T H E S I S

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# POLYEMBRYONY IN *LYCOPERSICON ESCULENTUM*

Submitted by

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# INTRODUCTION

## Need for study.

Twin plants, resulting from two embryos in the embryo sac, can be used to solve several genetic problems. Genetically identical twins provide the opportunity to study genetic-environmental relationships.

The incidence of twinning in different tomato varieties ranges between 0.1% and 1%. In order to increase the utility of twins in research, means should be found to increase the incidence and detection of twinning within seed lots. One possibility is by increasing the transparency of the seed coat and growing only those seeds which appear to contain twins.

Another way to detect twins more effectively within seed lots may be to separate the seeds into heavy and light classes and grow just the heavy seeds. It is probable that the incidence of twinning would be higher in heavy seeds than in lighter seeds.

The inheritance of frequent twinning is not known at present, but this knowledge would be helpful in incorporating frequent twinning into various stocks.

It should be known whether or not there is a

maternal modification of development of one member of the twin pair if genetically identical twins are to be used in genetic-environmental studies.

Objectives.

I. Study the incidence of polyembryony.

A. Inheritance

1. Determine whether or not a high incidence of twinning is inherited.

2. Determine the degree of dominance of the gene or genes determining frequent twinning.

B. Determine whether or not seed weight is related to the incidence of polyembryony.

C. Determine whether visual clarification of the seed coat helps to locate the seeds that contain polyembryos.

II. To investigate the development of twin plants and compare twins with normal plants from the same seed lot.

# Chapter II

## REVIEW OF LITERATURE

Polyembryony has been reported in many plant genera since the beginning of the eighteenth century. Maheshwari (22) cites Leeuwenhoek as the first man to discover its occurrence in 1719. In 1895, Jeffery (17) observed polyembryos in Erythronium americanum. Although many investigators became interested in the study of polyembryony after Jeffery's work, progress in genetic and cytogenetic studies was made mainly after 1930. In 1934, Namikawa and Kawakami (26) found polyembryony in Triticum. Three years later, Muntzing (24) reported multiple embryos in different genera of Gramineae and in Solanum.

The occurrence of polyembryony in tomatoes, however, was not reported until recently. Rick (28) reported twin seedlings in tomatoes in 1943. Marshall (23) and Foskett (12) made independent studies on polyembryony. They obtained different results regarding the nature of polyembryos. This necessitated further investigation of the essential characters of polyembryony in tomatoes.

### Types of Polyembryony

True and false polyembryony.--According to Webber (34), Earnst divides polyembryony into two types: true and false.

The development of more than one embryo within, or by projection into, a single embryo-sac is termed true polyembryony. Under false polyembryony he includes all those cases in which plural embryos arise from several embryo-sacs. The multiple embryos in the latter case may be derived from A) megasporocytes in different nucelli; B) two or more megasporocytes in the same nucellus; C) from the normal megasporocyte and apospory in the same nucellus. This classification, however, has been subjected to serious objections. Those cases in which two or more embryos arise from aposporic embryo-sacs are included under false polyembryony. Others which also develop from the tissues outside the embryo-sac but merely project into it are classed as true polyembryony. Webber (34) gives a more natural and logical classification of this process. He distinguishes four classes of polyembryony which are as follows:

Sporophytic polyembryony.--Adventitious embryos originate from the nucellus or from integuments by sporophytic budding. Maheshwari (22) states that the development of such embryos starts soon after flowering in enlarged cells of the integument or nucellus. These cells actively divide to form small groups of cells which push their way into the embryo-sac and grow further to form embryos. Frost (14) showed that in citrus, adventitious embryos develop from the nucellus by budding. An example of sporophytic embryos from integuments

was reported by Juliano (18) in Mangifera. The sporophytic embryos thus formed have been reported to replace the normal embryos in some cases, while in other cases the gametic embryo also develops. Since the adventitious embryos are maternal in derivation, the seedlings arising from them are genetically identical to the parent plant.

There has been a difference of opinion regarding the effect of pollination or fertilization on the development of sporophytic embryos. Maheshwari (22) thinks that such embryos may be completely independent of pollination and fertilization or may be stimulated by one or both of these factors. Wright (37) thought that this type of embryo developed without pollination and fertilization. Frost (14) and Wong (35) concluded that fruits might develop parthenocarpically, but pollination was necessary for seed formation.

Cleavage polyembryony.--Webber (34) describes this as the type of polyembryony in which the zygote or proembryo divides into two or more units, each of which develops into an independent embryo. This type of polyembryony, according to Webber (34) and Maheshwari (22), is common in gymnosperms. In angiosperms, its occurrence is sporadic. Jeffery (17) found this type of polyembryony in Erythronium americanum. It has also been reported in Tulipa, Crotallaria by Skovsted (29), in Nicotiana by Cooper (7), in Holcus by Webber (34),

and in many orchids. Orchidaceae has been reported (22) as the only family in which cleavage polyembryony seems to be the most frequent type.

Since the embryos in cleavage polyembryony originate from a single embryo, the resulting seedlings are genetically identical.

Simple polyembryony.--In this case, two or more eggs develop in a single embryo-sac. These eggs may arise from a single megaspore, or some of the synergids or antipodals may function as eggs. Webber (34) reports that in gymnosperms, several eggs are derived from a single megaspore and unite with the sperms. The sperms from the one to several microspores, which germinate within the micropyle, allow fertilization for several independent embryos in a single embryo-sac. In angiosperms neither plural eggs nor multiple sperms are produced; therefore, Webber (34) thinks that it is questionable whether this type of polyembryony occurs in angiosperms or not. In his opinion, however, the simple polyembryony in angiosperms might be the result of development of synergids into eggs. In Saccharum, however, Dutt and Krishnasuami (9) reported the occurrence of multiple sperms by the division of generative nucleus and in a later paper Dutt and Subba Rao (10) reported that adventitious embryos are derived from the fertilization of synergids. Several other cases have been cited by Maheshwari (22) in

which multiple embryos arise either from antipodals or from synergids. Marshall (23) found that in tomatoes, simple polyembryony occurs. However, he did not indicate whether the adventitious embryos developed from the synergids or from the division of the zygote. Moreover, Marshall's results are still to be confirmed as Foskett (12) found genetically identical twins, indicating cleavage embryony.

Euploid polyembryony.--Euploid polyembryony has been described by Muntzing (24) as the type of polyembryony in which multiple embryos may give rise to haploid, diploid, triploid, and tetraploid plants.

The development of euploid twins has been explained by Muntzing (24) after his studies of this type of seedlings in different genera of Graminae. He states that sometimes two megaspores develop in an ovule which give rise to haploid embryo-sacs, but occasionally an unreduced embryo-sac may arise from the accessory megasporocyte. The eggs in these embryo-sacs may give rise to twin embryos parthenogenetically or after fertilization. The triploid-triploid twins, according to him, are formed from unreduced sister megaspores while tetraploid twins develop by somatic chromosomes doubling after pollination or possibly by doubling at the beginning of the aposporic development.

Different types of euploid polyembryony have been reported in several angiosperm families. Rick (28) reported

seven diploids and one haploid in four pairs of twins in tomatoes while Marshall (23) and Foskett (12) found diploid-diploid twins. In pepper, which is also a solanaceous plant, haploid-diploid twins were reported by Christensen (5). In several plants, listed later in this review, haploid-diploid, haploid-triploid, diploid-triploid, diploid-tetraploid and triploid-triploid twins have been reported by various investigators.

Unclassified cases of polyembryony.--Several cases of polyembryony have been reported in which the origin of multiple embryos is not known. Webber (34) cited several such cases under families as Gramineae, Solanaceae, Cruciferae, Compositae, and Leguminoseae. Maheshwari (22) also gives a description of special cases of polyembryony in which nothing can be said with certainty about the nature and development of a multiple embryos.

## Occurrence of Polyembryony in Angiosperm Families

Polyembryony has been reported in many angiosperm families. A few of these are tabulated by types of polyembryony, together with literature citations.

I. Plant families and genera in which sporophytic polyembryony occurs:

A. Embryo develops from nucellus tissue.

1. Rutaceae -

Citrus, Poncirus, Fortunella, Frost (13)

2. Anacardiaceae -

*Mangifera*, Webber (32)

3. Malpighiaceae -

*Hiptage*, Subba Rao (31)

4. Myrtaceae -

*Eugenia*, Pijl (27)

5. Rosaceae -

*Potentilla*, Soueges (30)

6. Betulaceae -

*Alnus*, Woodworth (36)

B. Embryo develops from integument.

1. Myrtaceae -

*Eugenia*, Pijl (27)

2. Rosaceae -

*Potentilla*, Soueges (30)

II. Cleavage polyembryony occurs in the following families:

1. Loranthaceae -

*Loranthus*, Webber (34)

2. Liliaceae -

*Erythronium* and *Tulipa*, Webber (34)

3. Lobeliaceae -

*Lobelia*, Crete (4)

4. Leguminoseae -

*Trifolium*, *Medicago*, Skovsted (29)

III. Simple polyembryony has been reported in the following plant families and species:

1. Nyctaginaceae -

   Boerhaavia, Kajale (19)

2. Gramineae -

   Saccharum, Dutt and Subba Rao (10)

3. Liliaceae -

   Allium, Webber (34)

4. Betulaceae -

   Alnus, Woodworth (36)

5. Solanaceae -

   Lycopersicon, Marshall (23)

IV. Euploid polyembryony has been reported in the following:

A. Malvaceae -

   Gossypium: Haploid-diploid, Harland (15)
   Diploid-diploid, Webber (33)
   Diploid-diploid, Endrizzi (11)
   Diploid-triploid, Beasley (2)

B. Gramineae -

1. Dactylis: Haploid-diploid, Skovsted (29)

2. Triticum: Haploid-diploid, Muntzing (24)
   Triploid-triploid, Muntzing (24)
   Diploid-tetraploid, Muntzing (24)
   Triploid-diploid, Muntzing (24)

3. Poa: Diploid-triploid, Muntzing (24)
   Triploid-triploid, Muntzing (24)

4. Phleum: Diploid-triploid, Muntzing (24)

5. Lolium: Diploid-triploid, Muntzing (24)

6. *Avena*: Diploid-triploid, Muntzing (24)

C. Cruciferae -

*Brassica*: Diploid-tetraploid, Howard (16)

D. Solanaceae -

1. *Solanum*: Haploid-haploid, Muntzing (24)
Diploid-diploid, Muntzing (24)

2. *Capsicum*: Haploid-diploid, Christensen (5)
Diploid-diploid, Christensen (5)

3. *Lycopersicon*: Haploid-diploid, Rick (28)
Diploid-diploid, Foskett (12)

4. *Nicotiana*: Haploid-triploid, Cooper (7)

E. Rosaceae -

*Pyrus*: Diploid-triploid, Bergstrom (4)

V. Unclassified cases of polyembryony.

A. Gramineae -

1. *Elcusine*, Bayliss (1)

2. *Festuca*, Skovsted (29)

3. *Avena*, Skovsted (29)

4. *Alopecurus*, Skovsted (29)

5. *Lolium*, Skovsted (29)

B. Leguminoseae -

1. *Medicago*, Skovsted (29)

2. *Lotus*, Skovsted (29)

C. Solanaceae -

*Solanum*, Bayliss (1)

D. Cruciferae

*Brassica*, Skovsted (29)

E. Compositeae -

Zinnia, Skovsted (29)

## Uses of Polyembryony

Twin plants have been suggested for solving several botanical and agricultural problems. According to Muntzing (24), of all the methods of inducing polyploidy, the twin method is a relatively convenient means of obtaining heteroploid forms. Moreover, as euploid twins are known to occur in a large number of genera, twinning may be a more effective method of obtaining heteroploid forms than other methods. He further states that twinning may be useful for studying haploids and apomixis. Foskett (12) suggested that where twins are proved to be identical, it may be possible to use twin seedlings in research studies where completely identical geno-types are desired.

Furthermore, polyploid twins have been subjected to many biological (Lamm, 21), cytological (Krishnaswami, 20) and morphological studies (Muntzing, 24). From these studies and similar work with diploid plants, significant phylogenetic conclusions have been drawn.

## Polyembryony and Haploidy

Haploid plants are of paramount importance in

plant breeding. The chromosome number of haploid plants can be doubled by colchicine, or they may rarely produce seed by selfing. The progeny obtained would be completely homozygous for all genes, and thus genetically pure lines can be obtained immediately instead of by generations of inbreeding. Cook (6) suggests haploidy for producing pure lines in tomatoes.

Muntzing (25) describes twinning as one of the four methods of obtaining haploids in plants. The other methods being delayed pollination, use of pollens that have been exposed to heavy doses of X-ray for pollination, and the use of pollen of a different species. By twinning he obtained eleven haploid plants from six species. The species were ordinary wheat, barley, timothy, cocksfoot, Kentucky bluegrass, and potato. The twin method, according to him may give fairly good results; however, it is very laborious as very few of the observed twins were haploids in some species. Moreover, the frequency of the twins is very low.

# Chapter III

## MATERIALS AND METHODS

Inheritance studies.

These studies were made on the tomato varieties Red Jacket and Premier, which have a low incidence of twinning and on 54-3-4, a line of Beefsteak variety which produced a high incidence of twinning. In the early spring of 1961, six plants of each of the above mentioned varieties and lines were grown in the greenhouse. During the growth of the plants all of the axillary buds were removed and the plants were staked because of limited growing space. Each line was selfed and reciprocal crosses were made between lines believed to produce a high incidence of polyembryony and those believed to produce a low incidence.

Crosses were made by removing pollen from the pollen parent with a mechanical vibrator onto a glass slide and applying it to emasculated buds of the seed parent. Only one flower in each inflorescence was used on the seed parent and all other flowers and buds were removed before tagging. The following progenies were thus obtained:
(Selfs and crosses)

a. Red Jacket

b. Premier

c. 54-3-4

d. Red Jacket x 54-3-4

e. 54-3-4 x Red Jacket

f. Premier x 54-3-4

g. 54-3-4 x Premier

Seeds were collected from the mature fruits and all seeds of a given pedigree were bulked and saved to observe incidence of twinning and to grow $F_1$ plants.

$F_1$ plants of each cross, along with each parent, were grown in the field at Fort Collins during the summer of 1962. The plants were started in the greenhouse in clay pots and later transplanted to the field.

Seeds from the above mentioned $F_1$ and parent plants were collected in September 1962. Seeds from all plants of each pedigree were bulked together and saved to observe incidence of twinning in them.

$F_1$ seeds saved from greenhouse crosses along with selfed parent seeds and $F_2$ seed along with the seeds of parents obtained from the field were germinated in lots of 250 to 500 seeds each in the germinator on dark germination paper. The seeds were watered and checked for germination and for twins every day. Each batch of seeds was kept in the germinator for eleven days. The number of twins and per cent germination in each lot were recorded on the eleventh day and the seedlings discarded.

Seed weight experiment.

It was suspected that twinning occurs more frequently among heavy seeds within a given seed lot than among randomly selected seeds because of the presence of two embryos. To test this it was decided to separate seeds into different lots according to weight. Seeds of the following varieties were separated by an air column seed separator (figure 1) into three classes.

| Variety | Source |
|---|---|
| VF 145 B | Seed Research Service |
| Campbell 1402 | Clow Seed Company |
| VF 145 A | Clow Seed Company |
| Valient | Harris |
| Rutgers | Harris |

Campbell 1402 because of its greater seed size variability, was divided into four classes.

The seed classes will be referred to as 1, 2, 3, or 4, indicating the settings on the seed separator. These settings refer, in turn, to the air velocity which lifts the seed and results in the lighter seed being raised to the baffle plates located in the air column. While light seed is thus usually separated from heavier seed, the type of separation is such that flat, wide seed, which is more buoyant, is more likely to rise to the baffle plates than thicker and narrower seed of the same weight. The highest numbered weight class, normally the heaviest class,

Figure 1.--Air column seed separator used in seed weight experiment.

occasionally weighed less than the next lower numbered class, indicating that this phenomenon sometimes occurred.

A measured sample of seed, approximately one-half ounce, was put in the separator, which was timed for one minute of separating time to minimize differences due to a differential resistance of varying seed lot volumes and to different lengths of operating time. Each class was weighed to determine the distribution of classes within the entire population of each variety. Lots of 500 seeds were counted from each class and weighed to determine the average weight per seed. These seeds were germinated on germinating paper in a Mangelsdorf germinator at 80° F. (unless otherwise noted in the results) to observe the incidence of twinning and per cent germination in each class. The twins along with check non-twin plants, were then used for the variability studies.

Seed treatment experiment.

Chlorox, or a 5.25 per cent solution of sodium hypochlorite, is commonly used to kill bacteria on seed coats. This material also clarifies the seed coat tissue, enabling observation of the embryo outline *in vivo* with the naked eye. To test the effectiveness of Chlorox in increasing the detection of the number of polyembryonic seeds in a given seed lot, beyond using heavy seeds, the following treatments were included. The variety Beefsteak was used throughout

this experiment because of its high incidence of twinning. Where heavy seeds are mentioned, they were approximately one-third of the original population.

Treatment 1. Heavy seeds were soaked in Chlorox for five minutes and immediately washed thoroughly in tap water. While they were still wet the seeds were examined for indications of multiple embryos and those suspected of containing them were germinated. Twin embryos were not visible with the Chlorox treatment, although single embryo outlines could be discerned in most seeds. Treatment 1 consisted of those seeds in which the embryo outline was not clearly visible.

Treatment 2. This treatment consisted of heavy seeds, which were treated with Chlorox, but which clearly showed embryo outlines.

Treatment 3. Seeds were Chlorox treated but not weight separated.

Treatment 4. Heavy seeds which were not Chlorox treated.

Treatment 5. A check treatment, in which seeds were neither weight-separated nor Chlorox treated.

Except for treatments 1 and 2, all treatments contained 100 seeds in each of five replications. Treatments 1 and 2 together contained 100 seeds per replication but were separated into the two treatments on the basis of visual examination before germination.

Variability experiment.

Seeds which were observed to produce two roots in the seed weight experiment were placed on moist germination papers in covered petri dishes. Two seeds with single roots, to be used as control plants with each pair of twins, were also saved from the same lot of seeds. They were then exposed to light and held there until the complete emergence of seedlings. The seedlings were then planted in separate four-inch pots in the greenhouse and each member of a twin pair was marked as T-1-A, T-1-B, T-2-A, T-2-B, etc. The seedling of each pair of twins that first emerged completely from the seed was always designated as B. The respective non-twin control plants with each pair of twins were labeled as CT-1-A, CT-1-B, CT-2-A, CT-2-B, etc. The B and A labels were randomly alloted to the control plants.

Twins, along with control plants, were also obtained from the varieties Heinz 1370 and VR Moscow for variability studies.

The seedlings were transplanted into benches in the greenhouse when they were approximately five to six inches tall. About one square foot space was allowed for each plant. The plants were pruned to single stems by removing all the axillary buds and tied to stakes. The following observations were made in this experiment:

a. Date on which the first flower opened.

b. Height to the first flower.

c. Height of each of the four plants when the first flower opened on the first of the four plants.

d. Number of nodes on the main stem between cotyledons and the first inflorescence.

e. Number of flowers in the first inflorescence.

f. Number of flowers in the second inflorescence.

# Chapter IV

## RESULTS AND DISCUSSION

Inheritance.

Polyembryony was found to be heritable. In two trials line 54-3-4, a line derived from a twin plant of the variety Beefsteak, produced a greater incidence of twinning than all other lines tested.

In the first trial, 12 pairs of twins were found in 500 seeds produced by selfing plants of line 54-3-4 (table 1). The incidence in this case was 1 in 23 germinated seed or 1 in 31 when based on total number of seeds used. No twins were found in 500 seeds each from the varieties Premier and Red Jacket. All these seed lots were produced in the greenhouse at the same time and under similar conditions.

Plants from each of the above mentioned seed lots were grown in the field at Fort Collins during the summer of 1962. Seeds produced from these plants produced similar results (table 2). In 1,600 seeds of line 54-3-4, 24 pairs of twins were found. The incidence in this case was 1 in 60 germinated seeds or 1 in 67 when based on total number of seeds used. There were no twins in 2,250 seeds each from the varieties Premier and Red Jacket.

These data suggest that polyembryony is inherited. In order to gain more information concerning the type of

inheritance and also in order to begin incorporating a high incidence of twinning into other lines, crosses were made between line 54-3-4 and other varieties.

Because of the low incidence of twinning in most lines it is not possible to draw many conclusions regarding the results from $F_1$ seed (table 1). It is clear, however, that polyembryony is also not purely maternal. Both maternal and dominant inheritance would have resulted in a high incidence of twins in certain crosses. For maternal or simple dominant inheritance to have been the sole factor, the seed from the cross 54-3-4 x Red Jacket would have produced as many twins as 54-3-4 selfed, at least in homozygous plants.

The results from the $F_2$ seed, harvested from $F_1$ plants in the field, give us additional information. Aside from 54-3-4, the only seed lots that produced twins were 54-3-4 x Premier and the reciprocal cross. These two lots produced similar results: 1 in 375 and 1 in 563. The $F_1$ plants 54-3-4 x Red Jacket and Red Jacket x 54-3-4, on the other hand, produced no twins. There is an indication here that gene interactions are involved, since $F_2$ seeds of reciprocal crosses with Premier produced twins, while $F_2$ seeds from crosses with Red Jacket produced no twins.

The conclusions from this preliminary inheritance study, then, are that inheritance of a high incidence of

Table 1.--INCIDENCE OF TWINS FOUND IN SEED OF THREE TOMATO LINES AND IN $F_1$ PROGENY SEED (PLANTS FROM WHICH SEED WAS OBTAINED WERE GROWN IN GREENHOUSE).

| Seed Pedigrees | No. Seeds | No. Germinated | Per cent Germination | No. Twins | Incidence | |
|---|---|---|---|---|---|---|
| | | | | | Total Seeds | Germinated Seeds |
| 54-3-4 | 500 | 367 | 73.4 | 12 | 1:31 | 1:22 |
| Red Jacket | 500 | 421 | 84.2 | 0 | ---- | ---- |
| 54-3-4xR.J. | 500 | 401 | 80.2 | 2 | 1:250 | 1:200 |
| R.J.x54-3-4 | 500 | 486 | 97.2 | 0 | ----- | ----- |
| Premier | 500 | 400 | 80.0 | 0 | ----- | ----- |
| Premier x 54-3-4 | 500 | 490 | 98.0 | 1 | 1:500 | 1:490 |

Table 2.--INCIDENCE OF TWINS FOUND IN THREE TOMATO LINES AND IN $F_2$ PROGENY SEED (PLANTS FROM WHICH SEEDS WERE OBTAINED WERE FIELD GROWN).

| Seed Pedigree | No. Seeds | No. Germinated | Per cent Germination | No. Twins | Incidence | |
|---|---|---|---|---|---|---|
| | | | | | Total Seeds | Germinated Seeds |
| 54-3-4 | 1600 | 1446 | 90.3 | 24 | 1:66 | 1:60 |
| Red Jacket | 2250 | 2177 | 96.7 | 0 | ---- | ---- |
| 54-3-4xR.J. | 2250 | 2090 | 92.8 | 0 | ---- | ---- |
| R.J.x54-3-4 | 2250 | 2214 | 98.4 | 0 | ---- | ---- |
| Premier | 2250 | 1527 | 67.8 | 0 | ---- | ---- |
| 54-3-4x Premier | 2250 | 2173 | 96.3 | 6 | 1:375 | 1:362 |
| Premier x 54-3-4 | 2250 | 2125 | 94.4 | 4 | 1:562 | 1:531 |

twinning is not completely maternal, that it is not due to a single dominant gene (in fact the genes appear to be recessive) and that there appears to be an interaction of genes.

Seed weight.

Table 3 shows the number of twins found in seed lots of several tomato varieties after being separated into at least three weight classes each by an air column seed separator. Because of the low frequencies of twins occurring in these seed lots it is difficult to draw conclusions from these data. It should be noted, however, that in only one case, in Campbell 1402, a twin was found in the smallest seeded weight class. This fact gives us the first indication that seed weight is a factor in the detection of twins.

In table 4 the data from all varieties in table 3 were pooled to show the frequency of twins in the three weight classes 2, 3, and 4. Here the increase in frequency of twinning with increase in seed weight can easily be seen. Seed weight class 2 produced only 1 twin in 6,000 seeds. Germination was only 75.9 per cent in this class, but even calculating the incidence of polyembryos on the basis of number germinated, rather than on the basis of number of seeds used, the frequency is only 1 in 4566.

Seed weight class 3 produced 4 twins in 6,000 seeds, or 1 in 1,500. Seed weight class 4 with an average

Table 3.--FREQUENCY OF TWINS FOUND IN SEED LOTS OF FIVE VARIETIES AFTER SEPARATION INTO WEIGHT CLASSES BY AN AIR COLUMN SEED SEPARATOR.

| Variety | Weight Class | Total Class Weight | Average Weight/ Seed (mg) | No. of Seeds | No. Germinated | Percent Germinated | No. of Twins |
|---|---|---|---|---|---|---|---|
| VF145-B | 2 | 16.56 | 2.18 | 1000 | 922 | 92.2 | 0 |
| | 3 | 48.52 | 2.78 | 1000 | 936 | 93.6 | 0 |
| | 4 | 2.92 | 3.30 | 1000 | 944 | 94.4 | 6 |
| Campbell 1402 | 2 | 1.74 | 1.42 | 500 | 234 | 46.8 | 1 |
| | 3 | 14.02 | 2.76 | 500 | 428 | 85.6 | 0 |
| | 4 | 31.55 | 3.32 | 500 | 475 | 95.0 | 1 |
| | 5 | 3.30 | 3.52 | 500 | 464 | 92.8 | 0 |
| VF145-A | 2 | 4.14 | 1.72 | 2000 | 1563 | 78.1 | 0 |
| | 3 | 26.15 | 2.36 | 2000 | 1825 | 91.2 | 2 |
| | 4 | 15.84 | 2.74 | 2000 | 1924 | 96.2 | 2 |
| Valiant | 2 | 7.37 | 2.16 | 2000 | 1379 | 68.9 | 0 |
| | 3 | 50.59 | 2.56 | 2000 | 1800 | 90.0 | 2 |
| | 4 | 8.88 | 2.58 | 2000 | 1851 | 92.5 | 2 |
| Rutgers | 2 | 6.42 | 2.66 | 500 | 468 | 93.6 | 0 |
| | 3 | 17.57 | 2.82 | 500 | 472 | 94.4 | 0 |
| | 4 | 2.86 | 2.80 | 500 | 478 | 95.6 | 1 |

Table 4.--FREQUENCY OF TWINS FOUND IN SEED LOTS SEPARATED INTO THREE WEIGHT CLASSES (CONSOLIDATED FROM TABLE 3).

| Class of seed | Average Weight per Seed (mg) | No. of Seed | No. Germinated | Percent Germination | No. of Twins |
|---|---|---|---|---|---|
| 2 | 2.02 | 6000 | 4566 | 75.9 | 1 |
| 3 | 2.65 | 6000 | 5461 | 90.9 | 4 |
| 4 | 2.94 | 6000 | 5672 | 94.7 | 12 |

weight per seed of 2.94 mg. compared with 2.65 mg. in class 3, produced 12 twins in 6,000 seeds. Class 4 thus had a frequency of 1 twin in 500 seeds. The frequency of twinning in the entire population tested was 1 in 1058 similar to the frequencies found by Foskett (12).

Table 4 supports the above mentioned findings that twinning frequency is higher in heavy seeds. In the following discussion it is shown that another method further increases the chances of finding twins in a given seed lot, but weight separation alone was also found to increase the frequency in that experiment.

Chlorox treatment.

Soaking tomato seed in Chlorox for five minutes to clarify the seed coat tissue was found to be effective in facilitating the detection of polyembryos within heavy seed (table 5). The highest frequency of twinning was found by first separating a seed lot of the variety Beefsteak in an air column seed separator. The heavy seeds, comprising approximately one-third of the original lot by volume, were then kept and the rest discarded. Of these heavy seeds, 500 were treated with Chlorox and the clarified seeds were then examined for twin embryos. Two embryos could not be discerned but it was found that in some seeds the embryo outline could be clearly seen, whereas in others no clear line of demarcation was evident. These latter seeds were the

ones suspected of containing more than one embryo; and 87 of the 500 seed sample were germinated separately. These 87 seeds produced 5 twins, or a ratio of 1 in 17 seeds.

The remaining sub-lot of seeds, those in which the embryo outline was clearly visible, produced 4 twins from 413 seeds or a ratio of 1 in 103. Possibly with more careful observation at least some of these four twins could have been identified before germination. This entire seed lot of 500 heavy, Chlorox-treated seeds thus produced 9 twins, the same as the heavy but non-treated seeds.

From the randomly selected seed-lot, 42 seeds were suspected of containing twins. In this case, however, no twins were found among the seeds in which the embryos were not clearly visible. One reason for the failure might be that the frequency of twins in this seed lot was lower, 1 in 167, making the chance of finding them less likely. Another reason might be that embryos in this class are smaller and more difficult to see.

One lot of heavy seeds and one lot of randomly selected seeds were also germinated without previous treatment with Chlorox. This check served two purposes--first, it was meant to show whether or not germination was significantly lowered by treatment with Chlorox. There seemed to be a slightly lower germination percentage after treatment, although this decrease was not serious. Secondly, the check lots were included to demonstrate whether or not

Table 5.--THE EFFECT OF CHLOROX TREATMENT IN THE DETECTION OF TWINS IN BEEFSTEAK TOMATO; AND THE EFFECT OF CHLOROX ON GERMINATION.

| Treatment | Seed Lot | Visual Selection | No. of Seeds | Percent Germination | No. of Twins | Frequency of Twinning |
|---|---|---|---|---|---|---|
| Chlorox | Heavy | | 500 | | 9 | 1:56 |
| | | Embryo not visible | 87 | 92 | 5 | 1:17 |
| | | Embryo visible | 413 | 95 | 4 | 1:103 |
| | Randomly selected | | 500 | | 4 | 1:125 |
| | | Embryo not visible | 42 | 83 | 0 | 0 |
| | | Embryo visible | 458 | 95 | 4 | 1:114 |
| None | Heavy | | 500 | 97 | 9 | 1:56 |
| | Randomly selected | | 500 | 94 | 3 | 1:167 |

Chlorox would affect the frequency of twinning by being toxic to the weaker member of some pairs of embryos. It was found that the frequency of twinning in both treated and non-treated seed lots was approximately the same. Among the heavy seeds, treated and non-treated lots each produced 9 twins from 500 seeds. Among the randomly selected seeds, the treated and non-treated lots produced 4 and 3 twins respectively.

Aside from its value in detecting twin embryos, Chlorox treatment could well be useful in other research dealing with embryos.

Variability.

A number of measurements were taken to determine whether twin plants or randomly chosen pairs of plants from the same seed lot were more similar for several growth traits. Comparisons were made for number of days to flowering, total height when the first plant in the group of four plants flowered, height to first inflorescence, number of nodes to the first inflorescence, number of flowers in the first inflorescence, and number of flowers in the second inflorescence. The 31 twins used in this study were of several varieties (table 6). Since the largest number of twins were from the variety V. R. Moscow, statistical analyses were also made on this group of 14 twins and their checks for those characters which showed a significant

difference between the TA and TB groups in the entire twin population.

Twins were designated TA or TB on the basis of which seedling completely emerged first from the seed coat. The one that completely left the seed coat first was designated B. It was observed that the radicle of B was usually smaller than that of A when only the two radicles had emerged. The check pairs of plants accompanying each pair of twins were designated CTA and CTB, the A and B designation being given arbitrarily.

Days to flowering.--Twin plant B was found to have a later average flowering date than A. The average number of days to flowering for all TB plants was 58.5, while the average for TA plants was 56.2, a difference of 2.3 days (table 6). The CTA and CTB plants took 55.9 and 56.2 days respectively, a difference of only .3 day. For the V. R. Moscow plants, there was a highly significant difference between TA and TB, with an average difference of 1.8 days, whereas the average difference between CTA and CTB plants was .1 day. Again, the difference between the check plants was not statistically significant.

In the case of the entire population and in the case of the V. R. Moscow twins the greatest average number of days to flowering occurred in the TB plants. These plants not only took longer to flower than the TA plants but also were slower in flowering than the CTA and CTB groups. The

retarding effect that occurred on TB plants apparently was not great if present in TA plants, for the average number of days to flowering for the latter group was 56.2 and the average number of days for all plants in the CT group was 56.1. The same situation was true among V. R. Moscow plants. In this case the average number of days for the TA group was 58.2 and for the entire CT group it was 57.7.

Total height.--The height of each plant in every group of four, two twins and their two check plants, was measured in centimeters when the first plant in the group opened its first flower. Measurements were taken at this time rather than after a given number of days for all plants in the study because the twins used came from more than one variety with different flowering dates.

The average height of twin B was found to be less than A at the time of measurement. The average height for all TB plants was 31.8 cms., while the average height for all TA plants was 36.1 cms., a difference of 4.3 cms. (table 6). The CTA and CTB plants measured 39.0 and 36.9 cms. respectively a difference of only 2.1 cms. This difference was not statistically significant. There was a highly significant difference between the means of TA and TB plants for this character. Upon testing the 14 V. R. Moscow plants (table 7) it was found that there was also a significant difference, but only at the 5 per cent level, between TA

and TB plants with an average difference of 0.6 cms. The check plants exhibited a difference of 1.5 cms., but this was not significant. It will be noted that among the 14 V. R. Moscow sets of plants, 11 TB plants were smaller than their TA twins and 3 TB plants were larger than their twins. Conversely, the CTB plants were of larger average size than the CTA plants and there were 9 CTA plants of the 14 that were smaller than their CTB counterparts. A majority of the plants in this case showed a different response from that indicated by the CT means. The T plants were significantly different and the CT plants were not clearly so because the experimental variance was higher among the CT plants.

The means of the TA and TB plants were not significantly different for height to the first inflorescence, number of flowers in the first and second inflorescences, and number of nodes to the first inflorescence. There were also no significant difference between the means of the CTA and CTB plants for these same characters.

The main effect on the development of twin plants appears to be that one member of the pair is slower in development than the other. TB plants were smaller and flowered later than their twins. When the TB plants finally flowered, however, the height to the first inflorescence and number of nodes to the first inflorescence were approxi-

mately the same as in the TA plants, and there was no significant difference between the number of flowers per inflorescence.

Table 6.--AVERAGE MEASUREMENTS OF VARIOUS CHARACTERS IN 31 PAIRS OF YOUNG TWIN PLANTS, TA AND TB, AND RANDOMLY SELECTED PAIRED CHECK PLANTS, CTA AND CTB. STATISTICAL ANALYSES WERE BY MEANS OF t TESTS.

| Character | TA | TB | CTA | CTB | TA vs TB t | CTA vs CTB t |
|---|---|---|---|---|---|---|
| Days to flowering | 56.2 | 58.5 | 55.9 | 56.2 | 4.09** | 0.43 |
| Total height (cms.) | 36.1 | 31.8 | 39.0 | 36.9 | 4.18** | 1.30 |
| Height to 1st inflorescence (cms.) | 34.7 | 35.1 | 34.6 | 34.2 | 0.39 | 0.31 |
| No. flowers in 1st inflorescence | 9.3 | 9.3 | 9.0 | 10.1 | 0.1 | 1.09 |
| No. flowers in 2nd inflorescence | 10.3 | 7.2 | 8.6 | 9.1 | 1.62 | 0.69 |
| No. nodes below 1st inflorescence | 9.1 | 9.1 | 8.8 | 9.1 | 2.00 | 0.25 |

t values from table were .01 - 2.04 and .05 - 2.75

** highly significant difference

Table 7.--AVERAGE MEASUREMENTS OF VARIOUS CHARACTERS IN 14 PAIRS OF V. R. MOSCOW TWIN PLANTS, TA AND TB, AND RANDOMLY SELECTED PAIRED CHECK PLANTS, CTA AND CTB. STATISTICAL ANALYSES WERE MADE ONLY ON DAYS TO FLOWERING AND TOTAL HEIGHT.

| Character | TA | TB | CTA | CTB | TA vs TB t | CTA vs CTB t |
|---|---|---|---|---|---|---|
| Days to flowering | 58.2 | 60.0 | 57.6 | 57.7 | 3.4** | 0.19 |
| Total height (cms.) | 39.0 | 35.7 | 42.7 | 40.8 | 2.34* | 0.9 |

t values from table were .01 - 3.01 and .05 - 2.16

* significant difference (at 5 per cent level)

** highly significant difference (at 1 per cent level)

Table 8.--VARIETAL SOURCES OF TWINS USED IN THE VARIABILITY STUDY.

| No. | Variety | Twin No. |
|---|---|---|
| 1 | Beefsteak | 1-2 |
| 2 | " | 1-3 |
| 3 | " | 1-4 |
| 4 | " | 1-5 |
| 5 | " | 1-6 |
| 6 | " | 1-7 |
| 7 | Red Jacket | 2-1 |
| 8 | " | 2-2 |
| 9 | 145-B(SRS) | 4-1 |
| 10 | " " | 4-2 |
| 11 | Heinz 1370 (Clow) | 17-2 |
| 12 | " " " | 17-3 |
| 13 | V. R. Moscow | 21-2 |
| 14 | " | 21-3 |
| 15 | " | 21-4 |
| 16 | " | 21-5 |
| 17 | " | 21-6 |
| 18 | " | 21-7 |
| 19 | " | 21-8 |
| 20 | " | 21-9 |
| 21 | " | 21-10 |
| 22 | " | 22-5 |
| 23 | " | 22-7 |
| 24 | " | 22-8 |
| 25 | " | 22-13 |
| 26 | " | 22-15 |
| 27 | VF 145B(SRS) | 26-1 |
| 28 | " " | 26-2 |
| 29 | 1402 (Clow) | 29-1 |
| 30 | 145-A (Clow) | 33-1 |
| 31 | " " | 36-1 |

# Chapter V

## SUMMARY

It was found that means are available to increase the probability of finding polyembryonic seeds in tomato. The frequency of such seeds increased with increasing seed weight. The frequency of twinning was further increased by combining a weight separation with Chlorox treatment. The seeds were soaked in Chlorox for five minutes to clarify the seed coat tissue, and those seeds in which the embryo outline was not clearly visible through the clarified seed coat were found to contain a higher proportion of twins.

A high twinning rate was found to be an inherited character. Lines which produced a high percentage of twins in one generation also produced a high percentage in the progeny. Lines which produced a low percentage likewise produced a low percentage in the progeny. A high rate of twinning was found to be not maternally inherited, since $F_1$ seed of a cross with a high twinning line as the seed parent did not produce as many twins as the self pollinated seed parent.

A nutritional competition within polyembryonic seeds was shown by differences between the twin which completely emerged from the seed coat first and the one

which remained partly inside for a longer time. Differences were found to be highly significant for number of days to flowering and for total height of young plants. Differences were not significant for height to first inflorescence, number of flowers in first and second inflorescences, or number of nodes below first inflorence.

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*(Original not seen. Information taken from Webber, J. M. (1940), 37 above.)

# APPENDIX

APPENDIX.--ADDITIONAL PLANT MEASUREMENTS USED IN THE VARIABILITY STUDY MEASUREMENT FOR DAYS TO FLOWERING OF TWINS.

| Plant No. | Days to Flower TA | TB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 52 | 53 | -1 | 1 |
| 2-2 | 57 | 59 | -2 | 4 |
| 2-1 | 54 | 61 | -7 | 49 |
| 1-3 | 51 | 51 | 0 | 0 |
| 1-6 | 51 | 56 | -5 | 25 |
| 1-7 | 56 | 56 | 0 | 0 |
| 1-5 | 53 | 59 | -6 | 36 |
| 1-4 | 54 | 61 | -7 | 49 |
| 4-1 | 56 | 60 | -4 | 16 |
| 4-2 | 56 | 62 | -6 | 36 |
| 17-2 | 62 | 60 | 2 | 4 |
| 17-3 | 59 | 62 | -3 | 9 |
| 21-2 | 55 | 57 | -2 | 4 |
| 21-3 | 56 | 60 | -4 | 16 |
| 21-4 | 56 | 57 | -1 | 1 |
| 21-5 | 59 | 62 | -3 | 9 |
| 21-6 | 62 | 63 | -1 | 1 |
| 21-7 | 60 | 61 | -1 | 1 |
| 21-8 | 61 | 62 | -1 | 1 |
| 21-9 | 57 | 65 | -8 | 64 |
| 21-10 | 60 | 58 | +2 | 4 |
| 22-5 | 59 | 58 | 1 | 1 |
| 22-7 | 60 | 58 | 2 | 4 |
| 22-8 | 54 | 63 | -9 | 81 |
| 22-13 | 56 | 56 | 0 | 0 |
| 22-15 | 56 | 61 | -5 | 25 |
| 26-1 | 54 | 54 | 0 | 0 |
| 26-2 | 54 | 53 | 1 | 1 |
| 29-1 | 48 | 52 | -4 | 16 |
| 33-1 | 60 | 61 | -1 | 1 |
| 36-1 | 56 | 54 | 2 | 4 |
| Total | 1744 | 1815 | -71 | 463 |
| Average | 56.5 | 58.5 | 2.29 | |

$n = 31 \qquad \bar{d} = 71/31 = 2.29$

$$s^2 = \frac{s(d^2) - [s(d)]^2/n}{n-1} = \frac{463 - 162.61}{30} = 10.01$$

$$t = \frac{2.29}{0.56} = 4.09$$

APPENDIX.--MEASUREMENTS FOR DAYS TO FLOWERING OF THE CHECK PLANTS.

| Plant No. | Days to Flower CTA | CTB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 50 | 53 | -3 | 9 |
| 2-2 | 57 | 61 | -4 | 16 |
| 2-1 | 56 | 54 | 2 | 4 |
| 1-3 | 51 | 54 | -3 | 9 |
| 1-6 | 51 | 52 | -1 | 1 |
| 1-7 | 56 | 50 | 6 | 36 |
| 1-5 | 52 | 52 | 0 | 0 |
| 1-4 | 52 | 54 | -2 | 4 |
| 4-1 | 58 | 62 | -4 | 16 |
| 4-2 | 57 | 56 | 1 | 1 |
| 17-2 | 58 | 60 | -2 | 4 |
| 17-3 | 58 | 59 | -1 | 1 |
| 21-2 | 59 | 57 | 2 | 4 |
| 21-3 | 57 | 56 | 1 | 1 |
| 21-4 | 59 | 55 | 4 | 16 |
| 21-5 | 59 | 60 | -1 | 1 |
| 21-6 | 60 | 60 | 0 | 0 |
| 21-7 | 56 | 61 | -5 | 25 |
| 21-8 | 56 | 57 | -1 | 1 |
| 21-9 | 65 | 59 | 6 | 36 |
| 21-10 | 55 | 55 | 0 | 0 |
| 22-5 | 58 | 56 | 2 | 4 |
| 22-7 | 54 | 56 | -2 | 4 |
| 22-8 | 56 | 60 | -4 | 16 |
| 22-13 | 56 | 61 | -5 | 25 |
| 22-15 | 57 | 56 | 1 | 1 |
| 26-1 | 53 | 48 | 5 | 25 |
| 26-2 | 54 | 52 | 2 | 4 |
| 29-1 | 48 | 48 | 0 | 0 |
| 33-1 | 61 | 63 | -2 | 4 |
| 36-1 | 56 | 56 | 0 | 0 |
| Total | 1735 | 1743 | -8 | 268 |
| Average | 59.9 | 56.2 | 0.25 | |

$n = 31 \qquad \bar{d} = 0.25$

$$s^2 = \frac{\Sigma(d^2) - [\Sigma(d)]^2/n}{n-1} = \frac{268 - 2.06}{30} = \frac{265.94}{30} = 8.86$$

$$t = \frac{0.25}{0.52} = 0.48$$

APPENDIX.--MEASUREMENTS FOR TOTAL PLANT HEIGHTS (CMS) OF TWINS.

| Plant No. | Total height when first plant flowers TA | TB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 35.5 | 29.0 | 6.5 | 42.25 |
| 2-2 | 32.0 | 30.0 | 2.0 | 4.00 |
| 2-1 | 30.2 | 19.7 | 10.5 | 110.25 |
| 1-3 | 42.0 | 39.5 | 2.5 | 6.25 |
| 1-6 | 45.4 | 39.0 | 6.4 | 40.96 |
| 1-7 | 33.0 | 25.0 | 8.0 | 64.00 |
| 1-5 | 43.3 | 25.5 | 17.8 | 316.84 |
| 1-4 | 35.5 | 22.7 | 12.8 | 163.84 |
| 4-1 | 33.7 | 32.7 | 1.0 | 1.00 |
| 4-2 | 24.1 | 22.1 | 2.0 | 4.00 |
| 17-2 | 28.7 | 26.5 | 2.2 | 4.84 |
| 17-3 | 27.0 | 25.5 | 1.5 | 2.25 |
| 21-2 | 41.3 | 36.0 | 5.3 | 28.09 |
| 21-3 | 38.9 | 36.0 | 2.9 | 8.41 |
| 21-4 | 45.2 | 41.7 | 3.7 | 13.69 |
| 21-5 | 42.0 | 36.1 | 5.9 | 34.81 |
| 21-6 | 34.6 | 30.0 | 4.6 | 21.16 |
| 21-7 | 37.0 | 30.0 | 7.0 | 49.00 |
| 21-8 | 37.3 | 33.7 | 3.6 | 12.96 |
| 21-9 | 44.1 | 35.4 | 8.7 | 75.69 |
| 21-10 | 32.8 | 41.9 | -9.1 | 82.81 |
| 22-5 | 37.1 | 36.2 | 0.9 | .81 |
| 22-7 | 39.1 | 44.4 | -5.3 | 28.09 |
| 22-8 | 43.0 | 30.0 | 13.0 | 169.00 |
| 22-13 | 33.8 | 36.8 | -3.0 | 9.00 |
| 22-15 | 41.0 | 32.0 | 9.0 | 81.00 |
| 26-1 | 30.0 | 19.0 | 11.0 | 121.00 |
| 26-2 | 28.7 | 32.6 | -3.9 | 15.21 |
| 29-1 | 33.5 | 26.5 | 7.0 | 49.00 |
| 33-1 | 35.0 | 29.5 | 5.5 | 30.25 |
| 36-1 | 36.0 | 41.0 | -5.0 | 25.00 |
| Total | 1120.8 | 985.8 | 135.0 | 1615.46 |
| Average | 36.1 | 31.8 | 4.35 | |

$n = 31 \qquad \bar{d} = \frac{135}{31} = 4.35$

$$s^2 = \frac{\Sigma(d^2) - [\Sigma(d)]^2/n}{n-1} = \frac{1615.46 - 587.90}{30} = 34.25$$

$$t = \frac{4.35}{1.04} = 4.18$$

APPENDIX.--MEASUREMENTS FOR TOTAL PLANT HEIGHT (CMS.) OF CHECK PLANTS.

| Plant No. | Total Height When First Plant Flowers CTA | CTB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 40.0 | 33.5 | 6.5 | 42.25 |
| 2-2 | 32.5 | 30.0 | 2.5 | 6.25 |
| 2-1 | 31.5 | 30.5 | 1.0 | 1.00 |
| 1-3 | 41.0 | 29.0 | 12.0 | 144.00 |
| 1-6 | 46.3 | 39.5 | 6.8 | 46.24 |
| 1-7 | 33.8 | 36.7 | -.9 | .81 |
| 1-5 | 40.8 | 47.0 | -6.2 | 38.44 |
| 1-4 | 43.5 | 36.0 | 9.5 | 90.25 |
| 4-1 | 36.6 | 29.8 | 6.8 | 46.24 |
| 4-2 | 26.7 | 26.4 | .3 | .09 |
| 17-2 | 31.5 | 27.6 | 3.9 | 15.21 |
| 17-3 | 36.4 | 38.5 | -2.1 | 4.41 |
| 21-2 | 32.8 | 37.9 | -5.1 | 26.01 |
| 21-3 | 38.4 | 45.0 | -6.6 | 43.56 |
| 21-4 | 53.0 | 47.7 | 5.3 | 28.09 |
| 21-5 | 42.8 | 53.4 | -10.6 | 112.36 |
| 21-6 | 52.0 | 39.0 | 13.0 | 169.00 |
| 21-7 | 44.5 | 37.2 | 7.3 | 53.29 |
| 21-8 | 48.3 | 38.0 | 10.3 | 106.09 |
| 21-9 | 39.8 | 38.5 | 1.3 | 1.69 |
| 21-10 | 39.5 | 45.4 | -5.9 | 34.81 |
| 22-5 | 35.7 | 42.3 | -6.6 | 43.56 |
| 22-7 | 44.3 | 29.0 | 15.3 | 234.09 |
| 22-8 | 41.2 | 38.3 | 2.9 | 8.41 |
| 22-13 | 47.1 | 41.0 | 6.1 | 37.21 |
| 22-15 | 39.6 | 39.3 | .3 | .09 |
| 26-1 | 24.5 | 34.0 | -9.5 | 90.25 |
| 26-2 | 29.0 | 34.3 | -5.3 | 28.09 |
| 29-1 | 32.6 | 34.6 | -2.0 | 4.00 |
| 33-1 | 46.0 | 39.0 | 7.0 | 49.00 |
| 36-1 | 39.9 | 30.0 | 9.9 | 98.01 |
| Total | 1211.6 | 1144.4 | 67.2 | 1602.80 |
| Average | 39.0 | 36.9 | 2.16 | |

$n = 31 \qquad \bar{d} = 2.16$

$$s^2 = \frac{\Sigma(d^2) - [\Sigma(d)]^2/n}{n-1} = \frac{1602.80 - 145.67}{30} = \frac{1457.13}{30} = 48.57$$

$$t = \frac{2.16}{1.56} = 1.3$$

APPENDIX.--MEASUREMENT FOR HEIGHT TO FIRST INFLORESCENCE OF TWINS.

| Plant No. | Height to first Inflorescence TA | TB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 29.6 | 29.5 | 0.1 | 0.01 |
| 2-2 | 26.0 | 30.5 | -4.5 | 2.25 |
| 2-1 | 25.7 | 28.7 | -3.0 | 9.00 |
| 1-3 | 32.4 | 29.5 | 2.9 | 8.41 |
| 1-6 | 34.7 | 47.0 | -12.3 | 151.29 |
| 1-7 | 48.0 | 32.0 | 16.0 | 256.00 |
| 1-5 | 39.3 | 42.0 | -2.7 | 7.29 |
| 1-4 | 34.5 | 51.0 | -16.5 | 272.25 |
| 4-1 | 28.2 | 33.0 | -4.8 | 23.04 |
| 4-2 | 20.4 | 28.0 | -7.6 | 57.76 |
| 17-2 | 31.0 | 27.1 | 3.9 | 15.21 |
| 17-3 | 26.5 | 29.9 | -3.4 | 11.56 |
| 21-2 | 36.1 | 37.1 | -1.0 | 1.00 |
| 21-3 | 38.4 | 40.0 | -1.6 | 2.56 |
| 21-4 | 44.5 | 39.9 | 4.6 | 21.16 |
| 21-5 | 44.0 | 36.5 | 7.5 | 56.25 |
| 21-6 | 25.1 | 24.3 | .8 | .64 |
| 21-7 | 40.5 | 37.5 | 3.0 | 9.00 |
| 21-8 | 45.0 | 44.1 | .9 | .81 |
| 21-9 | 42.0 | 37.5 | 4.5 | 20.25 |
| 21-10 | 39.1 | 46.9 | -7.8 | 60.84 |
| 22-5 | 39.5 | 34.1 | 5.4 | 29.16 |
| 22-7 | 38.5 | 41.0 | -2.5 | 6.25 |
| 22-8 | 37.5 | 45.0 | -7.5 | 56.25 |
| 22-13 | 43.0 | 42.0 | 1.0 | 1.00 |
| 22-15 | 41.0 | 39.3 | 1.7 | 2.89 |
| 26-1 | 25.1 | 24.3 | .8 | .64 |
| 26-2 | 28.5 | 28.0 | .5 | .25 |
| 29-1 | 32.6 | 29.9 | 2.7 | 7.29 |
| 33-1 | 29.1 | 25.5 | 3.6 | 12.96 |
| 36-1 | 32.0 | 30.0 | 2.0 | 4.00 |
| Total | 1077.8 | 1091.1 | 13.3 | 1125.27 |
| Average | 34.7 | 35.1 | 0.42 | |

$$n = 31 \qquad \bar{d} = \frac{13.3}{31} = 0.42$$

$$s^2 = \frac{\Sigma(d^2) - /\Sigma(d)7^2/n}{n-1} = \frac{1125.27 - 57.03}{30} = 35.60$$

$$t = \frac{0.42}{1.06} = 0.39$$

APPENDIX.--MEASUREMENTS FOR HEIGHT TO FIRST INFLORESCENCE OF CHECK PLANTS.

| Plant No. | Height to first Inflorescence CTA | CTB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 33.0 | 28.0 | 5.0 | 25.00 |
| 2-2 | 27.0 | 31.0 | -4.0 | 16.00 |
| 2-1 | 30.0 | 26.0 | 4.0 | 16.00 |
| 1-3 | 28.0 | 25.2 | 2.8 | 7.84 |
| 1-6 | 32.3 | 34.0 | -1.7 | 2.89 |
| 1-7 | 47.5 | 28.8 | 18.7 | 349.69 |
| 1-5 | 28.5 | 33.8 | -5.3 | 28.09 |
| 1-4 | 35.0 | 32.3 | 2.7 | 7.29 |
| 4-1 | 32.5 | 31.5 | 1.0 | 1.00 |
| 4-2 | 25.8 | 21.6 | 4.2 | 17.64 |
| 17-2 | 27.3 | 29.0 | -1.7 | 2.89 |
| 17-3 | 30.1 | 35.9 | -5.8 | 33.64 |
| 21-2 | 36.2 | 39.3 | -3.1 | 9.61 |
| 21-3 | 41.0 | 39.8 | 1.2 | 1.44 |
| 21-4 | 47.0 | 44.5 | 2.5 | 6.25 |
| 21-5 | 37.0 | 49.5 | -12.5 | 156.25 |
| 21-6 | 25.5 | 29.5 | -4.0 | 16.00 |
| 21-7 | 39.5 | 44.0 | -4.5 | 20.25 |
| 21-8 | 42.3 | 40.3 | 2.0 | 4.00 |
| 21-9 | 47.5 | 41.6 | 5.9 | 34.81 |
| 21-10 | 38.9 | 42.1 | -3.2 | 10.24 |
| 22-5 | 34.0 | 34.0 | 0 | 0 |
| 22-7 | 38.5 | 35.0 | 3.5 | 12.25 |
| 22-8 | 40.1 | 47.0 | -6.9 | 47.61 |
| 22-13 | 42.0 | 41.0 | 1.0 | 1.00 |
| 22-15 | 36.5 | 39.5 | -3.0 | 9.00 |
| 26-1 | 25.5 | 29.5 | -4.0 | 16.00 |
| 26-2 | 28.5 | 28.1 | .4 | .16 |
| 29-1 | 31.0 | 30.6 | +.4 | .16 |
| 33-1 | 38.1 | 32.0 | 6.1 | 37.21 |
| 36-1 | 28.0 | 18.0 | 10.0 | 100.00 |
| Total | 1074.1 | 1062.4 | 11.7 | 990.21 |
| Average | 34.6 | 342 | 0.34 | |

$n = 31 \qquad \bar{d} = 0.34$

$$s^2 = \frac{s(d^2) - [s(d)]^2/n}{n-1} = \frac{990.21 - 4.41}{30} = 32.86$$

$$t = \frac{0.34}{1.09} = 0.31$$

APPENDIX.--MEASUREMENTS FOR NUMBER OF FLOWERS IN THE FIRST INFLORESCENCE OF TWINS.

| Plant No. | No. of Flowers TA | TB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 13 | 15 | -2 | 4 |
| 2-2 | 6 | 8 | -2 | 4 |
| 2-1 | 9 | 8 | +1 | 1 |
| 1-3 | 16 | 21 | -5 | 25 |
| 1-6 | 19 | 25 | -6 | 36 |
| 1-7 | 23 | 16 | +7 | 49 |
| 1-5 | 15 | 17 | -2 | 4 |
| 1-4 | 20 | 12 | +8 | 64 |
| 4-1 | 8 | 8 | 0 | 0 |
| 4-2 | 7 | 8 | -1 | 1 |
| 17-2 | 7 | 8 | -1 | 1 |
| 17-3 | 8 | 7 | +1 | 1 |
| 21-2 | 6 | 7 | -1 | 1 |
| 21-3 | 5 | 7 | -2 | 4 |
| 21-4 | 6 | 11 | -5 | 25 |
| 21-5 | 7 | 5 | +2 | 4 |
| 21-6 | 10 | 7 | +3 | 9 |
| 21-7 | 14 | 6 | +8 | 64 |
| 21-8 | 8 | 9 | -1 | 1 |
| 21-9 | 5 | 6 | -1 | 1 |
| 21-10 | 6 | 11 | -5 | 25 |
| 22-5 | 7 | 7 | 0 | 0 |
| 22-7 | 6 | 7 | -1 | 1 |
| 22-8 | 6 | 5 | +1 | 1 |
| 22-13 | 7 | 6 | +1 | 1 |
| 22-15 | 7 | 5 | +2 | 4 |
| 26-1 | 10 | 7 | +3 | 9 |
| 26-2 | 7 | 7 | 0 | 0 |
| 29-1 | 6 | 6 | 0 | 0 |
| 33-1 | 9 | 8 | +1 | 1 |
| 36-1 | 8 | 9 | -1 | 1 |
| Total | 291 | 289 | +2 | 342 |
| Average | 9.3 | 9.3 | 0.06 | |

$n = 31 \qquad \bar{d} = \frac{2}{31} = 0.06$

$$s^2 = \frac{S(d^2) - /S(d)/^2/n}{n-1} = \frac{342 - .129}{30} = 11.39$$

$$t = \frac{0.06}{0.6} = 0.1$$

APPENDIX.--MEASUREMENTS FOR NUMBER OF FLOWERS IN THE FIRST INFLORESCENCE OF CHECK PLANTS.

| Plant No. | No. of Flowers CTA | CTB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 12 | 32 | -20 | 400 |
| 2-2 | 12 | 15 | -3 | 9 |
| 2-1 | 9 | 7 | +2 | 4 |
| 1-3 | 13 | 14 | -1 | 1 |
| 1-6 | 13 | 21 | -7 | 49 |
| 1-7 | 16 | 19 | -3 | 9 |
| 1-5 | 16 | 23 | -7 | 49 |
| 1-4 | 19 | 8 | +11 | 121 |
| 4-1 | 7 | 8 | -1 | 1 |
| 4-2 | 8 | 6 | +2 | 4 |
| 17-2 | 7 | 8 | -1 | 1 |
| 17-3 | 7 | 8 | -1 | 1 |
| 21-2 | 7 | 5 | +2 | 4 |
| 21-3 | 6 | 6 | 0 | 0 |
| 21-4 | 7 | 6 | +1 | 1 |
| 21-5 | 8 | 6 | +2 | 4 |
| 21-6 | 9 | 8 | +1 | 1 |
| 21-7 | 8 | 7 | +1 | 1 |
| 21-8 | 6 | 6 | 0 | 0 |
| 21-9 | 6 | 12 | -6 | 36 |
| 21-10 | 5 | 6 | -1 | 1 |
| 22-5 | 7 | 6 | +1 | 1 |
| 22-7 | 7 | 6 | +1 | 1 |
| 22-8 | 3 | 7 | -4 | 16 |
| 22-13 | 9 | 7 | +2 | 4 |
| 22-15 | 8 | 6 | +2 | 4 |
| 26-1 | 9 | 8 | +1 | 1 |
| 26-2 | 7 | 24 | -17 | 289 |
| 29-1 | 7 | 5 | +2 | 4 |
| 33-1 | 10 | 8 | +2 | 4 |
| 36-1 | 10 | 8 | ±2 | 4 |
| Total | 279 | 316 | 37 | 1025 |
| Average | 9.0 | 10.1 | 1.19 | |

$n = 31 \qquad \bar{d} = \frac{37}{31} = 1.19$

$$s^2 = \frac{\Sigma(d^2) - [\Sigma(d)]^2/n}{n-1} = \frac{1025 - 44.16}{30} = \frac{980.84}{30} = 32.69$$

$$t = \frac{1.19}{1.09} = 1.09$$

APPENDIX.--MEASUREMENTS FOR NUMBER OF FLOWERS IN THE SECOND INFLORESCENCE.

| Plant No. | No. of Flowers TA | TB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 28 | 7 | 21 | 441 |
| 2-2 | 1 | 9 | -8 | 64 |
| 2-1 | 12 | 11 | 1 | 1 |
| 1-3 | 22 | 3 | 19 | 361 |
| 1-6 | 27 | 12 | 15 | 225 |
| 1-7 | 17 | 17 | 0 | 0 |
| 1-5 | 21 | 9 | 12 | 144 |
| 1-4 | 12 | 14 | -2 | 4 |
| 4-1 | 6 | 7 | -1 | 1 |
| 4-2 | 8 | 8 | 0 | 0 |
| 17-2 | 7 | 7 | 0 | 0 |
| 17-3 | 6 | 4 | 2 | 4 |
| 21-2 | 4 | 12 | -8 | 64 |
| 21-3 | 14 | 12 | 2 | 4 |
| 21-4 | 6 | 9 | -3 | 9 |
| 21-5 | 6 | 6 | 0 | 0 |
| 21-6 | 8 | 6 | 2 | 4 |
| 21-7 | 7 | 9 | -2 | 4 |
| 21-8 | 18 | 3 | +15 | 225 |
| 21-9 | 10 | 6 | 4 | 16 |
| 21-10 | 6 | 7 | -1 | 1 |
| 22-5 | 6 | 11 | -5 | 25 |
| 22-7 | 5 | 6 | -1 | 1 |
| 22-8 | 3 | 7 | -4 | 16 |
| 22-13 | 7 | 6 | +1 | 1 |
| 22-15 | 6 | 6 | 0 | 0 |
| 26-1 | 7 | 6 | 1 | 1 |
| 26-2 | 8 | 6 | 2 | 4 |
| 29-1 | 8 | 8 | 0 | 0 |
| 33-1 | 8 | 7 | 1 | 1 |
| 36-1 | 7 | 6 | 1 | 1 |
| Total | 311 | 247 | 64 | 1622 |
| Average | 11.9 | 9.5 | 2.06 | |

$n = 31 \qquad \bar{d} = \frac{64}{31} = 2.06$

$$s^2 = \frac{\Sigma(d^2) - /\Sigma(d)72/n}{n-1} = \frac{1622 - 132.12}{30} = \frac{1489.88}{30} = 49.66$$

$$t = \frac{2.06}{1.27} = 1.62$$

APPENDIX.--MEASUREMENTS FOR NUMBER OF FLOWERS IN THE SECOND INFLORESCENCE OF CHECK PLANTS.

| Plant No. | No. of Flowers CTA | CTB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 14 | 28 | -14 | 196 |
| 2-2 | 12 | 11 | 1 | 1 |
| 2-1 | 9 | 8 | 1 | 1 |
| 1-3 | 11 | 13 | -2 | 4 |
| 1-6 | 18 | 12 | 6 | 36 |
| 1-7 | 6 | 16 | -10 | 10 |
| 1-5 | 21 | 27 | -6 | 36 |
| 1-4 | 20 | 7 | 13 | 169 |
| 4-1 | 7 | 7 | 0 | 0 |
| 4-2 | 7 | 7 | 0 | 0 |
| 17-2 | 8 | 7 | 1 | 1 |
| 17-3 | 6 | 9 | -3 | 9 |
| 21-2 | 5 | 7 | -2 | 4 |
| 21-3 | 6 | 9 | -3 | 9 |
| 21-4 | 7 | 7 | 0 | 0 |
| 21-5 | 4 | 6 | -2 | 4 |
| 21-6 | 7 | 6 | 1 | 1 |
| 21-7 | 8 | 10 | -2 | 4 |
| 21-8 | 10 | 7 | 3 | 9 |
| 21-9 | 9 | 3 | 6 | 36 |
| 21-10 | 4 | 7 | -3 | 9 |
| 22-5 | 6 | 6 | 0 | 0 |
| 22-7 | 6 | 5 | 1 | 1 |
| 22-8 | 5 | 8 | -3 | 9 |
| 22-13 | 7 | 6 | 1 | 1 |
| 22-15 | 5 | 8 | -3 | 9 |
| 26-1 | 7 | 6 | 1 | 1 |
| 26-2 | 8 | 9 | -1 | 1 |
| 29-1 | 7 | 6 | 1 | 1 |
| 33-1 | 10 | 8 | 2 | 4 |
| 36-1 | 7 | 9 | -2 | 4 |
| Total | 268 | 285 | -17 | 570 |
| Average | 8.6 | 9.1 | 0.54 | |

$n = 31 \qquad \bar{d} = \frac{17}{31} = 0.54$

$$s^2 = \frac{S(d^2) - /S(d)7^2/n}{n-1} = \frac{570 - 9.32}{30} = \frac{560.68}{30} = 18.68$$

$$t = \frac{0.54}{0.78} = 0.69$$

APPENDIX.--MEASUREMENTS FOR NUMBER OF NODES TO THE FIRST INFLORESCENCE OF TWIN PLANTS.

| Plant No. | No. of Nodes TA | TB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 8 | 7 | +1 | 1 |
| 2-2 | 8 | 9 | -1 | 1 |
| 2-1 | 8 | 9 | -1 | 1 |
| 1-3 | 7 | 7 | 0 | 0 |
| 1-6 | 9 | 15 | -6 | 36 |
| 1-7 | 15 | 8 | 7 | 49 |
| 1-5 | 8 | 9 | -1 | 1 |
| 1-4 | 7 | 8 | -1 | 1 |
| 4-1 | 8 | 8 | 0 | 0 |
| 4-2 | 7 | 8 | -1 | 1 |
| 21-2 | 9 | 10 | -1 | 1 |
| 21-3 | 14 | 12 | 2 | 4 |
| 21-4 | 10 | 9 | 1 | 1 |
| 21-5 | 10 | 9 | 1 | 1 |
| 21-6 | 10 | 9 | 1 | 1 |
| 21-7 | 9 | 9 | 0 | 0 |
| 21-8 | 10 | 9 | 1 | 1 |
| 21-9 | 9 | 9 | 0 | 0 |
| 21-10 | 9 | 9 | 0 | 0 |
| 22-5 | 9 | 10 | -1 | 1 |
| 22-7 | 10 | 9 | 1 | 1 |
| 22-8 | 9 | 10 | -1 | 1 |
| 22-13 | 9 | 9 | 0 | 0 |
| 22-15 | 10 | 11 | -1 | 1 |
| 33-1 | 8 | 8 | 0 | 0 |
| 36-1 | 8 | 7 | 1 | 1 |
| Total | 238 | 237 | 1 | 105 |
| Average | 8.7 | 8.7 | 0.03 | |

$n = 26 \qquad \bar{d} = \frac{1}{26} = 0.03$

$s^2 = \frac{105 - 0.03}{25} = 4.19$

$t = 2.00$

APPENDIX.--MEASUREMENTS FOR NUMBER OF NODES TO FIRST INFLORSCENCE OF CHECK PLANT.

| Plant No. | No. of Nodes to First Flower CTA | CTB | d | $d^2$ |
|---|---|---|---|---|
| 1-2 | 8 | 8 | 0 | 0 |
| 2-2 | 8 | 10 | -2 | 4 |
| 2-1 | 9 | 8 | 1 | 1 |
| 1-3 | 7 | 7 | 0 | 0 |
| 1-6 | 8 | 9 | -1 | 1 |
| 1-7 | 12 | 8 | 4 | 16 |
| 1-5 | 7 | 8 | -1 | 1 |
| 1-4 | 8 | 8 | 0 | 0 |
| 4-1 | 8 | 10 | -2 | 4 |
| 4-2 | 9 | 8 | 1 | 1 |
| 21-2 | 10 | 10 | 0 | 0 |
| 21-3 | 6 | 9 | -3 | 9 |
| 21-4 | 9 | 11 | -2 | 4 |
| 21-5 | 9 | 11 | -2 | 6 |
| 21-6 | 10 | 9 | 1 | 1 |
| 21-7 | 10 | 10 | 0 | 0 |
| 21-8 | 10 | 10 | 0 | 0 |
| 21-9 | 10 | 9 | 1 | 1 |
| 21-10 | 10 | 9 | 1 | 1 |
| 22-5 | 8 | 10 | -2 | 4 |
| 22-7 | 10 | 9 | 1 | 1 |
| 22-8 | 10 | 10 | 0 | 0 |
| 22-13 | 10 | 10 | 0 | 0 |
| 22-15 | 9 | 10 | -1 | 1 |
| 33-1 | 9 | 8 | 1 | 1 |
| 36-1 | 7 | 8 | -1 | 1 |
| Total | 231 | 237 | -6 | 56 |
| Average | 8.5 | 8.7 | 0.23 | |

$n = 26 \qquad \bar{d} = \frac{6}{26} = 0.23$

$$s^2 = \frac{s(d^2) - /s(d)7^2/n}{n-1} = \frac{56 - 1.38}{25} = 2.19$$

$$t = \frac{0.23}{0.91} = 0.25$$

ABSTRACT OF THESIS

POLYEMBRYONY IN LYCOPERSICON ESCULENTUM

A study of polyembryonic tomatoes was conducted with two major objectives. The first was to determine means of obtaining a higher frequency of polyembryonic seeds. The second was to study various growth traits of twin plants, especially to observe differences between twins which might be attributable to a nutritional competition within polyembryonic seeds.

The variety Beefsteak, a seed lot of which was previously observed to produce a high incidence of twins, was found in this study to transmit this trait to its progeny. $F_1$ seed of reciprocal crosses with low incidence lines showed that inheritance of a high incidence of twinning was neither dominant nor maternal since in no case was there a high incidence of twinning in any of the progenies. Self pollinated seed taken from $F_1$ plants of these crosses indicated a more complex inheritance than would be afforded by a single gene. Reciprocal crosses between the Beefsteak line and Premier both produced a higher incidence than crosses between the same high twinning line and Red Jacket.

It was found that detection of polyembryonic seeds was better accomplished where seeds had been separated by weight. Heavy seeds were found to contain a higher proportion of twins. Detection of polyembryonic seeds was further

improved by soaking heavy seeds in Chlorox, a 5.25 per cent solution of sodium hypochlorite, for five minutes. Chlorox clarifies the seed coat tissue and enables the observer to see the outline of the embryo more clearly in single embryo seeds than in polyembryonic seeds. Chlorox treated, heavy seeds in which the embryo could not be clearly discerned through the seed coat produced twins at the rate of 1 in 17 seeds; whereas, non-treated and randomly selected seeds of the same seed lot produced twins at the rate of 1 in 125 seeds.

A distinction was made between each twin pair according to which seedling completely emerged first from the seed coat. It was found that the twin that first completely emerged was later in flowering and slower in growth. Differences between the twins were not significant for height to first inflorescence, number of flowers in first and second inflorescences, or number of nodes below first inflorescence.

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