Gibberellins And Morphogenesis Of The Stamenless-2 Mutant Of Tomato (lycopersicon Esculentum, Mill)

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GIBBERELLINS AND MORPHOGENESIS OF
THE STAMENLESS-2 MUTANT OF
TOMATO (LYCOPERSICON ESCULENTUM MILL.)

by

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Department of Plant Sciences

Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Canada
January, 1972

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ABSTRACT

This study was conducted in an attempt to interrelate gene
with hormone and hormone with stamen form in stamenless-2
($s_{12}/s_{12}$) mutant of tomato. The mutant plants differ phenotypi-
cally from the normal only in the stamen structure. Stamens in
the mutant range from those with anther differentiation and the
presence of external ovules to those resembling carpel-like organs
with internal ovules. Analysis of the ontogeny revealed that the
stamen primordia of $s_{12}/s_{12}$ and wild type flowers resembled each
other closely until about 100 $\mu$ in length, thereafter the develop-
mental pattern of the stamens in the two genotypes could be dis-
tinguished. A comparison of the relative growth rates of stamens
with sepals of $s_{12}/s_{12}$ and wild type showed no significant difference
in their relative growth rates, but some difference at the time of
or prior to stamen initiation was apparent.

Application of GA$_3$ (gibberellic acid) to young floral buds of
mutant, induced the production of stamens resembling those of nor-
mal ('phenocopies'). These stamens also bore viable pollen and
were useful in obtaining $s_{12}/s_{12}$ seeds through selfing. 'Pheno-
copies' of the normal could only be produced on the mutant plants
if GA$_3$ was applied to floral buds with stamen primordia not ex-
ceeding 0.1 mm in length. GA$_3$ applied to floral buds with stamen primordia from 0.15 to 0.6 mm induced some morphological changes while buds with stamens 0.7 mm or longer failed to respond to GA$_3$ applications. Alternatively, flowers produced on normal plants following CCC (an inhibitor of gibberellin synthesis) treatment exhibited some stamen abnormalities similar to the mutant. Application of GA$_3$ to normal plants however stimulated the ovary development by increasing the diameter and number of carpel and locules per ovary. Mutant plants treated with auxin at a critical stage of flower development produced flowers with carpel-like organs ('carpelloid stamens') in place of stamens. Extractions, purification through TLC, followed by bioassay revealed lower levels of gibberellin-like substances in both vegetative and floral parts of the mutant than in the wild type plants.

From these observations we suggest that the sl$_2$ allele could operate by affecting gibberellin synthesis which in turn affects the stamen form. Our data also sheds light on the role of plant hormones in sex expression and impinges on the problem of organogenesis in floral meristems.
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<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certificate of Examination</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>xv</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>17</td>
</tr>
<tr>
<td>A. Seed source</td>
<td>17</td>
</tr>
<tr>
<td>B. Chemical source</td>
<td>17</td>
</tr>
<tr>
<td>C. Plant culture technique</td>
<td>17</td>
</tr>
<tr>
<td>D. Rooting of cuttings</td>
<td>20</td>
</tr>
<tr>
<td>E. Microtechnique</td>
<td>21</td>
</tr>
<tr>
<td>F. Application of growth substances</td>
<td>21</td>
</tr>
<tr>
<td>G. Observations and photography</td>
<td>22</td>
</tr>
<tr>
<td>H. Extraction of gibberellins</td>
<td>22</td>
</tr>
<tr>
<td>I. Chromatography</td>
<td>23</td>
</tr>
<tr>
<td>J. Bioassays</td>
<td>24</td>
</tr>
<tr>
<td>a. Dwarf pea bioassay</td>
<td>24</td>
</tr>
<tr>
<td>b. Lettuce hypocotyl bioassay</td>
<td>25</td>
</tr>
</tbody>
</table>
RESULTS........................................................................................................26

I. QUALITATIVE AND QUANTITATIVE DESCRIPTION OF
THE ONTOGENY OF sl2 AND WILD TYPE FLOWERS..................26

A. Vegetative shoot apex.........................................................26
B. Inflorescence apex.........................................................27
C. Initiation of the flower and floral organs......................30
D. Development of floral organs...........................................36
E. Stamen development in wild type....................................38
F. Stamen development in sl2..............................................40
G. Growth correlation studies between sepals and stamens
   of sl2 and wild type........................................................50
H. Allometric growth relationships......................................54

II. ENVIRONMENTAL AND HORMONAL EFFECTS ON THE
DEVELOPMENT OF STAMENS IN sl2 AND WILD TYPE
FLOWERS.................................................................66

A. Environmental effects.....................................................66
B. Hormonal effects............................................................71
   1. Gibberellic acid (GA3)..................................................71
      a. Stamen development in sl2 flowers......................72
      b. Pollen development in sl2 flowers....................83
      c. Effect of GA3 application to floral buds
         of various sizes.............................................86
      d. Stem elongation...............................................93
      e. Leaf morphology..............................................94
   2. Gibberellin A4/A7........................................................94
   3. (-)-Kaurenoic acid....................................................98
4. (2-chloroethyl) trimethyl ammonium chloride (CCC) .................................................. 99

5. Amo-1618 .................................................................................................................. 103

6. 3-Indole acetic acid (IAA) ......................................................................................... 104

7. Effects of GA₃ on the flowers of wild type ................................................................. 109

III. ANALYSIS OF ENDOGENOUS LEVELS OF GIBBERELLINS IN s¹₂ AND WILD TYPE PLANTS ................................................................. 114

A. Extraction of gibberellins from vegetative parts ....................................................... 114

B. Extraction of gibberellins from flowers ................................................................. 123

DISCUSSION .................................................................................................................. 128

A. Plant hormones and sex organs ................................................................................. 129

B. Organogenesis in the floral meristem .................................................................. 136

C. Gibberellins and gene activity ................................................................................. 147

CONCLUSIONS AND PROSPECTS ..................................................................... 154

REFERENCES .............................................................................................................. 158

APPENDICES ............................................................................................................. 174

Appendix - A Procedure for gibberellin extraction ................................................. 174

Appendix - B Stamen characters of s¹₂ flowers grown under short day and long day conditions ................................................................. 175

Appendix - C A comparison of stem lengths of s¹₂ plants produced by the application of GA₃ and GA₄/A₇ ........................................................................ 176

VITA .............................................................................................................................. xvi
Table No. | Content                                                                 | Page
----------|------------------------------------------------------------------------|-----
9.        | Effects of different concentrations of GA₃ on the stamen characters of sl₂ plants | 80  
10.       | A comparison of stamen characters of sl₂ flowers produced following the application of GA₃ to those of wild type | 81  
11.       | Petal, stamen and ovary lengths, corresponding to various sepal lengths of the floral buds of sl₂ | 88  
12.       | Effect of GA₃ on the stamen characters of sl₂, applied to floral buds of different sepal lengths | 90  
13.       | A comparison of stamen characters produced in sl₂ plants following the application of GA₄/GA₇ and GA₃ | 97  
14.       | Effect of CCC on the stamen characters of wild type flowers | 102  
15.       | Effect of IAA on the stamen characters of sl₂ flowers | 107  
16.       | Effect of GA₃ on the number of carpels and locules in the ovary of wild type plants | 112  
17.       | Activity of the extracts from the vegetative parts of sl₂ and wild type plants on dwarf pea bioassay | 121  

x
<table>
<thead>
<tr>
<th>Table No.</th>
<th>Content</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.</td>
<td>Activity of the extracts from floral parts of s12 and wild type plants on dwarf pea bio- assay.</td>
<td>126</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>$s_{12}$ flower at anthesis</td>
<td>11</td>
</tr>
<tr>
<td>2.</td>
<td>Wild type flower at anthesis</td>
<td>11</td>
</tr>
<tr>
<td>3-8.</td>
<td>Variations in stamen from $s_{12}$ and wild type</td>
<td>13</td>
</tr>
<tr>
<td>9-14.</td>
<td>Vegetative shoot apex and stages in the development of inflorescence in normal tomato</td>
<td>29</td>
</tr>
<tr>
<td>15-20.</td>
<td>Initiation of floral organs on the apices of $s_{12}$ and wild type plants</td>
<td>32</td>
</tr>
<tr>
<td>21-24.</td>
<td>Stages in stamen development in $s_{12}$ and wild type flowers</td>
<td>35</td>
</tr>
<tr>
<td>25-28.</td>
<td>Stages in stamen development in $s_{12}$ and wild type flowers</td>
<td>42</td>
</tr>
<tr>
<td>29-32.</td>
<td>Cross sections of floral buds of $s_{12}$ and wild type through the stylar and ovary region</td>
<td>44</td>
</tr>
<tr>
<td>33-38.</td>
<td>Stages in micro- and megasporogenesis in $s_{12}$ and wild type flowers</td>
<td>47</td>
</tr>
<tr>
<td>39-40.</td>
<td>Median longissections through the flower buds (near anthesis) of $s_{12}$ and wild type plants</td>
<td>49</td>
</tr>
<tr>
<td>Figure No.</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>41.</td>
<td>Scatter diagram and regression lines on stamen and sepal lengths of ( s_2 ) and wild type flowers.</td>
<td>58</td>
</tr>
<tr>
<td>42.</td>
<td>A comparison of regression lines on stamen and sepal lengths calculated by model I and model II analyses.</td>
<td>58</td>
</tr>
<tr>
<td>43.</td>
<td>Growth correlations between sepal length and stamen length of wild type and ( s_2 ) flowers, with 95% confidence limits on regression lines.</td>
<td>60</td>
</tr>
<tr>
<td>44.</td>
<td>Scatter diagram and regression lines on the log values of stamen and sepal lengths of wild type and ( s_2 ) flowers.</td>
<td>63</td>
</tr>
<tr>
<td>45.</td>
<td>A diagrammatic comparison of the stamen characters of ( s_2 ) flowers grown under greenhouse (winter) and field (summer) conditions.</td>
<td>69</td>
</tr>
<tr>
<td>46-48.</td>
<td>Effect of ( GA_3 ) on the stamen development of ( s_2 ) flowers.</td>
<td>74</td>
</tr>
<tr>
<td>49.</td>
<td>Effect of different concentrations of ( GA_3 ) on the mean number of Y.P. and C.S. stamens per flower of ( s_2 ) flowers.</td>
<td>77</td>
</tr>
<tr>
<td>Figure No.</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>50.</td>
<td>Effect of different concentrations of GA₃ on the mature stamen length (mean) of sl₂ flowers</td>
<td>77</td>
</tr>
<tr>
<td>51.</td>
<td>Effect of different concentrations of GA₃ on the mean number of E.O. per stamen and per flower of sl₂ flowers</td>
<td>79</td>
</tr>
<tr>
<td>52-54.</td>
<td>Pollen of sl₂ and wild type flowers</td>
<td>85</td>
</tr>
<tr>
<td>55.</td>
<td>A diagrammatic representation of the effect of GA₃ on stamen development of sl₂ plants, applied to floral buds of various sizes</td>
<td>92</td>
</tr>
<tr>
<td>56-58.</td>
<td>Effect of GA₃ on the leaf morphology of sl₂ plants</td>
<td>96</td>
</tr>
<tr>
<td>59-62.</td>
<td>Effect of CCC on wild type flowers</td>
<td>101</td>
</tr>
<tr>
<td>63-65.</td>
<td>Effect of IAA on the stamen characters of sl₂ flowers</td>
<td>106</td>
</tr>
<tr>
<td>66-68.</td>
<td>Effect of GA₃ on wild type flowers</td>
<td>111</td>
</tr>
<tr>
<td>69.</td>
<td>Standardization of dwarf pea bioassay</td>
<td>117</td>
</tr>
<tr>
<td>70.</td>
<td>Activity of gibberellin-like substances on dwarf pea bioassay from the extracts of vegetative parts of wild type and sl₂ plants</td>
<td>119</td>
</tr>
<tr>
<td>71.</td>
<td>Activity of gibberellin-like substances on dwarf pea bioassay from the extracts of flowers of wild type and sl₂ plants</td>
<td>125</td>
</tr>
</tbody>
</table>
ABBREVIATIONS

Amo-1618 = 2 - isopropyl - 4 - dimethyl amino -
5 - methyl phenyl - 1 - piperidine carboxylate
methyl chloride.

CCC = (2 - chloroethyl) trimethyl ammonium chloride.

C.S. = Carpelloid stamen.

Cv. = Cultivar.

E.O. = External ovules.

EtOH = Ethyl alcohol.

GA$_3$ = Gibberellic acid.

HCl = Hydrochloric acid.

IAA = 3 - indoleacetic acid.

sl$_2$ = Stamenless - 2 mutant, homozygous recessive
($sl_2/sl_2$).

TLC = Thin layer chromatography.

wild type = Homozygous dominant (+/+)

Y.P. = Yellow and pubescent.
INTRODUCTION

Morphogenesis, the study of the origin of form, has long intrigued biologists and has been approached in different ways by various workers. With plant material, earlier studies included the description of morphological characters. Later attempts concentrated on relating physiological processes or biochemical reactions to the ultimate form (Sinnott, 1960; Wardlaw, 1968). Davis (1964) defined morphogenesis as the "aspect concerned with the shaping of three dimensional structure, at all levels, from one dimensional gene products". However, genes alone do not determine the final form but the surrounding environment plays a significant role as well. In fact the development and form at maturity of an organ or an organism is largely dependent upon the interaction of genes with the environment (both physical and biochemical). Despite our ability to recognize the problems associated with morphogenesis and despite considerable advances made in the understanding of structure, nature and function of the gene, only a few reports exist which attempt to bridge the gap between gene and form. A paucity of such information is felt especially in studies with higher plant organs (Stebbins, 1967; Nelson, 1967).
Of those research reports which have attempted to explore the basis of form, few exist which pertain to the role of genes. Most studies were conducted by subjecting a single genotype to a number of differing environmental factors, thus providing information on the role of environment alone. Studies which take into account two closely related genotypes (with small known genetic differences associated with distinct morphological characters) and their interaction with environmental stimuli are scarce. Such studies which could provide insights in the physio-genetic control of form are doubtlessly needed. Use of single gene mutants affecting the form of a particular organ or a step in the development of an organ are especially useful in such pursuits and have been advocated by a number of biologists (Wagner and Mitchell, 1955; Wardlaw, 1957; Sinnott, 1960; Hadorn, 1961; Waddington, 1962; Stebbins, 1965; Cutter, 1965; Meyer, 1966).

In higher plants, the first attempt to relate the role of genes with morphological characters was made by Wheadle (1909) with flower color mutants of several species. Lawrence and Price (1940) later reviewed the literature on the subject and suggested the possibility that genes supply enzymes which are involved in the reactions leading to the character. More recently, information on the synthesis
of carotenoids has been revealed by a study of the mutants affecting pigment formation in tomato fruit (see reviews by Goodwin, 1963 and Nelson, 1967).

Extensive study on a single gene mutant affecting the leaf form in tomato has been conducted by Mathan and Jenkins (1960, 1962), Mathan and Cole (1964), Stettler (1964) and Caruso (1968). Lanceolate mutant in the heterozygous condition (La$^+$/La) differs from the normal (La$^+$/La$^+$) in that it has simple entire leaves as compared to the odd-pinnately compound leaf. The homozygous mutant (La/La) expresses three different forms, ranging from a plant with small leaves to a type which grows into a column of tissue and lacks any foliar structure. Tyrosine supplied through the culture medium or sprayed on young homozygous mutant plants resulted in phenotypes similar to the heterozygous condition. On the other hand, gibberellic acid sprayed to normal plants resulted in morphological characters similar to those of heterozygous plants. The level of tyrosinase, laccase, peroxidase and catalase was higher in the mutant as compared to the normal and correlated with genetic dosage. Phenylboric acid which produced phenocopies of the mutant when applied to the normal plants also showed an increase in the level of these same enzymes (Mathan, 1965, 1967). With
increasing dose of La allele in the tetraploids produced by colchicine treatment (Stettler, 1964), there was a reduction in, a) total leaf length, b) the number and size of primary and secondary leaflets, c) the number of flowers per inflorescence and other modifications in foliar and flower structures. Caruso and Cutter (1970) showed that the proliferation of cells observed in the middle of the hypocotyl of the leafless form of this mutant could be prevented if the mutant hypocotyls were grafted below the normal shoot apices.

Another study which attempts to relate single gene differences to morphological characters is that of Yagil (1965). In barley, the dominant hooded gene (K/K) produces a hooded structure on the upper part of the lemma containing extra rudimentary florets as compared to the awned type (k/k) which produces a long awn. Short days and cold temperature treatments at a critical stage of hood development induce awn-like structures. A detailed account of the morphogenetic events leading to the hooded and awned characters is presented in Stebbins and Yagil (1966). Gupta and Stebbins (1969) reported a higher peroxidase activity in the hooded genotype as compared to the awned type particularly at the stage of transition from vegetative to the flowering condition. Injections of metabolic inhibitors (hydrazine, sodium azide and hydroxylamine) and phenylboric acid, to the hooded genotype (K/K) at the stage of young lemma
primordia altered the phenotypic expression to the awned type and also showed a decrease in peroxidase activity (Stebbins and Gupta, 1970). Although the hooded genotype is not distinguished from the normal until flowering, a significant difference in the free amino acids (glutamate, L-alanine, glutamine, asparagine, α-aminobutyrate and proline) between the two genotypes was observed as early as the seedling stage (Sarkissian et al., 1962). Similarly in 'agropyroides', a mutant in barley (which produces one functional spikelet per node in the upper nodes of the spike), differences in three amino acids (arginine, glutamine and glutamic acid) were observed from the normal, during the seedling stage. No morphological differences could be detected between the mutant and the normal at this stage (Wijewantha and Stebbins, 1964). However, at the stage when all the floral parts were differentiated differences in arginine alone were observed. If plants of 'agropyroides' were grown on a mineral nutrient solution supplemented with arginine, glutamic acid, tyrosine or adenine sulphate, near-normal spikes were produced.

Other, though not so extensive, studies of obvious gene controlled morphology include: ontogenetic development of two mutants 'ramosa' and 'polytypic' (affecting inflorescence development) in maize studied by Postlethwait and Nelson (1964). A similar developmental study on a 'Knotted' mutant in maize, which produces
finger-like structures on the abaxial surface of the leaf, was conducted by Gelinas et al. (1969). Angeles (1966) reported the morphogenetic events in the development of 'Corn grass' mutant in maize which affects the tillering habit of the plant. Morphological and histological study on a 'Curl' mutant in tomato affecting laminar structures (leaves, sepals, petals) was conducted by Smith and Stebbins (1971).

The involvement of growth hormones in the genetic control of form has been documented in a few instances. Dwarf mutants of several plant species, upon treatment with gibberellic acid, produce plants similar to the normal tall phenotypes (Phinney, 1956; Bukovac and Wittwer, 1956). One interpretation of these observations i.e. that the dwarf plants are deficient in the level of endogenous gibberellins has been tested in a few cases. In pea, Radley (1958) and Jones and Lang (1968) did not observe any difference in either extractable or diffusible gibberellins from tall and dwarf varieties. Phinney (1961) reported abundant gibberellin-like substances from tall maize plants, but their absence from plants of dwarf mutants d₃, d₅, a₁, and reduced amounts from dwarf mutants d₁ and d₂. Ogawa (1965) found lower levels of gibberellins in the seeds and seedlings of a dwarf variety of Pharbitis as compared to the tall variety. Suge and Murakami (1968) observed no gibberellin activity
from the shoot extracts of a dwarf rice mutant. The seeds of a radiation induced dwarf mutant in bean (Proano and Greene, 1968) contained less gibberellin activity than did the seeds of tall plants. Gotoh (1970) similarly observed reduced levels of gibberellins in the cotyledons of a dwarf variety in bean. Contrary to the above observations, Radley (1970) reported that gibberellic acid applied to the tall varieties of wheat stimulated growth of the seedling but was without effect on the dwarf varieties. Analysis of endogenous gibberellins revealed a higher amount collected from the dwarfs as compared to the tall varieties. Thus, the simplistic view that dwarf mutants are a result of gene-induced gibberellin deficiency is not universally true.

Despite the presence of information listed above and other literature which attempts to link genes with form and genes with hormones and hormones with form, genetic control of a number of developmental processes in higher plants is basically unexplored. One such unexplored area concerns the development and differentiation of floral organs. The need for information on the physio-genetic control on the development and differentiation of sex organs in the angiosperm flower has as well been stressed by Heslop-Harrison (1964), Wardlaw (1965) and Meyer (1966).

Tomato (Lycopersicon esculentum Mill.) provides a large number of single gene mutants affecting one or more than one organ in the flower. Besides possessing numerous floral mutants which
are readily available from tomato geneticists (refer to Tomato Genetics Cooperative Reports), tomato is good experimental material. It is easily cultured and can if necessary, be vegetatively propagated through cuttings. Thus plants possessing sterile flowers can be maintained as clones.

The tomato flower (Fig. 2) consists of:

a) short calyx tube with 5-6 sepals,

b) corolla also with a short tube terminating in 5-6 lobes,

c) stamens (usually 6) bearing short filaments with the anthers laterally joined to form a hollow cone,

d) a gynoecium consists of two to several carpels, a long style terminating in a stigma and contained within the encircling androecium.

This description is consistent with earlier descriptions by Cooper (1927), Smith (1935), Hayward (1938) and Luckwill (1943). The sequence of floral whorl initiation is centripetal starting with sepals in the outermost whorl, followed by petals, stamens and finally the pistil. Flowers are perfect, hypogynous and regular. The number of floral parts in each whorl is variable. Some authors (Cooper, 1927; Howlett, 1939) consider the tomato flower to be hexamerous, while others (Bailey, 1928) consider it to be pentamerous. Luckwill (1943) considers the corolla pentamerous except in cultivated species where it is hexamerous.
The mutant 'stamenless-2' \((s_1^2)\) first reported by Hafen and Stevenson (1958) was collected in the field from a progeny produced from a cross between Cvs. 'Early Chatham' and 'Bounty'. Their description of the mutant included; flowers with twisted, distorted anthers which were laterally free, and the stigma protruding beyond the anthers. Under our greenhouse cultural conditions, flowers with similar morphological characters were produced (Fig. 1). However, in addition, we observed the occurrence of naked external ovules (E.O.) associated with the stamens of this mutant (Sawhney and Greyson, 1969). These E.O. were present on the adaxial surface near the junction of the anther and the filament. Stamens of \(s_1^2\) were shorter in length than those of the wild type stamens (Table 3 and also compare Fig. 8 with Figs. 3-7). There was considerable variation in the stamen structure of \(s_1^2\) flowers produced not only on the same plant but as well within the same flower. In wild type flowers however, besides some variation in stamen length and stamen color no variation in organogenesis was observed. In \(s_1^2\) flowers, forms of stamens observed from both greenhouse and field grown plants include:

a) stamens with many (up to 80) E.O. (Fig. 3)
Fig. 1.  $s_{12}$ flower at anthesis (x 6), E.O. = external ovules.

Fig. 2.  Wild type flower at anthesis (x 6).
Figs. 3-8. Variations in stamens from sl₂ and wild type flowers (all x8).

Fig. 3. sl₂ stamen with many E. O. at the junction of anther and filament.

Fig. 4. sl₂ stamen with few E. O. and a flap-like structure proximal to E. O.

Fig. 5. sl₂ stamen with few E. O., long filament than in Figs. 3, 4, 6-8 and poor anther development.

Fig. 6. sl₂ stamen with broad basal end and cylindrical distal end. E. O. absent.

Fig. 7. 'Carpelloid' stamen of sl₂ with swollen basal end which sometimes contains internal ovules and a distal end resembling a style.

Fig. 8. Wild type stamen with well developed bilobed anther and a short filament.
b) stamens with few (up to 20) E.O. but possessing a flap like structure proximal to E.O. (Fig. 4)

c) stamens with few E.O. but bearing longer filament than others (compare Fig. 5 with Figs. 3, 4, 6-8) and poor anther development

d) stamens with no E.O. but a broader basal end (Fig. 6)

e) stamens may resemble carpels ('Carpelloid stamens') with swollen basal end which sometimes bear internal ovules, and the distal end resembling a style (Fig. 7)

In addition, stamens in all cases except sometimes those similar to Fig. 3 were pale yellow to green in color. By way of contrast, stamens from wild type (+/+ ) flowers possessed short filaments and long fully developed, bright orange-yellow anthers (Fig. 8). Besides the distinct alteration in the stamen morphology of this mutant, no other differences from the wild type in the floral or vegetative characters were observed.

Inheritance studies (Hafen and Stevenson, 1958) have shown that in breeding experiments sl₂ allele behaves as a single recessive gene and the morphological abnormality is expressed only in the homozygous (sl₂/sl₂) condition. The phenotype of the heterozygote (+/sl₂) is identical to the homozygous dominant (+/+ ) form. Since, the morphological characters produced by sl₂ mutant are attributed
to a single gene and since the mutant has a unique feature of
influencing both male and female characters on the same organ,
it potentially represents an excellent subject for experimentation
into the relationship between gene and character in the flower.
The reasons for calling the gene-affected organ, a stamen, are;

a) its position on the flower,
b) its ability to produce pollen,
c) its tendency to develop pigments similar to those of
   normal stamens.

Yet, this abnormal organ bears characters normally
associated with carpels;

a) presence of ovules (E.O.),
b) under certain conditions produces carpel-like structure
   (Fig. 7) in which the distal end resembles a style and
   the swollen basal end sometimes bears internal ovules.

Thus, evidently at some stage during the production of these
organs the normal sequence of reactions is disrupted and an abnor-
mal morphology results. Using this $sl_2$ mutant the writer has attemp-
ted to interrelate gene - hormone - character and the following thesis
includes:

a) qualitative and quantitative description of the ontogeny of
   the stamens of $sl_2/\text{sl}_2$ and how it deviates from the normal
genotype (+/+).
b) experimentation to modify the expression of $sl_2/sl_2$ by environmental and hormonal treatments.

c) analysis of the endogenous gibberellin levels of $sl_2/sl_2$ and wild type.
MATERIALS AND METHODS

A. Seed Source:

The original seed sample containing the sl\textsubscript{2} mutant, was received from Dr. C. D. Clayberg of the Connecticut Agricultural Experiment Station, New Haven, Connecticut. When sown, the seeds yielded approximately 3:1: wild type: mutant. Since homozygotes (+/+\text) and heterozygotes (+/sl\textsubscript{2}) are phenotypically indistinguishable, the genetic constitution of the two was checked by back crosses. In this study, in all the comparisons to be reported, wild type was +/+. Seeds of sl\textsubscript{2}/sl\textsubscript{2} genotype were obtained by selfing GA\textsubscript{3}-reverted stamenless plants (see section II, B. a. under Results). The sources of other seeds used in this project are listed in Table 1.

B. Chemical Source:

The chemicals used in this study and their suppliers are listed in Table 2.

C. Plant culture technique:

Seeds, sown in wooden flats containing a mixture of sand, loam and peat, were covered with fine sand and sprinkled with water. Young seedlings (approximately 3 weeks old) bearing 2-3 leaves were transferred to 3'" clay pots. Plants used for flower
Table 1

Various types of seeds used in this study and their sources.

<table>
<thead>
<tr>
<th>Seed type</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lycopersicon esculentum</em> Mill.</td>
<td>Stokes seed Ltd.</td>
</tr>
<tr>
<td>Cv.s. Vantage, Viceroy and Vendor</td>
<td>St. Catherines, Ontario.</td>
</tr>
<tr>
<td><em>L. esculentum</em> Mill.</td>
<td>Dominion Seed House</td>
</tr>
<tr>
<td>Cv.s. Early Chatham and Bounty</td>
<td>Georgetown, Ontario.</td>
</tr>
<tr>
<td><em>Lactuca sativa</em> L.</td>
<td>Stokes Seed Ltd.</td>
</tr>
<tr>
<td><em>Pisum sativum</em> L.</td>
<td>Asgrow Seed Co.</td>
</tr>
<tr>
<td>Cv. Progress No. 9</td>
<td>Bradford, Ontario.</td>
</tr>
</tbody>
</table>
Table 2  
Chemicals used in this study and their sources.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2-Chloroethyl) trimethyl ammonium chloride (CCC)</td>
<td>Eastman Organic Chemicals</td>
</tr>
<tr>
<td>Ethyl acetate ('Analar')</td>
<td>British Drug House</td>
</tr>
<tr>
<td>3-indoleacetic acid (IAA)</td>
<td>Eastman Organic Chemicals</td>
</tr>
<tr>
<td>2-isopropyl-4-dimethyl amino-5-methylphenyl-1-piperidine carboxylate methyl chloride (Amo-1618)</td>
<td>Calbiochem.</td>
</tr>
<tr>
<td>Gibberellic acid (GA₃)</td>
<td>Eastman Organic Chemicals</td>
</tr>
<tr>
<td>Giberellin A₄ and A₇</td>
<td>Courtesy of Dr. D. Broadent of I.C.I. London, U.K.</td>
</tr>
<tr>
<td>(-)-Kaurenoic acid</td>
<td>Courtesy of Dr. Jefferies of University of Western-Australia.</td>
</tr>
<tr>
<td>Precoated TLC silica-gel Chromatography plates</td>
<td>E. Merckag Darmastadt, Germany.</td>
</tr>
<tr>
<td>Tween-20</td>
<td>Nutritional Biochemicals Corp.</td>
</tr>
</tbody>
</table>
observation were maintained in these pots, while those saved for fruits were subsequently transferred to 8'' pots. Soil nutrients were supplemented weekly with a commercial fertilizer.

Plants raised as stock material for cuttings or those for fruit or seed collection were maintained in temperature controlled green houses. The normal daily illumination was supplemented by 'Gro-lux' tubes and incandescent bulbs and extended to 16 hrs. daylength. Temperature settings in these green houses were 24°C during the day and 18°C during the night, although in summer, temperature during the day occasionally reached 35°C. Plants selected for experimentation were transferred to 'Walk in' growth rooms with artificial light. Light at an intensity of 800 - 1,000 F.C. at pot level (measured by G.E. light meter) was supplied by both incandescent bulbs and 'Gro-lux' tubes. The temperature in these rooms was maintained at 23°C ± 2 during the day and 18°C ± 2 during the night.

D. Rooting of cuttings:

When vegetative propagation of tomato was required, stem cuttings with leaves were planted in wet sand. They received a water mist every day for 6 hrs. at one minute intervals. Within 10 - 14 days, each cutting had produced many roots, although no root stimulating hormones were applied.
E. Microtechnique:

Apices and floral buds were killed and fixed in Randolph's modified Navashin fluid (CRAF) (Johansen, 1940) for 24 hrs. and stored in 70% EtOH. Dehydration of the fixed material was done through tertiary butyl alcohol (TBA) series (Johansen, 1940) and the dehydrated material was subsequently embedded in 'Paraplast' (M.P. 56-57°C).

Embedded material was sectioned with a "Leitz Minot Microtome". Apices and floral buds were sectioned at 7μ. Staining procedures were a modification of Foster's tannic acid - iron chloride scheme (Greyson and Mitchell, 1969).

F. Application of growth substances:

In most experiments growth substances were applied from disposable micropipettes ('Microcaps', Drummond Co.) to the axil of the leaf closest to the shoot apex. This method was chosen since a known amount of substance in solution could be applied to a specific position on the plant. In a few experiments growth substances were applied to whole plants as a spray mist. Regardless of method of application, growth substances were prepared in distilled water with 0.05% Tween-20. Controls in all experiments were treated with equal amount of distilled water with 0.05% Tween-20.
G. **Observations and photography:**

Observations of apices and flowers were made with a Zeiss (Stereo Zoom-3) dissecting microscope. Photographs of fresh apices and flowers were taken through an Asahi Pentax Spotmatic camera attached to the dissecting microscope, on Plus X-35mm film and developed in Microdol diluted 1:3. Microphotography of anatomical slides was conducted through a Linhof (Technika) bellow camera attached to a Zeiss GFL microscope. Plus-X pan 120 Professional film loaded in a 'Graflex' film holder was used and films were subsequently developed in D-76 diluted 1:1.

H. **Extraction of Gibberellins:**

Extraction procedure for gibberellins was modified from Radley (1963) and is summarized in a flow chart in Appendix-A. Fresh materials (stems, leaves and flowers) were homogenized in a Virtis macro homogenizer (Model 45) in 75% EtOH and dry ice. Tissue was left overnight covered with 75% EtOH at room temperature and then filtered through Whatman No. 1 filter paper. The residue was extracted twice with 75% EtOH and filtered. Finally the filtrates were combined and concentrated at 36°C under vacuum. This concentrate was treated with a slurry of basic lead acetate and centrifuged at 10,000 x g for 10 minutes. Excess lead acetate was removed by treating with 5N HCl. The solution was filtered and adjusted to pH 2.5. The solution was then extracted
three times with equal volumes of deacidified ethyl acetate (deacidified with 5% sodium bicarbonate, 20 ml/l (Hartley et al., 1969)).

The ethyl acetate fractions were combined and concentrated to about 150 ml. This fraction was then extracted three times with equal volumes of 1% sodium bicarbonate. Three fractions were combined and acidified to pH 2.5 with 1N HCl and then re-extracted 3 times, with equal volumes of de-acidified ethyl acetate. These three fractions were combined and the water removed by freezing and filtering through a chilled funnel. Finally, this filtrate was evaporated to near dryness and the residue spotted on a TLC plate.

I. Chromatography:

The extracted residue was spotted on 20x20cm silica gel (0.25mm layer thickness) precoated TLC plates with the help of 50μl microsyringe (Hamilton Co.). TLC plates were developed at room temperature in a mixture of benzene: n-butanol: acetic acid (75 : 20 : 5) (Kagawa et al., 1963) in the saturated atmosphere of a glass chromatography tank. The solvent front ascended 15cm in 60-80 minutes. Plates were air dried and gibberellin spots were developed by spraying the plates with concentrated sulphuric acid and heating them in an oven at 110°C for 10 minutes.

For bioassays, plates were divided horizontally in 10 equal
fractions. Each fraction was scraped from the plate and eluted with water-saturated ethyl acetate. Silica gel was separated by filtration and the ethyl acetate evaporated. Each fraction was then dissolved in a known volume of distilled water.

J. Bioassays:

a) Dwarf pea bioassay:

A modified procedure of Hayashi and Rappaport (1966) was used for this bioassay. Dwarf peas (Pisum sativum var. Progress No. 9) were immersed in running tap water for 6-8 hours and then planted in vermiculite (wetted with tap water) and contained in plastic basins. These basins with seeds were moved to a dark room at 24°C. After 4 days, under low intensity green light, seedlings (3.25 - 3.5 cm high) were selected and transferred to water culture. 5μl of extract or known GA₃ prepared in 0.05% Tween-20, were applied to the apex of each seedling with a microsyringe. Control seedlings received 5μl of 0.05% Tween in distilled water. Eight seedlings were used for each treatment. Seedlings were transferred to a cabinet illuminated with red light. The red light source consisted of one red fluorescent tube (Westinghouse, F 15 T8/R) which was behind one sheet of Ruby (No. 14) Cinemoid acetate filter (Strand Electric Ltd., Toronto, Ontario) and a sheet of Whatman No. 1 filter paper. Spectroradiometric analysis (ISCO, model SR) of the filtered light indicated that there was no light transmitted below 600 and above 750
nm with a peak at 660 nm. After 5 days of exposure to red light the distance between the cotyledonary node and the hook of each seedling was measured.

b) **Lettuce hypocotyl bioassay**:

The procedure of Frankland and Wareing (1960) was followed with slight modifications. Lettuce seeds (Cv. Grand Rapids Forcing) were germinated in the dark at 25°C in petridishes on a filter paper (Whatman No. 1) moistened with distilled water. After 36-48 hours, seedlings with radicles 3-4 mm long were selected and planted in petri dishes on Whatman No. 1 filter paper moistened with 3 ml. of test solution or distilled water in the case of controls. Ten seedlings per treatment were used. The petridishes with seedlings were transferred to light (supplied by cool white fluorescent tubes) at 300 F.C. After 3 days at 25°C, the hypocotyl length of each seedling was measured to the nearest mm.
RESULTS

I. QUALITATIVE AND QUANTITATIVE DESCRIPTION OF THE
ONTOGENY OF s12 AND WILD TYPE FLOWERS.

In this section a detailed account of the qualitative and
quantitative (by growth correlation studies) description of the
ontogeny of the flowering apex and the floral organs of the two geno-
types will be presented. Since no morphological differences were
recognized in the vegetative shoot apex, the inflorescence apex,
the pattern of floral organ initiation, and the development of
sepals, petals and carpels of s12 and wild type, the ontogeny of
these will be considered together. As the development of the sta-
men is different in the two genotypes, it will be dealt with separat-
ely and in greater detail.

A. Vegetative shoot apex:

Vegetative shoot apex in tomato possessed a convex dome
(Figs. 9 and 10). The approximate diameter of the apex was 175 μ
and a height of 55 μ. Variations in the dimensions of the dome were
related to the leaf plastochron, apices from older plants being larg-
er. Median longitudinal sections indicate that the apical organisa-
tion consists of a biseriate tunica over a central corpus region
(Fig. 10). These observations are consistent with the detailed study
of Bedsem (1958).

B. **Inflorescence apex:**

In tomato, the number of leaves and nodes produced prior to floral differentiation varies not only with cultivar but also with the existing environment (see reviews by Phatak, 1964; Calvert, 1965; Wittwer and Aung, 1969). Observations of over 100 plants grown under our cultural conditions, revealed that after the initiation of 8 to 10 true leaves, the main vegetative apex elongated and flattened and gave rise to the inflorescence apex (Fig. 11). A new vegetative apex resulted from the outgrowth of the bud axillary to the leaf immediately below the inflorescence apex. By its subsequent growth, the vegetative apex assumed a terminal position and the inflorescence finally appeared lateral. These observations agree with those of Hayward (1938), Luckwill (1943), Went (1944), Malayer and Guard (1964) and Calvert (1965) but are contrary to those of Cooper (1927), Smith (1935) and Wittwer and Aung (1969) who considered the flowering apex to arise in the axil of the leaf. Our observations, documented with photographic illustrations rule out any other origin of inflorescence but from a vegetative apex. While the different interpretations could be related to different cultivars studied, our casual observations of 'Early Chatham', 'Bounty', 'Vantage' and 'Viceroy' support the interpretation on the inflorescence initiation espoused here.
Figs. 15 - 20. Initiation of floral organs on the apices of sl₂ and wild type plants.

Fig. 15. Floral bud with sepal primordia (x 40).

Fig. 16. Mid-longissection of floral bud with sepal primordia.
Note the enlarged epidermal cells on the abaxial surface of the sepal (x 235).

Fig. 17. Flower bud with sepals removed (except one) to show petal and stamen primordia (x 40). se = sepal, p = petal, st = stamen.

Fig. 18. Mid-longissection of the flower bud with sepals, petals, stamens and the central axial region where carpel primordia will initiate (x 175). se = sepal, p = petal, st = stamen.

Fig. 19. Mid-longissection of stamen primordia of sl₂ - 75 μ long (x 600).

Fig. 20. Mid-longissection of stamen primordia of wild type - 75 μ long (x 600).
the periphery. Subsequent primordia arose independently in a helical sequence at approximately 135° intervals (Fig. 15) until a ring of six sepal primordia was produced. This interpretation of organ initiation was based on both observations of fresh material and on microscopic slides of serial cross sections of at least two buds of each stage. This description of organ initiation disagrees with that of Cooper (1927) which suggested that primordia arise one after another until a ring of six primordia is produced. Since the sepal primordia start to grow soon after their initiation, the first sepal had grown considerably by the time the last primordium was initiated. Due to greater growth on the abaxial surface of sepal primordia they overarch the floral apex (Fig. 16) and the initiation of organs of inner whorls cannot be seen unless sepals are removed.

Petal primordia initiated central to sepals and in a similar helical manner, but they normally occupied alternate positions to them. Stamen primordia initiated centripetally, alternate with petals and opposite to sepals (Fig. 17). The innermost whorl of floral organs consisted of carpel primordia which arose around a central meristematic zone. The number of carpels varied with the cultivar and also between the flowers of the same inflorescence.
Fig. 21. Mid longisection of 225μ long (approx.) stamen of sl₂. Note the absence of sporogenous tissue on the abaxial surface (x 270).

Fig. 22. Mid longisection of 225μ (approx.) stamen of wild type. Sporogenous tissue present on both abaxial and adaxial surfaces (x 270).

Fig. 23. Mid longisection of 500μ long (approx.) stamen of sl₂. Note the differentiation of two separate zones (shown by arrows) of sporogenous tissue in the vertical direction on adaxial surface (x 200).

Fig. 24. Mid longisection of 500μ long (approx.) stamen of wild type. Note continuous zone of sporogenous tissue on either side of anther (x 200).
Similar to Hayward's (1938) observations, the position of the carpel primordia on the floral apex in relation to the rest of the organs varied with their number.

D. **Development of floral organs:**

The development of floral organs subsequent to their initiation is a slow process and extends up to 3 or 4 weeks. Observations reported here on the development of floral organs are based on an examination of over 50 young floral buds and for most stages at least two microscopic slides of cross and longisections. Sepals, the outermost organs, soon after their initiation grew more on their abaxial surface. This growth, which is in part, due to greater cell enlargement in the outer cell layers and particularly in the cells near the tip of the growing primordium, resulted in the curving of sepals towards the apex (Fig. 16). Soon, the basal ends of the sepals unite due to the growth of lateral margins to form a cup-like structure with free distal ends. Subsequent linear extension of the sepals by cell division and cell enlargement resulted in sepals up to 10 mm long. At anthesis, sepals were curled back with their basal end joined to form a calyx tube and the distal ends remaining free. They were deep green in colour and bore both unicellular and multicellular hairs. Structure of hairs is described in detail by Hayward (1938).
Petals did not show marked overarchign of apex at the early stages of development (Fig. 18) however, during the later extension their terminal ends were bent inwards towards the apex (Figs. 23 and 27). In a manner similar to that described for calyx, petals formed a basal corolla tube with the distal ends free for about one third of their length. Further linear extension of petals by cell division and cell enlargement produced petals up to 10 mm long. Near anthesis, due to their rapid extension the petals, grew out of the bud and covered the sepals.

Carpels were the last and inner-most organs to arise on the floral apex. Early growth of carpel primordia resulted in these structures bending towards the axis. The central axis which was flat or concave, enlarged and was thus enclosed by the growing carpels, the terminal ends of which subsequently met at the top of central axis. The central axis acted as the placental tissue and bore ovules (Fig. 25) while the carpels enlarged and connected with each other to form a smooth ovary wall. These observations on the cauline origin of placental tissue are in agreement with those of Cooper (1927), Hayward (1938) and Murray (1945). Subsequent growth of the distal ends of carpels resulted in a long style
with a capitate stigma.

E. **Stamen development in the wild type:**

The ontogeny of stamens is so strikingly different from the rest of the floral organs that it has led some workers with phylogenetic interests to believe that stamens arose from axillary buds as compared to the suggested foliar origin of the other floral organs (Satina and Blakeslee, 1941; Barnard, 1960; Sharman, 1960). In tomato, unlike the rest of the organs on the floral apex, stamen primordia grew erect with little or no hyponastic growth at early (Fig. 20) or later stages of development (Figs. 22, and 24). They were club-like structures with broad apical ends in contrast to the narrow end of the other floral organs.

First signs of stamen initiation were observed in the lower tunica (T2) layer which underwent periclinal divisions and together with the various periclinal and oblique divisions in the corpus gave rise to a small projection on the floral axis, centripetal to sepals and petals. The outer tunica (T1) divided only by anticlinal divisions forming the outer protective layer. This hypodermal pattern of stamen initiation is similar to that described for *Ranunculus* by Tepfer (1953) and for *Downingia* by Kaplan (1968). Further divisions in the apical end in the outer and inner tunica produced a small undifferentiated primordium (Fig. 20). At this stage when the
primordium was approximately 100μ long there was some procambial
development in the center. When the stamen primordium reached
about 225 μ in length some hypodermal cells both on the abaxial and
adaxial surface enlarged and stained deeply with safranin (Fig. 22).
These cells which later gave rise to the sporogenous tissue did not
extend along the entire length of the stamens. Besides the early
differentiation of sporogenous tissue there was no distinction be-
tween the filaments and anthers until this stage. During subsequent
growth, the lower portion of the stamen differentiated into a non-
sporogenous tissue which by intercalary growth gave rise to the
filament. Filaments which reached 1.5 mm in length at maturity
separated the stamens at the base. The upper anther region of
the stamens remained free during the early stages of development
but underwent cohesion near anthesis. Anthers were bilobed, with
a central connective and possessed four crescent shaped sporangia
(Fig. 30).

Microsporogenesis in the anthers as mentioned above, started
early in ontogeny of the stamens. Hypodermal cells (Fig. 22)
divided periclinaly to produce a wall of 3-4 layers and an innermost
layer having cells with deeply staining contents formed the arche-
sporial layer (Fig. 26). Periclinal divisions of the arche-
sporial layer produced a tapetal layer on the outside and primary
sporogenous cells on the inside. The tapetal layer ultimately be-
came binucleate. The innermost vegetative layer adjacent to the
primary sporogenous cells on the other side differentiated into an inner tapetum. Primary sporogenous cells (Fig. 33) or pollen mother cells after meiotic division produced tetrads (Fig. 35) of microspores. The pollen or male gametophytes were produced from the microspores by the nuclear division of the microspores which gave rise to a generative and a vegetative nucleus. These observations on microsporogenesis in the normal tomato are similar to those of Smith (1935).

F. Stamen development in sl$_2$:

The position and pattern of initiation of stamen primordia on the floral apices of sl$_2$ plants is basically identical to that described for wild type flowers. At the early stages of development, stamen primordia of sl$_2$ (Fig. 19) and wild type (Fig. 20) resembled each other very closely. However, thereafter their developmental pattern was different. Unlike the normal stamens, those of sl$_2$ flowers did not grow erect but underwent greater growth on the abaxial surface which appears convex as compared to the straight adaxial surface (Fig. 23). Differentiation of sporogenous tissue generally takes place only on the adaxial surface (Fig. 21). There was considerable variation however, in the amount of anther tissue involved with microsporogenesis and unlike the four sporangia produced in the wild type, the number of sporangia in sl$_2$ stamens varied
Figs. 25-28. Stages in stamen development in s1₂ and wild type flowers.

Fig. 25. Mid-longissection of stamen of s1₂ showing the two zones of sporogenous tissue. Distal zone (1) will give rise to microspores and proximal zone (2) to E.O. Also note in this figure the ovules (ov) of the ovary borne on the central axis surrounded by developing carpel (x 135).

Fig. 26. Mid-longissection of stamen of wild type with archesporial cells and a wall of 3-4 layers (x 150).

Fig. 27. Mid-longissection of flower bud of s1₂ approx. 4 mm long. Stamens show microsporogenous (→) tissue in the distal end and E.O. at the junction of anthers and filament (x 35).

Fig. 28. Mid-longissection of flower bud of wild type (approx. 4 mm long). Anthers show a single row of primary sporogenous cells (→) on adaxial and abaxial surface (x 35).
Fig. 29. Cross section of flower bud of wild type (approx. 4 mm long) near the upper portion of the ovary. Note the filaments (F) of the stamens in this region (x 45).

Fig. 30. Cross section of same flower bud as in Fig. 29 through the stylar region. Note the presence of four crescent shaped sporangia (sp) in each anther (x 45).

Fig. 31. Cross section of flower bud of sl² (approx. 4 mm long) near the upper portion of the ovary. Note the presence of many E.O. on the adaxial surface of stamens (x 45).

Fig. 32. Cross section of same flower bud as in Fig. 31 through the stylar region. The number and shape of sporangia varies in each anther (x 45).
from 3 to none (compare Fig. 32 with Fig. 30). In addition, the sporogenous tissue did not extend along the entire length of the anther, but early in ontogeny two distinct zones of sporogenous tissue were observed on the adaxial surface (Fig. 23). This differentiation of two zones was maintained during later development (Fig. 25) and finally while the distal region underwent microsporogenesis, the lower region produced naked external ovules (E.O.) (Figs. 27 and 39). Cross sections through the buds of the two genotypes of the same age show that while a section near the top of the ovary of wild type (Fig. 29) showed only filaments of the stamens, that of \( s_{12} \) (Fig. 31) exhibited numerous E.O. facing the ovary. Cross sections of the buds through the stylar region of wild type (Fig. 30) and \( s_{12} \) (Fig. 32) however, both show microsporogenous tissue in the anther region. Mid-longissections through the buds of the two genotypes (Figs. 27 and 28) and also of buds near anthesis (Figs. 39 and 40) show the position and arrangement of microspores and E.O. in \( s_{12} \) stamens as compared to the wild type. Although no quantitative measurements were made upon the proportion of anther tissue involved with microsporogenesis of \( s_{12} \), there is no doubt that it is much less in \( s_{12} \) stamens than in stamens of wild type. The number of pollen grains
Fig. 33. Primary sporogenous cells from the anther of flower bud of wild type (x 1000).

Fig. 34. Megaspore mother cell in the ovule of the ovary (x 800).

Fig. 35. Tetrad of microspores in the anthers of s12 flower bud (x 1000).

Fig. 36. Megaspore mother cell in E.O. (x 600).

Fig. 37. Microspores from the anthers of wild type flower bud (x 1000).

Fig. 38. Embryo sac in the ovule of the ovary (x 800).
Fig. 39. Median longisection of flower bud of s12 near anthesis (x 32).

Fig. 40. Median longisection of flower bud of wild type near anthesis (x 32).
produced per stamen or even per flower in $s_{12}$ flowers is therefore less than in wild type flowers. Due to the difficulty of removing pollen from the anthers of $s_{12}$ flowers, no quantitative comparisons could be made on pollen grain number in the two genotypes. Similar steps of microsporogenesis as those described for the wild type were observed in $s_{12}$ stamens. Microspores (Fig. 37) were produced and while in some cases apparently normal pollen existed, in other stamens microspores were shriveled. In the stamens where no anther differentiation takes place (Figs. 6 and 7) no signs of sporogenous tissue were observed.

External ovules, which are the striking character in $s_{12}$ stamens, exhibit great resemblance to ovules in the ovary. The differentiation of a megaspore mother cell in an E.O. was frequently observed (Fig. 36) and resembled that one in the ovules of the ovary (Fig. 34). However, normal embryo sacs (Fig. 38) as found in normal ovules were not observed in E.O. All attempts to fertilize these ovules by dusting them with pollen failed.

G. Growth correlation studies between sepals and stamens of $s_{12}$ and wild type:

Earlier ontogenetic description has shown that the stamens of $s_{12}$ and wild type not only differ at maturity but also at some of the stages during their development following inception. A quanti-
tative estimate was conducted to determine; a) how soon the
differences in the form of stamens of the two genotypes are
established and b) what differences in their relative growth
rates from initiation to maturity could be detected. Since
the stamen length and not the sepal length of the $s_{12}$ mutant
and the wild type are significantly different at maturity (Table
3, and compare Fig. 8 with Figs. 3-7) a comparison of growth
correlation between sepals and stamens of the two genotypes
was carried out.

Stamen and sepal lengths from the flower buds of various
sizes were measured with the help of an ocular micrometer from
prepared slides of longitudinal sections. For each stamen length,
sepal length was measured from the same individual flower. In
Table 4, mean values of 3 organs of each stage are presented.
Regression analysis on these data was conducted using both Model I
(Woolf, 1968) and Model II using Bartlett's three group method
(Sokal and Rohlf, 1969), since values of both stamen and sepals
length are independent variables. In Table 5 and Fig. 42 a compar-
ision of the regression statistics computed by the two methods and
the best fitting lines using the two methods are presented. Since
the values calculated by the two methods with these data are rather
similar and since with Model II test of significance of regression
Table 3

A comparison of sepal and stamen length of mature wild type and $s_{12}$ flowers.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sepal length$^a$ (mm)</th>
<th></th>
<th>Stamen length$^a$ (mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>$\pm$</td>
<td>Confidence$^b$ interval</td>
<td>Mean</td>
</tr>
<tr>
<td>Wild type</td>
<td>9.82</td>
<td>0.50</td>
<td></td>
<td>9.88</td>
</tr>
<tr>
<td>$s_{12}/s_{12}$</td>
<td>9.62</td>
<td>0.48</td>
<td></td>
<td>7.64**</td>
</tr>
</tbody>
</table>

$^a$ = sample number 20

$^b$ = confidence interval 95%

$**$ = difference significant from wild type at p$\leq 0.01$
Table 4

Stamen length* and sepal lengths* of the $s_{12}$ and wild type flowers.

<table>
<thead>
<tr>
<th>Flower No.</th>
<th>Sepal length (mm)</th>
<th>Stamen length (mm)</th>
<th>Flower No.</th>
<th>Sepal length (mm)</th>
<th>Stamen length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.49</td>
<td>0.16</td>
<td>1</td>
<td>0.49</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>0.86</td>
<td>0.22</td>
<td>2</td>
<td>0.62</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>0.93</td>
<td>0.24</td>
<td>3</td>
<td>0.80</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>1.61</td>
<td>0.49</td>
<td>4</td>
<td>0.93</td>
<td>0.23</td>
</tr>
<tr>
<td>5</td>
<td>1.89</td>
<td>0.74</td>
<td>5</td>
<td>1.63</td>
<td>0.31</td>
</tr>
<tr>
<td>6</td>
<td>2.35</td>
<td>0.81</td>
<td>6</td>
<td>2.11</td>
<td>0.54</td>
</tr>
<tr>
<td>7</td>
<td>2.72</td>
<td>0.89</td>
<td>7</td>
<td>2.58</td>
<td>0.66</td>
</tr>
<tr>
<td>8</td>
<td>4.13</td>
<td>1.50</td>
<td>8</td>
<td>2.96</td>
<td>0.74</td>
</tr>
<tr>
<td>9</td>
<td>6.01</td>
<td>2.44</td>
<td>9</td>
<td>4.51</td>
<td>1.22</td>
</tr>
<tr>
<td>10</td>
<td>6.67</td>
<td>3.10</td>
<td>10</td>
<td>6.58</td>
<td>2.06</td>
</tr>
<tr>
<td>11</td>
<td>7.99</td>
<td>3.94</td>
<td>11</td>
<td>7.99</td>
<td>2.72</td>
</tr>
</tbody>
</table>

* Each value represents a mean length of 3 organs from the same flower.
coefficients cannot be performed (Simpson et al., 1960) regression statistics of Model I will be presented here.

Scatter diagram and regression lines for the stamens versus sepals of wild type (line I) and $s_{12}$ (line II) are shown in Fig. 41. In Table 6, correlation coefficient ($r$), regression coefficient ($b$) and error mean square ($d^2$) for each genotype is presented. There is a high correlation between the linear extension of stamens with sepals of the two genotypes. The 't'-test of significance (Woolf, 1968) performed on the regression coefficient ($b$) showed that slopes of the two lines are significantly different. Next, 95% confidence limits were calculated for the two lines and are shown in Fig. 43. It can be seen that the upper limit of line II ($s_{12}$) and lower limit of line I (wild type) overlap until stamen length is 0.7 mm and the corresponding sepal length is 2.5 mm. Beyond this point the two lines are independent. Thus, the significant difference in the stamen lengths of the two genotypes at maturity can be traced back to when stamens are approximately 0.7 mm long. Earlier than this however, the stamen lengths of the two genotypes are not significantly different at least as can be determined from these data.

H. Allometric growth relationships:

The striking initial similarities between the primordia of the two genotypes (Figs. 19 and 20) and the additional observation
Table 5

A comparison of Model I and II regression analysis on the sepal and stamen length of wild type and \( s_{12} \) flowers.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Model I.</th>
<th></th>
<th></th>
<th>Model II.</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression formula</td>
<td>Regression coefficient (b)</td>
<td>Y intercept (a)</td>
<td>Regression formula</td>
<td>Regression coefficient (b)</td>
<td>Y intercept (a)</td>
</tr>
<tr>
<td>Wild type</td>
<td>( Y = -0.27 + 0.49X )</td>
<td>0.49</td>
<td>-0.27</td>
<td>( Y = -0.2 + 0.47X )</td>
<td>0.47</td>
<td>-0.2</td>
</tr>
<tr>
<td>( s_{12}/s_{12} )</td>
<td>( Y = -0.15 + 0.33X )</td>
<td>0.33</td>
<td>-0.15</td>
<td>( Y = -0.15 + 0.31X )</td>
<td>0.31</td>
<td>-0.15</td>
</tr>
</tbody>
</table>
### Table 6

Regression statistics on the sepal and stamen length of wild type and *sl₂* flowers.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Regression formula</th>
<th>Correlation coefficient (r)</th>
<th>Y intercept (a)</th>
<th>Error mean square (d²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>$Y = -0.27 + 0.49X$</td>
<td>0.99</td>
<td>-0.27</td>
<td>0.29</td>
</tr>
<tr>
<td><em>sl₂/sl₂</em></td>
<td>$Y = -0.15 + 0.33X$</td>
<td>0.99</td>
<td>-0.15</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Fig. 41. Scatter diagram and regression lines on stamen and sepal lengths of wild type [line I (●)] and s₁₂ [line II (○)]. Data plotted from Table 4.

Fig. 42. A comparison of regression lines on stamen and sepal lengths calculated by model I [line a (—)] and model II [line b (-----)] for both wild type (I) and s₁₂ (II). Regression statistics in Table 5.
Growth correlations between sepal length and stamen length of wild type (line I) and s12 (line II). 95% confidence limits on regression line I (-----) and line II (---) indicates that the two lines are not different until sepal length is approximately 2.25 mm and stamen length is 0.7 mm.
that the earlier stages of stamen elongation of \( sl_2 \) and wild type are not different (as shown above) suggest that differences in the stamens of the two genotypes are established sometime after their initiation. A comparison on the relative growth rates of stamens with sepals of the two genotypes was conducted, to obtain further insights on the growth pattern of the stamens of \( sl_2 \) and wild type.

Growth correlations between two dimensions of an organ, or between two organs of an organism or between a part and the whole of an organ are best described by the equation:

\[
Y = bX^k \quad \text{(Huxley, 1932)}
\]

where \( X \) and \( Y \) are two variables, \( K \), a constant representing a ratio between the geometric growth rates of the two variables and \( b \), a constant, is the value of \( Y \) when \( X \) is equal to zero. In most biological materials, the ratio of two dimensions of an organ, or the ratio between two organs of an organism is constantly changing, while the ratio of their growth rates is constant. They are thus said to grow allometrically or heterogonically (Simpson et al., 1960).

This allometric expression can also be written as:

\[
\log Y = \log b + K \log X
\]

Using this equation, the allometry of growth between sepals
Fig. 44. Scatter diagram and regression lines on the log values of stamen and sepal lengths of wild type [line I (●)] and sl₂ [line II (○)]. Regression statistics in Table 7.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Regression formulae</th>
<th>Regression coefficient (K)</th>
<th>Y-intercept (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>log Y = -0.52 + 1.19X</td>
<td>1.19</td>
<td>-0.52 ± 0.039a</td>
</tr>
<tr>
<td>sl₂/sl₂</td>
<td>log Y = -0.65 + 1.17X</td>
<td>1.17</td>
<td>-0.65 ± 0.047a</td>
</tr>
</tbody>
</table>

a = 95% confidence interval.
and stamens of the two genotypes was conducted. Scatter diagram and regression lines of the log values of stamens and sepals are presented in Fig. 44, and regression statistics in Table 7. A high correlation coefficient (r) between the relative growth of stamens with sepals is shown for both genotypes. The test of significance on the regression coefficient (K) for the two genotypes indicates that the two are not significantly different. There is however, a significant difference in the Y intercept (Table 7) of the two lines and the level of the stamen - sepal line is different throughout the linear extension of the organs.

Thus the above analysis suggests that although relative growth rates of stamens of wild type and s1\textsubscript{2} are not different at any stage during their development, some differences during the initiation of these organs is apparent.
II. ENVIRONMENTAL AND HORMONAL EFFECTS ON THE DEVELOPMENT OF STAMENS IN s12 AND WILD TYPE FLOWERS

Since, as was suggested in the introduction, both the environment and hormones can play a significant role in the phenotypic expression, we studied the effects of different environmental conditions and exogenously applied growth substances on the development of stamens in s12 and wild type plants in an attempt to interrelate gene with hormone and hormone with form. Results of the experiments will be presented in this section.

A. Environmental effects:

Flowers of s12 mutant, developed under different cultural conditions and at different times of the year, exhibited considerable variation in stamen characteristics. In plants grown in summer at the experimental field station, significant differences in flowers were observed as compared to the flowers from plants cultivated at the greenhouse in winter. A comparison of four stamen characters viz; 'carpelloid stamens' (C.S.), yellow and pubescent stamens (Y.P.), the number of E.O. per flower and the number of stamens per flower bearing E.O. under these two conditions is presented in Table 8 and in a diagramatic polygonal
representation in Fig. 45. In plants grown under field conditions, the number of C.S. per flower was greater than in those grown under greenhouse conditions, while the Y.P. stamens which resemble the wild type were greater in number per flower in plants grown in the greenhouse as compared to those grown at the field. The number of E.O. per flower however, was greater under greenhouse conditions and so was the number of stamens bearing E.O. per flower. Thus, the greenhouse conditions in winter which favour the production of E.O. are similar to those which promote the formation of yellow and pubescent stamens. The summer conditions on the other hand which promote the formation of 'carpelloid stamens', also influence reduction in the number of naked external ovules. Although some ovules are present within the basal swollen end of C.S., the opposite response of these two characters usually interpreted as being feminine, is intriguing. Further discussion on this topic will be dealt with later.

The above observations suggested that the two basic environmental conditions; light (both duration and intensity) and temperature play an important role in the expression of stamen characteristics of flowers from sl2 plants. In summer, long days and presumably high temperature promoted the carpellization of stamens, while in winter short days and cool temperature favoured the production of
Fig. 45. A diagrammatic comparison of the stamen characters of $s_{12}$ flowers grown in greenhouse (winter) and at the field station (summer). Four axes of the polygons from the central point represent; E. O. = external ovules; S. E. = number of stamens per flower bearing E. O.; C. S. = number of carpelloid stamens per flower; Y. P. = number of yellow and pubescent stamens per flower. Data from table 8.
Table 8

Stamen characters of sl2 flowers grown under field (summer) and greenhouse (winter) conditions.

<table>
<thead>
<tr>
<th>Cultural condition</th>
<th>Number of E. O. per flower Mean(^{a} \pm ) C.I.(^{b})</th>
<th>Number of stamens per flower with E. O. Mean(^{a} \pm ) C.I.(^{b})</th>
<th>Number of Y. P. stamens per flower Mean(^{a} \pm ) C.I.(^{b})</th>
<th>Number of C. S. stamens per flower Mean(^{a} \pm ) C.I.(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field (summer)</td>
<td>24.15 16.51</td>
<td>1.44 0.83</td>
<td>0.20 0.41</td>
<td>2.00 0.83</td>
</tr>
<tr>
<td>Greenhouse (winter)</td>
<td>86.90** 28.11</td>
<td>5.25** 0.80</td>
<td>1.55** 0.85</td>
<td>0.05** 0.10</td>
</tr>
</tbody>
</table>

** = difference significant from field conditions at \( p \leq 0.01 \)

a = sample size 20.

b = 95% confidence interval.
yellow pubescent stamens which in some instances even produced viable pollen. Experiments conducted in growth chambers with 9 hr. day length (short days) and 15 hrs. day length (long days) at light intensity (600-800 F.C.) and temperatures (62°F night and 72°F day) yielded results (Appendix-B) consistent with the suggestion that long days promote carpelization of stamens while short days influenced morphological similarities to wild type. All subsequent experiments attempting to produce phenocopies of normal from the mutant were conducted under short day conditions at 72°F during the day and 62°F during the night.

B. Hormonal effects:

1. Gibberellic acid (GA₃):

Some research workers have previously demonstrated that the exogenous application of GA₃ to young monoecious and gynoecious plants promotes the development of male organs (Galun, 1959; Peterson and Andher, 1960; Mitchell and Wittwer, 1962). In-vitro experiments on floral buds (Blake, 1969) have also shown that the presence of GA₃ in the culture medium stimulates pollen development. Phatak et al., (1966) reported that stamenless - 1 (s₁₁) mutant of tomato when supplied with GA₃ through solution culture exhibited pollen development although the flowers produced did not
resemble the normal type. Rana and Jain (1969) similarly reported stamen development in a stamenless mutant of *Cosmos* following GA$_3$ application. Thus, based upon these and other reports both in higher and lower plants which demonstrate that gibberellins promote maleness, we conducted experiments to study the effects of GA$_3$ on the development of stamens in s$_{12}$ and wild type flowers.

Various concentrations of GA$_3$ (10$^{-8}$M to 10$^{-2}$M) were prepared in 0.05% Tween 20 and as mentioned in the Methods, a known volume (10μl) of the solution was applied in the axil of the leaf nearest to the apex, at a stage when the apex was in transition to flowering. Control plants were s$_{12}$ or wild type plants treated with same amount of Tween – 20 alone. This method of application was preferred to spraying or supplying through solution culture, since a known amount of the substance could be applied to the plant at a specific location.

a). *Stamen development in s$_{12}$ flowers:*

Flowers produced on s$_{12}$ plants following one application of GA$_3$ (10μl of 10$^{-3}$M) resembled very closely those of the wild type flowers (compare Fig. 46 with Fig. 2). Stamens formed a regular cone around the pistil by the fusion of anthers along the margin and were yellow and pubescent. No E.O. were observed on the adaxial surface of the stamens from GA$_3$ treated flowers (Fig. 47), unlike
Figs. 46-48. Effect of GA$_3$ on the stamen development of sl$_2$ flowers.

Fig. 46. Flower produced on a sl$_2$ plant subsequent to GA$_3$ treatment. Note the fusion of anthers (x 5).

Fig. 47. Stamens of the flower in Fig. 46 showing their adaxial surfaces. All the stamens are Y.P. and none bears E.O. (x 6).

Fig. 48. Stamens of an untreated sl$_2$ flower, showing their adaxial surfaces. Each stamen bears numerous E.O. and is shorter than those shown in Fig. 47 (x 6).
the stamens of the mutant (Fig. 48). In Table 9, stamen characters of the first three flowers produced on the inflorescence initiated subsequent to the application of different concentrations of GA₃ are presented. The analysis of variance and multiple range test (Student - Newman - Keul test) was performed for comparison of individual means. As is evident from Table 9, the greatest response in all the characters was obtained with the application of a solution of 10⁻³M conc. solution of 10⁻²M conc. was toxic, since it suppressed the development and final size of the flowers. With 10⁻⁴M conc. although the response was significantly different from controls with respect to most characters, the difference was not as great as with 10⁻³M. The experiment was repeated at least three times and consistent results were obtained.

A staminal cone was observed in all the first three flowers produced on a plant treated with a solution of 10⁻³M GA₃, while with 10⁻⁴M conc., on average 50% of the first three flowers developed the staminal cone. With 10⁻⁵M and controls (s1₂ plants treated with Tween - 20 only) no fusion of anthers was observed (Table 9). The number of Y.P. stamens also increased significantly with 10⁻⁴ and 10⁻³M concentrations (Table 9 and Fig. 49). With 10⁻³M the number of Y.P. stamens was not significantly different from
Fig. 49. Effect of different concentration of GA$_3$ on the number (mean) of yellow and pubescent (Y. P.) and carpelloid (C. S.) stamens per flower of $s_{12}$ flowers. C = control, $s_{12}$ flowers treated with Tween - 20 only. W = wild type. Data taken from Table 9.

Fig. 50. Effect of different concentrations of GA$_3$ on the length (mean) of mature stamens of $s_{12}$ flowers. C = control ($s_{12}$) W = wild type. Numerical values in Table 9.
Fig. 51. Effect of different concentrations of GA$_3$ on the number (mean) of E.O. per flower (■) and the number (mean) of E.O. per stamen (●●●). Note the change in scale of right ordinate. C = control (sl$_2$), W = wild type. Data from Table 9.
Table 9
Effects of different concentrations of gibberellic acid (GA3) on the stamen characters of sl2 plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean number of E. O. per flower¹</th>
<th>Mean number of E. O. per stamen²</th>
<th>Mean number of Y. P. per flower¹</th>
<th>Mean number of C. S. per flower¹</th>
<th>Mean final stamen length³ (mm)</th>
<th>Percent flowers with staminal cone</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻³ M</td>
<td>2.50ᵃ</td>
<td>0.35ᵃ</td>
<td>6.60ᵃ</td>
<td>0.00ᵃ</td>
<td>9.30ᵃ</td>
<td>100%</td>
</tr>
<tr>
<td>10⁻⁴ M</td>
<td>59.80ᵇ</td>
<td>12.20ᵇ</td>
<td>4.00ᵃ</td>
<td>0.80ᵃ ab</td>
<td>8.20ᵇ</td>
<td>50%</td>
</tr>
<tr>
<td>10⁻⁵ M</td>
<td>130.80ᶜ</td>
<td>14.55ᵇ</td>
<td>1.20ᵇ</td>
<td>1.80ᵃ ab</td>
<td>7.90ᵇ</td>
<td>0%</td>
</tr>
<tr>
<td>Control</td>
<td>145.33ᶜ</td>
<td>24.95ᶜ</td>
<td>1.80ᵇ</td>
<td>2.20ᵇ</td>
<td>7.80ᵇ</td>
<td>0%</td>
</tr>
</tbody>
</table>

Means followed by the same letter in a column not different at 5% level.
1 = sample size 6 flowers.
2 = sample size 20 stamens.
3 = sample size 5 stamens.
Table 10

A comparison of stamen characters of sl$_2$ flowers produced following the application of GA$_3$ (10 $\mu$l of 10$^{-3}$M), to those of wild type.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mean stamen length.$^1$ (mm)</th>
<th>Mean number of sporangia per anther.$^2$ (from cross sections)</th>
<th>Mean number of Y.P. stamens per flower.$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>sl$_2$</td>
<td>7.80$^a$</td>
<td>1.83$^a$</td>
<td>1.80$^a$</td>
</tr>
<tr>
<td>sl$_2$ + GA$_3$</td>
<td>9.30$^b$</td>
<td>3.83$^b$</td>
<td>6.60$^b$</td>
</tr>
<tr>
<td>wild type</td>
<td>9.80$^b$</td>
<td>4.00$^b$</td>
<td>6.36$^b$</td>
</tr>
</tbody>
</table>

Means followed by the same letter in a column not different at 5% level.  
$^1$ = sample size 5 stamens or flowers.  
$^2$ = sample size 6 anthers.
the wild type (W) flowers (Table 10 and Fig. 49). The number of carpelloid stamens (C.S.) per flower on the other hand, was reduced with GA$_3$ treatment (Table 9 and Fig. 49) and the flowers produced following 10$^{-3}$M treatment lacked such stamens. The number of E.O. per flower or per stamen was also reduced with GA$_3$ treatment (Table 9 and Fig. 51). Although the number of E.O. per stamen on average was 0.35 with 10$^{-3}$M GA$_3$ treatment, most stamens lacked any E.O. With 10$^{-4}$ and 10$^{-5}$M concentrations, the number of E.O. per stamen was higher from those with 10$^{-3}$M but, still significantly different than untreated controls. The total number of E.O. per flower similarly was much reduced with 10$^{-3}$M (with most flowers completely devoid of any E.O.) and with lower concentrations the number of E.O. per flower increased. As has been shown earlier (Table 3), the stamens of flowers from $s_{12}$ plants are shorter in length at maturity as compared to the wild type. The application of GA$_3$ resulted in an increase in stamen length in the flowers produced following the treatment (Table 9 and Fig. 50). With 10$^{-3}$M, the stamen length of $s_{12}$ flowers was not different from those of wild type (Table 10 and Fig. 50).

In summary, with the application of appropriate concentrations of GA$_3$ to young $s_{12}$ plants, flowers with yellow pub-
escent stamens which fuse to form a staminal cone can be produced. These stamens lack any E.O. and are as long as those of wild type. All these characters resemble those of wild type stamens and may therefore be considered as their 'phenocopies'.

b). Pollen development in sl₂ flowers:

It was documented earlier that the pollen produced in flowers from sl₂ plants was both shriveled and aborted. In addition, microsporogenesis did not extend along the entire length of anthers and the number of sporangia per anther was also less than that of wild type stamens. As shown in section 1a (above), the application of GA₃ to sl₂ plants stimulates the production of yellow and pubescent stamens in the flowers. Microscopic examination (cross sections) of at least ten anthers of the stamens produced following GA₃ treatment revealed that they bore pollen (Fig. 53) unlike that of sl₂ flowers (Fig. 52) but resembling that of wild type flowers (Fig. 54). Pollen was also differentiated along the entire length of anthers in the stamens produced after GA₃ treatment, as well the number of sporangia per anther was not different from the wild type (Table 10). Although no direct tests were conducted to study percentage germination of the pollen produced in sl₂ flowers after GA₃ treatment, attempts to pollinate the ovaries of sl₂ flowers with this pollen (selfing) were successful
Figs. 52-54. Pollen of $s_{12}$ and wild type flowers (All x 200).

Fig. 52. A portion of a cross-section of an anther from untreated $s_{12}$ flower. Note that the pollen grains are shrunken.

Fig. 53. A portion of a cross-section of an anther from a flower produced following GA$_3$ treatment. Note the resemblance to pollen in Fig. 54.

Fig. 54. A portion of a cross-section of an anther of a wild type flower.
and fruits with seeds were produced. These seeds were viable and upon germination produced plants which on flowering exhibited the sl₂ characteristics. Thus, the pollen produced in sl₂ flowers following GA₃ treatment resembled that of normal pollen and was also viable. This treatment was used in obtaining homozygous (sl₂/sl₂) seeds.

c. **Effect of GA₃ application to floral buds of various sizes:**

In the experiments reported above the application of GA₃ to floral buds of sl₂ plants produced stamens which resembled those of wild type. However, the response was observed in the flowers initiated subsequent to the treatment. Young floral buds in an inflorescence which were already initiated at the time of treatment failed to produce 'phenocopies' of the wild type. Experiments were next designed to obtain more precise information on, a): the stage or stages of floral bud development which were most sensitive to GA₃ treatment and b): the stage after which GA₃ was without effect.

One way of obtaining such information is by applying GA₃ to the floral buds with various stamen sizes, starting from floral transition apex to the stage when stamens are near anthesis. However, since the stamens of tomato are enclosed within the outer covering of sepals at most stages of floral development, direct measurements of stamens could not be achieved without injuring
sepals and petals especially at the early stages. We therefore measured the lengths of outer sepal and corresponding stamens from microscopic slides with the help of an ocular micrometer.

In Table 11, petal, stamen and ovary lengths for buds with various sepal sizes are presented. Each value in Table 11 is a mean length of 3 organs.

Gibberellic acid (10µl of 10⁻³M) was applied to plants with floral buds at various developmental stages. The length of outer sepals was measured through a dissecting microscope at the time of treatment and GA₃ was applied with a micropipette near the base of the floral bud. The development of treated floral buds was observed until maturity. The results obtained from the experiments are presented in Table 12 and in a diagrammatic presentation in Fig. 55. GA₃ applied to floral buds with sepals up to 0.3 mm long, resulted in the production of stamens which resembled the normal wild type. No E.O. developed on the stamens and all the stamens in the flower were yellow and pubescent (Y.P.). Final stamen length was similar to the wild type and they also fused to form a staminal cone. At the stage when sepals were 0.3 mm long, stamens were a small projection on the floral axis, while the petal primordium on average were 0.1 mm (Table 11). When GA₃ was applied to younger floral buds with only sepal primordium (S) which
<table>
<thead>
<tr>
<th>Sepal length (mm)</th>
<th>Petal length (mm)</th>
<th>Stamen length (mm)</th>
<th>Ovary length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>less than 0.1</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>0.3</td>
<td>0.11</td>
<td>0.08</td>
<td>---</td>
</tr>
<tr>
<td>0.5</td>
<td>0.15</td>
<td>0.08</td>
<td>---</td>
</tr>
<tr>
<td>0.8</td>
<td>0.33</td>
<td>0.22</td>
<td>0.15</td>
</tr>
<tr>
<td>1.0</td>
<td>0.34</td>
<td>0.23</td>
<td>0.17</td>
</tr>
<tr>
<td>1.5</td>
<td>0.55</td>
<td>0.31</td>
<td>0.26</td>
</tr>
<tr>
<td>2.0</td>
<td>0.80</td>
<td>0.59</td>
<td>0.28</td>
</tr>
<tr>
<td>2.5</td>
<td>0.95</td>
<td>0.67</td>
<td>0.45</td>
</tr>
<tr>
<td>3.0</td>
<td>1.07</td>
<td>0.77</td>
<td>0.50</td>
</tr>
<tr>
<td>4.5</td>
<td>1.70</td>
<td>1.60</td>
<td>1.03</td>
</tr>
<tr>
<td>6.0</td>
<td>3.19</td>
<td>2.20</td>
<td>1.88</td>
</tr>
<tr>
<td>8.0</td>
<td>3.33</td>
<td>2.72</td>
<td>1.92</td>
</tr>
<tr>
<td>10.5</td>
<td>6.01</td>
<td>4.79</td>
<td>4.70</td>
</tr>
</tbody>
</table>

* Each value is a mean of 3 observations (measurements made on microscopic slides). Statistics on sepal and stamen lengths in Table 6.
were 0.1 mm long, or when the apex was bolting (BO) i.e. at the transition phase (Fig. 55), responses similar to floral buds with 0.3 mm long sepals were produced. However, the application of GA₃ to floral bud when sepals were 0.5 mm long and stamens about 0.15 mm, resulted in stamens with few E.O., although all the stamens were Y.P., the stamen length was significantly greater and the stamens formed a staminal cone (Table 12 and Fig. 55). At the stage when sepals were 0.8 mm long and stamens were 0.2 mm, the ability to form the staminal cone was lost and also the number of Y.P. stamens per flower was reduced. GA₃ application, when sepals were from 1.5 mm to 2.0 mm long and mean stamen length was between 0.3 mm to 0.6 mm, a significant increase in the filament length was observed as compared to stamens which resemble the wild type or the stamens which were unaffected by GA₃ application (Table 12). The total length of the stamens with long filaments however, was less than in phenocopies of GA₃ treatment, but was more than those produced in the untreated controls or those produced in the flowers which received GA₃ treatment after the sepals reached a length of 3.0 mm (Table 12). These stamens did not form a staminal cone but they bore few E.O. at the junction of anther and filament. Application of GA₃ to buds with 3.0 mm long sepals and mean stamen length of 0.77 mm and
Fig. 55. Diagrammatic representation of the effects of GA$_3$ on stamen development of sl2 plants applied to floral buds of various sizes. Bolting floral apices (BO) or buds with sepal primordia alone (S) or buds with sepals up to 0.3 mm in length upon GA$_3$ treatment produced stamens resembling wild type with no E.O. If GA$_3$ was applied to buds with 0.5 - 1.0 mm long sepals, few E.O. were produced. Floral buds with sepals ranging from 1.0 - 2.0 mm length upon GA$_3$ treatment produced long filaments with few E.O. GA$_3$ application to buds with 3.0 mm long sepals or more was without effect. For quantitative data on this Figure see Table 12.
to buds with sepals longer than 3.0 mm, failed to produce any significant difference in any of the stamen characters (Table 12 and Fig. 55). Thus, the ability of sl₂ flowers to produce stamen characters similar to those of wild type is restricted to very early stages of floral development. In sl₂ floral buds, it is at the stage when the stamen primordia are initiating and are only a mound of a few cells (up to 0.1 mm in length) at the floral apex. After this stage, although the course of development of the primordium for some time can still be partially altered for a few characters by the hormonal treatment, the primordium seems determined for most other characters.

d). Stem elongation:

As is widely known, application of GA₃ to plants stimulates stem elongation (Bukovac and Wittwer, 1956) and within limits the response is concentration dependent. GA₃ applied to sl₂ plants similarly stimulated the stem elongation (Appendix-C). Since the amount of solution applied in this work was very small (10μl) no significant difference in plant height was observed with concentration less than 10⁻⁴M (0.35μg per plant). Above this concentration however, significant stem elongation was observed both in sl₂ and wild type plants.
e). **Leaf morphology:**

Application of GA₃ to young tomato plants, stimulates the production of leaves with entire margin (Gray, 1957). With the application of 10⁻⁴ and 10⁻³M solution of GA₃ similar effects were observed in sl₂ plants (compare Fig. 58 with Fig. 56) in the leaves initiated after the treatment. However, the leaves which were already initiated at the time of treatment and were enlarging, developed sharp pointed leaf margins (Fig. 57). Responses similar to those of Gray were observed with wild-type plants.

2. **Gibberellin A₄/GA₇**

Pike and Peterson (1969) showed that in the gynoecious cucumber, application of a mixture of GA₄/GA₇ was effective in inducing staminate flowers at much lower concentrations than those of GA₃. We therefore conducted experiments to check the effectiveness of the combination of these compounds on sl₂ plants at various concentrations. The mixture of GA₄/GA₇ produced typical GA₃ responses in stem elongation (Appendix-C), however, with the same concentrations different responses were observed in stamen development. In Table 13, a comparison of stamen characters produced by the application of 5µg per plant of GA₄/GA₇ and GA₃ is presented. The number of E.O. per flower and per
Figs. 56-58. Effect of GA$_3$ on the leaf morphology of sl$_2$ plants (all approximately 1/2).

Fig. 56. Untreated leaf of a sl$_2$ plant.

Fig. 57. Leaf form produced as a result of GA$_3$ application during the development of the leaf. Note the sharp pointed ends of the leaf margin.

Fig. 58. Leaf form produced as a result of GA$_3$ application prior to or at the early stages of initiation. Note the entire margin of the leaf.
Table 13

A comparison of stamen characters produced in s12 plants following the application of 5 μg of GA4/A7 and GA3.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean number of E. O. 1 per flower. 1</th>
<th>Mean number of E. O. per stamen. 2</th>
<th>Mean number of Y. P. stamens per flower. 3</th>
<th>Mean number of c. s. stamens per flower. 3</th>
<th>Percentage of flowers with staminal cone.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA4/A7</td>
<td>24.0**</td>
<td>3.4**</td>
<td>4.8*</td>
<td>0.0</td>
<td>20%</td>
</tr>
<tr>
<td>GA3</td>
<td>2.3</td>
<td>0.7</td>
<td>6.4</td>
<td>0.0</td>
<td>100%</td>
</tr>
</tbody>
</table>

1 = sample size 3 flowers.
2 = sample size 10 stamens.
3 = sample size 5 flowers.
* = difference significant from GA3 at p ≤ 0.05.
** = difference significant from GA3 at p ≤ 0.01.
stamen was greater in plants which received the GA₄/GA₇ treatment. Also, while no carpelloid stamens (C.S.) were induced by either compound, the number of yellow and pubescent stamens (Y.P.) per flower was less with the GA₄/GA₇ treatment. The number of flowers with staminal cones was also few following GA₄/GA₇ application as compared to GA₃ application. Thus, although there is no significant difference in stem elongation caused by GA₄/GA₇ and GA₃, contrary to observations from cucumber, GA₄/GA₇ is less effective than GA₃ in inducing stamen development in sl₂ plants.

3. (-)-Kaurenoic acid:

Kaurenoic acid, an intermediate in gibberellin synthesis (Graebe et al., 1965), which itself is produced from mevalonic acid (Graebe, 1968) has been shown to be incorporated into gibberellic acid (Barendse and Kok, 1971). It also produces responses similar to gibberellic acid, viz; stimulating growth in dwarf mutants of maize (Katsumi et al., 1964) and releasing α-amylase in the embryo - less halves of barley seeds (Petridis et al., 1966). Kaurenoic acid (10⁻³ and 10⁻⁴M) applied to young sl₂ plants however, did not produce any significant effect in the stamen morphology. It is possible that much higher concentrations of the
compound might be required to produce any significant effect.

4. (2-Chloroethyl) trimethyl ammonium chloride (CCC):

CCC, one of the growth retardants (see review by Cathey, 1964) has been shown to cause dwarfing effects on plants. That this dwarfing effect is due to an inhibition of gibberellin bio-
synthesis was shown by Harada and Lang (1965) in Gibberella and
in higher plants by Zeevaart (1966). Since the exogenous appli-
cation of \( \text{GA}_3 \) to \( \text{s}_{12} \) mutant plants stimulates the production of
flowers which resemble wild type flowers, one could postulate
that the mutant may not be synthesizing enough gibberellin and
that the administration of CCC (or other substances attributed
to block gibberellin synthesis) to the wild type should therefore
result in some abnormality in the stamen development.

CCC (10\(^{-4}\) to 10\(^{-2}\)M) was applied in the form of a spray
to the leaves and stems of young wild type plants, at the stage
when the apex was in a transition phase to flowering. In the
flowers produced subsequent to the treatment no significant effect
on the floral organs was observed with any of the concentrations,
although some stunting of plants was apparent. However, three
applications (at an interval of 5 days) of foliar spray of 10\(^{-2}\)M
conc. resulted in a number of flowers with altered stamen mor-
Figs. 59-62. Effect of CCC on wild type flowers.

Fig. 59. Flower produced following CCC application. Note the anthers are separated near the base (x 5).

Fig. 60. Stamen of a flower in Fig. 59 showing the adaxial surface. Note the difference in stamen texture from the normal stamen in Fig. 8 (x 9).

Fig. 61. Sepals from an untreated flower (A) and CCC treated flower (B). Sepals in 61 B are longer and reduced in width near the base (x 2.5).

Fig. 62. A portion of a cross-section of an anther from CCC treated flower. Note the presence of few shrunken pollen and also reduced size of sporangia as compared to wild type in Fig. 57 (x 200).
Table 14

Effect of CCC (3 sprays of $10^{-2} \text{M}$, at an interval of 5 days) on the stamen character of wild type flowers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean final stamen length. 1 (mm)</th>
<th>Mean number of sporangia per anther. 1</th>
<th>Mean number of E.O. per stamen. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCC</td>
<td>9.20**</td>
<td>1.20</td>
<td>0.00</td>
</tr>
<tr>
<td>Control</td>
<td>10.25</td>
<td>4.00**</td>
<td>0.00</td>
</tr>
</tbody>
</table>

1 = sample of 5 stamens or anthers.
** = difference significant from control at $p \leq 0.01$. 
phology (Figs. 59 and 60). The anthers of these stamens were not fused along the entire length (Fig. 59). The stamens were also shorter, (Table 14) and thinner as compared to untreated controls (compare Fig. 60 with Fig. 8). Cross-section of the anthers revealed that the thin texture of the anthers could be in part due to a reduction in the number of sporangia per anther (Table 14) particularly on the abaxial surface. There was little pollen produced in these sporangia (Fig. 62) and the pollen also appeared shriveled. There were no E.O. present on the stamens however, and also no carpelloid stamens similar to those found in flowers from sl₂ plants, were observed in any of the CCC treated flowers. Besides the changes in stamen structure, the sepals (Fig. 61) and petals produced in these flowers were narrow and longer than the controls.

Thus, with the application of CCC to the wild type plants, although stamens similar to sl₂ plants were not produced, some characters reminiscent of sl₂ flowers; i) reduction in stamen length, ii) tendency of stamens to separate, iii) few sporangia per anther, iv) few pollen per sporangia, were produced.

5. Amo - 1618:

Amo - 1618, another growth retardant and a compound which
has also been shown to inhibit gibberellin biosynthesis (Baldev et al., 1965) was used to study its effect on the stamen development in wild type plants. Application of 10μl of 10^{-2} and 10^{-3}M of Amo-1618, applied when the apex was in a transition stage to flowering, was without effect on the stamen morphology of wild type plants.

6. **Indole acetic acid (IAA):**

In section 'c' under GA₃ effects, we demonstrated that prior to or at the time of stamen initiation in sl₂ flower buds, application of GA₃ resulted in the production of stamens which resembled the wild type flowers instead of the mutant. This suggested that at these stages the stamen primordia are uncommitted or undetermined to follow a particular series of reactions and a change in the hormonal level might alter the developmental pathway. Since it is reported that auxins promote the initiation and development of female organs (Heslop-Harrison, 1956, 1959 and Galun et al., 1963), and also since, it was shown above that certain environmental conditions promote the production of 'carpelloid stamens' (C.S.) in sl₂ flowers, the effect of IAA on the young floral buds of sl₂ plants (applied at or before the stamen initiation) was studied. IAA was applied with the help of a micropipette (25μl of 10^{-3} and 10^{-4}M) in the axil of the leaf closest to the apex.
Figs. 63-65. Effect of IAA on the stamen character of $s_{12}$ flowers.

Fig. 63. Flower produced following IAA application to $s_{12}$ plants. Note the absence of stamens, and the presence of additional carpel-like structures (carpelloid stamens - C.S.) adhering to the ovary (x 4).

Fig. 64. Carpelloid stamens separated from the flower in Fig. 63 (abaxial view) showing basal swollen region and distal region resembling a style (x 5).

Fig. 65. Mid-longitudinal section of a flower produced after IAA treatment. C.S. (shown by arrow) bear ovules in their basal swollen region (x 200). $se = sepal, p = petal.$
Table 15

Effect of IAA (2 applications of 25 μl of 10^{-4}M) on the stamen characters of sl₂ flowers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean number of E. O. per flower. a</th>
<th>Mean number of E. O. per stamen. a</th>
<th>Mean number of stamens per flower with E. O. a</th>
<th>Mean number of C. S. per flower. a</th>
<th>Mean number of Y. P. per flower. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>11.37**</td>
<td>4.25**</td>
<td>1.62**</td>
<td>4.25**</td>
<td>0.25**</td>
</tr>
<tr>
<td>Control</td>
<td>143.50</td>
<td>36.75</td>
<td>4.87</td>
<td>0.50</td>
<td>2.25</td>
</tr>
</tbody>
</table>

a = sample size 8 flowers.

** = difference significant from control at p ≤ 0.01.
In the flowers produced subsequent to IAA treatment, organs resembling the carpels were produced in place of stamens (Fig. 63). The basal ends of these 'carpelloid stamens' were swollen like the carpels and the distal ends resembled the style (Fig. 64). These supernumerary carpels adhered closely to the central pistil and along with it appeared as a multicarpellary ovary.

The number of C.S. per flower was greatest following the applications of 25µl of $10^{-4}$ IAA, applied at an interval of 5 days. In Table 15 a comparison of some of the stamen characters of IAA treated flowers as compared to the controls (sl₂ flowers) is presented. While the number of C.S. per flower was significantly greater in IAA treated flowers as compared to the controls, the number of yellow and pubescent (Y.P.) stamens per flower was less. Also, the number of E.O. per flower and per stamen and the number of stamens per flower bearing E.O. was also less in the flowers produced after IAA treatment as compared to the controls. Mid-longitudinal section through these flowers (Fig. 65) showed that in the basal ends of these 'carpelloid stamens' ovules were present and the distal end which morphologically resembled the styles also lacked any signs of microsporogenesis. Thus, IAA applied at a critical stage of stamen initiation, induced the
development of carpel-like organs in place of stamens.

7. **Effects of GA₃ on the flowers of wild type:**

   Although GA₃ promotes stamen development in the female flowers of cucumber and in stamenless mutants as shown above, it is reported to have stimulated the female organs in some hermaphroditic species (Resende and Viana, 1959) and in monoeccious corn (Nickerson, 1959) and castor bean (Shifris, 1961). Effect of GA₃ on the hermaphroditic flowers of normal wild type tomato plants was thus studied.

   Young wild type plants were administered two applications of GA₃ (10μl of 10⁻³M) at an interval of five days, prior to flower initiation. In the flowers produced subsequent to the treatment, ovaries having a larger diameter than those of untreated control plants were produced (Fig. 66). The number of carpels per ovary (observed as ridges on the ovary) was significantly greater than controls (Fig. 66) and Table 16. Cross sections through these ovaries also showed an increase in the number of locules per ovary (Compare Fig. 67 with Fig. 68 and Table 16). The size of the locules was smaller in treated flowers and although no data was collected on the number of ovules per locule, the total number of ovules per flower may not be different from controls. The styles produced in the treated flowers were also thicker and shorter.
Figs. 66-68. Effect of GA$_3$ on wild type flowers.

Fig. 66. Ovaries of GA$_3$ treated (left) and untreated (right) flowers (rest of the floral organs removed). Compare the number of carpels and the diameter of the GA$_3$ treated ovary with the untreated. Also, note the difference in thickness and length of the style of GA$_3$ treated ovary (x 6).

Fig. 67. Cross section of the ovary of untreated flower (x 25).

Fig. 68. Cross section of the GA$_3$ treated ovary. Note the increase in number of locules as compared to Fig. 67 (x 25).
Table 16

Effect of GA$_3$ on the number of carpels and locules in the ovary of wild type plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of carpels per flower $^a$ Mean $^b$ C. I.</th>
<th>Number of locules per flower $^a$ Mean $^b$ C. I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA$_3$</td>
<td>8.1** 0.95</td>
<td>8.6** 1.24</td>
</tr>
<tr>
<td>Control</td>
<td>4.5 0.82</td>
<td>4.5 1.00</td>
</tr>
</tbody>
</table>

$a$ = sample size 10.

$b$ = confidence interval at 95%

**$^a$ = difference significant from control at $p \leq 0.01$
Extension of these observations to commercial varieties was conducted and the results published (Sawhney and Greyson, 1971 a). The multilocular ovaries produced through this treatment, upon pollination produced fruits which were larger both in size and fresh weight (Sawhney and Greyson, 1971 b). The practicality of these observations on a commercial scale needs to be tested.

Thus, in the hermaphroditic flowers of normal wild type tomato plants, GA$_3$ stimulates the ovary development while in the $s_{12}$ mutant it induces the normal development of stamens.
III. ANALYSIS OF ENDOGENOUS LEVELS OF GIBBERELLINS IN sl₂ AND WILD TYPE PLANTS

In the previous section it was demonstrated that following the application of GA₃ to sl₂ plants, flowers which resembled the wild type, both morphologically and functionally, were produced instead of sl₂ flowers. Similarly, the application of CCC to wild type plants resulted in some abnormalities in stamen morphology similar to those of sl₂ flowers. It can thus be interpreted that the mutant plant might have; either a deficiency in gibberellin levels, possibly through a blockage in gibberellin synthesis or although the gibberellin synthesis may be unaffected, the utilisation of gibberellins may be blocked. In order to resolve the two interpretations gibberellins were extracted, purified and bioassayed from sl₂ mutant and wild type plants.

A. Extraction of gibberellins from vegetative parts:

In a preliminary test 500 gms of tissue from leaves and stem portions of young plants were extracted through a procedure described in methods and outlined in a flow sheet (Appendix-A). Dry residues were separated on a TLC plate (as described under methods). None of the ten horizontal fractions of the chromato-
gram exhibited a stimulatory response on either the dwarf pea or the lettuce hypocotyl bioassay. Thus, it was decided to use much higher amounts of tissue for extraction of gibberellins.

During 1970 summer, sl₂ and wild type plants were grown at the experimental field station, and 3 kilograms of tissue (leaves and stems) were harvested from each of the two genotypes. Tissues of sl₂ and wild type were homogenised separately in 6 liters of 75% ethanol. Further extraction of gibberellins was followed in a similar manner as outlined in Appendix-A. Dry extracts which represent the acidic fraction, were redissolved in water-saturated ethyl acetate and spotted on a TLC plate. After developing the chromatograms in benzene: butanol: acetic acid solvent system (as described in methods) chromatograms were dried and divided horizontally into ten equal parts. Each fraction was eluted with ethyl acetate, filtered and the ethyl acetate evaporated. Dry residue was dissolved in 0.2 ml of distilled water and 5μl per plant was used for the dwarf pea bioassay (see methods for the description and Fig. 69 for the sensitivity response of bioassay). Eight seedlings were used for each fraction and after five days of exposure to red light, epicotyl lengths of seedlings were measured. Mean epicotyl lengths measured after
Fig. 69. Standardization of dwarf pea bioassay. Epicotyl elongation produced after 5 days of GA$_3$ treatment to dwarf pea plants (Progress No. 9) grown under red light. 5 μl of GA$_3$ of different concentrations was applied at the apex of young seedlings. Data on some points in Table 17.
five days of the application of different fractions from the extracts of the two genotypes are presented in Fig. 70. The fraction between Rf's 0.4-0.5 from the extracts of wild type plants shows a strong stimulation of epicotyl elongation (Fig. 70A). At the same Rf position stimulation of epicotyl length was also observed in extracts from sl₂ plants (Fig. 70B) significantly higher than controls (dotted lines) but less than the wild type (Table 17). A slight increase in epicotyl length with the fractions between Rf's 0.3-0.4 and 0.2-0.3 of wild type plants (Fig. 70A) is not significantly different from the response in similar fractions of sl₂ plants (Fig. 70B). There is also no significant stimulatory or inhibitory response in any other fractions from the extracts of both the genotypes. For comparisons, stimulation of epicotyl length by the application of 0.01 and 0.001μg GA₃ is also presented in Fig. 70A and Table 17.

The activity of the extracts from wild type and sl₂ plants was also checked on lettuce hypocotyl bioassays. The remaining extract of each fraction after treating the dwarf peas was dried and redissolved in 3 ml of distilled water. Germinated lettuce seeds were placed in petridishes (10 seeds per dish) with a filter paper (Whatman No. 1) and 3 ml of test solution (see methods for
Table 17

Activity of the extracts (fraction between Rf 0.4 - 0.5) from the vegetative parts of sl₂ and wild type plants on "dwarf pea" bioassay.

<table>
<thead>
<tr>
<th>Material</th>
<th>Epicotyl length (cm)</th>
<th>% of control</th>
<th>Total wt. of the fraction (µg.) (equivalent to 0.01 µg GA₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean⁸ ± C.I. b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sl₂</td>
<td>7.26** 0.45</td>
<td>113.00</td>
<td>0.174</td>
</tr>
<tr>
<td>Wild type</td>
<td>9.83** 0.51</td>
<td>153.35</td>
<td>0.236</td>
</tr>
<tr>
<td>GA₃ (0.001 µg)</td>
<td>7.82 0.61</td>
<td>122.65</td>
<td>---</td>
</tr>
<tr>
<td>GA₃ (0.01 µg)</td>
<td>16.67 1.27</td>
<td>260.15</td>
<td>---</td>
</tr>
<tr>
<td>Control</td>
<td>6.41 0.25</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

** = significantly different from control and from each other at p = 0.01.
a = sample size 8.
b = 95% confidence interval.
the description of bioassay). Hypocotyl lengths were measured after 3 days. Only the fraction (Rf 0.4-0.5) from the extracts of wild type plants stimulated a significant increase in hypocotyl length (115% approximately) over the controls. The same fraction from $s_{12}$ extracts or any other fraction from both the genotypes failed to produce any significant increase in hypocotyl length. Thus the fraction of the same Rf value (0.4-0.5) produced stimulatory response in both the dwarf pea and lettuce hypocotyl bioassay. As well, with both the bioassays, the extracts from wild type plants produced a significantly greater effect compared to the extracts from $s_{12}$ plants.

The Rf position of standard GA$_3$ on the TLC plate was between 0.4-0.5. This was recorded by applying GA$_3$ on one end of the same TLC plate as the extracts and was thus developed under similar conditions. Since the two plates with extracts of two genotypes were developed at the same time in a single chromatography tank and since the activity of the extracts on bioassays was observed in the fraction with Rf value similar to that of GA$_3$, it is suggestive that this stimulatory response of the extracts is possibly due to GA$_3$. However, since information on the chemical structure of these extracted substances is lacking they may only
be referred to as 'gibberellin-like substances' (Paleg, 1965).

It can thus be concluded that tissues from vegetative parts of wild type plants contain higher levels of gibberellin-like substances than do comparable tissues of sl₂ plants.

B. **Extraction of gibberellins from flowers:**

Gibberellin-like substances were also extracted from flowers of sl₂ and wild type plants. Two hundred and fifty flowers at anthesis of each genotype were extracted for gibberellins in a similar manner as described above for vegetative parts. After purification, extracts were separated on a TLC plate and activity of the ten fractions for each genotype was checked on the dwarf pea bioassay.

Epicotyl lengths were recorded 5 days after the treatment with various fractions of the two genotypes and are illustrated in Fig. 71. Unlike the extracts from vegetative parts no significant increase in epicotyl length was observed in the fraction between Rf's 0.4-0.5 in either of the two genotypes. However, with the fraction between Rf 0.3-0.4 of wild type flowers significant increase in epicotyl length was recorded (Fig. 71A, Table 18). The fractions between Rf 0.2-0.3 and 0.1-0.2 of wild type flower extracts also showed a significant increase in epicotyl length
Fig. 71. Activity of gibberellin-like substances on dwarf pea bioassay, from the extracts of flowers of wild type (A) and sl2 (B) plants. Dotted line represents control.
Table 18

Activity of the extracts (fractions between Rf 0.1 - 0.4) from floral parts of sl2 and wild type plants on dwarf pea bioassay.

<table>
<thead>
<tr>
<th>Material</th>
<th>Rf</th>
<th>Mean Epicotyl length (cm)</th>
<th>% of control</th>
<th>Total wt. of the fraction (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sl2</td>
<td>0.1 - 0.2</td>
<td>6.70</td>
<td>104.68</td>
<td>0.160</td>
</tr>
<tr>
<td>wild type</td>
<td>0.1 - 0.2</td>
<td>6.90*</td>
<td>107.81</td>
<td>0.165</td>
</tr>
<tr>
<td>sl2</td>
<td>0.2 - 0.3</td>
<td>6.63</td>
<td>103.59</td>
<td>0.159</td>
</tr>
<tr>
<td>wild type</td>
<td>0.2 - 0.3</td>
<td>6.94*</td>
<td>108.43</td>
<td>0.166</td>
</tr>
<tr>
<td>sl2</td>
<td>0.3 - 0.4</td>
<td>6.78a</td>
<td>105.93</td>
<td>0.162</td>
</tr>
<tr>
<td>wild type</td>
<td>0.3 - 0.4</td>
<td>7.36*b</td>
<td>115.00</td>
<td>0.176</td>
</tr>
<tr>
<td>control</td>
<td>--</td>
<td>6.40</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

* = significantly different from control at p = 0.05.
a, b = significantly different from each other at p = 0.05.
1 = sample size 8.
as compared to the controls (Table 18). However, the response of the same fractions from sl₂ flower extracts was not significant. Other fractions of both the genotypes did not produce any statistically significant stimulatory or inhibitory response.

Thus the stimulatory response of some of the fractions from the extracts of wild type flower is suggestive of gibberellin-like activity. Also, since the Rf values of these fractions are not similar to GA₃ but correspond to GA₁ and GA₂, the extracted gibberellin-like substances may be a mixture of GA₁, GA₂ and even GA₃, unlike the extracts from vegetative parts. No matter what the qualitative character of these gibberellins might be, quantitatively they are higher in wild type as compared to sl₂ flowers.
DISCUSSION

The present study was conducted in an attempt to interrelate gene with hormone and hormone with stamen form in stamenless - 2 mutant of tomato. Different aspects of this study have revealed the following:

a). The analysis of the ontogeny of stamens showed that at early stages of development, stamen primordia of sl2 and wild type resembled each other closely. The developmental pattern however, could be distinguished in the two genotypes after the primordia reach about 100μ in length. The extent of this divergence is exhibited in the final stamen form of the two genotypes, and the production of naked ovules and aberrations in microsporogenesis in sl2 stamens.

b). On comparing the relative growth rates of stamens with sepals of sl2 and wild type flowers, no significant difference in their regression coefficients (allometric constants) was noted. However, some differences prior to or coincident with stamen initiation seem to exist in the two genotypes.

c). The exogenous application of growth hormones induced alterations in the sex expression of both sl2 and wild type flowers. GA3 applied to floral buds of sl2 plants, when stamen primordia
were up to 0.1 mm long, resulted in stamens resembling those of wild type flowers ('phenocopy'). Alternatively, in wild type plants, flowers produced following CCC application exhibited some abnormalities in stamen morphology similar to sl₂ flowers. However, GA₃ applied to wild type flowers, stimulated ovary development, with no significant effect upon stamens. IAA application, on the other hand, induced carpel-like structures in place of stamens in sl₂ flowers.

   d). Bioassay of extracted and purified residues from sl₂ and wild type plants (extracted both from vegetative parts and floral parts), revealed higher amounts of extractable gibberellin-like substances in wild type plants in comparison to sl₂ mutant.

   The significance of these results will be discussed under following headings:

   A. **Plant hormones and sex organs**

   Plant hormones have been implicated in the initiation and development of sex organs many times. Gibberellins have most commonly been associated with the development of male organs (Peterson and Anhder, 1960; Mitchell and Wittwer, 1962; Phatak et al., 1966; Greyson and Tepfer, 1967; Blake, 1969; Pike and Peterson, 1969 and others). On the other hand auxins have been
reported to promote the development of female organs (Heslop-Harrison, 1956; Galun et al., 1963). Our data on the effects of GA$_3$ and IAA on sl$_2$ and wild type flowers, in part support these general interpretations.

GA$_3$ application to the young floral buds (with stamen primordia up to 0.1 mm in length) of sl$_2$ plants induced the production of stamens resembling those of wild type flowers (compare Fig. 47 with Fig. 48). All stages of microsporogenesis were completed in sl$_2$ flowers and fertile, apparently normal pollen was produced (Figs. 53 and 54). That GA$_3$ is presumably involved with stamen development was further substantiated by treating the wild type plants with CCC (an inhibitor of gibberellin synthesis). Van Bragt (1969) demonstrated that in tomato one application of CCC lowered the levels of GA$_1$ and GA$_3$-like substances when plants were harvested five days after the treatment, although endogenous gibberellins were higher in CCC treated plants after four weeks.

In our studies, three applications of CCC at five day intervals to the wild type plants resulted in some stamen abnormalities similar to sl$_2$ mutant (Figs. 59, 60, 62 and Table 14). Atsmon et al. (unpublished results) also observed a reduction in male tendency following CCC treatment to plants of *Cucumis*. However, the interpreta-
tion on the role of gibberellins in maleness seems over simplified since GA$_3$ application to the wild type plants, promoted feminization by inducing multilocular ovaries and ovaries of larger diameter at anthesis as compared to the untreated controls (compare Fig. 67 with Fig. 68). Other reports in the literature have also documented stimulation of female organs over male organs by added gibberellins (Nickerson, 1959; Resende and Viana, 1959; Shifris, 1961).

Thus, GA$_3$ application promotes both male or female tendencies in different systems.

These apparently contradictory observations on the sexuality of plants by the exogenous application of GA$_3$ can be explained as follows. In systems, exhibiting abnormalities in the development of male organs e.g. sl$_2$ mutant and other stamenless mutants reported by Phatak et al. (1966) and Rana and Jain (1969) or in basically hermaphroditic flowers with suppressed stamen primordia e.g. female flowers of Cucumis (Atsmon and Galun, 1960; Peterson and Anhder, 1960), GA$_3$ application stimulates normal stamen development. On the other hand, systems in which normal development of both male and female organs exists e.g. hermaphroditic flowers of wild type tomato reported in this dissertation and bisexual flowers of Hyoscyamus (Resende and Viana, 1959) or in a system with normal development of stamens but under-
developed female organs, e.g. in the tassel of Zea (Nickerson, 1959), or the male flowers of Ricinus (Shifris, 1961), application of GA$_3$ shifts the balance towards increased femaleness. That this could possibly be through a GA$_3$ stimulated increase in endogenous auxin levels is suggested by some workers. An increase in the diffusible auxin levels following the application of GA$_3$ has been demonstrated by Kuraishi and Muir (1963) in Hyoscyamus and by Sastri and Muir (1963) in tomato. That GA$_3$ operates in a similar manner in wild type and other commercial varieties has also been postulated by Sawhney and Greyson (1971 a). In s$_1^2$ plants however, addition of GA$_3$ promotes stamen development alone in the first few flowers produced following treatment (presumably, by overcoming the deficiency of endogenous gibberellins), while multilocular ovaries are observed in the flowers produced later. These observations suggest that GA$_3$ is itself utilised initially, in a system inadequate in gibberellins while later on it operates affecting the auxin content. If application of GA$_3$ exerts its influence through auxin levels, then applied auxins should produce similar results on tomato flowers. IAA application to wild type plants however, did not induce multilocular ovaries. That replacement of GA$_3$ by the application of IAA
is successful in some systems and not in others has been discussed by Cleland (1969). Thus, while in systems which exhibit either an inhibition or an abnormality of stamen development (possibly due to lower levels of endogenous gibberellins) application of GA$_3$ promotes the development of male organs, in systems with normal stamen development, application of GA$_3$ stimulates the development of female organs.

The involvement of auxins in the development of female organs was investigated on sl$_2$ plants. IAA applied to the young floral buds of sl$_2$ mutant at a stage prior to stamen initiation induced the formation of carpel-like organs (carpelloid stamens) in positions normally occupied by stamens (Fig. 63). Besides the resemblance of these organs to carpels they also bore ovules inside the basal swollen end (Fig. 65). These observations corroborate the generalization of the role of auxins in feminization.

Environment is also known to play a role in the sex expression of plants. In most systems, long days promote the development of male organs while short days influence the carpel development (see review by Heslop-Harrison, 1957). In sl$_2$ plants however, while the number of carpelloid stamens per flower was higher in plants cultivated under long days as compared to those grown under short days, the opposite response was observed with respect
to number of yellow - pubescent stamens (like normals) in the two growing conditions. If carpellization of stamens is to be considered an increase in female tendency of flowers then our observations on sl₂ are contrary to the above generalization. In some other systems however, e.g. Spinach (Thompson, 1955); Silene (Heslop-Harrison and Heslop-Harrison, 1958), Ricinus (Shifris, 1961); and Begonia (Heide, 1969), responses similar to those observed in sl₂ plants were reported. It is possible that promotion of stamens under short day conditions in sl₂ flowers is through higher levels of gibberellins while carpellization of stamens under long days is through higher auxin levels. It can thus be assumed that a balance between gibberellins and auxins preceding the initiation of organs in the floral apex determines the sex expression in plants.

The above information gathered from the plants which had been treated exogenously with growth substances and environmental conditions is important but only suggestive of the possible mechanisms in the development of sex organs. Thus, an analysis of endogenous hormones in sl₂ mutant and wild type plants was conducted. Extractions followed by chromatography and bioassay of both vegetative as well as floral parts of sl₂ and wild type plants consistently
showed lower levels of endogenous gibberellin-like substances in the mutant as compared to the wild type. This information which is based on bioassays (Figs. 70 and 71) is of quantitative nature and although there are indications from TLC that Rf positions of active fractions of the extracts which correspond to GA$_3$ exist in vegetative parts and a mixture of GA$_1$, GA$_2$ and GA$_3$ in floral parts, the chemical nature of these substances remains unknown. Despite the fact that the chemical identification of these gibberellin-like substances was not obtained the correlation between stamen development and level of gibberellins supports the general theory that sl$_2$ mutant is a product of altered gibberellin metabolism, possibly levels.

Correlations between sex expression and endogenous hormones have been demonstrated in some other instances. In *Cucumis sativus*, Atsmon et al. (1968) reported higher levels of gibberellins (quantified by bioassays) in the diffusates and exudates of monoecious as compared to the gynoecious plants. Hayashi et al. (1971) performed a qualitative analysis on the same material with the help of gas chromatography and showed lower levels of GA$_1$ in the gynoe- cious line than in the monoecious one. Auxins, on the other hand were higher in hermaphrodite plants of cucumber as compared to
the andromonecious plants (Galun et al., 1965). Conrad and Mothes (1961) similarly reported that female plants of *Cannabis* contained higher auxins than male plants. Sladky' (1969), in his analysis of *Zea* reported higher levels of gibberellins and lower levels of auxins prior to tassel differentiation while the reverse situation was true before ear differentiation. Other morphological characters e.g. dwarfing in plants have also been correlated with levels of hormones (for a review see Introduction).

Thus, information from analyses of endogenous growth substances is consistent with earlier conclusions on the effects of applied growth substances on sexuality of plants. While gibberellin levels are crucial prior to the initiation and subsequent development of male organs, auxins play an important role in the initiation and development of female organs.

B. **Organogenesis in the floral meristem**

Descriptive studies on the initiation and development of floral organs have been carefully conducted by a number of workers (Engard, 1944; Douglas, 1944; Boke, 1949; Tepfer, 1953; Barnard, 1960; Kaplan, 1968 and others). Such studies are a prerequisite before any questions on the underlying factors of organogenesis can be attempted. A study conducted on the ontogeny of stamens of *sl₂* and wild type revealed that at initiation, stamen primordia
of the two genotypes resembled each other closely (Figs. 19 and 20). However, thereafter, the developmental pattern of the two could be distinguished and ultimately at maturity the \( s_{12} \) stamens did not exhibit regular microsporogenesis, were not fused to each other, were shorter than wild type stamens and besides other differences, bore external ovules. These observations led us to believe that at early stages of initiation the stamen primordia of \( s_{12} \) and wild type flowers undergo similar kinds of events (up till approximately 100 \( \mu \) in length), beyond this stage some changes in the sequence of events take place which alter the course of development resulting in two dissimilar organs. Some of the factors that might be involved in the organogenesis of stamens in \( s_{12} \) and wild type flowers and the stages at which these factors are most critical will be discussed below.

Basically, two general theories have been advanced which impinge on the nature of mechanisms involved in floral organogenesis. Based upon Turing's (1952) concept of a 'physico-chemical reaction system', Wardlaw (1957) proposed that the floral meristem be viewed as a 'reaction system'. According to this interpretation reacting substances or their products could become concentrated at regular intervals in the floral apex and thus give rise to growth centers. The basic constituents of this reaction
system would be carbohydrates, amino acids, enzymes, etc. and more specifically 'gene determined substances' which are involved in the reactions at specific times. It was envisaged that at the onset of flowering, besides a change in the working of a reaction system operative during the vegetative phase, an orderly sequence of reactions is initiated which is in part due to 'turning on' of some specific genes. The activity of the reaction system results in evenly spaced growth centers on helices or in whorls and these primordia result in sepals or in some cases bracts or bracteoles. Changes resulting in the 'sepal phase' affect the new specific genes which lead to new growth centers resulting in petals and similarly changes taking place during the 'petal phase' would 'turn on' new genes causing new growth centers for stamens and so on. Thus, according to this theory, organogenesis in the floral meristem can be attributed to specific gene activity prior to the initiation of a set of primordia, feeding into the reaction system of some 'gene determined substances', affecting a change in the reaction system and finally resulting in new growth centers. In addition, changes taking place during one phase of organogenesis in some way affecting the next set of organs.

In a similar vein, Heslop Harrison (1964) proposed a relay system in the flowering apex. According to him the flowering pro-
cess is initiated in the presence of an effector substance (flowering stimulus) which activates a gene complex responsible for the first lateral appendages (sepals). Sepals in turn activate the next gene complex responsible for petals through an inducer, and petals in turn, through another inducer, activate a gene complex for stamens and so on. Both this and Wardlaw's model take into account the activity of specific genes prior to the initiation of each type of floral organ. This gene activity is dependent upon some stimulus and in turn leads to the activation of next gene complex.

Although very little data exists which supports these hypotheses, our experiments, conducted on the young floral buds of sl₂, can be interpreted in terms of these models and have yielded insights on some of the factors regulating organogenesis. Stamen primordia of sl₂ which if left untreated would have produced abnormal stamens, upon GA₃ treatment produced 'phenocopies' of normal stamens. It is conceivable that GA₃ or other gibberellins may be part of the 'gene determined substances' as envisaged by Wardlaw and play an important role prior to stamen initiation. If gibberellins are indeed crucial for stamen initiation and development, then it can be expected that lower available
gibberellins could cause poor stamen development. Since, analysis of the endogenous gibberellins has revealed lower levels of gibberellin-like substances in sl₂ plants as compared to wild type plants the above contention seems more plausible. Furthermore by applying an inhibitor of gibberellin synthesis to normal plants, some abnormalities in stamens similar to the sl₂ mutant were induced. These results can also be viewed in terms of Heslop-Harrison's relay system of the floral apex. If gibberellins are considered to be responsible in some way for derepressing the operon prior to stamen initiation (whether they were themselves a product of the transcription of gene complex for petals or operated in conjunction with other products as inducers) then a reduction in their level would cause disordered transcription thus disturbing the sequence of events which were to follow.

Experiments involving the application of IAA can shed light on these general hypotheses. In sl₂ plants carpel-like structures can be induced in place of stamens if treated with IAA at an appropriate stage of development. Presumably, the application of IAA completely suppresses the information leading to stamen formation. According the Heslop-Harrison's model it can be envisioned that application of IAA derepresses the information for carpel-like structures thus the information for stamens remains
inactive. If this speculation is true then it raises an important
question: Is the initiation and development of carpels dependent
upon stamen initiation? Our results positively show that in the
sl2 mutant the development of carpel-like organs can take place
in the absence of stamens or their primordia and thus the two
organ types are developmentally independent. It can thus be
summarized that in the floral apex levels of gibberellins are
most critical prior to stamen initiation while auxins in some
way play a definite role before carpel initiation. It can be specu-
lated that these substances either themselves act as inducers
activating a new set of genes or they operate in conjunction with
the other products (possibly proteins as suggested by Matthysse
and Phillips, 1969). However, since the development of carpel-
like organs can take place in the absence of stamens, our data
rejects the hypotheses of the dependence of an inducer from the
organs of a previous whorl as proposed by Wardlaw (1957) and
Heslop-Harrison (1964).

The above discussion only took into account some of the
factors or reactions taking place within the floral apex at the
time of or prior to organ initiation. A number of question regard-
ing organ primordia following initiation remain unanswered. For
example; how soon, following initiation is a primordium determined to follow an irreversible pathway. According to Hadorn (1965):

"Determination is a process which initiates a specific pathway of development by singling it out from among various possibilities for which a cellular system is competent".

Heslop-Harrison (1959) suggested two alternative pathways a primordium might follow, following its inception. On the one hand, development and differentiation of a primordium could be 'autonomous' after early determination (self determination) or the final form could result from various stimuli acting upon the developing primordium (dependent differentiation).

In the sl₂ mutant since the application of GA₃ induces a change in the morphological character it is implied that at some developmental stage the stamen primordium is undetermined. Application of GA₃ at that time directs the course of development in one direction thus acting as a determinant. In order to obtain information on the critical stage or stages ('effective periods' as referred by Mather, 1965) at which the stamen primordia are determined or committed to follow a definite pathway, GA₃ was applied to floral buds with various stamen sizes. The results indicated (Table 12 and Fig. 55) that if GA₃ was applied until the stage when
stamens were 0.1 mm long, stamens resembling the normal instead of abnormal stamens were produced. After the stamens reach 0.15 mm in length and until 0.6 mm, GA$_3$ application induced some morphological changes, while stamens 0.7 mm in length or longer failed to respond to GA$_3$ application. Thus, the 'effective period', at which GA$_3$ can act as a determinant in stamen development of sl$_2$ flowers is from inception until primordia are 0.1 mm long. In other words, stamen primordia at this stage are not irrevocably committed to develop into either an abnormal or a normal stamen. This conclusion correlates with the observation on the ontogeny of stamens, exhibiting similarity in stamen primordia of sl$_2$ and wild type up till they are approximately 100 $\mu$ in length (Figs. 19 and 20). After the primordia reach approx. 0.15 mm in length, while some of the reactions are initiated and fixed, others can still be altered, therefore some morphological changes can be induced. By the time stamens attain 0.7 mm length, the developmental pathway of stamens as a whole is fixed and GA$_3$ treatment does not alter the established course of events, and the stamens are thus said to be determined.

In another study, Heslop-Harrison and Heslop-Harrison (1957) reported information on the critical stage of floral development in Cannabis sativa which was open to experimental manipulation.
One percent carbon monoxide (CO) treatment to the genetically male floral buds at the stage when the floral meristem is flattening, induced intersexuality. CO treatment prior to or later than this stage was without effect. In an entirely different system, with surgical experiments on the shoot apices of a fern *Dryopteris aristata*, Cutter (1956) demonstrated that the three youngest leaf primordia (P₁ - P₃) if isolated before the formation of an enlarged lenticular leaf apical cell, can be induced to develop into buds. Primordia isolated after the formation of this cell develop into a leaf. Steeves (1961), similarly demonstrated in *Osmunda cinnamomea* that excised young leaf primordia if cultured in-vitro could give rise to buds instead of leaves. Older primordia however, gave rise predominantly to leaves.

All these examples point to one conclusion. Young primordia at early stages of inception are undetermined or uncommitted to follow a definite pathway. They however, pass through a phase of competence and are subsequently determined and follow a fixed developmental pathway into an organ of a particular kind.

Our studies suggested another question related to floral ontogeny. In stamens of *s₁₂* and wild type (two morphologically distinct organs at maturity), are the differences established at
inception or do they gradually diverge along the developmental pathway starting from similar patterns of initiation? For an answer to this question, growth correlations between stamens and sepals of the two genotypes were conducted. Regression analyses performed on the log values of stamen length and sepal length of the two genotypes showed no significant difference in the regression coefficients of $sl_2$ and wild type. There was however, a significant difference in the $Y$ intercept (Table 7 and Fig. 44). These results indicated that the final length of stamens in $sl_2$ and wild type is not due to differences in their relative growth rates (assuming that the sepals in the two flowers grow similarly), but perhaps due to some differences at or prior to initiation. One such difference may be a delay in stamen initiation with respect to sepal initiation in $sl_2$ flowers. Further work is required to identify the nature of these differences.

In another allometric comparison conducted by Stebbins and Yagil (1966), on lemma and anther length in 'awned' and 'hooded' strains of barley, a different kind of relationship than the one we observed in tomato was reported. While the allometric relationship of lemma and anther length, for the 'awned' variety yielded a straight line, that of 'hooded' could be resolved
into two separate lines each with its own growth constant. The regression representing initial growth was identical to 'awned' while the second line representing later development of the 'hood' intersected the first at a point which corresponded to the stage at which mitotic activity was resumed in the hooded lemma.

Thus, in this case differences in the lemma of the two genotypes are not apparent at early stages but are switched on part way along the developmental pathway. In our example however, differences seem to exist at the earliest visible stages.

In his studies on *Cucurbita*, Sinnott (1936) came to the conclusion that the shape index of fruits could be affected by: a) differences during the early stages of ovary development, b) differences in the relative rates of growth in length and width during the development or c) differences in fruit sizes at which dimensional growth rates are unequal. In the flowers of *Nigella damascena* we have reported (Greyson and Sawhney, 1972) that the differences between petals and stamens at maturity, can be related to a difference in the relative growth rates beginning from initiation and extending throughout the development of organs. Thus, in all the examples cited, except in the study by Stebbins and Yagil, differences between the organ types seem to be established at very early stages.
of development.

Thus, although observations on the description of the ontogeny of stamens in $sl_2$ and wild type suggested some similarities in the young primordia (primordia up till 100 $\mu$ in length), from the allometric analysis we generated the concept that some differences existed prior to or at the time of stamen initiation in the two genotypes. These conclusions lend support to the interpretations of the general hypotheses by Wardlaw and Heslop-Harrison described earlier.

C. Gibberellins and gene activity:

In general, all morphological characters and developmental processes have some genetic basis. In a number of situations a single gene is known to be involved with a character or a step in the development of a character. However, the question as to how a gene is related to form is still poorly understood and this is especially true in higher organisms. We can pose a similar question with our material, since the $sl_2$ mutant is different from wild type in a single gene (Hafen and Stevenson, 1958). How and when does the $sl_2$ allele operate causing the abnormality in stamen structure? It should be indicated in the beginning that no definite data on the mechanisms of gene action were obtained by the writer. However, based upon information from other systems some postulates in this area will be offered.
The \( s_{12} \) allele seems active only at the time of flowering since the mutant differs phenotypically from the wild type only in stamen structure. Our data however, does not lend support to this hypothesis. An analysis of endogenous gibberellins revealed that the mutant has a lower level of gibberellins not only in the flowers but also in the vegetative parts. In addition, the following preliminary observations strengthen our argument:

a) Germination of \( s_{12} \) seeds is delayed as compared to the wild type seeds.

b) In young seedlings, leaves and stems of \( s_{12} \) plants are darker green (due to higher levels of chlorophyll) than are the wild type plants.

c) Stem cuttings of \( s_{12} \) plants produced more adventitious roots as compared to the cuttings from wild type plants.

All these observations which if supported by more extensive studies are consistent with an interpretation that \( s_{12} \) plants contain lower levels of gibberellins and suggest that in the mutant the \( s_{12} \) allele is operative throughout the vegetative growth of the plant although the morphological effect of the allele is mainly apparent at the time of flowering. Thus, the \( s_{12} \) mutant does not represent gene activity operative only at the flowering stage but at all stages of development starting from germination. Sarkissian et al. (1962) similarly
reported a significant difference in a number of amino acids at the seedling stage in the 'hooded' genotype in barley as compared to the normal type, although the two genotypes are indistinguishable until flowering. In another study, on 'yg-6' mutant in tomato besides the expression of three characters viz; yellow green leaves, elongated hypocotyl and reduced anthocyanin, a number of pleiotropic characters were reported by Perez et al. (1971). The 'yg-6' mutant also contained three times as much gibberellins as the wild type. The lower levels of gibberellins throughout the growth of sl2 plants raises the next question: How does the sl2 gene operate? The process by which information coded in a stretch of DNA (gene) is transcribed and translated into protein synthesis is now widely accepted (Jacob and Monod, 1961). Although this interpretation is mainly based upon studies in micro organisms, there is little doubt that similar events take place in higher organisms. Some of the several possibilities by which a reduction in the gibberellin levels may be brought about are, because the sl2 allele is;

i) a basic modification in the structural gene affecting the synthesis of enzymes required in gibberellin synthesis.

ii) a modification in the structural gene, inducing some enzymes which convert the gibberellins to bound form in the mutant and
therefore a lower level of extractable (acidic) gibberellins.

iii) a modification in the structural gene inducing the synthesis of some other enzymes and compounds which affect the gibberellin metabolism.

iv) a modification in the regulator gene which in turn affects the operator and the structural gene in ways described in i, ii and iii.

Experiments by the application of CCC (an inhibitor of gibberellin synthesis) on wild type plants showed that, morphological characters similar to the sl₂ mutant were induced. Since, Van Bragt's (1969) experiments show that in tomato, following CCC treatment, there is an initial reduction in GA₃-like substances and since our analysis of endogenous hormones further documents lower levels of gibberellins in the mutant it suggests more strongly that in the mutant inhibition of gibberellin synthesis occurs, rather than conversion of gibberellin to another form or a difference in its metabolism as suggested in ii) and iii) above. However, whether modification of either a structural or a regulator gene or both exists is difficult to establish. Either way, reduction in gibberellin content occurs possibly by affecting some enzymes (as yet unknown) which are crucial for gibberellin synthesis.
After speculating on some of the mechanisms by which gibberellin levels in the sl₂ mutant might be reduced, we now consider the stage or stages in the biosynthetic pathway of gibberellins which might be affected (for biosynthetic pathway of gibberellins see Lang, 1970). Although this is another area where specific information is lacking, our observations on lower levels of carotenoids in sl₂ stamens (preliminary results) suggest that the blockage of gibberellin synthesis is prior to geranyl-geranyl pyrophosphate. Failure by the application of kaurenoic acid to induce normal stamen development also suggests that the enzymes required for the conversion of kaurenoic acid to gibberellin are either absent or latent. Thus, although it appears that blockage in the synthesis of gibberellins is at early stages it also has a definite effect on later steps. Use of radioactive precursors and intermediates in the gibberellin pathways could further help to provide precise information on this topic.

The next question, that we might consider in this general area is; how lower levels of gibberellins might affect stamen structure? One such possibility (also considered earlier as proposed in the models by Wardlaw and Heslop-Harrison) is that gibberellin 'turns on' new gene activity in the floral apex resulting in the induction of new enzyme synthesis. Filner et al. (1969) described 'de novo' synthesis of α-amylase in aleurone layers of barley in the presence of GA₃. In the same system Varner and Chandra
(1964) reported the incorporation of many labelled amino acids in \( \alpha \)-amylase. The \( \alpha \)-amylase synthesis was inhibited in the presence of actinomycin - D, an inhibitor of DNA dependent RNA synthesis. Broughton (1969) similarly reported in pea internodes, twice as much total RNA and proteins following GA\(_3\) treatment as compared to the control. In isolated pea nuclei, Johri and Varner (1968) showed the enhancement of RNA synthesis in the presence of GA\(_3\). An increase in RNA, DNA and protein synthesis influenced by gibberellins has also been shown in other systems (see reviews by Trewavas, 1968; Key, 1969; Filner et al., 1969; Marcus, 1971). Although no such information was obtained prior to stamen initiation in sl\(_2\) and wild type floral apices, it can be postulated that synthesis of m - RNA and proteins is induced. Presumably this is a suitable material for investigating differences if any, in protein synthesis prior to or at the time of stamen initiation in the mutant and normal.

However, whether gibberellins induce enzyme synthesis by derepressing some genes prior to stamen initiation or act at the transcription level, or at some stage of protein synthesis (translation) or they merely act by activating some latent enzymes, it is conceivable that lower levels of gibberellins in sl\(_2\) plants would affect the level of enzyme production or activation, thus upsetting
the series of reactions to follow. The new proteins (enzymes) would subsequently play an important role in cell division and expansion leading to organ formation.

In summary, it is thus postulated that the sl₂ allele (which is either a modification of the structural or a regulator gene) operates through some enzyme systems thus affecting gibberellin synthesis. This gene is active throughout the development of the plant rather than only at the time of flowering. In the flowering apex, prior to stamen initiation, lower levels of gibberellins presumably affect specific enzyme synthesis, and thus disrupt the organogenic events which ultimately result in a modification of stamen structure.
CONCLUSIONS AND PROSPECTS

Stamenless-2 mutant in tomato possesses a unique feature of bearing both the male and female characteristics on the same organ. Although there are other reports describing such features, the present work to our knowledge is one of the two which attempts to relate the gene-directed floral morphology with endogenous hormones. We have demonstrated that the floral abnormality in the mutant correlates with lower levels of endogenous gibberellins not only in the floral parts but also in the vegetative parts.

Although these results can be interpreted in many ways, the most plausible explanation in our view is that the $s_{12}$ allele operates by affecting gibberellin synthesis which in turn affects the stamen form. This interpretation is strengthened by the following observations:

a) Application of $GA_3$ to the mutant floral buds induces phenocopies of normal flowers.

b) Application of CCC (an inhibitor of gibberellin synthesis) to the normal plants influences some stamen abnormalities similar to the mutant.

c) Some preliminary observations, e.g. delay in germination of $s_{12}$ seeds in comparison to wild type; higher levels of
chlorophyll and lower levels of carotenoids in the mutant than in the wild type plants, can also be related to lower levels of gibberellins in the mutant.

It was also suggested that the sl₂ allele expresses itself throughout the vegetative growth of the plant although its effect on the morphology of the plant is not apparent until the time of stamen development.

This study also elucidates the role of plant hormones in sex expression. Our data supports in part the generally accepted view that gibberellins promote maleness and auxins favour femaleness in plants. Since GA₃ application stimulates stamen development in the mutant but promotes femaleness in wild type plants, we have suggested that GA₃ induces the development of male organs in systems exhibiting some stamen abnormality but promotes femaleness (possibly through an increase in endogenous auxin content) in systems with normal stamen development.

The present work also has a direct bearing upon organogenesis in the floral meristem. By comparing the relative growth rates of stamens with sepals of sl₂ and wild type we concluded that, despite some similarities at early stages of development in the stamens of the two genotypes, there are some differences established at or prior to initiation. Although the nature of the differences is not known these observations suggested some uniqueness in the primordia of each kind from the beginning thus lending support to some interpretations of the models.
proposed by Wardlaw and Heslop-Harrison.

The question of how soon a primordium on the floral apex is irrevocably determined was resolved by applying GA$_3$ to stamens of various sizes of sl$_2$ flowers. It was concluded from the results that a primordium at early stages of inception can be experimentally manipulated and their course of development altered. After the stamen primordia reach 0.7 mm in length they cannot be reverted and thus are determined to follow a particular pathway. Beside, the Heslop-Harrison and Heslop-Harrison (1957) study on Cannabis, to our knowledge no other report exist on the 'determination' of floral organs.

Another interesting observation in this work and possibly the only one of its kind in the flower literature, shows that carpel-like organs can be experimentally induced in place of stamens in sl$_2$ flowers. This observation throws doubt upon the validity of the concept that carpel formation is dependent upon the presence of stamens as suggested by both Wardlaw and Heslop-Harrison.

This study has opened many avenues for further research. By using other floral mutants in tomato, insights into some of the factors underlying the sequence of genetic control of organogenesis in the flower might be obtained. With sl$_2$, a number of questions remain unanswered, the solutions of which would be especially helpful in linking sl$_2$ gene action to stamen form. For example:

a) How do gibberellins operate prior to stamen initiation?

Do they indeed induce some enzyme synthesis and if so
what differences in proteins occur in the sl2 mutant and wild type?
b) What is the chemical nature of gibberellin-like substances extracted from sl2 and wild type plants? Besides the differences in the acidic fraction of the extracted material, are there any differences in neutral and basic fractions?
c) What step or steps in the biosynthesis of gibberellins are effected by the sl2 allele?
d) Extensive studies on the pleiotropic effects of the sl2 allele.
REFERENCES


__________ and __________. 1971b. Fruit size increase in tomato (Lycopersicon esculentum) through gibberellic acid treatment. Amer. J. Bot. 58: 460.


Appendix - A

PROCEDURE FOR GIBBERELLIN EXTRACTION
(modified from Radley, 1963)

Fresh material homogenised in 75% EtOH & dry ice
Left overnight at room temperature in EtOH
Filtered through Whatman No.1

Residue
Washed twice with 75% EtOH

Residue (Discard)
EtOH fraction concentrated at 36°C under vacuum
Concentrate treated with slurry of basic lead acetate
Centrifuged at 10,000 x g - 10 min.

Precipitate
Supernatant
Treated with 5N HCl to remove excess of lead acetate
Centrifuged at 10,000 x g - 10 min.

Precipitate (Discard)
Supernatant adjusted to pH 2.5 with 1N HCl
Partitioned with deacidified ethyl acetate (3 times)

Aqueous phase
Ethyl acetate fraction
Partitioned with 1M NaHCO₃ (3 times)

Ethyl acetate fraction
Adjusted to pH 2.5 with 1N HCl
Partitioned with deacidified ethyl acetate (3 times)

Aqueous phase
Ethyl acetate fraction
Chilled at -5°C
Filtered in cold room

Frozen water
Filtrate
Evaporated to near dryness at 36°C under vacuum
Spotted on TLC plate
Appendix - B

Stamen characters of sl_2/sl_2 flowers grown under 9 hr. day length (short day) and 15 hr. day length (long day) (15 hrs.) conditions, in the growth chambers. Light intensity of 800 - 1,000 f. c. was supplied by Gro-Lux tubes and incandescent bulbs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean number of E. O. per flower^a</th>
<th>Mean number of stamens per flower with E. O.^a</th>
<th>Mean number of yellow and pubescent stamens per flower^a</th>
<th>Mean number of carpelloid stamens per flower^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short day</td>
<td>184.4**</td>
<td>5.7**</td>
<td>0.8**</td>
<td>0.0**</td>
</tr>
<tr>
<td>Long day</td>
<td>57.7</td>
<td>4.5</td>
<td>0.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

^a = sample size 20 stamens or flowers.

** = difference significant from long day at p ≤ 0.01
Appendix - C

A comparison of stem length (in cms.) of s12 plants, after 15 days of treatment with various molar concentrations of GA3 and a mixture of GA4/GA7. Chemicals applied as a drop (5 μl) near the apex of a young seedling. Each value in the table is a mean of 5 samples.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Chemical</th>
<th>GA3</th>
<th>GA4/GA7</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^{-3} M</td>
<td></td>
<td>22.5^a</td>
<td>23.8^a</td>
</tr>
<tr>
<td>10^{-4} M</td>
<td></td>
<td>14.7^b</td>
<td>14.9^b</td>
</tr>
<tr>
<td>10^{-5} M</td>
<td></td>
<td>11.4^c</td>
<td>11.3^c</td>
</tr>
<tr>
<td>Tween -20</td>
<td></td>
<td>10.45^cd</td>
<td>10.5^cd</td>
</tr>
</tbody>
</table>

Means followed by the same letter in a column not different at 5% level.