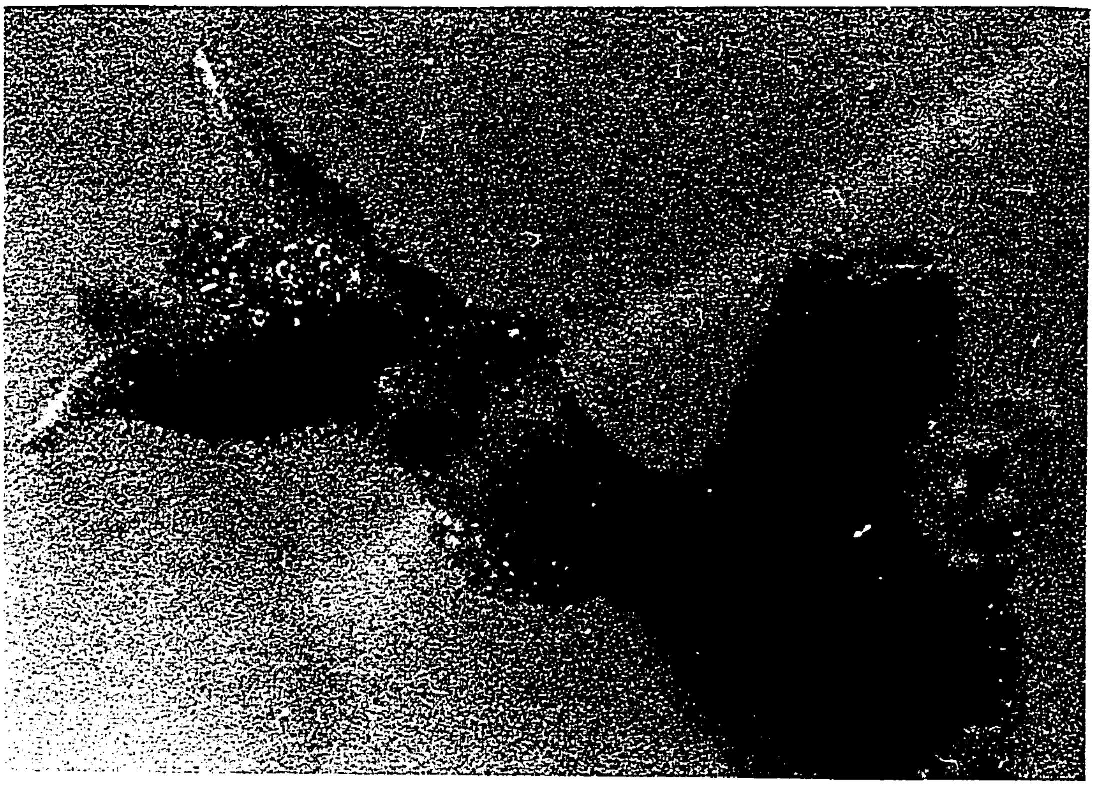


Fig. 51. Flower bud formation from the thin-layer explant with abscission zone under tissue culture. The explant was obtained from the "F-portion" of a green fruit. It was cultured in MS salts with 30g / L sucrose, 100mg / L myoinosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L KIN and 10 g / L agar, pH at 5.7 in a baby-food jar covered with aluminum foil and sealed with parafilm. The photo was taken at 20 days of culture.

Fig. 52. Flower bud formation from the thin-layer explant with abscission zone under tissue culture. The explant was obtained from the "F-portion" of a green fruit. It was cultured in MS salts with 30g / L sucrose, 100mg / L myo-inosital, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L KIN and 10 g / L agar, pH at 5.7 in a baby-food jar covered with aluminum foil and sealed with parafilm. The photo was taken at 15 days of culture.

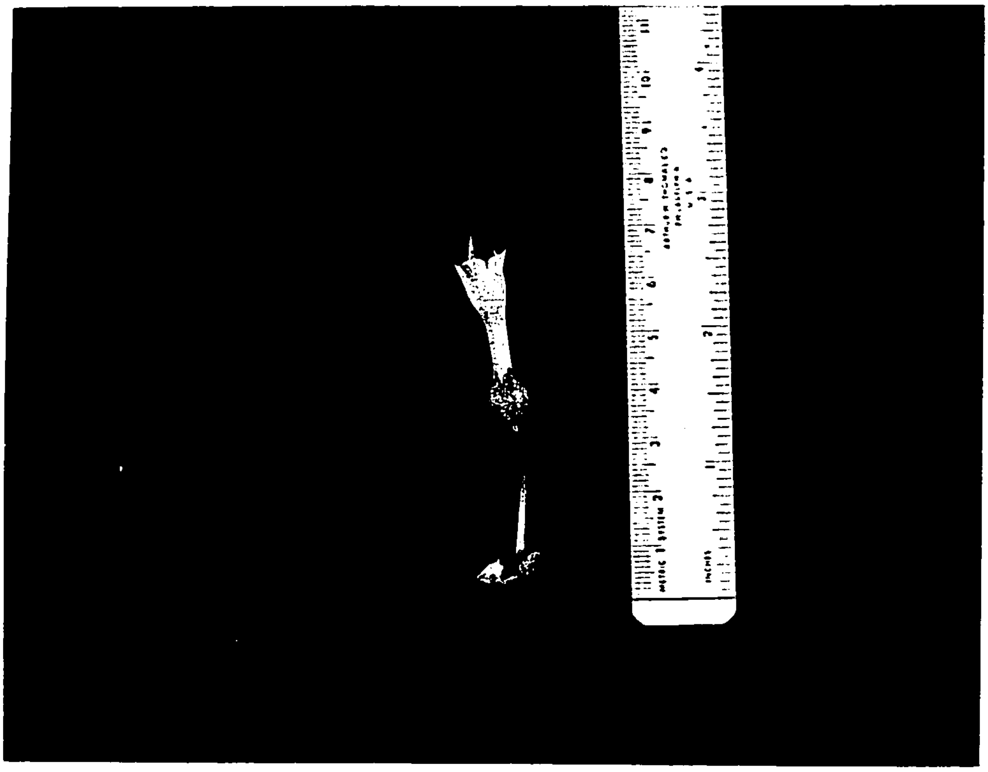


Fig. 53. Flower bud formation from the thin-layer explant with abscission zone under tissue culture. The explant was obtained from the "F-portion" of a flower or a green fruit. It was cultured in MS salts with 30g / L sucrose, 100mg / L myoinosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L KIN and 10 g / L agar, pH at 5.7 in a baby-food jar covered with aluminum foil and sealed with parafilm. The photo was taken at 25 days of culture.

Fig. 54. Flower bud formation from the thin-layer explant with abscission zone under tissue culture. The explant was obtained from the "F-portion" of a flower or a green fruit. A: Explant was cultured in MS salts with 30g / L sucrose, 100mg / L myoinosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L KIN and 10 g / L agar, pH at 5.7 in a baby-food jar covered with aluminum foil and sealed with parafilm. The photo was taken at 15 days of culture. B: Explant was cultured in the same medium as in "A"except that the growth regulators were 0.186 mg / L NAA and 0.225 mg / L BAP, and photo was taken 37 days of culture.


A
B

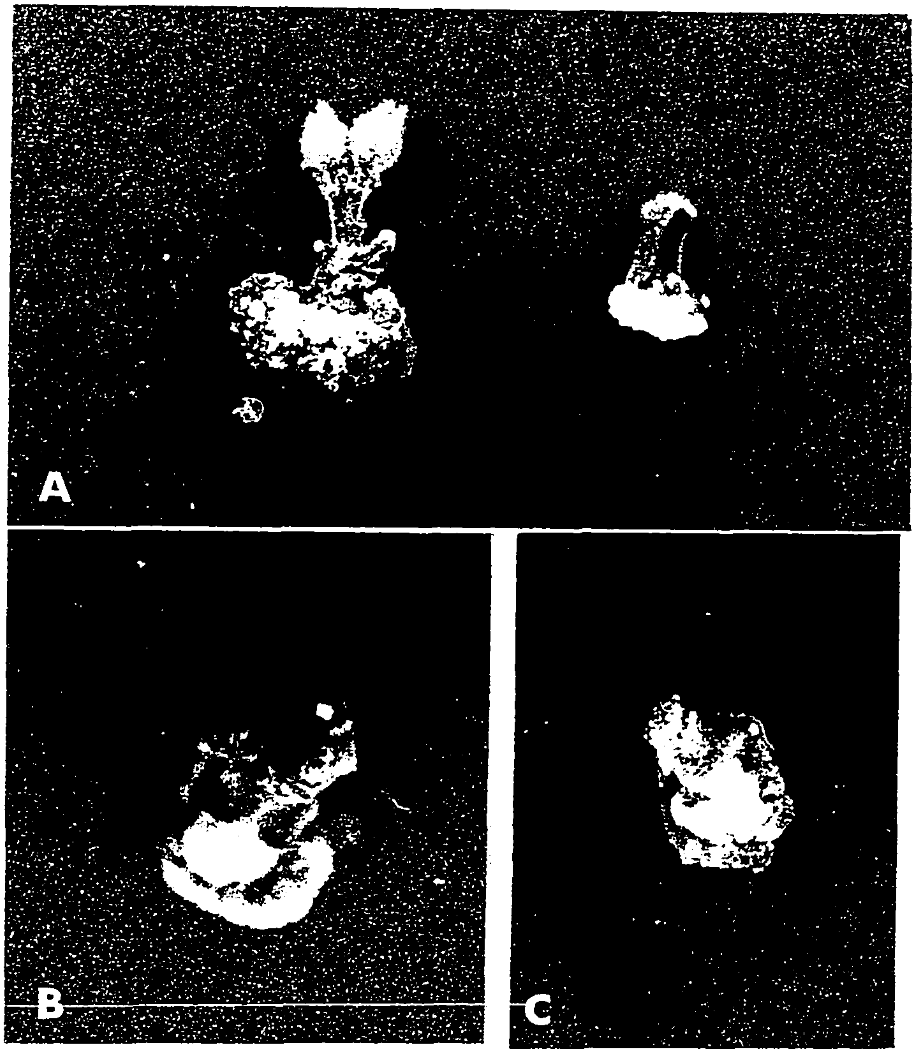


Fig. 55. Differences in flower bud formation on the pair of thin-layer explants with or without abscission zone from the same flower. The explant with abscission zone was obtained from the "F-portion" of a flower or a green fruit. The explant without abscission zone was obtained from the pedicel. A: The pair of explants were cultured in the same baby-food jar with the medium containing MS salts with 30g / L sucrose, 100mg / L myoinosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L kinetin and 10 g / L agar, pH at 5.7, photo was taken 32 days of culture. B and C: another pair of explants. They was cultured in one baby-food jar with same medium as in "A" except that the growth regulators were 0.186 mg / L NAA and 0.225 mg / L BAP, and photos was taken 37 days of culture.

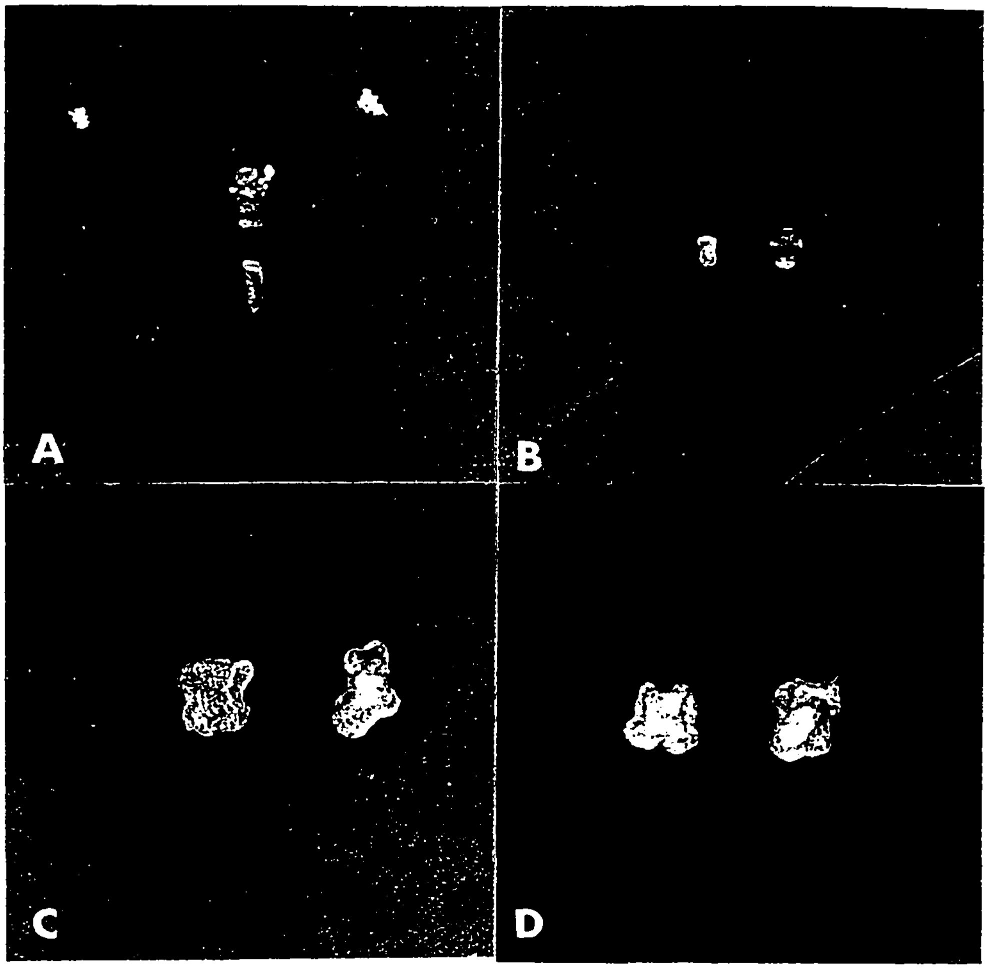
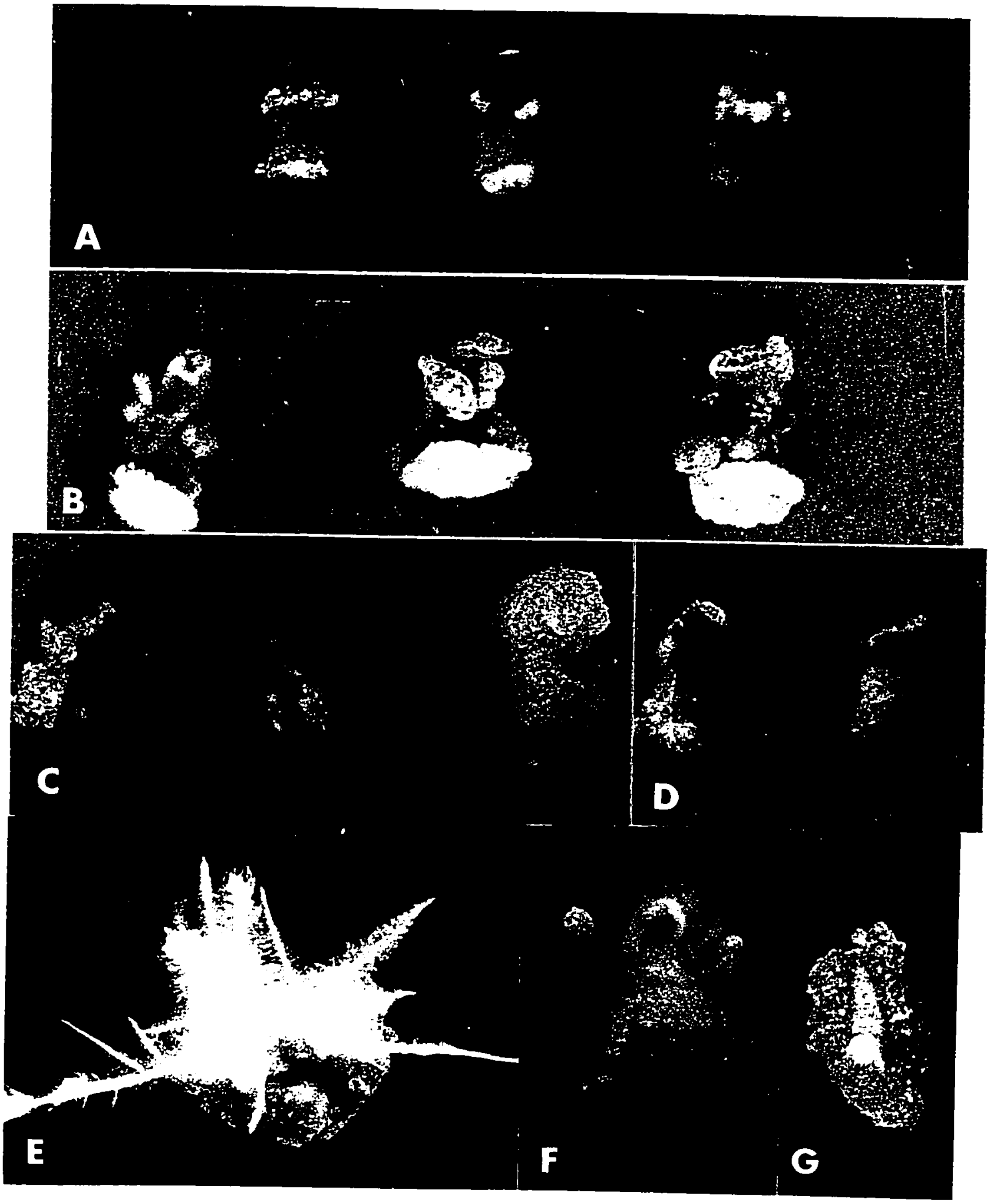


Fig. 56. Difference in flower bud formation of the pair of thin-layer explants with or without abscission zone from the same flower. The explants with abscission zone were obtained from the "F-portion" of a flower or a green fruit. The explants without abscission zone were obtained from the pedicel. A and B: Two pairs of explants were cultured in the medium with MS salts with 30g / L sucrose, 100mg / L myo-inosital, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L KIN and 10 g / L agar, pH at 5.7, photo was taken10 days of culture. C and D: Two pairs of explants were cultured in the same medium as in A and B except that the growth regulators were 0.186 mg / L NAA and 0.225 mg / L BAP, and photo was taken 15 days of culture.

Fig. 57. Differential organogenesis from the thin-layer explants with abscission zone. All explants were obtained from the "F-portion" of a flower or a green fruit. The culture conditions were A: MS salts with 30g / L sucrose, 100mg / L myo-inosital, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA 2.16m mg /L Kinetin and 10 g / L agar, pH at 5.7, constant lighting. B: MS salts with 30g / L sucrose, 100mg / L myo-inosital, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA 0.216m mg /L Kinetin and 10 g / L agar, pH at 5.7, constant lighting. C and D: MS salts with 30g / L sucrose, 100mg / L myo-inosital, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA 0.022 mg /L Kinetin and 10 g / L agar, pH at 5.7, constant lighting . E, F, G: MS salts with 30g / L sucrose, 100mg / L myo-inosital, 0.1 mg / L thiamine.HCl, 1.75 mg / L IAA 0.022 mg /L Kinetin and 10 g / L agar, pH at 5.7, constant dark. The photographes show, A: vegetative bud developed from the abscission zone, B: flower bud developed from the abscission zone , C: Callus developed from the abscission zone, D: no organogenesis, E: root developed from the abscission zone, F:flower bud developed from the abscission zone, G: callus developed from lower side of the explant.


A
B
C
D
E
F
G

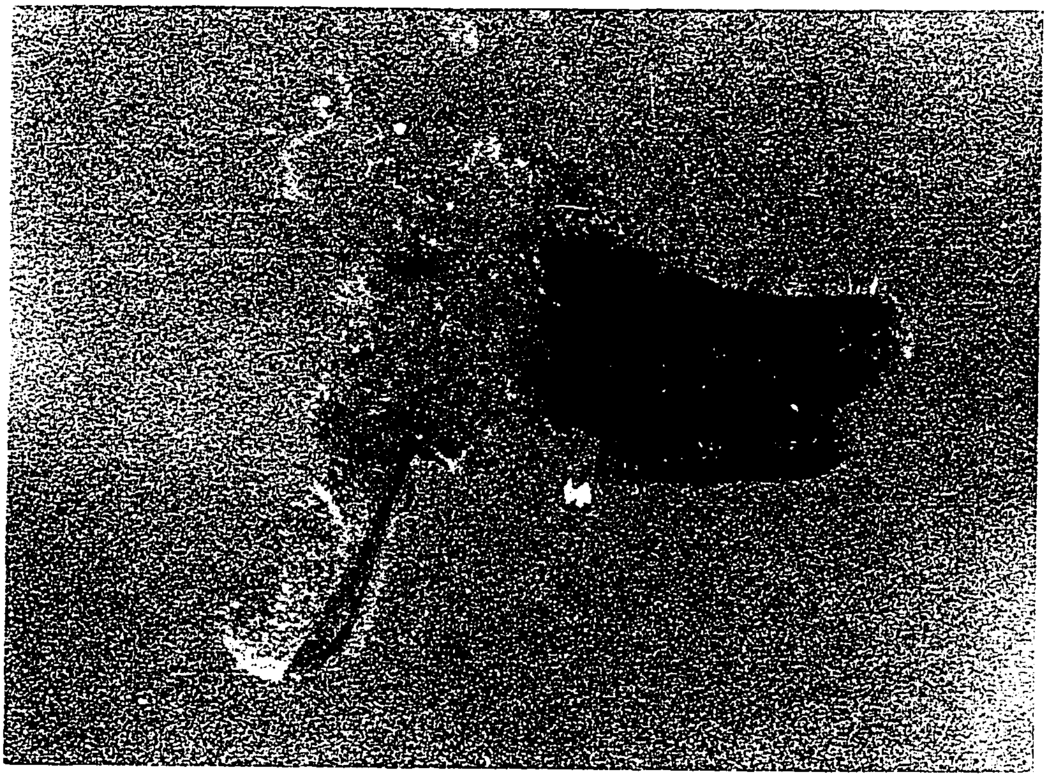


Fig. 58. Callus formation from the thin-layer explant with abscission zone under tissue culture. The explant was obtained from the "F-portion" of a green fruit. It was cultured in MS salts with 30g / L sucrose, 100mg / L myo-inosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L KIN and 10 g / L agar, pH at 5.7 in a plastic petri dish sealed with parafilm. The photo was taken at 20 days of culture.

Fig. 59. Daily observation of flower bud formation from the thin-layer explants with abscission zone under tissue culture (0 to 7 days). The explant was obtained from the "F-portion" of a flower or a green fruit. It was cultured in MS salts with 30g / L sucrose, 100mg / L myoinosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L KIN and 10 g / L agar, pH at 5.7 in a baby-food jar covered with aluminum foil and sealed with parafilm. A: 0 days of culture, B: 1 days of culture, C: 2 days of culture, D: 3 days of culture, E: 4 days of culture, F: 5 days of culture, G: 6 days of culture, H: 7 days of culture.

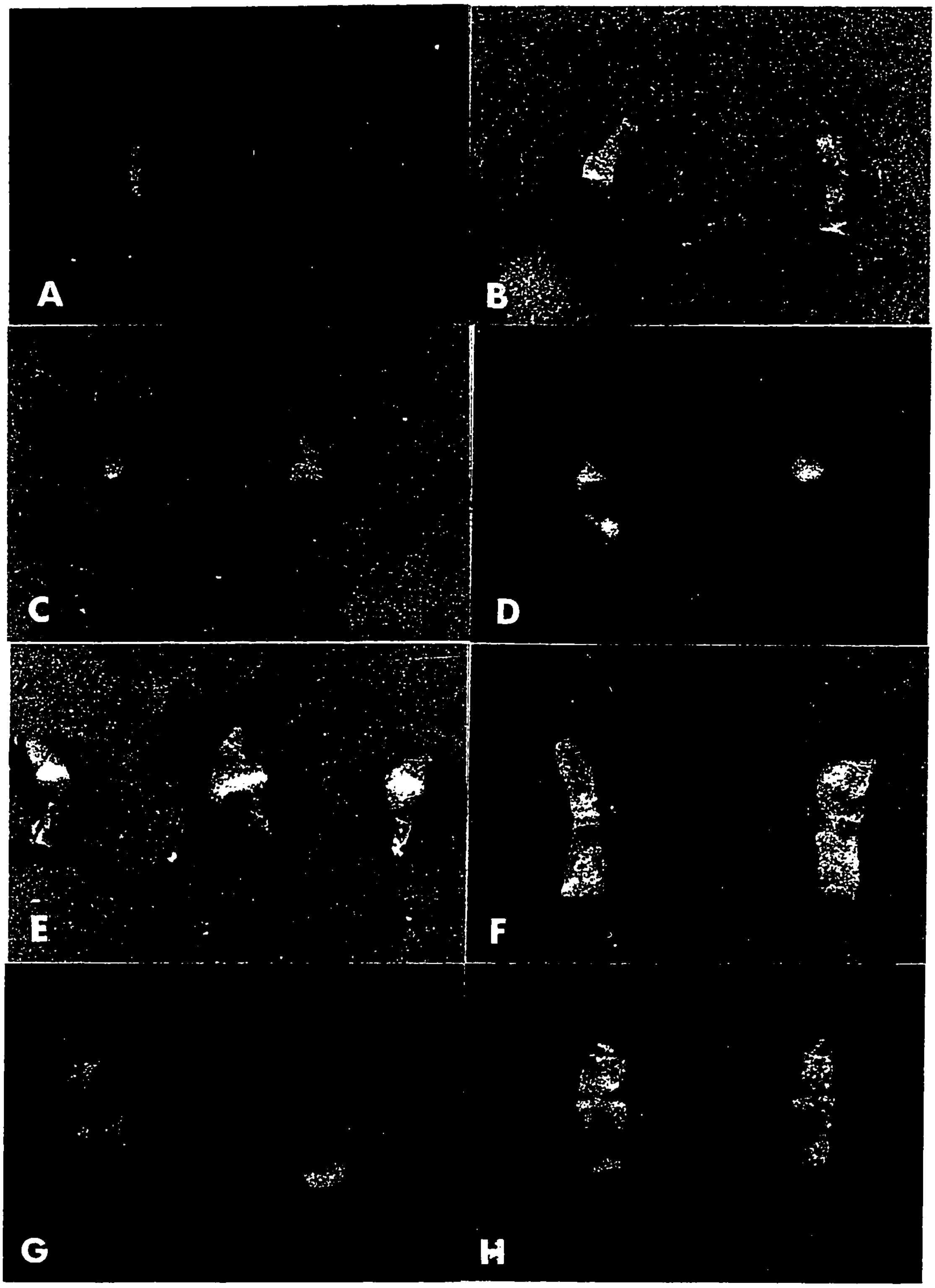
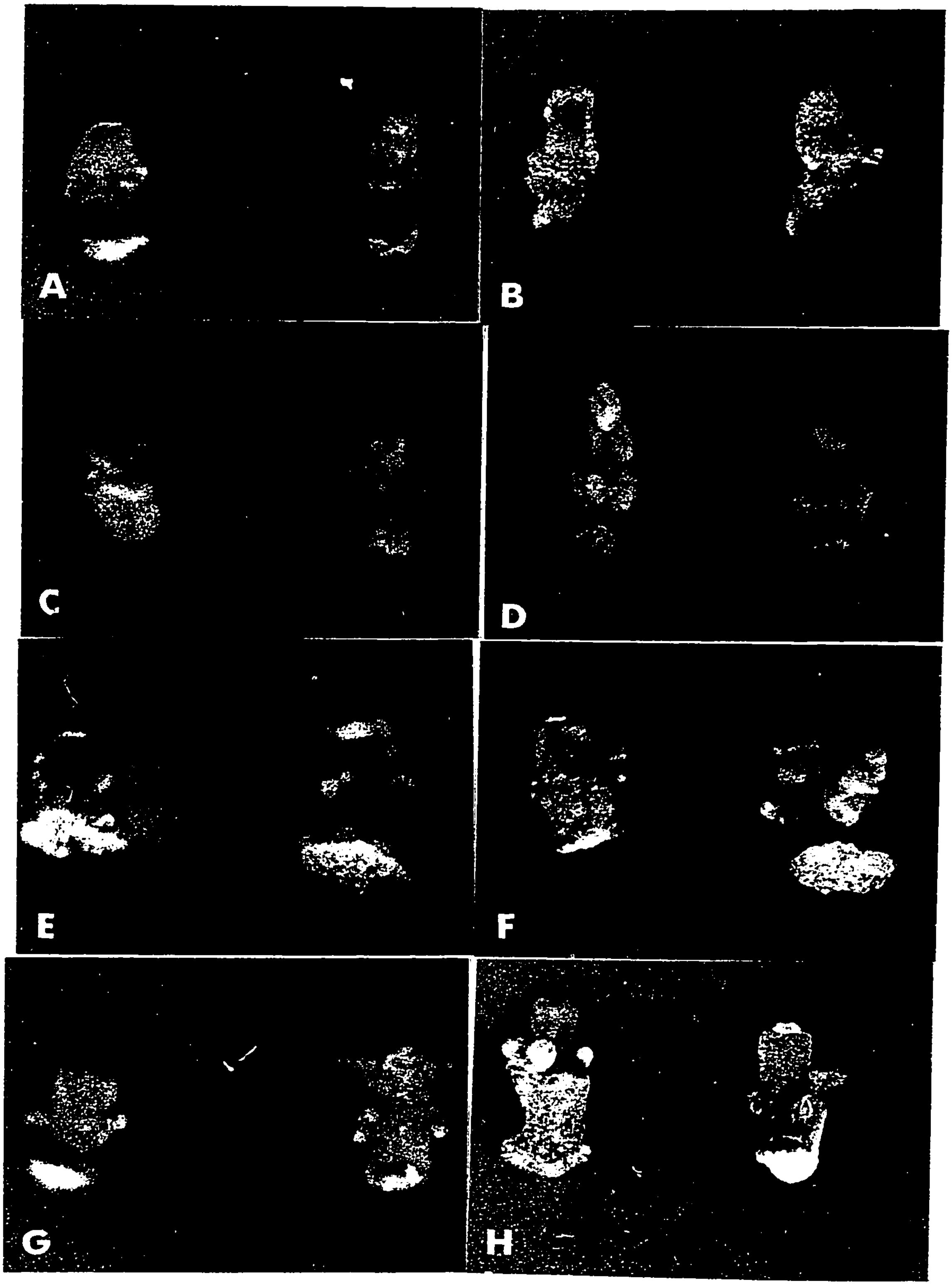

A
B
C
D
E
F
G
H

Fig. 60. Daily observation of flower bud formation from the thin-layer explants with abscission zone under tissue culture (8 to 15 days). The explant was obtained from the "F-portion" of a flower or a green fruit. It was cultured in MS salts with 30g / L sucrose, 100mg / L myoinosital, 2 mg / L glycine, 0.5 mg / L nicotinic acid, 0.5 mg / L pyridoxine.HCl, 0.1 mg / L thiamine.HCl, 0.175 mg / L IAA, 0.216 mg /L KIN and 10 g / L agar, pH at 5.7 in a baby-food jar covered with aluminum foil and sealed with parafilm. A: 8 days of culture, B: 9 days of culture, C: 10 days of culture, D: 11 days of culture, E: 12 days of culture, F: 13 days of culture, G: 14 days of culture, H: 15 days of culture.


A
B
C
D
E
F
G
H

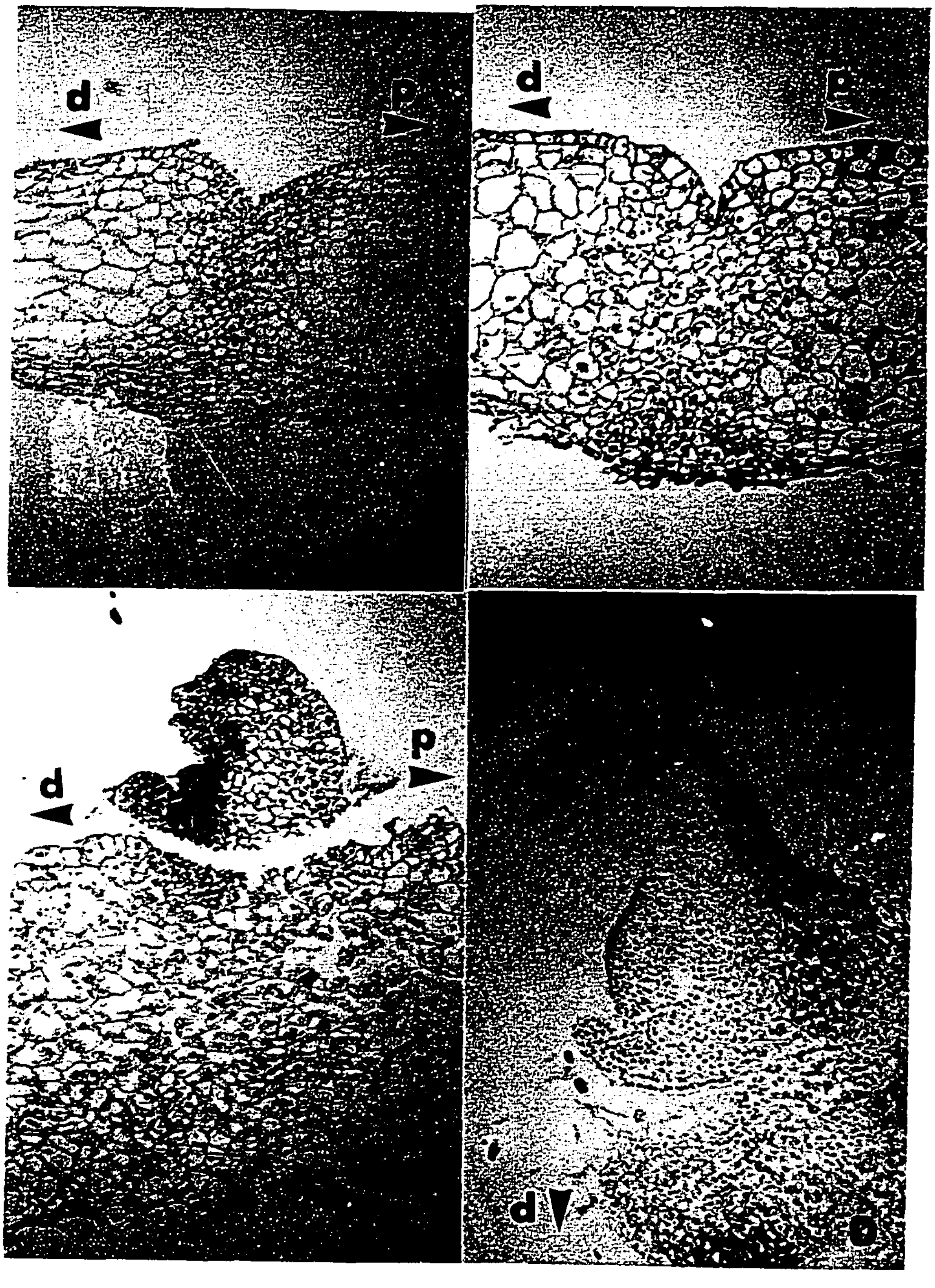


Fig. 61. Paraffin sections of the thin-layer explants during the flower bud formation period. A: Before culture; B: Three days after culture; C: Six days after culture. D: Eight days after culture, arrow d: distal, arrow p: proximal. 80X, 1 cm bar = 120 μm.

Appendix A

Glossary

Abscission zone, the localized portion of an organ where cellular activities take place if the abscission occurs.

Abscission pattern, the percentage of flowers abscised in each group of flowers with certain induction conditions.

Abscission, a physiological process occurs at a localized area (abscission zone) of an organ that causes the organ to abscise from the plant body.

Bract-monochasium, the monochasium branch type with a bract under the branch.

Bract-unequal trichasium, the trichasium branch type where the three subbranches are not the same size and one, two or three of the subbranches are with a bract.

Bract-unequal dichasium, the dichasium branch type where the two subbranches are not the same size and one or two of the subbranches are with a bract.

Break strength, the force (in gram) required to separate an organ (flower) from the plant body.

Determinate, a type of inflorescence where the terminal flower from the apical meristem first differentiates into a single flower.

Dichasium, a branch type where two subbranches develop from a subterminal node when the apical meristem differentiates to a single flower.

Egase, the enzyme endo-β-1,4-glucanase, a cellulase.

Equal dichasium, a dichasium branch type where the two subbranches are the same size.

Equal trichasium, a trichasium branch type where the three subbranches are the same size.

F-portion, a portion of tobacco axial including the abscission zone, partial pedicel and partial internode (See Fig. 5).

Flower node, a node under a flower in an inflorescence.

Histograph, a bar diagram showing the frequency distribution.

Indeterminate, a type of inflorescence with a terminal inflorescence meristem continuously developing the inflorescence

Inflorescence, a reproductive structure where flowers aggregate together to form a particular pattern.

Internode, the axial part of plant shoot system that between any two adjacent nodes.

Lateral inflorescence, an inflorescence developed from a lower node in contrast to terminal inflorescence; it usually has some very well developed leaves preceding to the flower development.

Monochasium, a branch type with only one single subbranch developed from the node.

Node, the position on the stem where leaf is located. In the tobacco inflorescence it is the branching point of the inflorescence.

Open-system of growth, the developmental model in plants where unlimited nodes and internodes can develop gradually and indefinitely.

Pedicel, the stalk of a single flower or fruit in an inflorescence.

R square, correlation coefficient, a statistic value in correlation analysis.

Subbranch, the continuous growth of branch from the subterminal tissue where the apical meristem differentiates to a flower.

Terminal inflorescence, an inflorescence develops from the terminal node and several subterminal nodes. In tobacco plant, the apical four nodes develop the terminal inflorescence and other nodes may develop lateral inflorescence.

Thin-layer explant, the explant contains epidermis and several subepidermal layers of cells from shoot of tobacco plant for tissue culture.

Trichasium, a branch type where three flowers develop from the node.

Two-AZ trichasium, a branch type where two flowers each with an abscission zones share a common subbranch and where two more small subbranches develop from each of the abscission zones.

Unequal trichasium, a trichasium branch type where the three subbranches are different in size.

Unequal dichasium, a dichasium branch type where the two subbranches are different in size.

BIBLIOGRAPHY

Abeles, F.B. (1968) Role of RNA and protein synthesis in abscission. Plant Physiol. 43:1577-1586

Addicott, F. T. (1982) Abscission. University of California Press, Berkeley

Aghion-Prat, D. (1965) Neoformation de fleurs *in vitro* chez *Nicotiana tabacum* L. Physiol. Veg 3:229-393

Altamura, M. M., and F. Capitani (1992) The role of plant hormones on morphogenesis of thin layer explants from normal and transgenic tobacco plants. Physiologia plantarum 84: 555-560

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

Bailey, L.H.(1949) Manual of cultivated plants, Revised Edition. The MacMillan Company, New York

Baird, L.M. and P.D. Reid (1992) Cellulase activity and localization during induced abscission of Coleus blumei. J. Plant Growth Regul. 11: 129-134

Barendse, G.W.M., A.F. Croes, M. Bosveld, W. M. Van Der Krieken, and G. J. Wullems (1987) Uptake amd metabolism of NAA and BAP in explants of tobacco in relation to *in vitro* flower bud formation. J. Plant Growth Regul. 6:193-200

Battey, N. (1996) And in the end ... a terminal flower! Trends in Plant Science 1:167-168

Bell, A. D. (1991) Plant Form. Oxford University Press. Oxford

Berger, R.K, and P.D. Reid (1979) Role of polygalacturonase in bean leaf abscission. Plant Physiol. 63:1133-1137

Bonhomme, F., H. Sommer, G. Bernier, A Jacqmard (1997) Characterization of SaMADS D from sinapis alba suggests a dual function of the gene: in inflorescence development and floral organogenesis. Plant Mol. Biol. 343:573-582

Bradley, D., C. Vincent, R. Carpenter and E. Coen (1996 a) Control of inflorescence architecture in *Antirrhinum.* Nature 379:791-797

Bradley, D. C., Vincent, R. Carpenter, E. Coen (1996 b) Pathways for inflorescence and floral induction in *Antirrhinum*. Development 122:1535-1544

Bradley, D., O. Ratcliffe, C. Vincent, R. Carpenter, E. Coen (1997) Inflorescence commitment and architecture in Arabidopsis. Science 275:80-83

Campbell, D. R. (1989) Inflorescence size: test of the male function hypothesis. Amer. J. Bot. 76:730-738.

Chen, L., J. C. Cheng, L. Castle, Z. R. Sung (1997) EMF genes regulate Arabidopsis inflorescence. Plant Cell 9:2011-2024

Crookes , P.R., and D. Grierson (1983) Ultrastructure of tomato fruit ripening and the role of polygalacturonase isoenzymes in cell wall degradation. Plant Physiol. 72:1088-1093

Croes, A. F., T. Creemers-Molenaar, G. van den Ende, A. Kemp, G. W. M. Barendse (1985) Tissue age as an endogenous factor controlling in vitro bud formation on explants from the inflorescence of Nicotiana tabacum L. J. Exp. Bot. 36:1771-1779

Cronquist, A. (1988) The evolution and classification of flowering plants (second Edition). The New York Botanical Garden, Bronx, New York

del Compillo, E., M. Durbin, L. N. Lewis (1988) Changes in two forms of membrane-associated cellulase during ethylene induced abscission. Plant Physiol. 88:904-909

del Compillo E., and L. N. Lewis (1992) Occurrence of 9.5 cellulase and other hydrolases in flower reproductive organs undergoing major cell wall disruption. Plant Physiol. 99:1015-1020

Eberhard, S., N. Doubrava, V. Marfa, D. Mohnen, A. Southwick, A. Darvill, P. Albersheim (1989) Pectic cell wall fragments regulate tabacco thin-cell-layer explant morphogenesis. Plant Cell 1:747-755

Emms, S. K., D. A. Stratton, A. A. Snow (1997) The effect of inflorescence size on male fitness: experiments tests in the andromonocious lily, Zigadenus paniculatus. Evolution 51:1481-1489

Fernald, M. L.(1950) Gray's manual of botany (eightth edition). American Book Company, New York

Foster, A. S. And E. M. Gifford, Jr. (1974) Comparative morphology of Vascular plants

2nd ed. W. H. Freeman and Company, San Francisco

Gleason, H. A., and A. Cronquist (1963) Manual of vascular plants of northeastern United States and Adjacent Canada. D. Van Nostrand Company, INC.

Gleason, H. A., and A. Cronquist (1991) Manual of vascular plants of northeastern United States and Adjacent Canada. (second Edition). The New York Botanical Garden, Bronx, New York

Goldberg, R. B. (1988) Plants: novel developmental processes. Science 240:1460-1467

Greyson R. I. (1994) The development of flowers. Oxford University press, Oxford

Hannig, E. (1913) untersuchung uber das abstossen von Bluten u.s.w., Zeitschr. Bot., 5: p. 417

Heylen, C. And J. C. Vendrig (1988) The influence of different cytokinins and auxins on flower neoformation in thin layers of *Nicotiana tabacum* L.. Plant Cell Physiol. 29:665-671

Hill, S. E., A. D. Stead, R. Nichols (1987) Pollination-induced ethylene and production of 1-aminocyclopropane-1-carboxylic acid by pollen of Nicotiana tabacum cv white burley. J. Plant Growth Regul. 6:1-13

Jackson, M. B., and D. J. Osborne (1970) Ethylene, the natural regulator of leaf abscission. Nature 225:1019-1022

Jacobs, W. P. (1968) Hormonal regulation of leaf abscission. Plant Physiol. 43: 1480-1495

Jensen T. E., and J. G. Valdovinos (1967) Fine structure of abscission zones I. Abscission zones of the pedicels of tobacco and tomato flowers at anthesis. Planta 77:298-318

Jensen T. E., and J. G. Valdovinos (1968) Fine structure of abscission zones III. Cytoplasmic changes in abscising pedicels of tobacco and tomato flowers. Planta 83:303-313

Kanchanapoom, M. L., and J. F. Thomas (1987a) Stereological study of ultrastructural changes in the shoot apical meristem of nicotiana tabacum during floral induction. Amer. J. Bot. 74:152-163

Kanchanapoom, M. L., and J. F. Thomas (1987b) Quantitive ultrastructural changes in tunica and corpus cells of the shoot apex of nicotiana tabacum during transition to flowering . Amer. J. Bot. 74:241-249

Kaur-Shawney, R., A. F. Tiburcio, A. W. Galston (1988) Spermidine and flower-bud formation differentiation in thin-layer explants of tobacco. Planta 173:282-284

Kaur-Shawney, R., G. Kandpal, B. McGogigle, A. W. Galston (1990) Further experiments on spermidine-mediated floral-bud formation in thin-layer explants of Wisconsin 38 tobacco. Planta 181:212-215

Kay, L. E., and D. V. Basile (1987) Specific peroxidases isoenzymes are correlated with organogenesis. Plant physiol. 84:99-105

Kelly A. J., M. B. Bonnlander, D. Ry Meeks-wagner (1995) NFL, The tobacco homolog of FLORICAULA and LEAFY, is transcriptionally exoressed in both vegetative and floral meristems. The plant cell 7:225-234

Kendall, J. N. (1918) Abscision of flowers and fruits in the Solanaceae, with special reference to nicotiana. Univ. of calif. Publ. In Botany 5:347-428

Kinet, J. M., R. M. Sachs, G. Bernier (1985) The physiology of flowering, Vol. III: The development of flowers. CRC Press, Inc. Boca Raton, Florida

Kual, R. B. (1995) Reproductive structure and organogenesis in a cottonwood, Populus deltoides (Salicaceae). Int. J. Plant Sci. 156:172-180

Lashbrook, C. C., C. Gonzalez-Bosch, A. B. Bennett (1994) Two divergent endo-B-1,4-glucanase genes exibit overlapping expression in ripening fruit and abscising flowers. Plant cell 6:1485-1493

Le Corff, J. (1993) Effects of light and nutrient availability on chasmogamy and cleistogamy in an understory tropical herb, *Calathea micans* (Marantaceae). Amer. J. Bot. 80:1392-1399

Lewis, L. N., and J. E. Varner (1970) Synthesis of cellulase during abscission of Phascolus vulgaris leaf explants. Plant Physiol. 46:194-199

Lieberman, S. J., J. G. Valdovinos, T. E. Jensen (1983) A morphometric study on the effects of ethylene treatment in promoting abscission of tobacco flower pedicels. Plant Physiol. 72:583-585

Lieberman, S. J. (1984) A physiological and ultrastructural study of cellulase in tobacco abscission cells. Doctoral Dissertation, The City University of New York

Lieberman, M. (1979) Biosynthesis and action of ethylene. Ann. Rev. Plant Physiol.30:533-591

Linnaeus, Carolus (1753) Species plantarum.

Loewi, E. (1907) Blattablossung und verwandte ercheinungen. Proc. Akad. W. Math-nat. Kl., 166: p. 983

Lovett Doust J. (1989) Plant reproductive strategies and resource allocation. Tree, 4:230-233.

Luft, J. H. (1961) Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9:409-414

McDaniel, C. N., K. A. Sangrey, D. E. Jegla (1989) Cryptic floral determination: stem explamts from vegetative tobacco plants have the capacity to regenerate floral shoots. Dev. Biol. 134:473-478

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

Mohl, H. (1860) Ueber den ablosungsprozess saftiger pflanzenorgane. Bot. Zeit., 18: P. 273

Morre, D. J. (1968) Cell wall dissolution and enzyme secretion during leaf abscission. Plant Physiol. 43: 1545-1559

Muir, R. M. (1942) Growth hormones as related to the setting and development of fruit in *Nicotiana tabacum*. Amer. J. Bot. 29:716-720

Murashige, T. and F. Skoog (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures.Univ of Wisconsin. Phyiologia plantarum. vol.15,pp473-497.

Rajeevan, M. S., and A. Lang (1993) Flower-bud formation in explants of photoperiodic and day-neutral *Nicotiana* biotypes and its bearing on the regulation of flower formation. Proc. Natl. Acad. Sci. USA 90:4636-4640

Reid, P. D., E. del Campillo, L. N. Lewis (1990) Anatomical changes and immunolocalization of cellulase during abscission as observed on nitrocellulose tissue prints. Plant Physiol. 93:160-165

Reid, M. S., D. W. Fujino, N. E. Hoffman, C. S. Whitehead (1984) 1-Aminocyclopropane-1-carboxylic acid (ACC)--The transmitted stimulus in pollinated flowers? J. Plant Growth Regul. 3:189-196

Reinold, S., K. D. Hauffe, C. J. Douglas (1993) Tobacco and parsley 4-coumarate: coenzyme A ligase genes are temporally and spatially regulated in a cell type-specific manner during tobacco flower development. Plant Physiol. 101:373-383

Richett, H. W. (1944) The classification of inflorescences. The botanical review 10:187-231

Rivers, B. A., and M. Marcotrigiano (1994) Floral determination in adventitious shoot meristems regenerated from leaves of day-neutral tobacco. Amer. J. Bot. 81:85-88

Robinson, B. L., and M. L. Fernald (1908) Gray's new manual of botany (Seventh edition-- Illustrated). American Book Company

Shannon, S., and D. R. Meeks-Wagner (1993) Genetic interactions that regulate inflorescence development in Arabidopsis. Plant Cell 5:639-655

Singer, S. R. and C. N. McDaniel (1986) Floral determination in the terminal and axillary buds of *Nicotiana tabacum* L. Dev. Biol. 118:587-592

Singer, S. R. and C. N. McDaniel (1987) Floral dtermination in internode tissues of day-neutral tobacco first occurs many nodes below the apex. Proc. Natl. Acad. Sci. USA 84:2790-2792

Stebbins, G. L. (1974) Flowering plants evolution above the species level. The Belknap Press of Harvard University Press, Cambridge, Ma

Sexton, R. (1982). Cell biology of abscision.Stirling univ,UK.Ann. Rev. Plant Physiol.vol.33, pp133-62.

Tiburcio, A. F., R. Kuar-Sawhney, A. W. Galston (1988) Polyamine biosynthesis during vegetative and floral bud differentiation in thin-layer tobacco tissue cultures. Plant Cell Physiol. 29:1241-1249

Tran Thanh Van, M. (1973a) *In vitro* control of *de novo* flower, bud, root, and callus differentiation from excised epidermal tissues. Nature 246:44-45

Tran Thanh Van, M. (1973b) Direct flower neoformation from superficial tissue of small explants of *Nicotiana tabacum* L. Planta 115:87-92

Tran Thanh Van, M., N. Thi Dien, A. Chlyah (1974) Regulation of organogenesis in small explants of superficial tissue of *Nicotiana tabacum* L. Planta 119:149-159

Trull, M. C., and R. L. Malmberg (1994) Puzzle Box, a tobacco line with flowers that mix floral and inflorescence characteristics. Americam J. of Botany 81:582-588

Tso, T. C. (1972) Physiology and biochemistry of tobacco plants. Dowden, Hutchinson and Ross, Inc. Stroudsburg, Pa.

Tucker, M., Baird,S ;Sexton,R (1991) Bean leaf abscission:Tissue-specific accumulation of a cellulase mRNA.Plant MOlecular Biology Lab.,U.S.Dept of Agricultural Research Service, Beltsville,Maryland. Planta.Vol.186,pp52-57.

Tucker, M. L., R. Sexton, E. Del Campillo, L. N. Lewis (1988) Bean abscission cellulase. Characterization of a cDNA and regulation of gene expression by ethylene and auxin. Plant Physiol 88:1257-1262

Valdovinos, J. G., T. E. Jensen, S. L. Lieberman (1985) An ultrastructural and physiological study of the effects of actinomycin D and cycloheximide on abscision of tobacco flower pedicels. Bot. Gaz. 146:308-314

Valdovinos, J. G., T. E. Jensen, L. M. Sicko (1972) Fine structure of abscission zone IV. Effects of ethylene on the ultrastructure of abscission cells of tobacco flower pedicels. Planta 102:324-333

Valdovinos, J. G., and T. E. Jensen (1968) Fine structure of abscission zone II. Cell wall changes in abscising pedicels of tobacco and tomato flowers. Planta 83:295-302

van den Ende, G., A. F. Croes, Anke Kemp, and G. W. M. Barendse (1984) Development of flower buds in thin-layer cultures of flower stak tissue from tobacco: Role of hormones in different stages. Physiol. Plant. 61:114-118. Copenhagen

Weberling, F. (1965) Typology of inflorescences. J. Linn. Soc. (Bot.) 59:215-221

Weberling, F. (1989) Morphology of flowers and inflorescences. Cambridge University Press, Cambridge

Weigel, D., J. Alvarez, D. R. Symth, M. F. Yanofsky, E. M. Meyerowitz (1992) Leafy controls floral identity in Arabidopsis. Cell 69:843-859

Wiggins, I. L. and D. M. Porter (1971) Flora of the Galapagos Islands. Stanford University Press, Stanford, California

Williams, A. G., and T. G. Whitham (1986) Premature leaf abscission: An induced plant defense against Gall aphids. Ecology 67:1619-1627

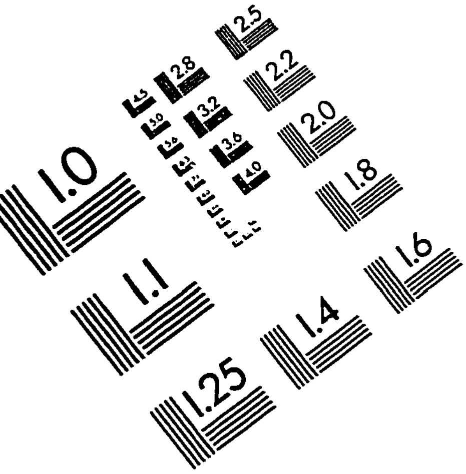
Yager, R.E. (1960) Possible role of pectic enzymes in abscission. Plant Physiol. 35:157-162

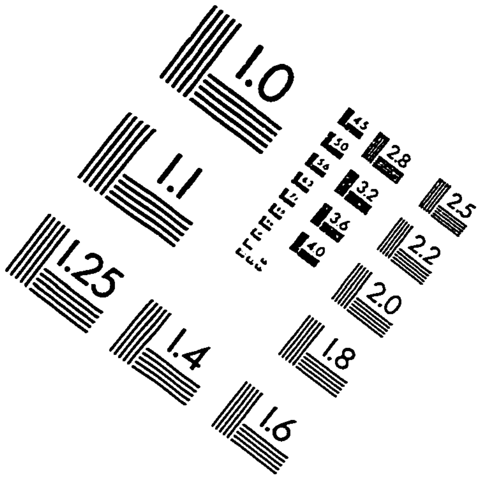
Yager, R. E. (1957) The mechanism of floral abscission in Nicotiana. Doctoral Dissertation. The State University of Iowa

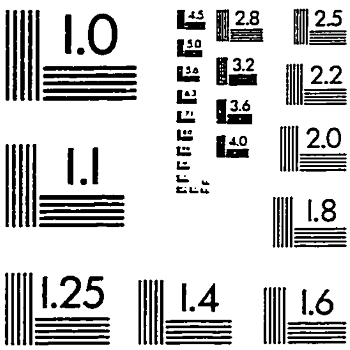
Yang, S. F. And N. E. Hoffman (1984) Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35:155-189

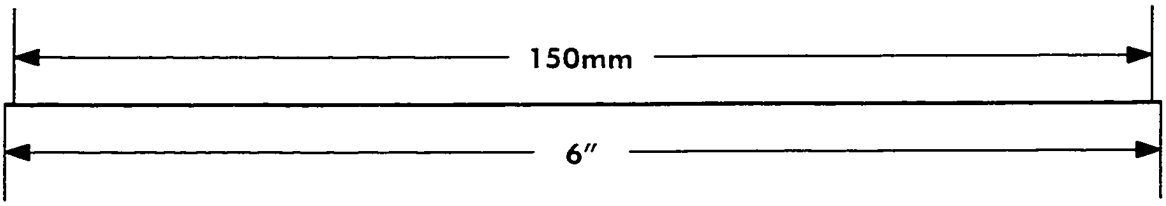
Zhang, Y. and J. G.Valdovinos (1995) Organization of the tobacco terminal inflorescence. The 59th Annual Meeting of the Northeast Section of the American Society from Plant Physiologists, Kingston, RI (poster)

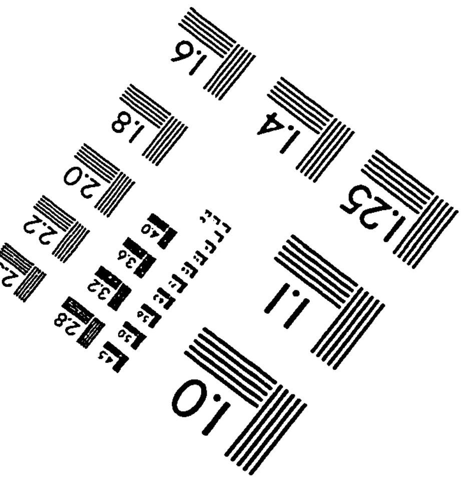
IMAGE EVALUATION TEST TARGET (QA-3)

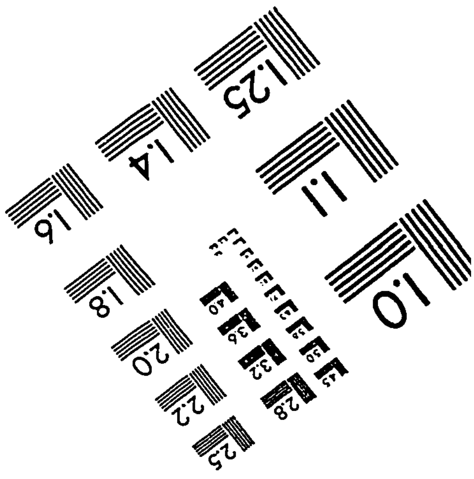












APPLIED IMAGE . Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989