1975

Several inheritance and related studies in the tomato.

Paul Brent Howes
University of Massachusetts Amherst

SEVERAL INHERITANCE AND RELATED STUDIES IN THE TOMATO

A Thesis Presented

By

PAUL BRENT HOWES

B.S., University of Massachusetts

Submitted to the Graduate School of the
University of Massachusetts in
partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

September, 1975

Major Subject Plant and Soil Sciences

SEVERAL INHERITANCE AND RELATED STUDIES IN THE TOMATO

A Thesis Presented

By

PAUL BRENT HOWES

Approved as to style and content by:

(Chairman of Committee)

(Head of Department)

(Member)

(Member)

ACKNOWLEDGEMENTS

Gratitude is here expressed to Professor William H. Lachman for the professional guidance and personal friendship he afforded me during my graduate studies.

Sincere thanks are extended to Dr. Allen V. Barker and Dr. Harold Rauch for their service on my thesis committee.

My appreciation goes to Mr. John L. Sullivan for his generous assistance in the greenhouse.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

I N T R O D U C T I O N

From the rediscovery and early application of Mendel's laws, to hybrid corn, to the continuing green revolution, plant improvement efforts of this century have significantly benefited mankind. Research in plant breeding and genetics is necessarily pursued at the basic level and at the level of practical application. The present study was undertaken to provide experience in the art of plant improvement and in both basic and applied scientific endeavor through the inquiry into relevant problems.

PART I

TITLE: A STUDY OF THE HERITABILITY OF AMMONIUM TOLERANCE IN THE TOMATO.

ABSTRACT: Testcrosses involving the ammonium tolerant lines yellow-green-5 and neglecta-1 with their respective parent lines susceptible to ammonium injury failed to produce normal plants in which the ammonium tolerance persisted.

INTRODUCTION

Fertilizers containing ammonium as a nitrogen source are frequently applied to commercial vegetable plantings. Several vegetable species including bean, sweet corn, cucumber, pea, eggplant, and tomato are susceptible to ammonium toxicity when cultured with an ammonium form of nitrogen (6,7). Ammonium injury to field-grown tomato plants has also been observed (3).

Other studies have shown that when certain commercially important tomato (Lycopersicon esculentum Mill.) cultivars are subjected to high soil levels of ammonium in the absence of adequate available potassium, necrotic lesions may appear on the plant stem, petiole, and lamina (1,8). Among cultivars and noncommercial lines there is a considerable range of response from extreme susceptibility to ammonium injury in V. R. Valiant and Heinz 1350 to high resistance to lesion formation in the mutant strains yellow-green-5 (yg_5) and neglecta-1 (neg-1) (4,9). The present study was undertaken to determine if a factor for ammonium tolerance could be transferred out of the mutant lines.

MATERIALS AND METHODS

The ammonium tolerant lines yg_5 and neg-1 were used as a basis for this investigation. The yg_5 mutant was obtained following irradiation of the seed of Stock 018, a Red Cherry inbred (2). This mutant is characterized by yellow cotyledons and leaves and by extremely slow growth. The neg-1 mutant was obtained following irradiation of seed from the cultivar Condine Red (12). This mutant is characterized as a small, weakly branched plant with leaves becoming progressively necrotic.

A preliminary study was completed using the various stocks involved. Six plants each of $\underline{yg}_5$, $\underline{neg}$-1, Stock 018, Condine Red, Stock 018 x $\underline{yg}_5$, Condine Red x $\underline{neg}$-1, and V. R. Valiant were tested. Eight-week old plants of each type were transplanted to 12.7 cm clay pots containing a medium consisting of soil, peat, and sand in the ratio 7:3:2 by volume. An addition 56.7 g of 20% superphosphate and 56.7 g of magnesian limestone was made to each 0.036 cu. m of medium. One week following transplanting, treatment with 0.04N $(NH_4)_2SO_4$ at the rate of 100 ml/pot/day for five consecutive days of every seven-day period was initiated. Plant response was recorded following three weeks of treatment. Classifications are based on lesions observed: 0 rating for no lesions to a 3 rating for severe lesion formation. The data were statistically analyzed using the analysis of variance and differences among means were tested by Duncan's new multiple range test (11).

In the testcross study, plants of $\underline{yg}_5$ and $\underline{neg}$-1 were crossed by their respective parents. The plants from the resulting F_1 generation were crossed back to the mutants (see Table 2). From the resulting two segregating generations normal phenotype plants were saved and subjected to the ammonium nutritional regimen described above.

Plants from the testcrosses that did not show lesion development were selfed. The resulting seed was harvested, and plants of normal phenotype from this generation were also subjected to the ammonium test.

RESULTS AND DISCUSSION

Table 1 gives the results of the preliminary study of the tomato lines involved. As expected, $\underline{neg}$-1 and $\underline{yg}_5$ demonstrated significant

ammonium tolerance over all genotypes tested.

Table 2 shows the segregation data resulting from the testcross. The low chi-squares obtained give a good indication that the crosses were not contaminated and that the mutant and normal plants had similar viability.

The segregants displaying $\underline{yg}_5$ and neg-1 phenotypes were discarded because of space limitations. Since ammonium-tolerant plants of normal phenotype were desired, the sacrifice of the mutant plants was not considered a fault in the experimental design.

The effect of the ammonium test on the normal testcross segregants is given in Table 3. Approximately 3% of all plants tested displayed no toxicity symptoms.

Table 4 shows the results of the ammonium test applied to plants grown from the selfed normal, lesionless plants. Only two plants of the neg-1 testcross were included because seeds from the remaining plants could not be obtained soon enough. Only one of the thirty-six plants tested, or about 3%, remained lesionless.

The fact that a consistent but random 3% of the plants tested remained lesionless following ammonium treatment may be attributed to a penetrance effect. A simple heritable factor for ammonium tolerance would be expected to appear in all replicates of neg-1 testcross plant number 5.

The one plant remaining lesionless was saved and selfed. Progeny of this plant will be subjected to the ammonium test.

The data obtained in this study provide evidence that the factor for ammonium tolerance in neg-1 and $\underline{yg}_5$ is probably conditioned by the

same genes responsible for the characteristic mutant phenotypes. However, the resolving power of this experiment was not strong enough to eliminate a strong intergenic linkage (10). If a 1% linkage exists, two or three plants showing recombination could be expected using the present design. A linkage of 0.5% or tighter could very probably go unbroken within the population examined here.

Previous tests have shown that the factors governing ammonium tolerance in neg-1 and yg_5 are not allelic (5), providing additional evidence consistent with a pleitropic effect.

It appears that a heritable factor affording ammonium tolerance in the tomato is not easily, if at all, separable from the mutated loci studied here.

Table 1. The expression of NH_4^+ toxicity symptoms in selected tomato lines.

Line	Toxicity Rating *	Statistical Significance**
neg-1	0.17	a
yg_5	0.33	a
Condine Red	2.00	b
Condine Red x *neg*-1	2.00	b
Stock 018	3.00	b
Stock 018 x yg_5	3.00	b
V. R. Valiant	3.00	b

* 0 no lesions; 3 most severe lesion formation; means six replicates.

** Means not followed by the same letter are significantly different. (P = 0.05)

Table 2. The calculation of chi-square for testcross ratios.

Testcross	Expected Ratio	Observed Mutant	Observed Normal	χ^2
(Stock 018 x $\underline{vg}_5$) x $\underline{vg}_5$	1:1	346	330	0.38
(Condine Red x <u>neg</u>-1) x <u>neg</u>-1	1:1	238	230	0.14

Table 3. The effect of NH_4^+ treatment on segregating populations.

Subject Population	Number of Plants With Lesions	Number of Plants Without Lesions	% Plants Lesionless
(Stock 018 x $\underline{yg}_5$) x $\underline{yg}_5$ green segregants	312	10	3.1
(Condine Red x $\underline{neg}$-1) x $\underline{neg}$-1 normal segregants	223	7	3.0

Table 4. The effect of NH_4^+ treatment on certain selfed lines.

Line			Replication Number With Lesion Rating		
			1	2	3
<u>yg_5</u>	testcross	1	3	3	3
"	"	2	2	3	3
"	"	3	3	2	3
"	"	4	2	3	3
"	"	5	3	2	3
"	"	6	3	1	3
"	"	7	3	3	3
"	"	8	3	2	3
"	"	9	3	3	2
"	"	10	3	3	3
<u>neg</u>-1	testcross	5	3	0	3
"	"	6	2	3	3

LITERATURE CITED

1. Barker, A. V., D. N. Maynard, and W. H. Lachman. 1967. Induction of tomato stem and leaf lesions, and potassium deficiency, by excessive ammonium nutrition. Soil Sci. 103:319-327.

2. Burdick, A. B. 1960. New gene symbols. Tomato Genet. Coop. Rept. 10:9.

3. Lachman, W. H. 1975. (Personal communication).

4. _____ and A. V. Barker. 1973. Tolerance of some tomato mutants to ammonium fertilization. Tomato Genet. Coop. Rept. 23:22-23.

5. _____ and _____. 1974. Testing for allelism among three mutants for tolerance to ammonium injury. Tomato Genet. Coop. Rept. 24:13-14.

6. Maynard, D. N. and Allen V. Barker. 1969. Studies on the tolerance of plants to ammonium nutrition. J. Amer. Soc. Hort. Sci. 94:235-239.

7. _____ and _____. 1970. Studies on the ammonium tolerance of some cultivated Solanaceae. J. Amer. Soc. Hort. Sci. 95:345-348.

8. _____, _____, and W. H. Lachman. 1966. Ammonium-induced stem and leaf lesions of tomato plants. Proc. Amer. Soc. Hort. Sci. 88:516-520.

9. _____, _____, and _____. 1966. Variation among tomato lines with respect to ammonium tolerance. HortScience 1:17-18.

10. Pontecorvo, G. and J. A. Roper. 1956. Resolving power of genetic analysis. Nature 178:83-84.

11. Steel, R. G. O. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw Hill Book Company, Inc., New Hork.

12. Stubbe, H. 1957. Mutanten der Kulturtomate Lycopersicon esculentum Miller I. Kulturpfl. 5:190-220.

PART II

TITLE: A STUDY OF THE EFFECT OF GRAFTING ON EXPRESSION OF AMMONIUM TOXICITY SYMPTOMS IN THE TOMATO.

ABSTRACT: Reciprocal grafts between V. R. Valiant, a cultivar susceptible to ammonium injury, and the neglecta-1 mutant, a line tolerant of ammonium, revealed that the root by itself does not appear to afford ammonium tolerance.

INTRODUCTION

Severe necrotic lesions commonly develop on the stems, petioles, and laminae of tomato (Lycopersicon esculentum Mill.) plants grown in soil fertilized heavily with ammonium (4). A continuum of response has been shown to exist among tomato lines tested from extreme susceptibility to high tolerance (5). A significant negative correlation between symptom development and potassium concentration in stem tissue has been found (1,6).

Reciprocal grafts between two soybean lines, one efficient and the other inefficient at uptake and translocation of iron, showed that the efficient rootstock was able to supply the scion from an inefficient plant with adequate iron. However, an inefficient rootstock caused an iron deficiency in the efficient scion (2). The present study was undertaken to determine if a similar relationship exists with respect to ammonium toxicity expression in the tomato.

MATERIALS AND METHODS

The cultivar V. R. Valiant has been shown to be highly susceptible to ammonium toxicity (3). The mutant stock neglecta-1 (neg-1) is known to be tolerant of ammonium fertilization (3). The neg-1 mutant was obtained by Stubbe from irradiated seed of the cultivar Condine Red (7). Stubbe characterized the mutant as being a small, weakly branched plant with leaves becoming progressively necrotic and prematurely dropping.

Reciprocal saddle grafts were made between V. R. Valiant and neg-1 eight weeks from seed sowing. V. R. Valiant scions were grafted to V. R. Valiant rootstocks also. The graft was made high enough on the

stem of the stock to allow three leaves to remain on the rootstock. Grafted and ungrafted plants were placed in a humidity chamber until graft unions were secure.

All plants were shifted to 12.7 cm clay pots following removal from the humidity chamber. The growing medium consisted of soil, peat, and sand in the ratio 7:3:2 by volume. An addition of 56.7 g of 20% superphosphate and 56.7 g of magnesian limestone was made to each 0.036 cu. m of medium.

Treatment with 0.04 N $(NH_4)_2SO_4$ at the rate of 100 ml/pot/day for five consecutive days of every seven day period was initiated when plants were eleven weeks old. The treatment was terminated after three weeks, and lesion development was observed.

RESULTS AND DISCUSSION

Table 1 shows the results obtained using reciprocal grafts. As expected, lesion development was severe on the ungrafted plants of V. R. Valiant and symptoms failed to appear on the neg-1 plants. Not surprisingly, lesions developed on the grafted plants having V. R. Valiant as both stock and scion.

Contrary to expectations, with V. R. Valiant as the rootstock and neg-1 as the scion, no lesions were present on either the stock or the scion. When the rootstock was neg-1 and the scion was V. R. Valiant, the stock remained lesionless, but the scion developed severe lesions. These results contraindicate a simple mechanism in the root that governs response to ammonium fertilization.

Table 1. The effect of grafting on expression of NH_4^+ toxicity symptoms.

Number Observed	Rootstock	Scion	Observation
3	V. R. Valiant	V. R. Valiant	lesions on stock and scion
5	V. R. Valiant	neglecta-1	no lesions
3	neglecta-1	V. R. Valiant	lesions on scion only
3	V. R. Valiant	(ungrafted)	lesions
3	neglecta-1	(ungrafted)	no lesions

The brittle stem (btl) mutant of the tomato has been shown to lack the ability to adequately mobilize boron to the stems of rapidly growing plants (8). A possible explanation for the results obtained in the present investigation is that a substance is translocated basipetally from neg-1 leaves or stems to the roots. This substance may allow for greater mobility of K^+ in the presence of excess ammonium. The lack of this hypothesized substance in V. R. Valiant scions may cause ammonium injury to occur. A determination of the K^+ concentration in roots versus stems of neg-1 and V. R. Valiant plants might provide evidence to test this hypothesis.

LITERATURE CITED

1. Barker, A. V. 1974. (Unpublished data).

2. Brown, J. C., R. S. Holms, and L. O. Tiffin. 1958. Iron chlorosis in soybeans as related to genotype of rootstock. Soil Sci. 86:75-82.

3. Lachman, W. H. and A. V. Barker. 1973. Tolerance of some tomato mutants to ammonium fertilization. Tomato Genet. Coop. Rept. 23:22-23.

4. Maynard, D. N., A. V. Barker, and W. H. Lachman. 1966. Ammonium-induced stem and leaf lesions of tomato plants. Proc. Amer. Soc. Hort. Sci. 88:516-520.

5. _____, _____, and _____. 1966. Variation among tomato lines with respect to ammonium tolerance. HortScience 1:17-18.

6. Steel, R. G. O. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw Hill Book Company, Inc., New York.

7. Stubbe, H. 1957. Mutanten der Kulturtomate Lycopersicon esculentum Miller I. Kulturpfl. 5:190-220.

8. Wall, J. R. and C. F. Andrus. 1962. The inheritance and physiology of boron response in tomato. Amer. J. Bot. 49:758-762.

PART III

TITLE: LINKAGE RELATIONS IN THE INDEHISCENS MUTANT OF THE TOMATO.

ABSTRACT: Assignment of the gene indehiscens (id) to linkage group 11 is confirmed. The locus of id is established at 74.4 on chromosome 11.

INTRODUCTION

The indehiscens (*id*) mutant of the tomato (*Lycopersicon esculentum* Mill.) was described by Stubbe, who reported its appearance in a population grown from irradiated seed of the cultivar Rhinelands Ruhm (3). Stubbe characterized the mutant as having broad leaf segments, flowers with connate sepals, and irregularly cracked fruit.

Stubbe grew an F_2 population which segregated 119 normal to 39 mutant phenotypes, establishing *id* as a monogenic recessive trait. Hansen, Rick, and Boynton assigned *id* to linkage group 11 (2). Their data indicate that *id* is linked with anthocyaninless-1 (a_1) on chromosome 11.

METHODS

The three-point testcross method was used to confirm the results of Hansen *et al*. (1) and to determine the locus of *id* on chromosome 11.

The compound recessive was constructed by crossing Stubbe's *id* stock to Rick's chromosome 11 tester stock jointless-1, hairless, and anthocyaninless-1 (j_1 *hl* a_1), and selecting in the F_2 generation. Plants of the *hl* a_1 *id* phenotype were crossed by Denby's V. R. Valiant, a cultivar homozygous for the normal type. The F_1 generation of this cross was testcrossed to the triple recessive. The segregating generation of plants was grown in the greenhouse in 7.6 cm peat pots. Plants in the four *hl a* classes were identified in the seedling stage, and final classification for each plant was completed at the flowering stage.

RESULTS AND DISCUSSION

Figure 1 shows the connate sepals characteristic of the mutant id. The fusion of the calyx segments is apparent.

Figure 2 illustrates the appearance of id on a mature fruit. The pressure of the developing berry has longitudinally severed the fused calyx in three places. Cracking of the fruit is not evident.

Table 1 gives the crossover data from which the segment of chromosome 11 can be represented as in Figure 3. Crossing over between the tester loci hl and a_1 is found to be 20.7% in this testcross, corresponding well with the standard hl-a_1 distance of 20 units (1). The data establishes id at locus 74.4 on chromosome 11. Accordingly, id should show tight linkage with mps located at locus 74 (1).

Figure 1. Flowers of the mutant indehiscens on the left with a normal flower on the right.

Figure 2. Mature fruit of the mutant indehiscens on the left with a normal fruit on the right.

Table 1. Summary of id three-point testcross data.

				Number Observed	Crossover Totals	% Crossovers
Parental Types	+	+	+	282		
	hl	a_1	id	248		
Region I Crossovers	+	a_1	id	88	173	20.74
	hl	+	+	71		
Region II Crossovers	+	+	id	45	145	17.39
	hl	a_1	+	86		
Double Crossovers	hl	+	id	12	14	1.67
	+	a_1	+	2		
			TOTAL	834		

* Total of single and double crossover types.

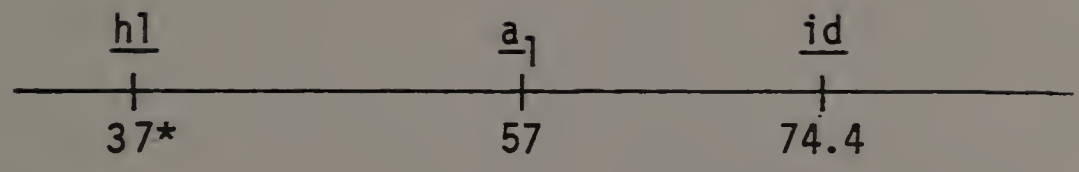

Figure 3. Diagrammatic section of chromosome 11 of Lycopersicon esculentum illustrating the suggested position of id; asterisk indicates distance from Cf_3.

LITERATURE CITED

1. Anonymous. 1971. Interim report of the linkage committee. Tomato Genet. Coop. Rept. 21:10-11.

2. Hansen, D. C., C. M. Rick, and J. E. Boynton. 1962. Linkage tests with mutants of Stubbe group III. Tomato Genet. Coop. Rept. 12:28-29.

3. Stubbe, H. 1959. Mutanten der Kulturtomate Lycopersicon esculentum Mill. III. Kulturpfl. 7:82-112.

PART IV

TITLE: A STUDY INVOLVING THE ALBO-PUNCTATA MUTANT OF THE TOMATO.

ABSTRACT: A three-point testcross was made in an attempt to determine the linkage relations of the gene albo-punctata (apn). The chromosome 11 tester genes hairless (hl) and anthocyaninless-1 (a_1) showed crossing over of approximately 50%, indicative of a chromosomal aberration. Pollen grains were examined, and pollen mother cells were studied to detect possible abnormalities, but nothing unusual was observed. The position of the apn locus remains uncertain.

INTRODUCTION

The albo-punctata (*apn*) mutant of the tomato (*Lycopersicon esculentum* Mill.) was obtained from EMS (ethyl methanesulfonate) treatment of the highly inbred line VF 36 (3). The *apn* mutant is characterized by a fine white speckling spread evenly over seedling leaves, diminishing in later leaves. Mature plants have reduced, wilty leaves and greatly reduced flower size. Preliminary linkage results place *apn* on chromosome 11 (3).

METHODS

The three-point testcross method was used to confirm the assignment of *apn* to linkage group 11 and to better define the locus of *apn* on chromosome 11.

The compound recessive was constructed by crossing the *apn* stock to Rick's chromosome 11 tester stock jointless-1, hairless, and anthocyaninless-1 (j_1 *hl* a_1) and selecting in the F_2 generation. A plant of the *apn hl* a_1 genotype was crossed by Denby's V. R. Valiant, a cultivar homozygous for the normal type. The F_1 of this cross was testcrossed by the triple recessive. The segregating population of plants was classified in the seedling stage.

Pollen analysis of the heterozygote *apn hl* a_1/+ + + was performed using acetocarmine and cotton blue stains and examining under the microscope.

Pollen mother cells were examined using acetocarmine and Feulgen methods (2).

RESULTS AND DISCUSSION

Table 1 shows the crossover data. The crossovers between the two tester loci hl and a_1 of 50% is evidence of a chromosomal aberration. The standard hl-a_1 distance is 20 crossover units (1). An inversion within chromosome 11 or a reciprocal translocation are two possible explanations for the crossover descrepancy (4,5).

Pollen analysis revealed no abnormalities in pollen appearance. Cytological study of pollen mother cells at pachytene and metaphase I of meiosis did not show any inversion or translocation figures. It is possible that a translocation complex or inversion figure was present but not observed.

Table 1. Summary of apn three-point testcross data.

				Number Observed	Crossover Totals	% Crossovers
parental types	+	+	+	448		
	apn	hl	a_1	399		
Region I Crossovers	apn	+	+	28	129*	7.08
	+	hl	a_1	34		
Region II Crossovers	+	+	a_1	509	914*	50.14
	apn	hl	+	338		
Double Crossover	+	hl	+	31	67	3.68
	apn	+	a_1	36		
			Total	1823		

* Total of single and double crossover types.

Opena et al. (2) obtained an apn-hl crossover distance of 12.5 and an apn-a_1 distance of 39.5. Their data would place apn to the left of hl on chromosome 11 at the uncertain position of 21, based on an apn-hl distance of 16 —— the mean of 12.5 and 19.5 (39.5 minus the standard hl-a_1 distance of 20 units).

The apn-hl distance of about 7 units obtained in the present study is inconsistent with the preliminary results of Opena et al. The present study is based on an observed population of about four times that of preliminary linkage work.

The apn study must be repeated from the beginning, reuniting apn with a normal tester complement and completing the three-point testcross. A more intensive cytological study may also elucidate the possible aberration in the chromosome complement used in the present testcross.

LITERATURE CITED

1. Anonymous. 1971. Interim report of the linkage committee. Tomato Genet. Coop. Rept. 21:10-11.

2. Klekowski, Edward. 1975. (Personal communication).

3. Opena, R. T., F. Borgnino, R. W. Zobel, and C. M. Rick. 1972. Segregation and linkage relations of 4 EMS mutants. Tomato Genet. Coop. Rept. 22:18-20.

4. Rick, C. M. 1974. (Personal communication).

5. Swanson, C. P. 1974. (Personal communication).