

THE MORPHOGENETIC EFFECTS OF THE DOUBLE
FLOWERING GENE IN PETUNIA HYBRIDA HORT

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ABSTRACT

THE MORPHOGENETIC EFFECTS OF THE DOUBLE FLOWERING GENE IN PETUNIA HYBRIDA HORT.

By

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Biological investigation of the mode of action of any genetically controlled character first requires an understanding of the phenotypic response of that gene. This study was conducted to provide a basis for future research and a thorough understanding of flower doubleness as it is expressed morphogenetically in Petunia hybrida Hort. Flower doubleness in petunia is controlled by a single dominant gene, D, which on a gross morphological basis appears as the proliferation of petals and fertile stamens accompanied by the production of a diminutive, malformed and sterile pistil.

The objective of this research was to determine the effect of the double allele, D, on the morphology and development of the apical meristem and flower in the homozygous, DD, and heterozygous, Dd, genotypes as compared to the single, dd. These studies were conducted on vegetative and flowering shoot tips and bud sections of three inbred

multiflora lines and one hybrid which differed genetically for the double flower character.

The vegetative apical meristem of the petunia, regardless of flower type, possesses a biseriate tunica and an organized corpus consisting of small cells which radiate laterally and downward to a depth of six to eight cells.

In the single, dd, the initiation of floral parts is acropetal. Floral differentiation and development follow the same pattern. The results show that in the presence of the D allele the normal sequence of initiation is disturbed. Following the initiation of the petal and stamen primordia, additional primordia proliferate over the remaining axial region of the apex converging toward the center. The additional primordia differentiate into petals and stamens with an ill-defined transitional zone, i.e. those primordia situated closest to the corolla become petals, those centrally located become stamens and the primordia between these develop into antheroid-petals and petaloid-anthers according to their location.

Determination is not inhibited in the double since all floral parts are found even though the pistil is undeveloped or malformed. The heterozygote, Dd, was noted to produce a few fertile pistils whereas the homozygote, DD, was never observed to possess fertile pistils.

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I. INTRODUCTION

The petunia, Petunia hybrida Hort. ($n = 7$), is an economically important member of the Solanaceae family. At present, it is the most popular annual bedding plant in the United States. Of the two flower forms available, single and double, the multi-petaled double type accounts for approximately 25 percent of the petunia market (Kline, 1968). The double flowered, female sterile phenotype is controlled by a single dominant gene. Therefore, only the homozygous dominant genotype can be used as the pollen parent for the production of double flowering hybrid seed.

Investigation of doubleness by morphological, cytological and biochemical methods has not been conducted. Whether the double flower phenotype is a product of stimulatory processes and/or inhibitory processes has yet to be determined. Of primary importance is the action of a single gene which alters a single perfect petunia flower into a double flower which exhibits a profusion of petals and of anthers which are functional and the loss of female fertility through the malformation or total absence of a functional pistil. An understanding of the site, time and mode of action of the gene causing doubleness during

floral development is primary to interpretation of gene action and as a basis for future biochemical and growth regulator investigations.

The purpose of this study was to conduct a morphological investigation of the double flowering character in petunia. The objectives were to determine the gross morphology of the vegetative shoot apical meristem of the seedling and mature non-flowering petunia, and to determine the morphological development of the double and single petunia flower genotypes from floral initiation to anthesis.

II. LITERATURE REVIEW

The common petunia, Petunia hybrida Hort. ($n = 7$), extensively used in ornamental horticulture and biological research has been noted by many to be a hybrid of at least two Petunia species, (Bailey, 1947; Bailey and Bailey, 1959; Darlington and Wylie, 1955; and Derman, 1931). Bailey, in 1901, stated that Petunia hybrida was first reported to be in European gardens in 1837. He proposed that it was the progeny of an interspecific cross of at least two species then known: P. axillaris BSP ($2n = 14$, Kostoff, et al., 1955) an upright herbaceous annual with white flowers and yellow pollen that originated from Argentina and was introduced into Europe in 1823; and P. violaceae Lindl. ($2n = 14$, Derman, 1931) a sprawling herbaceous annual with purple flowers and blue pollen that was also native to Argentina and had been transported to Europe in 1830.

The flower of the single petunia is typical of the Solanaceae. It is a perfect and regular flower with a calyx deeply lobed into five sepals. The corolla is sympetalous and tubular, terminating in five lobes. The five hypogynous stamens are adnate and alternate with the lobes of the corolla. The pistil is composed of a superior

ovary which contains two locules and a single style which terminates into a bi-lobed stigma, (Robbins, et al., 1961; Cronquist, 1961; and Bensen, 1967).

In one of the earliest studies of the double flower form, Saunders (1910) noted that this doubleness is easily recognized in the bud stage when the bud is short and blunt whereas the single bud is long and slender. She further reported that in the fully expanded double flower the corolla tube surrounds a profusion of additional petals and anthers, and that the number of stamens and petals are variable. Kline (1967) suggests that this variability may be due to modifying genes and/or environmental interactions. Most of the petals are adherent at their bases and partly along their sides and many of the anthers are adnate to the petals at various points along the filament. A functional gynoecium is rarely found. More commonly, the pistil is malformed or completely lacking. When it is present the carpel is usually incomplete and may contain antheroid tissue with functional pollen (Saunders, 1910). The style and stigma, if at all present, are reduced and distorted.

In 1937, Scott reported the inheritance of the double and single flower types. He showed that doubleness was controlled by a single dominant gene, D, which was allelic to the single flowering character, d.

The genetics of flower doubleness and the effect on floral fertility have been studied in a number of plants:

Saunders (1917), Meconopsis, Althaea, and Dianthus; Philip and Huskins (1931), Mathiola; and Eyster and Burpee (1936), Nasturium. It should be noted that these as well as other studies reviewed did not contain a microscopic investigation of the development of the double versus the single phenotypes.

A series of studies in the development of the inflorescence by Philipson (1946, 1947, 1948) was concerned primarily with the early transformation of the vegetative meristem into a capitulum in the Compositae. Similarly, studies have been reported on the initiation and ontogeny of the inflorescence with no data presented concerning the development and final morphology of the floral appendages: Popham and Chan (1952), Chrysanthemum morifolium; Tepfer (1954), Aquilegia formosa var. truncata and Ranunculus repens; Gifford and Tepper (1961), Chenopodium album; Miksche and Brown (1965), Arabidopsis thaliana; Nougarede, et al. (1965), Nicotiana sp.; Hodgson (1966), Gramineae.

At present there are a number of theories concerning cellular organization in the shoot apical meristem of higher plants. Meristem organization is interpreted by means of correlating meristematic regions, in either general or rigidly defined terms, to the ontogeny of plant structures or simply through designation of histological definable areas. Hanstein, in 1868, according to Esau (1953),

advanced the Histogen Theory. He proposed that specific plant tissues or parts originated from specific meristematic zones in the shoot apex. This theory was eventually shown to be difficult to apply in light of later advances in anatomical and histological research, especially those which concerned cytochimeras, (Esau, 1953). In 1924, Schmidt proposed the Tunica-Corpus Theory, (Gifford, 1954).

"According to the tunica-corpus theory, the shoot apex consists of meristematic tissue arranged more or less distinctly in two major parts--a central core, or corpus, sheathed by one or more external layers, the tunica" (Eames, 1961). Soon it became evident that this concept did not provide for certain deviations subsequently found, notably in the variation of tunica layers found within a species and in a single plant according to position, vigor or season. In 1950, Popham and Chan proposed the Mantle-Core Theory. This concept was so structured as to allow for the variations already reported. Their theory relaxed the restrictive rule that stated the cells of the tunica divide only anticlinally, and at the same time attacked the ambiguity of the theory, noting that the "Tunica and Corpus are at best arbitrary topographic terms and indicate nothing as regards ontogeny" (Popham and Chan, 1950). They, in turn, defined a mantle which included all of the distinct peripheral layers of cells in the apex and was maintained by anticlinal divisions but not devoid of periclinal

divisions which may add to the core. The core is analogous to the corpus.

According to Gifford (1954) three other theories have been advanced: 1.) The Histogenic Concept by Derman which is similar to Hanstein's theory without the implication of predetermined tissue origin; 2.) The "L' Anneau Initial" Theory by Plantefol which incorporates into the tunica-corpus theory an additional region of lateral self perpetuating initials; 3.) The "Meristeme D'Attente" Theory by Buvat which proposes the existence of an inactive axial region in the tunica and corpus which only becomes active after flower induction and is the sole origin of the flower parts.

This author found that most current ontogenetic and anatomical studies of the shoot apical meristem use primarily the tunica-corpus concept in the presentation of data with the incorporation of the mantel-core or the histogenic layers concept.

III. MATERIALS AND METHODS

A. Plant Material

The genetic lines used in this study were multi-flora inbreds obtained from the Pan-American Seed Company. The homozygous double line, DD, designated MSU-488 was selected from the fourth selfed generation of a heterozygous double. The two heterozygous double lines, Dd, employed were MSU-506, a sister line to MSU-488, and MSU-509, which is an F_1 hybrid known commercially as "Plum Double." The single flowered line, dd, MSU-507, had been inbred for three generations and then the seed was massed. The later two lines were obtained as seed.

B. Cultural Practices

For vegetative propagation, stem cuttings were taken from stock plants, treated with Hormodin No. 2 rooting compound, placed in a flat of sterilized sand which was then covered with a pane of glass and fertilized with 100 ppm of 20-20-20 every seven days. To prevent chlorosis, the cuttings were treated with an EDTA 12 percent NaFe iron chelate drench at one tablespoon per gallon. After the cuttings were rooted, they were transplanted into 2-1/4 inch

square peat pots using a soil mixture consisting of soil, German peat and Perlite in a 1:1:1 ratio by volume.

Seeds were germinated on horticultural grade Vermiculite or Peat-lite. The germinating medium was watered one day prior to sowing the seeds. The seeded flats were covered with a pane of glass and a few sheets of newspaper. The seeds germinated in three to five days. Within seven days after germination the young seedlings were transplanted into 2-1/4 inch square peat pots using the same soil mixture used for cuttings.

When the seedlings or rooted cuttings attained the eight to twelve leaf stage they were transplanted into five or six inch clay pots. The soil mix used was a 1:1:1 ratio by volume of soil, German peat and Turface.

All plants in the peat pots and clay pots received a weekly application of 600 ppm of a 20-20-20 fertilizer.

During the winter months flowering was inhibited by maintaining an eight hour photoperiod. The plants were covered with black cloth from 4 P.M. to 8 A.M. To obtain flowering plants during the winter, 100 watt incandescent light bulbs with twelve inch reflectors were placed four feet above the plants and spaced four feet apart. The plants were illuminated nightly from 10 P.M. to 2 A.M.

All plants were grown in a greenhouse with a 65 F night and 70 F day temperature.

For a single experiment, seeds were sown on filter paper and placed into a sterile petri dish to which 10 ml. of water was added. The seeds germinated in three to five days at room temperature. The seedlings were prepared for histological observation by the same method employed for the stem tips.

C. Histological Technique

Meristematic tip sections, 1 cm. long, and flower buds at various stages of development were removed from the plants and treated with FAA killing and fixing solution which consisted of ten parts 37 per cent Formalin, five parts glacial acetic acid, fifty parts 95 percent ethyl alcohol and thirty-five parts distilled water (Sass, 1958). They were evacuated at 35 mm. vacuum for one hour and then returned to atmospheric pressure for an additional twenty-four to seventy-two hours. The tertiary butyl alcohol (TBA) series employed for dehydration and the infiltration method used were according to the procedures of Knobloch and Mericle (1967). The specimens were imbedded into Tissuemat and sectioned with a rotary microtome at 5-7 microns. Selected sections of ribbon were mounted on slides with Haupt's adhesive (Johansen, 1940) and allowed to dry at room temperature for twenty-four hours.

Two staining procedures were used in this study: a general histological stain, Chlorazol Black E for the

flower buds and an Aniline Blue-Hematoxylin combination for stem tip sections.

The Chlorozol Black E schedule was a modification of Darrow's (1940) and was as follows.

- | | |
|--|---------------------------|
| 1. Xylene | at least 10 minutes |
| 2. Xylene and absolute ethyl alcohol (1:1) | 10 minutes |
| 3. Absolute ethyl alcohol | 5 minutes |
| 4. 95 percent alcohol | 5 minutes |
| 5. 70 percent alcohol | 5 minutes |
| 6. 1 percent Chlorozol Black E in 70 percent alcohol | 5 minutes |
| 7. 70 percent alcohol | Differentiate 2-3 minutes |
| 8. 95 percent alcohol | 5 minutes |
| 9. Absolute ethyl alcohol | 2-3 minutes |
| 10. Xylene and absolute ethyl alcohol (1:1) | 5 minutes |
| 11. Xylene | 5 minutes |
| 12. Xylene | 5 minutes |
| 13. Permatize | |

The second procedure was a modification of Vaughan's (1955) Hematoxylin-Aniline Blue schedule. The 0.5 percent aqueous hematoxylin stain was made according to Johansen (1940). The aniline blue stain was a saturated solution of the dye powder in methyl cellosolve, (Vaughan, 1955). The iron alum mordant used was according to Sass (1958).

Differentiation of the hematoxylin was achieved with a two percent aqueous solution of FeCl_3 (Johansen, 1940). The schedule was as follows:

- | | |
|--|------------------------------|
| 1. Xylene | at least 10 minutes |
| 2. Xylene and absolute ethyl alcohol (1:1) | 10 minutes |
| 3. 95 percent alcohol | 10 minutes |
| 4. 70 percent alcohol | 10 minutes |
| 5. 50 percent alcohol | 10 minutes |
| 6. 35 percent alcohol | 5 minutes |
| 7. Rinse in distilled water | |
| 8. Iron Alum mordant | 30 minutes |
| 9. Rinse in distilled water | |
| 10. 0.5 percent hematoxylin | 12 hours |
| 11. Rinse in distilled water | |
| 12. 2 percent aqueous FeCl_3 | Differentiate
3-5 minutes |
| 13. Wash thoroughly in running tap water | 30 minutes |
| 14. 50 percent alcohol | 5 minutes |
| 15. 70 percent alcohol | 5 minutes |
| 16. 95 percent alcohol | 5 minutes |
| 17. Absolute ethyl alcohol | 5 minutes |
| 18. Absolute ethyl alcohol | 5 minutes |
| 19. Aniline blue stain | 3 minutes |

20. Rinse in absolute ethyl alcohol
21. Methyl cellosolve: xylene:
absolute ethyl alcohol
(1:1:2) 10-15 minutes
22. Methyl cellosolve: xylene:
absolute ethyl alcohol
(2:1:1) 10-15 minutes
23. Xylene and absolute ethyl
alcohol (1:1) 5 minutes
24. Xylene 5 minutes
25. Xylene 5 minutes
26. Permatize

The stained slides were permatized with a sixty percent Harleco resin solution in xylene. Weights were placed on the cover slips of the slides and drying was accomplished on a 43 C warming table for forty-two to seventy-two hours. Final drying was in a 58 C paraffin oven for three to five days.

Observations and photomicrographs were made with a Spencer A&O dissecting microscope and a Carl Zeiss GFL microscope.

IV. RESULTS AND DISCUSSION

A. The Shoot Apical Meristem of the Seedling Petunia

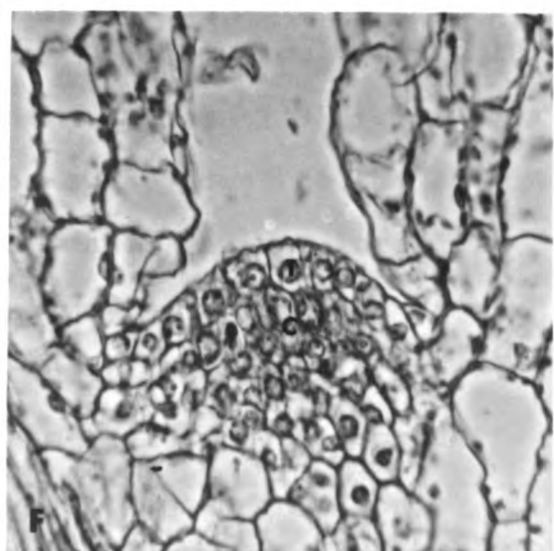
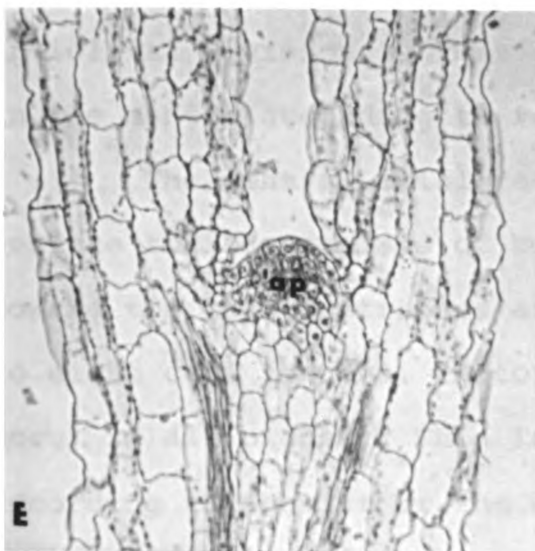
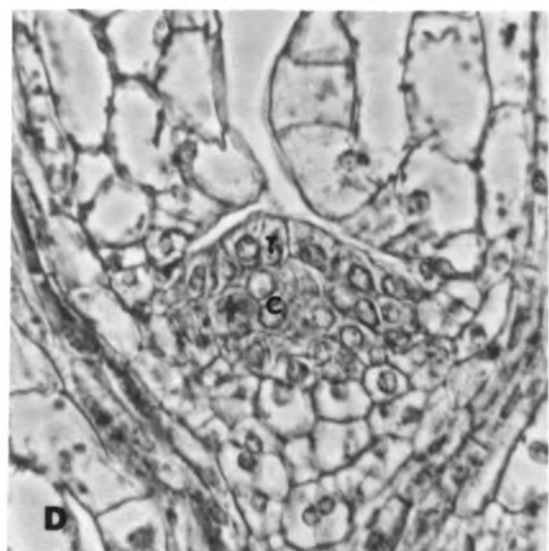
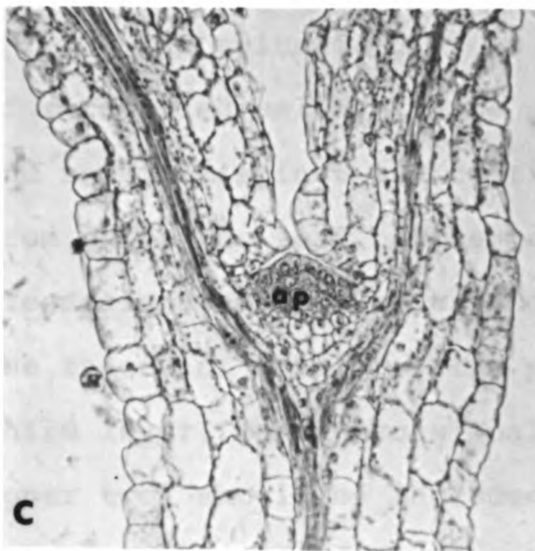
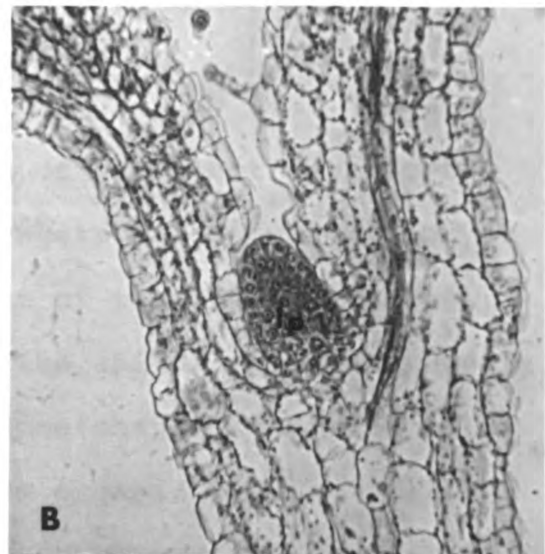
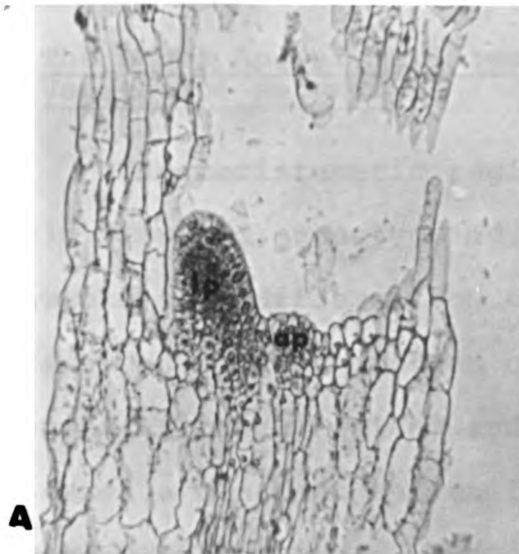
These results are from observations of the inbred single line MSU-30 and the hybrid line MSU-509.

Twenty-four hours after germination the seedling possessed an elongated hypocotyl, 2-4 mm long, and two small but fully expanded cotyledons. In transverse section the apical region reveals two leaf primordia about the central meristem which seem to be oriented nearly opposite one another and at right angles to the cotyledons, (Figures 1 A and B). Constantin and Mullenax (1966) reported the existence of a similar condition in Lactuca sativa; they showed that these first leaf primordia were already initiated in the ungerminated but mature seed.

In a transverse median section of the apex, the meristematic region appears to be a slightly conical mass of cells. This tissue stains much lighter than the leaf primordia. A single uniform layer of cells, extending over the entire meristematic region, was present. At this stage the petunia possesses a uniseriate tunica. The underlying cells, or corpus, exhibited no apparent organization, (Figures 1 C to F).

Figure 1. The shoot apical meristem of the seedling
petunia twenty-four hours after germination.
Apical meristem - ap; leaf primordium - lp;
tunica - T; corpus region - C.

- A. Single flower, genotype - dd. Transverse
section. x408
- B. Single flower, genotype - dd. Transverse
median section. x408
- C. Single flower, genotype - dd. Transverse
median section. x408
- D. Greater magnification of Figure 1-C. x1020
- E. Double flower, genotype - Dd. Transverse
median section. x408
- F. Greater magnification of Figure 1-E. x1020



B. The Shoot Apical Meristem of the
Vegetative Petunia

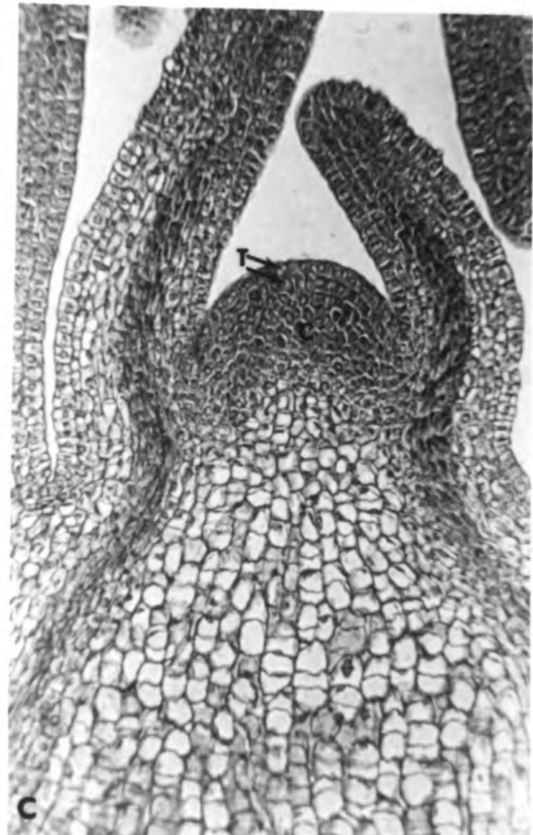
The meristematic region of the shoot apex in the single flowered, genotype dd, vegetative petunia, four to six weeks old, differs from that of the young seedling. A transverse median section of the apical meristem reveals a convex, slightly raised and dome-shaped structure which possesses a constant and uniform organization of cells, (Figure 2 A).

The apical region is composed of two uniform layers of stratified cells. These extend over the entire meristem and remain distinct and continuous with the lateral areas from which the leaf buttresses originate. These two layers are designated as a biseriate tunica as in accordance with the theory of Schmidt. The possibility that a subtending third layer, which occasionally appears as uniform as the upper two, should be included in the tunica is negated because of the sporadic occurrence of periclinal as well as anticlinal divisions. This layer of cells would be included in the mantle according to Popham and Chan (1950).

The central axial region subadjacent to the biseriate tunica consists of cells which radiate laterally toward the leaf buttresses and downward to a depth of six to eight cell lengths. Mitotic divisions in this area occur in all planes. This is the region of the corpus according to Schmidt or the core of Popham and Chan's

Figure 2. The shoot apical meristem of the vegetative
petunia. Transverse median sections.
Biseriate tunica - T; Region of the corpus - C.

A. Single flower, genotype - dd. x408
B. Double flower, genotype - Dd. x408
C. Double flower, genotype - DD. x408



theory. Stain intensity increases in all directions as the distance of the cells increases from the uppermost central area in this tissue. This lighter staining region as well as the axial portion of the tunica would probably be designated as the "Meristeme D'Attente" as theorized by Buvat, but no mitotic frequency counts were made. Without this information it would only be speculation as to the relevance of this theory to the petunia.

Leaf buttresses originate laterally from the meristem in an alternate phyllotaxis.

No morphological differences, from that of the single, were noted in the vegetative shoot apical meristems of the homozygous, DD, and heterozygous, Dd, double flowering plants, (Figures 2 B and C).

C. The Morphological Development of the Flower

1. The single flower, genotype - dd

After floral induction the meristem undergoes a series of ordered morphological changes which conducts the apical meristem from a vegetative condition, (Figure 3A), into a flower.

It should be noted that once flowering commences the gross morphology of the leaves produced along a flowering shoot changes and is distinct from that produced by the vegetative shoot. These non-floral organs are much smaller, entirely sessil and lanceolate. A flowering branch

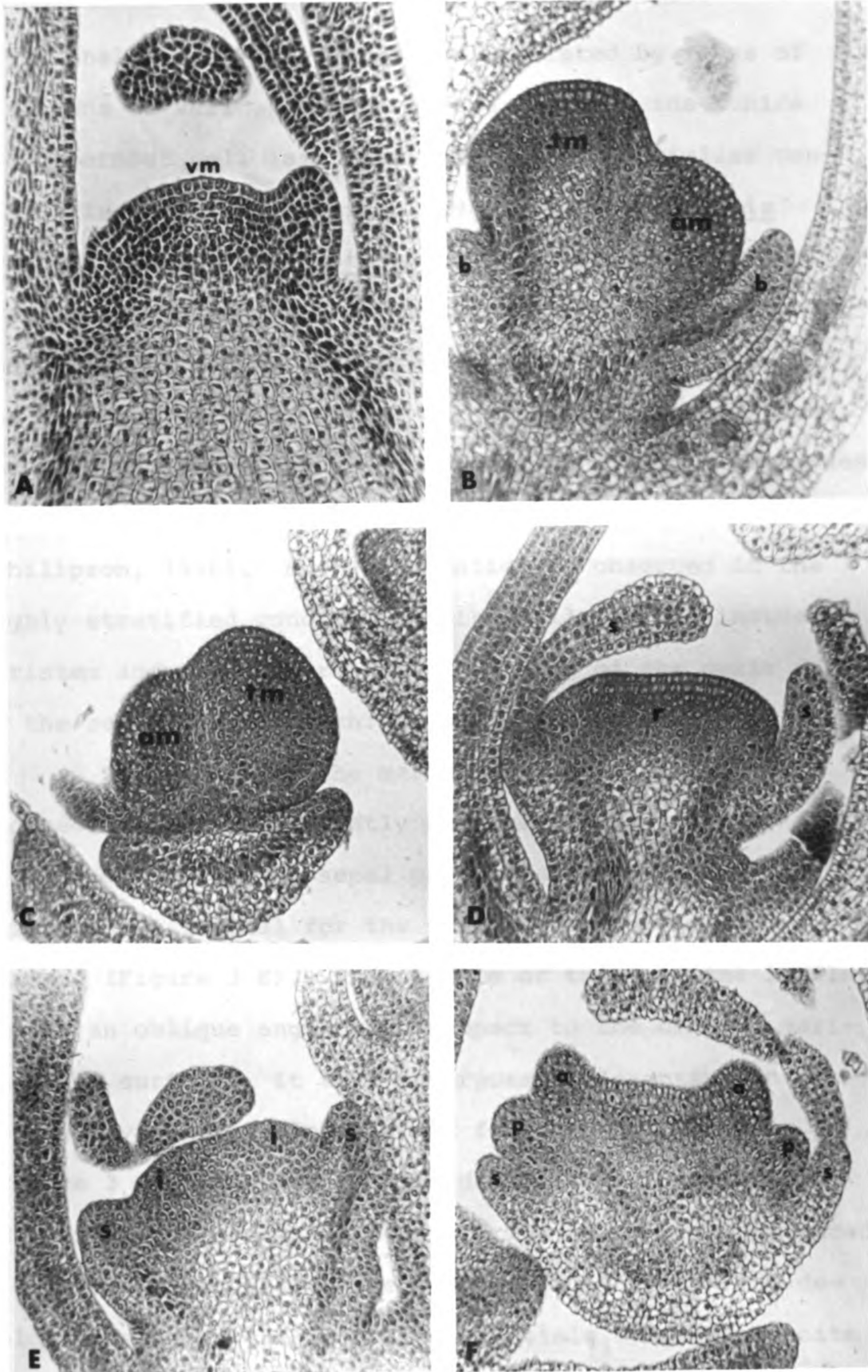
possesses two of these leaf-like structures nearly opposite one another at the base of each rachis. Typical vegetative leaves are alternate, nearly sessil, larger and lanceolate or oblate. The latter variation is probably due to genetic control, (Kline, 1967). Vegetative leaves may be found on a flowering plant, but they occur only on lower axillary shoots. The meristem of these shoots appears similar to the apical meristem on a vegetative plant, and produces four to six normal vegetative leaves prior to the initiation of a flower. Because of this morphological change these non-floral organs which are produced on a flowering shoot shall be referred to as bracts.

With the induction of flowering the apical meristem initiates two bracts. The central meristem rises above the bract primordia and initiates an axillary meristem which is noted to arise between the central meristem and the last bract initiated, (Figures 3 B and C). The exact origin of this second meristem was not clear, but in further discussion it shall be referred to as an axillary meristem.

The cells of this axillary meristem are mitotically active and also supercede the bract primordia in total growth and size. While the axillary meristem is thus developing, the central meristem broadens and initiates five sepal primordia around its periphery, (Figure 3 D). At this stage the major portion of the meristem has been transformed into the receptacle of the flower. All

Figure 3. Single flower, genotype - dd, floral initiation and development. Vegetative meristem - vm; transitional meristem - tm; axillary meristem - am; bract primordium - b; receptacle - r; sepal primordium - s; petal primordium - p; stamen primordium - a; petal-stamen initial - i.

- A. Shoot apical meristem in vegetative condition. Transverse median section. x408
- B. Transitional meristem with axillary meristem to the right. Transverse median section. x 408
- C. Similar to Figure 3-B. Lateral meristem to the left. Non-median section. x408
- D. Sepal primordia initiated around periphery of receptacle. Transverse median section. x408
- E. Petal-stamen initial appears adjacent to sepal primordia. Transverse median section. x408
- F. Petal and stamen primordia develop individually. Transverse section. x408



additional floral appendages are initiated by means of divisions in various planes of the cells in the tunica and uppermost cell layers of the corpus. A similar condition is noted by Philipson (1946, 1947) for Bellis perennis and Succisa pratensis. He describes a gradual transition taking place in which the peripheral cells of the corpus extend downward replacing the central zone of the corpus with a peripheral meristem which becomes continuous with the tunica. Thus the tunica and the extended peripheral zone of the corpus become a "meristematic mantel," (Philipson, 1946). Such a situation is observed in the highly stratified condition of the cells in the induced meristem and the uniform heavy staining of the cells down to the region of the pith.

The center of the meristem diminishes in height and becomes flat to slightly concave. Subsequently, just within the developing sepal primordia a continuous ring, which is the initial for the petals and the stamens, is formed, (Figure 3 E). The surface of this initial is flat and at an oblique angle with respect to the central meristematic surface. It then undergoes differentiation producing five petal primordia and five stamen primordia, (Figure 3 F). The petal primordia are alternate and adjacent to the sepal primordia. The stamens are produced within and alternate to the petals. Just within the developing stamens the two carpel initials develop opposite

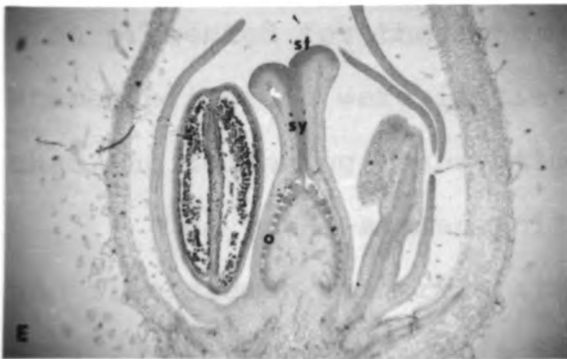
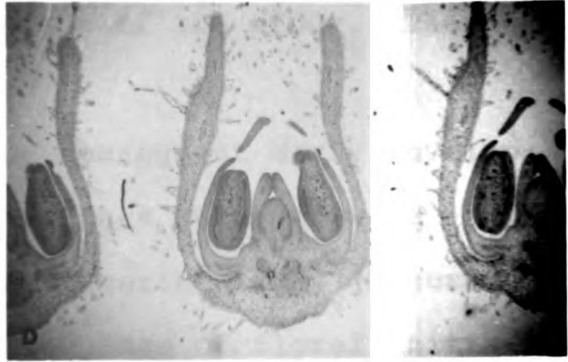
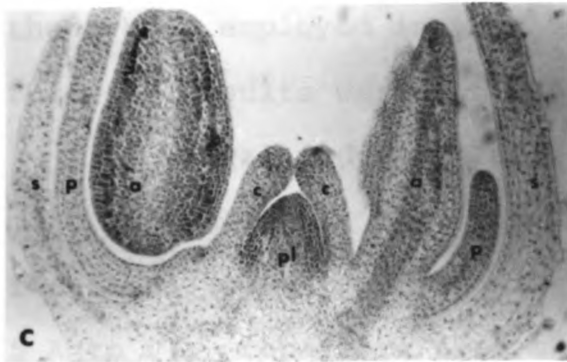
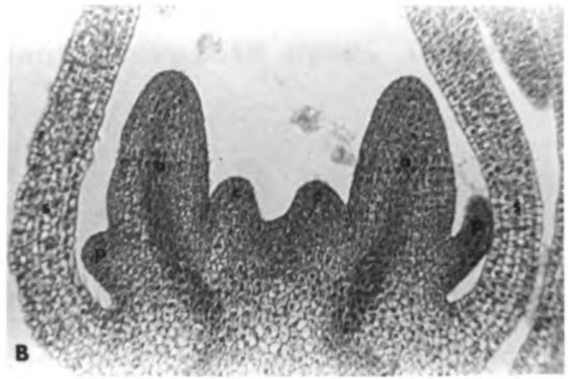
one another, (Figures 4 A and B). They increase in height with cellular division and elongation and meet over the placenta primordium which has arisen from the remaining area in the center of the meristem, (Figure 4 C). The carpel primordia then coalesce over the placenta and continue their vertical growth forming a single, slender style and a bi-lobed stigma, (Figure 4 D). The carpel primordia also develop laterally around the placenta coalescing with the placental wall at their point of meeting to form the two locules.

The stamen primordia develop into large anthers which appear to be almost sessile to the flower receptacle at this stage, (Figure 4 E). The filaments elongate after all floral development and microsporogenesis and megasporogenesis have occurred.

The axillary meristem that was initiated prior to the central meristem's transition into a flower now becomes the shoot's central meristem. This meristem produces two alternate bract buttresses, the first of which is opposed to the flowering meristem. Following initiation of the second bract buttress another axillary bud becomes active between this bract and the present central meristem. The central meristem initiates sepals and then proceeds in the development of a flower while the latest formed axillary meristem commences further growth in the identical pattern just described.

Figure 4. Single flower, genotype - dd, floral initiation and development continued. Sepal primordium - s; petal primordium - p; stamen primordium - a; carpel primordium - c; placenta primordium - pl. Transverse median sections.

- A. Carpel initials first evident within stamen primordia. x320
- B. Developing carpel primordia. x320
- C. Carpel primordia growing toward each other over placenta primordium. x320
- D. Carpel primordia meet and coalesce over placenta. x320
- E. Single flower bud. Stigma - st; style-sy; ovary - o. x320
- F. Two floral meristems from same shoot. Meristem A, the oldest, at stage comparable to Figure 4-B. Meristem B, originally the axillary meristem to A, at stage similar to Figure 3-D. x320.



It is through this mode of floral development that the inflorescence in the petunia, for both single and double genotypes, is a determinant scorpioid cyme, (Figure 4 F).

2. The double flower, genotype - Dd

There appeared to be no morphological differences in flower development between the heterozygous inbred and the hybrid employed in this section of the study. Therefore the results were combined.

Floral induction and the subsequent early development of the sepals in the double lines observed were identical to that of the single flowering line, (Figure 5 A to D). Departure from the sequence of floral initiation presented for the single occurred after the petal and stamen primordia were initiated. It is from this stage that the following observations are reported.

The petal-stamen initial develops into five petal and five stamen primordia similar to the single, (Figure 5 E). The petal primordia differentiate and develop into a tubular corolla. However, these stamen primordia remain undifferentiated while additional primordia are initiated over the entire surface of the receptacle in a random manner but converging toward the center, (Figure 6 A to F). These primordia seem to be produced rapidly and usually completely fill the surface of the receptacle before the

Figure 5. Double flower, genotype - Dd, floral initiation and development. Vegetative meristem - vm; transitional meristem - tm; axillary meristem - am; bract primordium - bp; bract initial - bi; receptacle - r; sepal primordium - s; petal primordium - p; stamen primordium - a. Transverse median sections.

- A. Shoot apical meristem in vegetative condition. x320
- B. Transitional meristem with first bract primordium to left and second bract but-
tress to the right. x320
- C. Transitional meristem with axillary
meristem to the right. x320
- D. Sepal primordia initiated on floral
meristem. x320
- E. Sepal, petal and stamen primordia initiated
on receptacle. x320

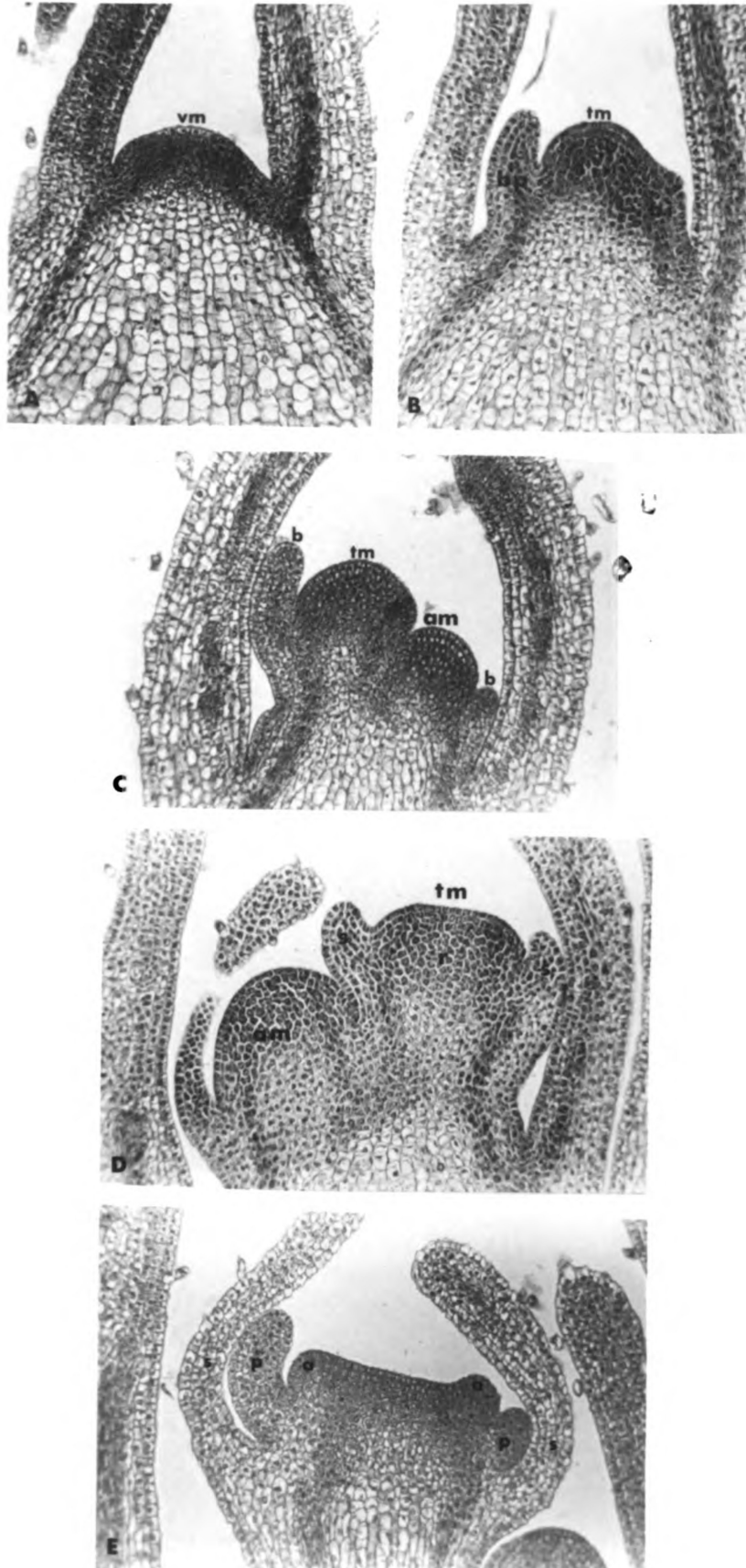


Figure 6. Double flower, genotype - Dd, advanced floral initiation and development. Additional primordia proliferate over the remaining surface area of the receptacle converging to the center. Sepal - s; petal primordium - p; additional primordium - +.

A. x280

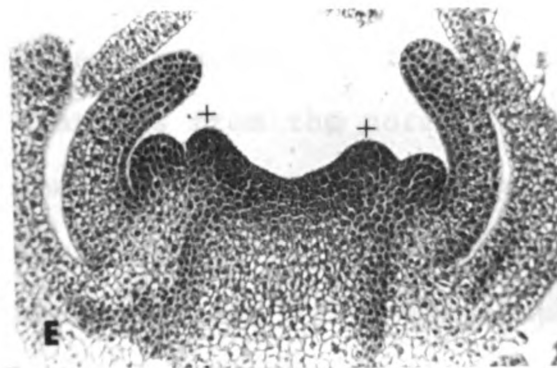
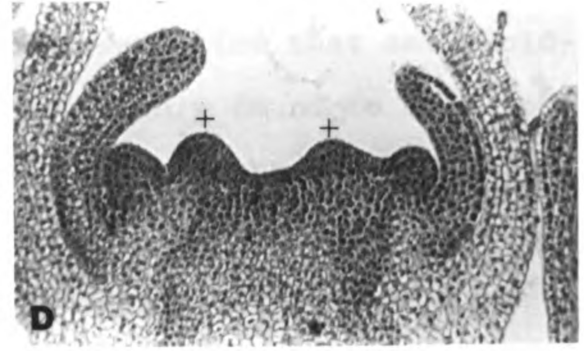
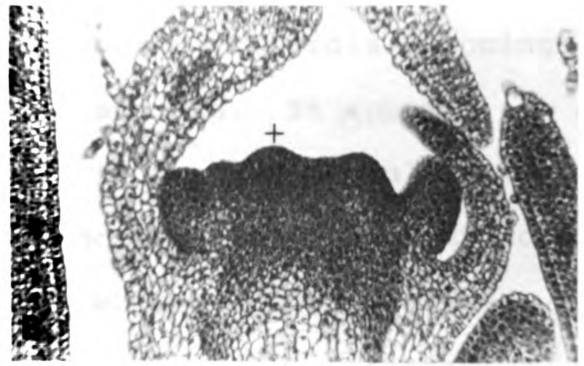
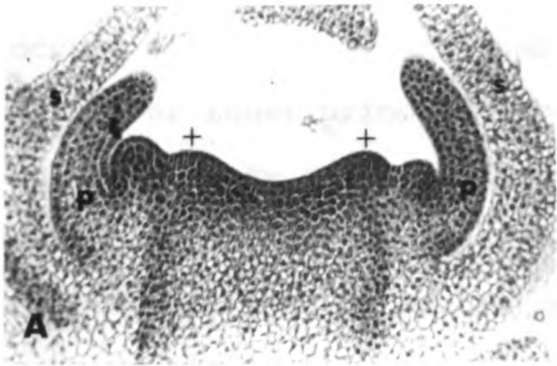
B. x280

C. x280

D. x280

E. x280

F. x280

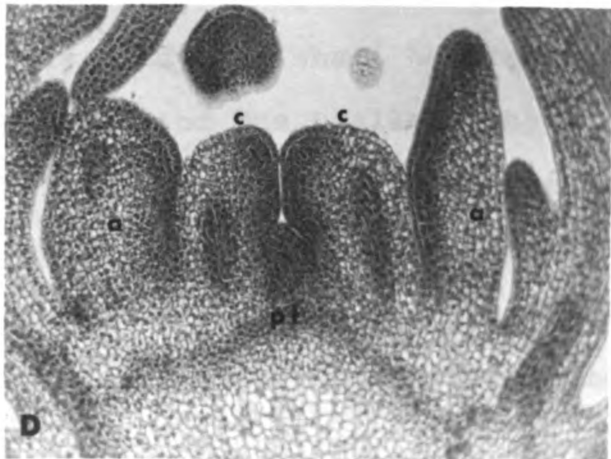
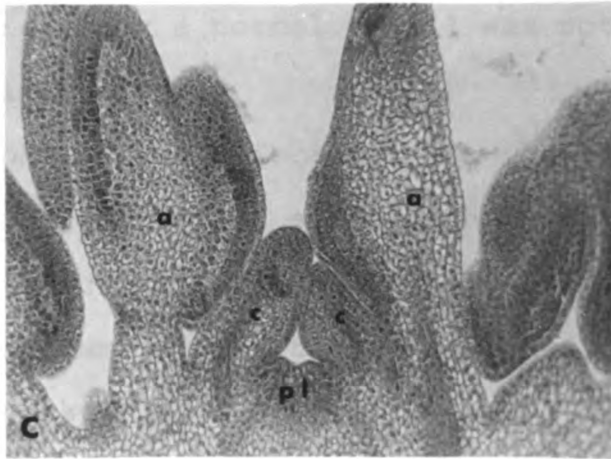
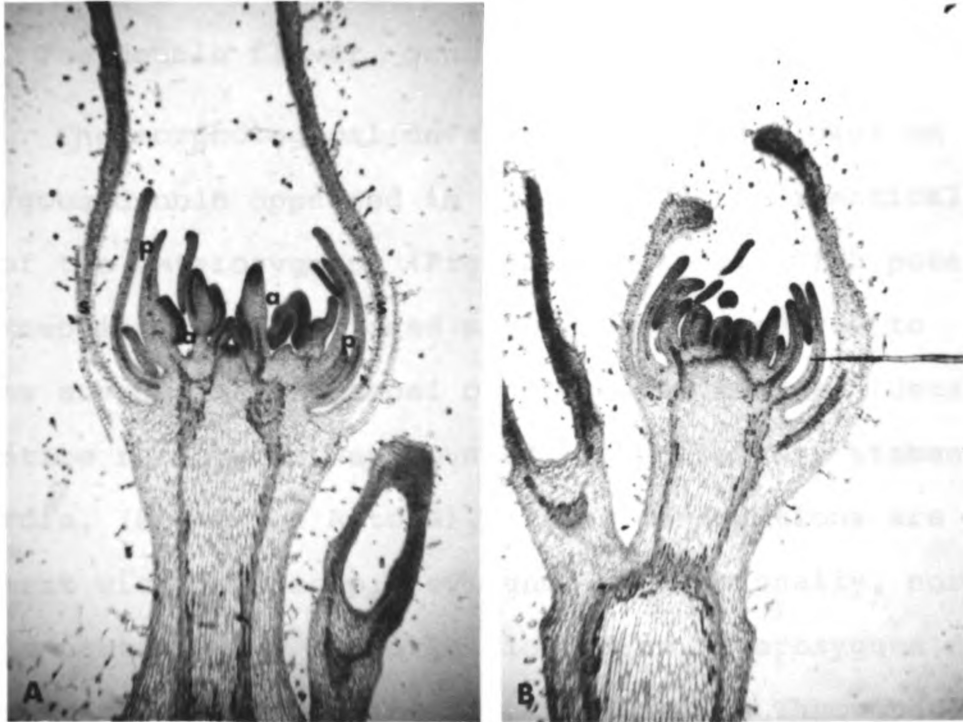


carpel or placenta initials are discernable. Subsequent development of the primordia into petals and stamens occurs with no definite number of outer primordia becoming petals or inner primordia becoming stamens. It appears that proximity is the determining factor for the ultimate morphological development, i.e. those initials closest to the corolla tend to become petals while those primordia more centrally situated become stamens. This interpretation is further verified by the observation that antheroid-petals and petaloid-anthers are commonly found to make up a transition zone midway between the periphery and the center of the flower.

Carpel and placenta primordia were found in many transverse median sections (Figures 7 A and B). It was noted, however, that early in their development major deviations from the normal development existed (Figures 7 C and D). Therefore, it was assumed that these primordia would give rise to non-functional and malformed pistils. Occasionally, a flower receptacle was noted which possessed neither carpel nor placenta primordia. These flowers were completely female sterile, possessing at anthesis only an abundance of petals and anthers which converged in the center into a tight mass of antheroid tissue with no prominent filaments.

Figure 7. Double flower buds, genotype - Dd, with malformed pistils.

- A. Double flower bud. x102
- B. Double flower bud. x102
- C. Malformed pistil in bud of Figure 7-A.
x342
- D. Malformed pistil in bud of Figure 7-A.
x342



3. The double flower, genotype - DD:

The morphological development of the flower on the homozygous double appeared in all cases to be identical to that of the heterozygous, (Figures 8 A to F), with possibly one exception. No sectioned specimen was observed to possess any definable carpel or placental tissue. Usually the entire receptacle was covered with petal and stamen primordia, (Figures 9 A to D). These observations are in agreement with macroscopic evidence. Occasionally, normal fertile pistils were found in flowers on heterozygous double plants which when pollinated produced seed. Throughout the two years of this study a normal pistil was not found in the homozygous line.

The morphological effects of the D allele have been shown with regard to time and site on the floral meristem. The precise anatomical condition of the cells in the differentiating meristem and the biochemical basis of this gene remain to be studied. However, several current concepts of gene action in morphogenesis have relevance to the results of this study and shall be presented.

In the presence of the D allele the floral phenotype is aberrant in all stages of its ontogeny. A possible approach to discussing the ontogeny of the petunia flower is to divide it into three stages designated as initiation, determination, and differentiation and development.

Figure 8. Double flower, genotype - DD, floral initiation and development. Vegetative meristem - vm; transitional meristem - tm; bract initial - b; axillary meristem - am; sepal promordium - s; petal primordium - p; additional primordia - +. Transverse median sections.

- A. Shoot apical meristem in vegetative condition. x184
- B. Transitional meristem with two bract primordia. x184
- C. Transitional meristem with axillary meristem to the right. x184
- D. Sepal and petal primordia initiated around periphery of receptacle. Stamen primordia evident but irregular. x184
- E. Similar to Figure 8-D. Additional primordia initiated. x184
- V. Similar to Figure 8-D and 8-E. x184

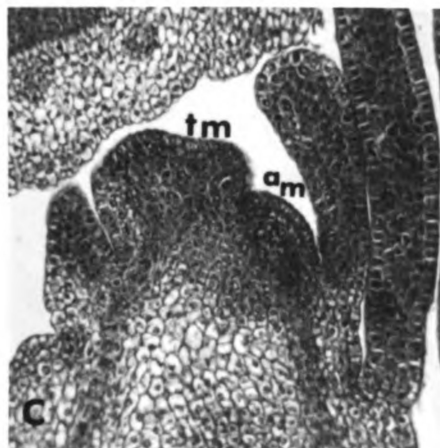
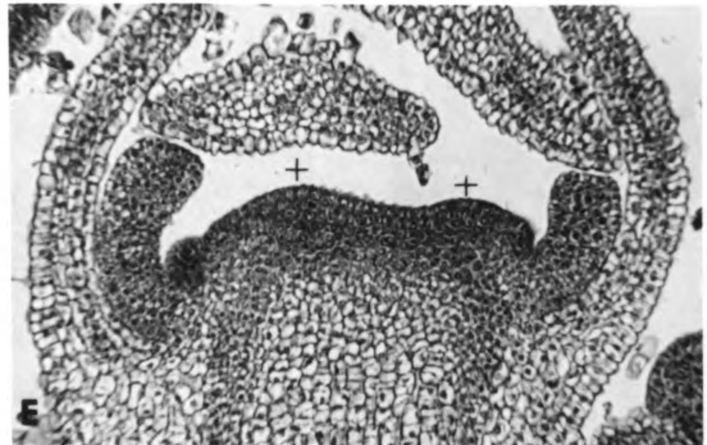
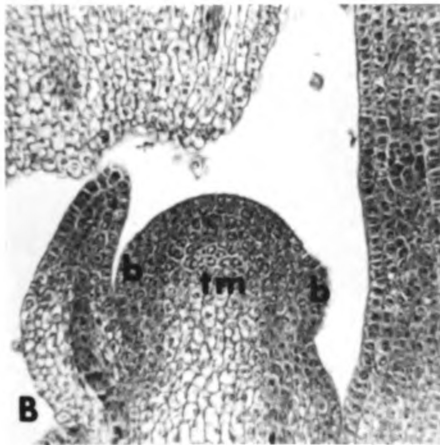
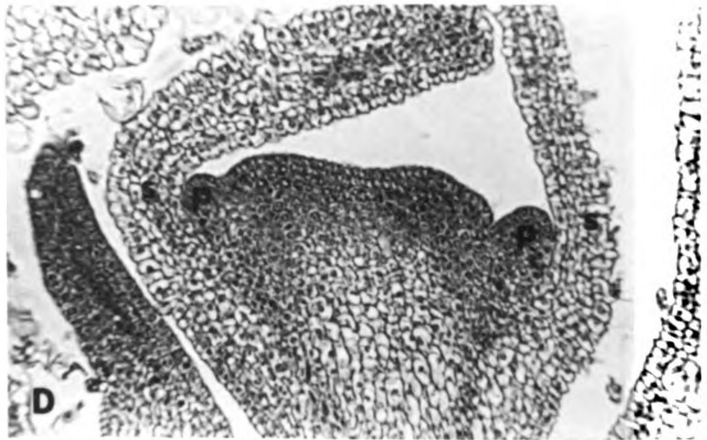
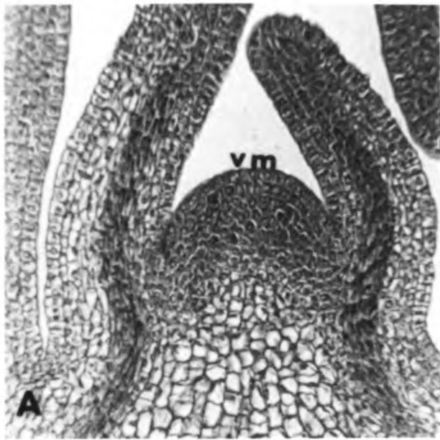


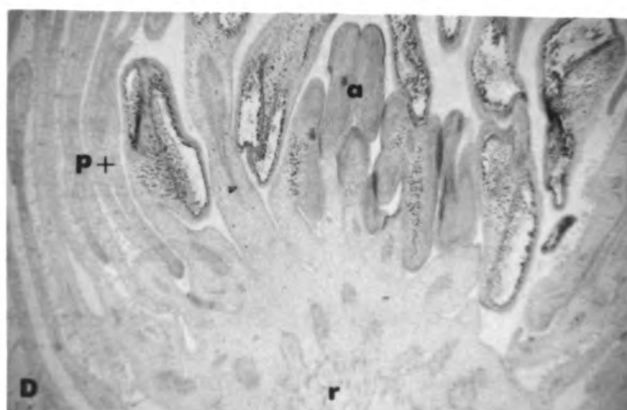
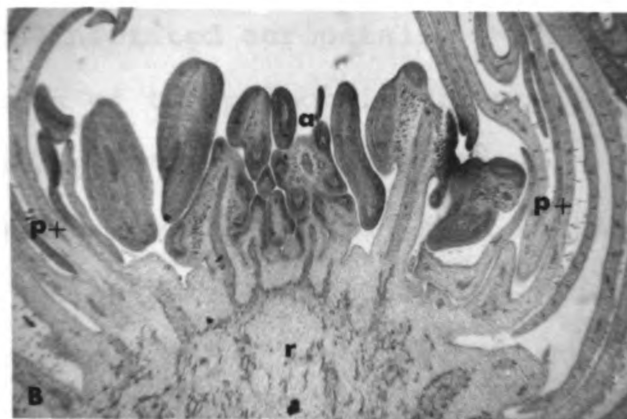
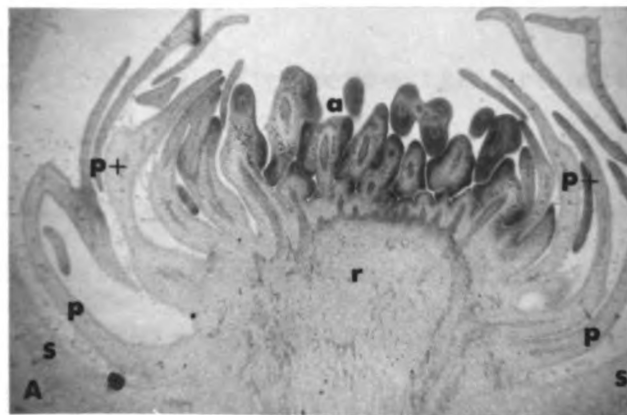
Figure 9. Double flower, genotype - DD, flower buds with a total absence of pistillate tissue. Stamens and petals in various stages of development. Sepal - s; Corolla petal - p; additional petals - p+; stamen - a; receptacle - r.

A. x55

B. x55

C. x55

D. x55



7

During initiation we observe the production of all primordia on the reproductive meristem. This process includes the activation of specific tunica and possibly corpus cells and their subsequent generation of the floral primordia. In the single petunia the product of this stage is a series of five concentric and circular primordial regions which are initiated acropetally. Since initiation as well as position of the primordia are normally constant they are obviously gene regulated phenomena.

Determination is the second stage in the ontogenesis of the flower. Heslop-Harrison (1963) in studies concerning sex expression in flowers has suggested a "chain of events" in the determination of floral parts. Using the repression-derepression mechanisms proposed by Jacob and Monod (1961), he has presented a series of active gene complexes which determine the subsequent differentiation of the floral primordia. This inductive sequence was the result of observations concerning determination, as effected by exogenous substances and photo-period, in a number of plant genera, Melandrium, Silene, Cannabis, Zea, Cucumis, and Cucurbita, (Heslop-Harrison, 1963). He proposed that some stimulus activates a gene complex within the reproductive meristem which brings about determination of the first group of floral primordia. Subsequently, some early product of the first induced system forms an inducer, of unspecified nature, ". . . which diffuses to the next group of

primordia . . ." where it activates a second gene complex, and so on. Initiation is separate from and precedes determination in the petunia. This is evidenced by a floral mutant found in petunia that possesses a second calyx in place of the corolla. Although no histological studies have been made, it is likely that the aberrant calyx is developed from the petal primordia.

The third phase is differentiation and development. These three stages are by no means intimated to occur independently in the meristem at any one time. They may all, in fact, be found on the meristem simultaneously. They are distinguished in that for any one set of primordia they must occur separately and in sequence.

In the case of the double flower type, D-, determination or sex expression is not the primary effect of this gene since neither the sequence nor the expression of any floral parts are totally altered or completely suppressed. Determination is completely functional, but is presented with ill-defined histological regions. Hence, differentiation in the double phenotype is expressed as transitional for petals and stamens.

The deviation from the normal type can be traced to the initiation stage. As noted above, two controlling factors are operative: primordium initiation and primordium localization. It is suggested that in the petunia the loss of control over localization at some early time in the

ontogeny of the flower can ultimately produce the observed phenotype.

Stebbins and Yagil (1966) have reported on a floral mutant, hooded, in barley. They have traced the aberrant phenotype, which is controlled by a single dominant gene, to the onset of a precocious synthesis of deoxyribonucleic acid (DNA) in certain cells of the reproductive meristem. They have reasoned from their data, that a reduction in the G_1 stage of the mitotic cycle reduces the length of time for cellular elongation and maturation. Elongation is a prerequisite for the development of the normal type since further divisions are at right angles to the long axis of the cells. In the absence of sufficient cellular elongation subsequent divisions are in various planes. The immediate product of such a condition is a maintenance of meristematic activity beyond that normally found.

A similar condition is possible in the double petunia. An overabundance of initials may be due to the excessive stimulation of mitoses or the loss in control of localization. Either could explain the observed situation. Mitotic frequency counts and determination of cell size for the three genotypes would be necessary to ascertain if such a condition exists. Autoradiographic techniques would define the areas of increased mitotic activity if they were randomly dispersed. Another approach to the problem would be with the application of exogenous substances.

Inhibitors of protein and DNA synthesis and mitotic poisons could cause a reversion to singleness. Auxins and anti-auxins may produce a similar effect. For example, recent unpublished results from a growth regulator study, have shown that an antiauxin, triiodobenzoic acid, can cause a decrease in the number of stamens and petals in the double flowered petunia.

V. CONCLUSIONS AND SUMMARY

The shoot apical meristem of the seedling petunia is conical in shape and possesses no definite cellular organization within the corpus. It does exhibit a uniseriate tunica. The first two leaf primordia are initiated early in the growth of the seedling and are well developed within the first twenty-four hours after germination.

The shoot apical meristem in a mature vegetative plant possesses a biseriate tunica and a corpus consisting of cells which radiate laterally and downward to the pith. Leaves are initiated alternately.

The flower of the petunia develops directly from the apical meristem. Additional growth beyond the flower is accomplished with the development of an axillary meristem between the central meristem and the last bract initiated. All floral appendages in the single flowered type are initiated acropetally. The petal and stamen primordia arise from the same initial.

The floral initiation and development of the double flowering type is similar to the single until the stamen primordia are produced. A departure from this sequence occurs with a proliferation of additional primordia across

the surface of the receptacle. The differentiation of these primordia into petals and stamens is probably determined by a position effect. Thus initiation of the carpels and placenta is defective in its normal development because of the intrusion of the additional primordia. It was noted that the heterozygous genotype, Dd, was capable of producing some functional pistils whereas the homozygous genotype, DD, was always lacking a normal pistil. The gene, D, therefore has 100 percent penetrance but less than 100 percent expressivity.

Some possibilities for the primary activity of the D allele have been discussed and a few suggestions presented concerning further research into the subject.

1

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