DEVELOPMENTAL CHANGES IN NITROGEN AND PROTEIN OF TALL AND DWARF TOMATO SEEDLINGS, *LYCOPERSICON ESCULENTUM* MILL.

by

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I would like to especially thank my wife, Lisa, to whom this thesis is dedicated, for her loyalty, patience and understanding.

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INTRODUCTION

Dwarf and tall tomato plants differ in their morphology and growth rates (Houghtaling, 1940; Bindloss, 1942; Figs. 1, 2 and 3). The dwarf seedlings are characterized by shorter, thicker hypocotyls and darker green cotyledons than the normal (tall) seedlings. In older plants, the dwarf leaves are darker green, smaller and have puckered rugose laminae, in contrast to the lighter green, larger and smoother laminae of the tall type (Rick and Butler, 1956). Genetically, the dwarf gene (d_1) is recessive to the tall gene (D_1) and segregates in the F_2-generation as 3 tall to 1 dwarf (Young and MacArthur, 1947).

Although distinct genetic and morphological characteristics are recognized between the tall and dwarf types, the underlying chemical differences between them are not understood. Bindloss (1942) suggested that endogenous auxins may differ between the tall and dwarf tomatoes, but recent studies (Plummer and Tomes, 1958; Brown *et al.*, 1968; Aung and Byrne, 1976) gave evidence that auxins play a lesser role in tomato shoot elongation.

Nitrogen and protein are important determinants of the metabolic processes of growth (Clark, 1936; Nightingale, 1937; Chibnall, 1939; McKee, 1949; Steward and Bidwell, 1962), and the contents of these substances in relation to growth of tall tomatoes have been investigated (Gates, 1957; Hall and Cocking, 1966; Bora and Selman, 1969), but the changes of these substances in

the dwarf tomato have not been studied. It is conceivable, however, that differences between the tall and dwarf tomatoes may be related to their endogenous nitrogen and protein contents, or in the manner in which these substances are utilized by the two types.

The purpose of this study is to determine the changes in total nitrogen and soluble protein contents of the leaves, hypocotyl and roots of tall and dwarf tomatoes during early seedling development.

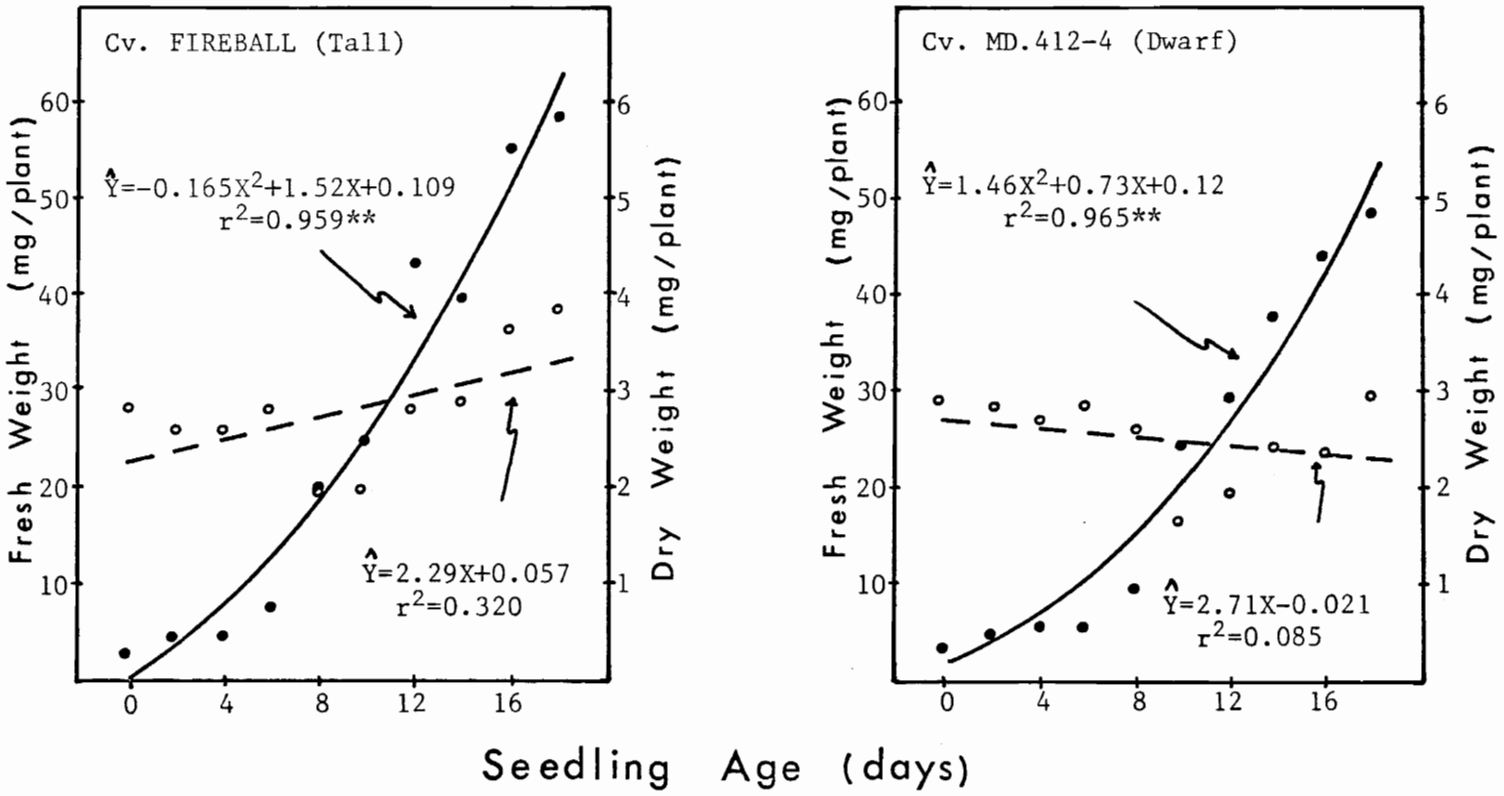

Fig. 1. Changes in fresh and dry weights of tall and dwarf tomatoes during early seedling development. Fresh weight, (●—●); dry weight, (o---o). (**) denotes significant correlation at the 1% level of probability.

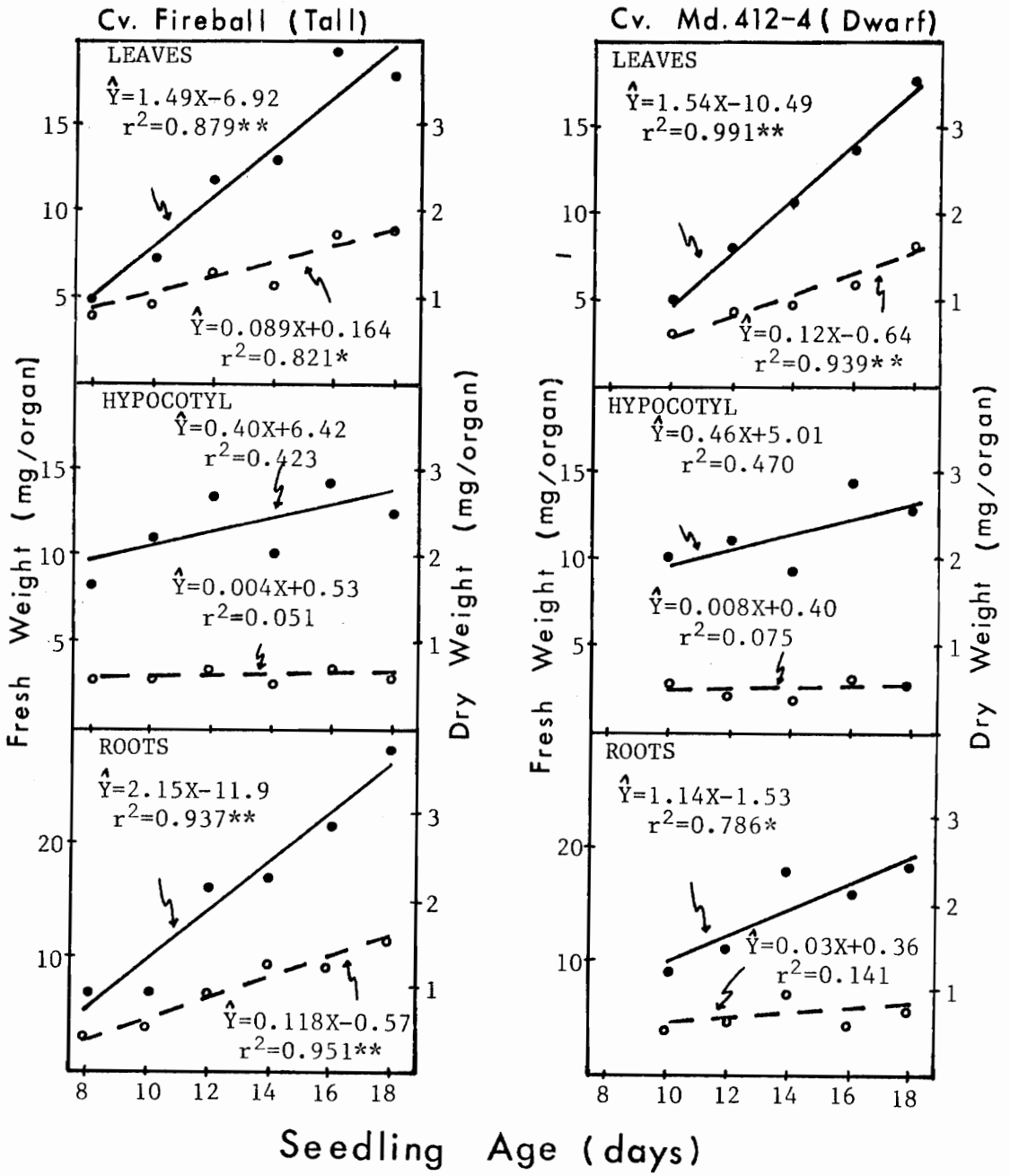

Fig. 2. Fresh and dry weight changes of the roots, hypocotyl and leaves of tall and dwarf tomatoes during development. Fresh weight, (●—●); dry weight (o---o). (*) and (**) denote significant correlation at the 5% and 1% levels of probability, respectively.

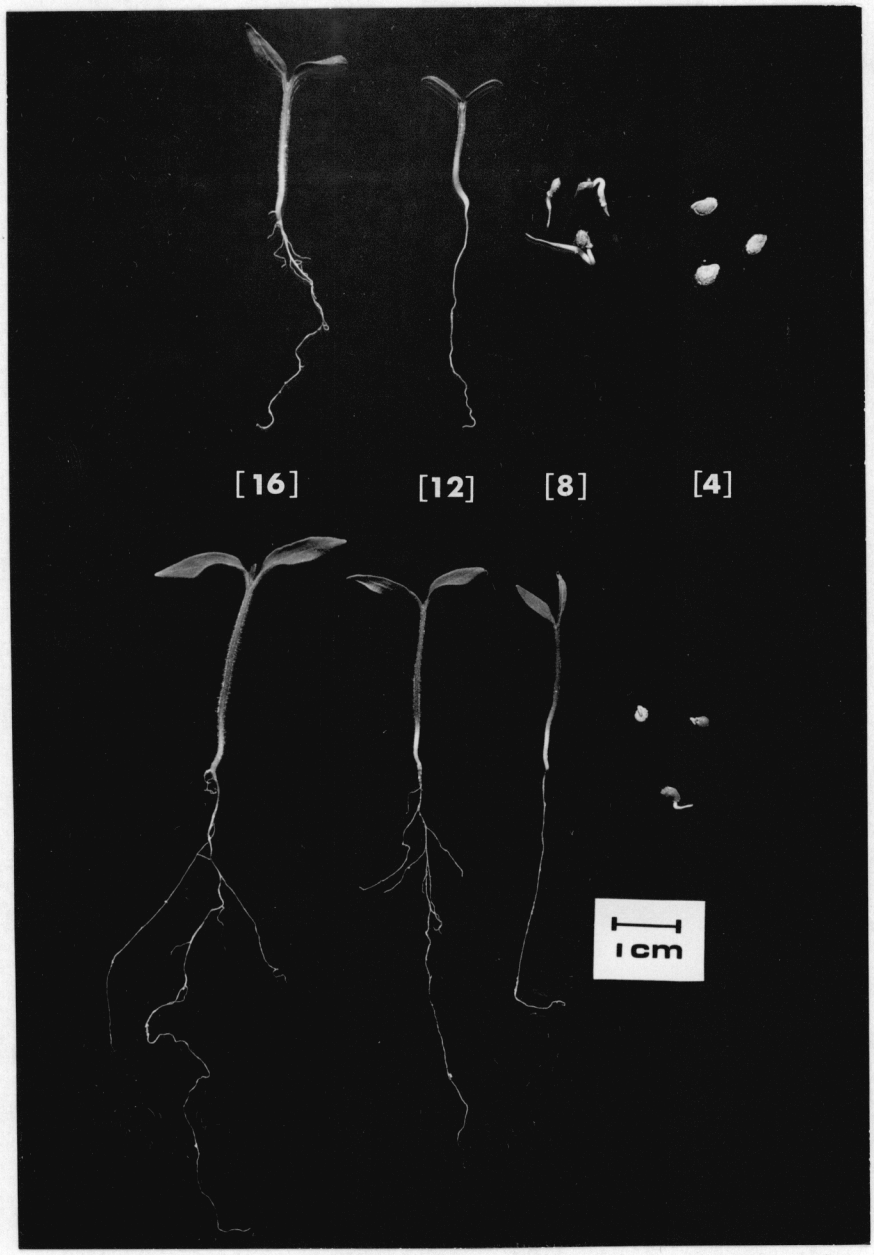

Fig. 3. Dwarf tomato cv. 'Md. 412-4' (Top) and tall cv. 'Fireball' (Bottom) during 4 stages of development. Age (days) of seedlings indicated in parentheses.

LITERATURE REVIEW

The common tomato belongs to the species *esculentum* and the genus *Lycopersicon* (Muller, 1940). Within the genus, many economically important species exist. In the species *esculentum*, numerous cultivars with differing growth habits are recognized (Luckwill, 1943). The plant is mainly grown for its fruit. From a biological standpoint, however, the tomato is a useful test plant for many developmental and mineral nutrition studies (Kraus and Kraybill, 1918; Hoagland and Arnon, 1950).

Factors affecting the absorption and assimilation of nitrogen- The tomato plant has been used extensively as an experimental object in nutritional studies. Clark and Shive (1934) found 'Marglobe' tomato seedlings grown in nutrient solution absorbed ammonium nitrogen (NH_4-N) faster at pH 7 than at pH 4, whereas the converse was found for the uptake of nitrate nitrogen (NO_3-N). However, in older plants pH exercised a lesser influence on the absorption of these ions. In these plants, NO_3-N absorption rates increased while those of NH_4-N decreased.

Arrington and Shive (1935) also reported that the absorption of NO_3-N and NH_4-N from nutrient solutions varied significantly in tomato seedlings at different stages of development.

In addition to pH and seedling age, the aeration of the nutrient solution was found to be an important factor in determining nitrogen uptake. Arrington and Shive (1936) found

that vigorously growing plants rapidly depleted the oxygen supply in non-aerated nutrient solutions with a concomitant decrease in NH_4-N and NO_3-N absorption.

The chemical composition of the tomato plant can be greatly altered by nutrition. Clark (1936) reported that the leaves of tomato plants grown in NH_4-N were higher in total-N and soluble-N than the leaves of NO_3-N plants. In contrast, the stems and roots of NH_4-N fed plants were lower in total-N and soluble-N than those fed with NO_3-N. It was also observed that the NH_4-N plants were higher in amino-N but lower in organic acids than the NO_3-N fed plants. Margolis (1960) found N-deficient tomato plants were low in soluble-N, glutamine and asparagine. Upon addition of NO_3-N, soluble-N, glutamine, glutamic and aspartic acids were increased. When NH_4-N was supplied, the N-deficient plants showed a large increase in glutamine.

Similarly, Hoff *et al.* (1974) observed a large increase in glutamic and aspartic acids in the roots of NH_4-N grown tomato plants and suggested that the roots were the main sites of synthesis of these amino acids under NH_4-N nutrition.

Woolhouse and Hardwick (1966), on the other hand, found that NH_4-N grown tomato plants had a lower content of potassium in the leaves, stems and roots than the NO_3-N grown plants. Kirkby and Mengel (1967) reported that tomato plants maintained their cationic and anionic balance (1:1) under NH_4-N or NO_3-N nutrition. The balance was maintained by a removal of anions under NO_3-N nutrition,

and a removal of cations under NH_4-N nutrition from the roots.

Environmental factors are also important in determining the absorption and assimilation of nitrogen by tomato plants. Nightingale (1933) found that nitrate absorption was very rapid in tomato plants grown at 13°, 21° or 35° C. However, plants grown at 13° C had a low nitrate assimilation rate and a high carbohydrate content. At 21° C the plants assimilated NO_3 rapidly and contained less carbohydrates than plants grown at 13° C, The growth of these plants was vigorous and an abundance of nitrogenous compounds was seen. At 35° C NO_3 absorption was rapid and the carbohydrate content was low. The rapid depletion of carbohydrates was partly due to increased respiration.

Tomato plants grown under low humidity in controlled chambers were higher in carbohydrates than plants grown at high humidity. When NO_3-N was supplied to plants grown at low humidity the content of protein was increased, while the carbohydrate content decreased. In contrast, addition of NO_3-N to plants grown under high humidity resulted in an increase of amino acids and amides, but the protein content was low (Nightingale and Mitchell, 1934).

Gates (1957) reported that the uptake of nitrogen and phosphorus by tomato plants was impaired by a short period of moisture stress. Under moisture stress, phosphorus and nitrogen were translocated from the leaves to the stem. Upon rewatering, however, nitrogen and phosphorus were translocated to the leaves from the stem and the uptake of both nitrogen and phosphorus was also increased.

Losses of nitrogen from plants- In studies on nitrogen metabolism, the uptake of nitrogen or nitrogenous compounds is generally stressed. However, the loss of these substances from plants also occurs (Pearsall and Billimoria, 1937; Rovira, 1969). In several tomato studies, root exudation of amino acids and sugars was found to be influenced by plant age, temperature, light and plant nutrition. Subba-Rao *et al.* (1962) reported that the tomato cultivar 'Bonney Best' released more exudates than the cultivar 'Geneva II.' Went (1944) found that nitrogen losses occurred from tomato plants by guttation.

Nitrate reduction- The NO_3-N absorbed by tomato plant roots must be reduced before assimilation into organic nitrogenous constituents. Eckerson (1924) has shown that juices extracted from the leaves of 'Bonney Best' tomato plants were capable of reducing NO_3-N. Later work (1932) showed that 'reducase activity' (now known as 'reductase') was present in both the tops and roots of the tomato. Sanderson and Cocking (1964a) confirmed the findings of Eckerson, and reported that light increased nitrate reductase activity in tomatoes. Subsequently Sanderson and Cocking (1964b) found that a nitrite reductase system also operates in the tomato. Thus an enzyme system exists in the tomato for the reduction of nitrates to nitrites and thence to ammonia, which can be combined with carbohydrates to form organic nitrogenous compounds.

The role of carbohydrates in nitrogen metabolism- Kraus and Kraybill (1918) demonstrated the importance of nitrogen and carbohydrates in the growth and reproduction of tomato plants. They

found that growth and fruitfulness were associated with a balance of nitrogen and carbohydrates. With an abundance of available nitrogen the tomato plants were strongly vegetative and unfruitful, higher in moisture, total nitrogen and NO_3-N and low in carbohydrates. However, when plants were grown in low nitrogen, the plants were weakly and also unfruitful. They were lower in moisture, total nitrogen and NO_3-N but were higher in total carbohydrates than plants supplied with adequate N.

Nightingale *et al.* (1928) also reported that tomato plants were vegetative when the content of amino acids and soluble nitrogen was high, and carbohydrates were low. However, when the content of soluble nitrogenous compounds and carbohydrates were moderate the plants were fruitful.

Amide formation in plants requires carbohydrates and NH_4-N. Chibnall (1939) indicated that the amides asparagine and glutamine were synthesized from carbon skeletons and NH_4-N. MacVicar and Burris (1948) showed that NH_4-N as $(^{15}NH_4)_2SO_4$ was rapidly used for amides formation in the roots of 6-week-old tomato seedlings. High amounts (8%) of the labeled NH_4-N were found in glutamine and asparagine of root tissue.

Protein metabolism- There are many reviews in the past and current literature concerning plant proteins (Vickery, 1945; Pirie, 1959; Stahman, 1963) and protein synthesis (Chibnall, 1939; Petrie, 1943; Zalik and Jones, 1973) but there are relatively few specific studies on the proteins of tomatoes.

Johns and Gersdorff (1922) reported that α- and β-

globulins were present in tomato seed-cake and found the protein content (N x 6.25) of the seed to be 36.9%. Protein extraction by a 4% sodium chloride solution yielded 21% protein which constituted 57% of the total seed protein. Evans and Alldridge (1965) extracted peroxidases from mature tissues of both dwarf and normal tomato plant. Peroxidase activity was found to be three times higher in the pith, cortex, and leaves of the dwarf than in the same tissues of the normal (tall) plant.

Ulrich *et al.* (1964) reported that tomato plants were unable to assimilate high molecular weight proteins. However, when a mixture of amino acids was supplied to the roots in soil cultures, the plants were only able to assimilate the amino acids to a limited extent.

Hall and Cocking (1966) found that the rate of protein synthesis in the tomato leaves was higher than the rate of synthesis in the cotyledons. It was further observed that the leaves of light-grown seedlings incorporated greater amounts of amino acids into protein.

Davies and Cocking (1967) also found that plastids isolated from tomato fruit locular tissues possessed the ability to synthesize protein.

Hormonal responses of tall and dwarf tomatoes- Aung (1974) found that tall and dwarf tomatoes responded differentially to different concentrations of abscisic acid (ABA). Apical applications of 10^{-8} M ABA significantly promoted the growth of

the tall cultivar 'Fireball', but not the dwarf cultivar 'Epoch'. Aung and Bryan (1974) have shown that the tall and dwarf tomato seedlings contained similar amounts of total gibberellins, but 93% of the gibberellin of the dwarf resided in the basic fraction while 64% of the tall gibberellins resided in the bound fraction. It was also noted that the tall plants had several more GA components than the dwarf.

Bora and Selman (1968) observed that exogenous GA caused significant growth increases of the roots, stems, and leaves of the tall tomato under adequate nitrogen nutrition. In contrast, Rajagopal and Rao (1974) found nitrogen deficient 'Marglobe' tomato plants contained lower gibberellin-like and auxin-like activities than non-deficient nitrogen controls.

MATERIALS AND METHODS

Plant material- Seeds of the tomato (*Lycopersicon esculentum* Mill.) cultivars 'Fireball' (tall) and 'Md. 412-4' (dwarf) were sown in plastic trays (22 x 15 x 5 cm) containing a low nutrient medium of vermiculite and fine white sand (1:1 v/v). They were germinated in a growth chamber at 15 ± 2^{o} C night and 25 ± 2^{o} C day temperature. The 12-hour photoperiod was provided by a mixture of fluorescent and incandescent lamps with a radiant energy flux of 109 micro-einsteins/m^2/ sec PAR[1]. Relative humidity was 45% in the day and 60% at night. Seedlings were watered with tap water.

Seedlings were harvested at 2-day intervals from 0 to 18 days. Sand was washed from the roots with tap water and the seedlings were blotted lightly, separated into roots, hypocotyls and leaves, and their fresh weights determined accurately to $\pm$ 2 mg. Samples were then placed in small vials (10 organs/vial) and frozen at -15^{o} C. Samples at 0-day age consisted only of dry (unimbibed) seeds.

Samples of seedlings which had not grown sufficiently were not separated into roots, hypocotyls and leaves for chemical analyses. When dry weights were determined, samples were harvested at 1-day intervals, dried in a forced draft oven at 80^{o} C for 24

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[1]PAR refers to the photosynthetic active radiation, 400 nm-700 nm.

hours and weighed accurately to $\pm$ 0.05 mg.

Nitrogen determination- Kjeldahl nitrogen was determined using a modified micro-Kjeldahl direct-Nesslerization technique adapted from the methods of Thompson and Morrison (1951), Yuen and Pollard (1952), Middleton (1960) and Nelson and Sommers (1973).

Briefly, the modified method was as follows:

Samples (3-35 mg dry wt., or 30-600 mg fresh wt.) were placed in thick-walled test tubes (150 x 18 mm) and 1 ml of concentrated sulfuric acid added. The tubes were capped with a marble and allowed to reflux in a nitrogen furnace at 260° C for 90 minutes. After cooling the digested samples, 0.5 ml of 50% hydrogen peroxide was added and the samples were then reheated to 200° C for 5 minutes.

If the samples remained cloudy after this treatment, addition of hydrogen peroxide was continued at 0.1 ml increments until clear samples resulted. The pH of the samples was adjusted to pH 9 with sodium hydroxide and appropriate dilutions were made before Nesslerization.

The Nesslerization reaction mixture consisted of 2 ml of digested sample to which 0.8 ml of fresh gum arabic was added. The gum arabic was prepared by dissolving 1 g gum arabic in 50 ml of cold water and adding 1 ml of Nessler's reagent.[2] The solution was then filtered through glass wool, made up to 70 ml

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[2]Fisher Scientific Co.

with water and centrifuged for 10 minutes at 900 x g in a IEC centrifuge. Two ml of 10% sodium potassium tartrate (w/v) was then added to the reaction mixture with shaking. After the addition of 4 ml of Nessler's reagent the mixture was mixed thoroughly and allowed to stand at room temperature (21° C) for 30 minutes and the absorbance read at 435 nm on a Spectronic 20 spectrophotometer. Nitrogen was calculated from a calibration curve using ammonium sulfate as a standard.

Protein extraction- Samples of frozen root, hypocotyl and leaf tissues of varying fresh weights (30-600 mg) and ages were used for protein extraction. Each of the samples was homogenized with 10 ml of cold Tris[3] buffer in a glass-to-glass tissue grinder for one minute. All samples were maintained at 5±3° C during extraction. The homogenates were rinsed into 50 ml centrifuge tubes with 10 ml of Tris buffer and centrifuged. Seed samples were similarly treated except that they were ground up using a chilled mortar and pestle.

The homogenized samples were centrifuged at 48,300 x g for 30 minutes at 5° C using a Beckman J-21 refrigerated centrifuge. After centrifugation the supernatant fraction was decanted and the protein in it precipitated by the addition of trichloroacetic acid (TCA) to 10% (v/v). The resultant pellet obtained following centrifugation was designated protein pellet I. The remaining

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[3]Tris (hydroxymethyl) aminomethane, pH 7.5, 0.04 M.

supernatant of this centrifugation was again precipitated with TCA to 20% (v/v), centrifuged and the pellet obtained designated as protein pellet II. The original pellet containing cellular debris was suspended in 20 ml of Tris buffer and centrifuged. The protein of the supernatant was precipitated with TCA (10% v/v) and the resultant pellet obtained after centrifugation was designated as protein pellet III. The supernatant was then discarded. The protein extraction procedure which is a modification of the method used by Smillie and Krotkov (1961), is shown in fig. 4.

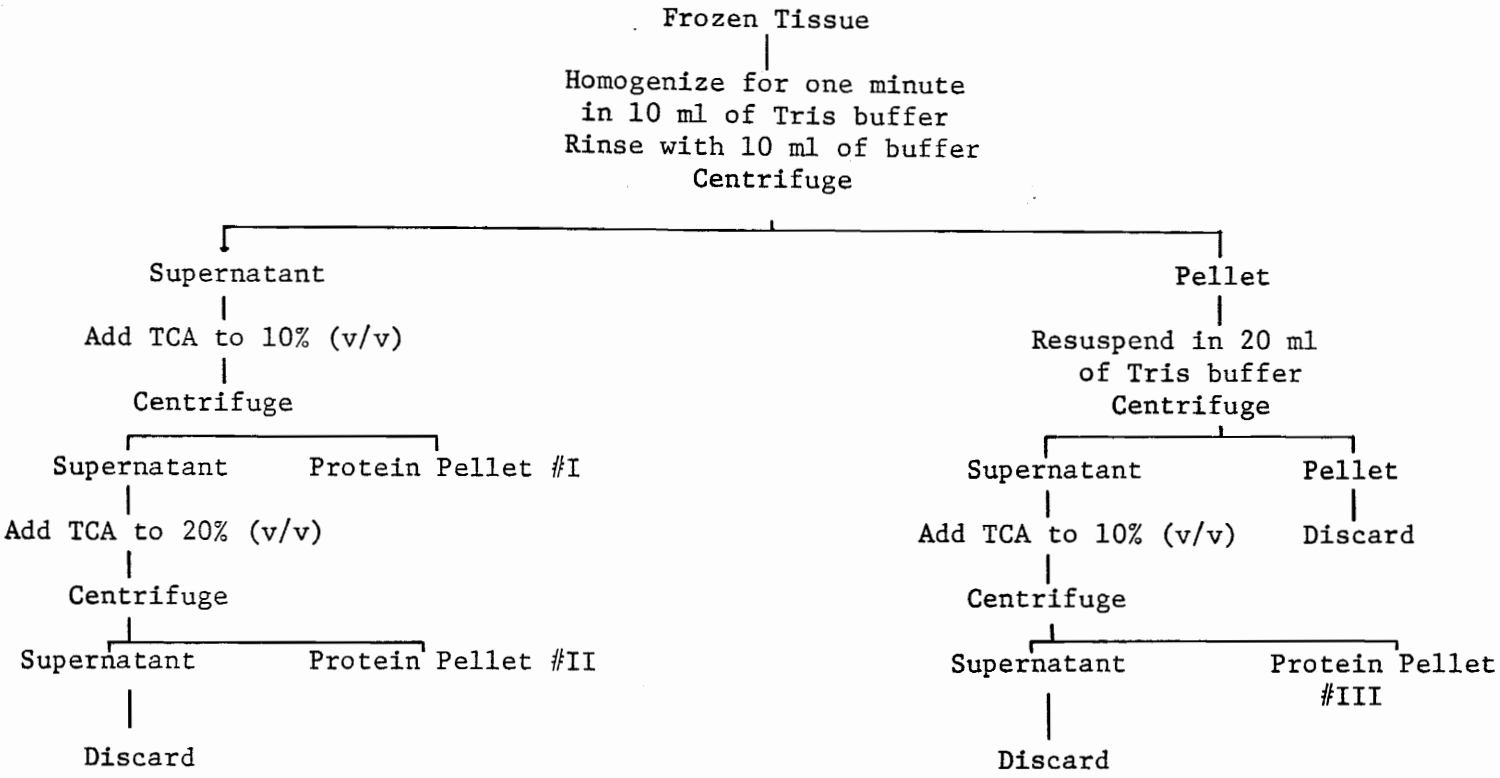

Fig. 4. Flow-chart of protein extraction procedure used in the determination of tomato protein.

Protein determination- The method of Lowry *et al.* (1951) was used for determining the soluble protein of the tomato samples. 0.5 ml of 1 N NaOH was added to each centrifuge tube containing 5-100 µg of precipitated protein. The mixture was stirred and placed in a water bath (30° C) for 30 minutes. For samples containing more than 100 µg of protein, aliquots were taken after appropriate dilutions.

Reagents:

A. 2% Sodium carbonate ($NaCO_3$)

B. 0.5% Cupric sulfate ($CuSO_4 \cdot 5H_2O$) in 1% sodium citrate[1]

C. 50 ml Reagent A plus 1 ml Reagent B

D. Folin Phenol Reagent diluted 1:2 with distilled water (Fisher Scientific Co.)

Reagent C (5 ml) was added to the tube containing the dissolved protein and the mixture was stirred and allowed to react for 10 minutes at room temperature.

After the 10 minutes, Reagent D (0.5 ml) was added while mixing on a rotary mixer.

After the 30 minutes, the absorbance was read on a Spectronic 20 spectrophotometer at 750 nm. Protein was determined from a calibration curve using bovine serum albumin (Sigma Chemical Co., St. Louis) as a standard.

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[1]Sodium citrate is used in place of sodium potassium tartrate, (Bailey, 1962) to give a more stable reagent.

Statistics and curve fitting- Analysis of variance was made on all data using a completely random design. Duncan's Multiple Range test was used to determine significant differences between ages within a cultivar. Significant differences between cultivars within an age were determined by t-tests (Snedecor and Cochran, 1967; Steel and Torrie, 1960).

Regression lines, predicted equations and correlation coefficients were determined by a computer using the methods of Service (1972).

RESULTS

Total Nitrogen (N)- In a preliminary experiment, based on limited plant samples, the concentration of N (mg/g fresh wt.) of 10-18 day old dwarf plants was shown to be greater than that of the tall plants (Appendix, Table I). Subsequently, more thorough experiments, employing a greater number of plant samples, showed that the early results were not reproducible (Table II). The N concentration of the seedlings, which ranged in age from 0-18 days, and their organs (roots, hypocotyl and leaves) did not differ significantly between the tall and dwarf tomatoes. In both cultivars, nitrogen declined sharply with seedling development, but the rate of decline was faster in the tall than the dwarf. The content of N per plant was significantly higher in the tall compared to the dwarf. The N content of the two types, however, did not change appreciably during seedling development (Figs. 5, 6, 7, Table III.)

On a unit dry weight basis, the N concentration of the tall plants was significantly higher than the dwarf plants only during early seedling development, but not at later stages of growth. The N contents in the roots, hypocotyl and leaves of the tall tomato were significantly greater than those of the dwarf (Table IV). During seedling development, the nitrogen of the cultivars decreased, and the rate of decrease was much greater in the tall tomato than in the dwarf (Fig. 8). On the other hand, the tall tomato utilized nitrogen more efficiently in the production of fresh weight than

did the dwarf (Fig. 9). The decline in N of the two cultivars coincided with an increase in the rate of development of both the tall and dwarf tomatoes. The dwarf, however, developed at a slower rate. The rates of decline in N of the roots, hypocotyl and leaves of the tall plants were also greater than corresponding organs of the dwarf plants. Although the rates of dry weight accumulation were quite similar, the total amounts of dry weight were generally higher in the tall than in the dwarf plants (Fig. 10).

Table II. Total nitrogen (mg/g fresh wt.) in the organs of tall and dwarf tomatoes during seedling development.[1]

Cultivar	Seedling Age (Days)					Total[2]	Interaction
	10	12	14	16	18		
				Total			
Fireball	11.6	9.0	7.8	6.9	6.1	8.3	ns
Md. 412-4	11.4	9.4	7.1	6.9	6.2	8.2	
				Leaves			
Fireball	7.7	5.7	5.0	4.2**	3.9	5.3	ns
Md. 412-4	7.7	6.4	4.7	4.7	4.1	5.5	
				Hypocotyls			
Fireball	1.6	1.1	1.0	1.0	0.8	1.1	ns
Md. 412-4	1.6	1.3	1.0	0.8	0.8	1.1	
				Roots			
Fireball	2.3	2.2	1.8	1.7	1.4	1.9	ns
Md. 412-4	2.1	1.7	1.4	1.4	1.3	1.6	

[1]March 1974 experiments. Values represent duplicate analyses of each of 3 samples containing 10 organs each.

[2]Differences between cultivars not significant (ns) at the 5% level of probability.

**Denotes significant differences between cultivars at a particular age at the 1% level of probability.

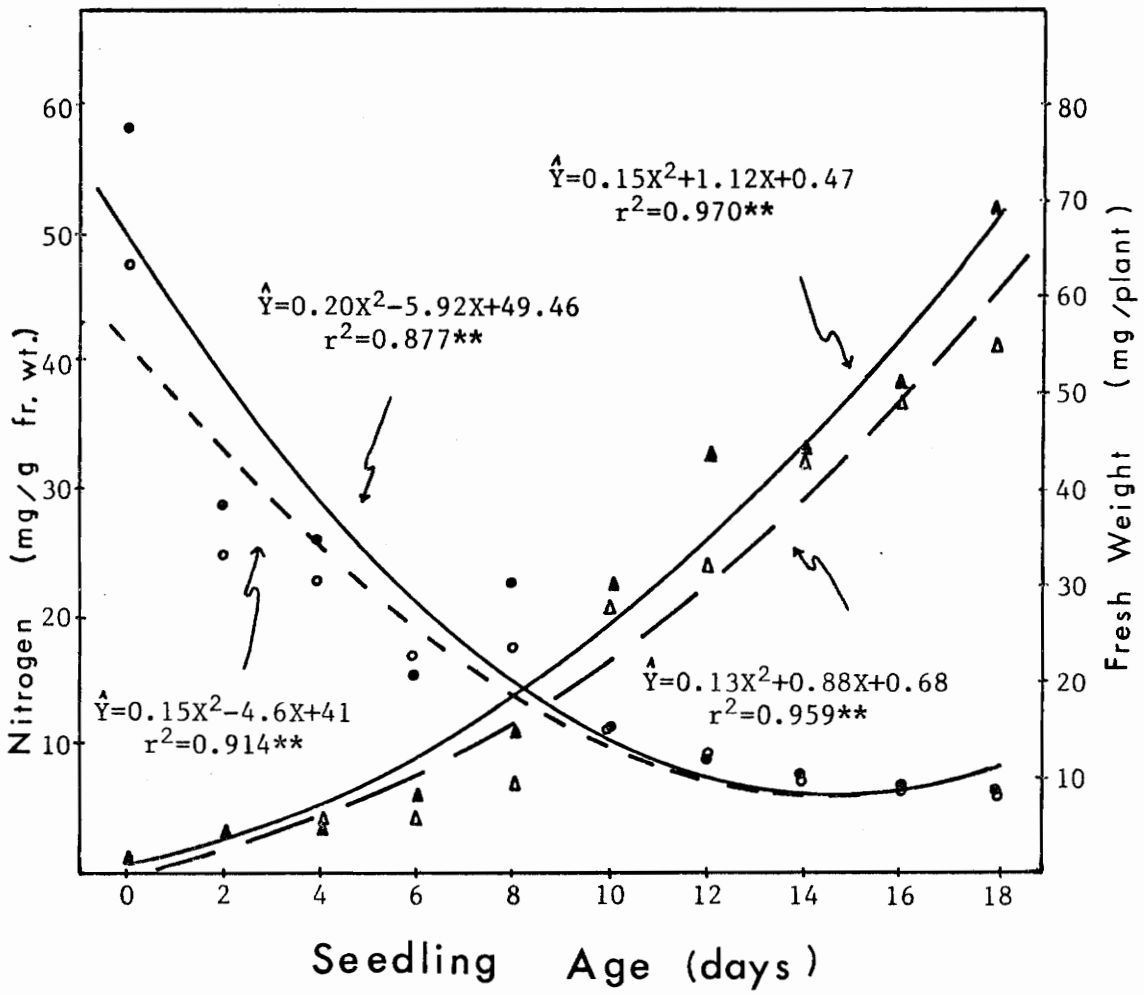

Fig. 5. Changes in total nitrogen and fresh weights of tall and dwarf tomatoes during development. Tall 'Fireball' nitrogen, (●—●); dwarf 'Md. 412-4' nitrogen, (o---o); tall fresh weight , (▲—▲); dwarf fresh weight, (Δ---Δ). (**) denotes significant correlation at the 1% level of probability.

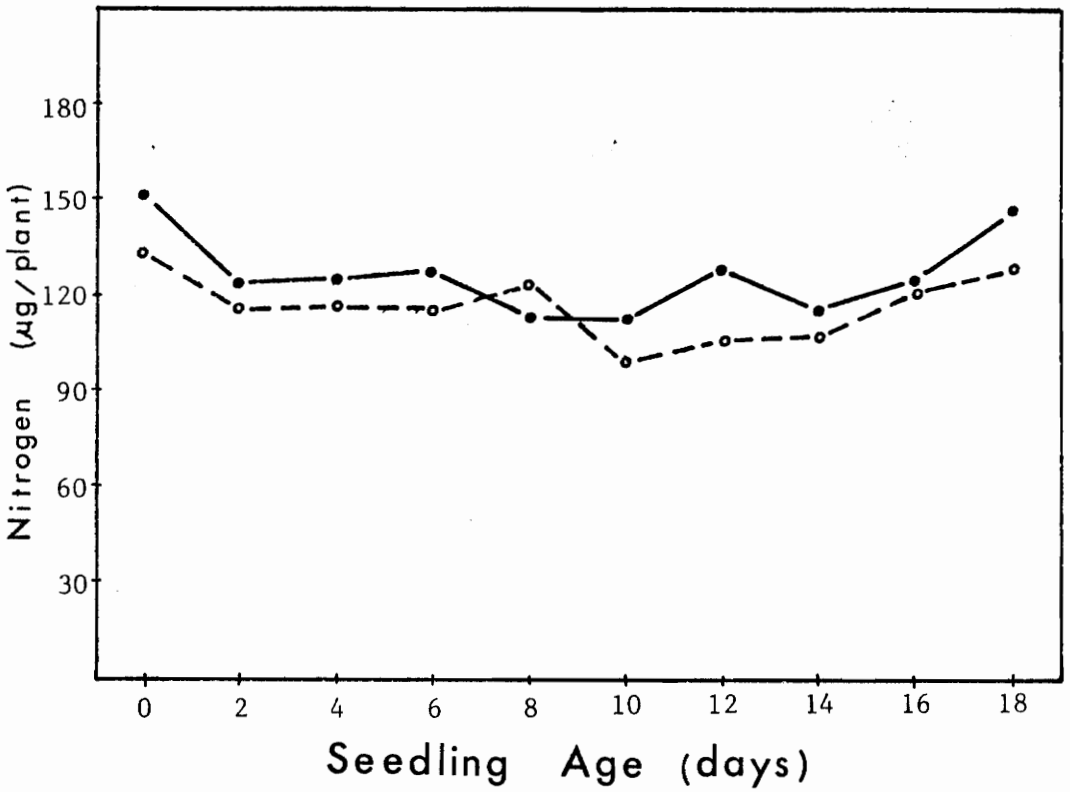

Fig. 6. Changes in total nitrogen content of tall and dwarf tomatoes during development. Tall 'Fireball', (●—●); dwarf 'Md. 412-4', (o---o). See Table III for sample size and significant differences between cultivars and between ages.

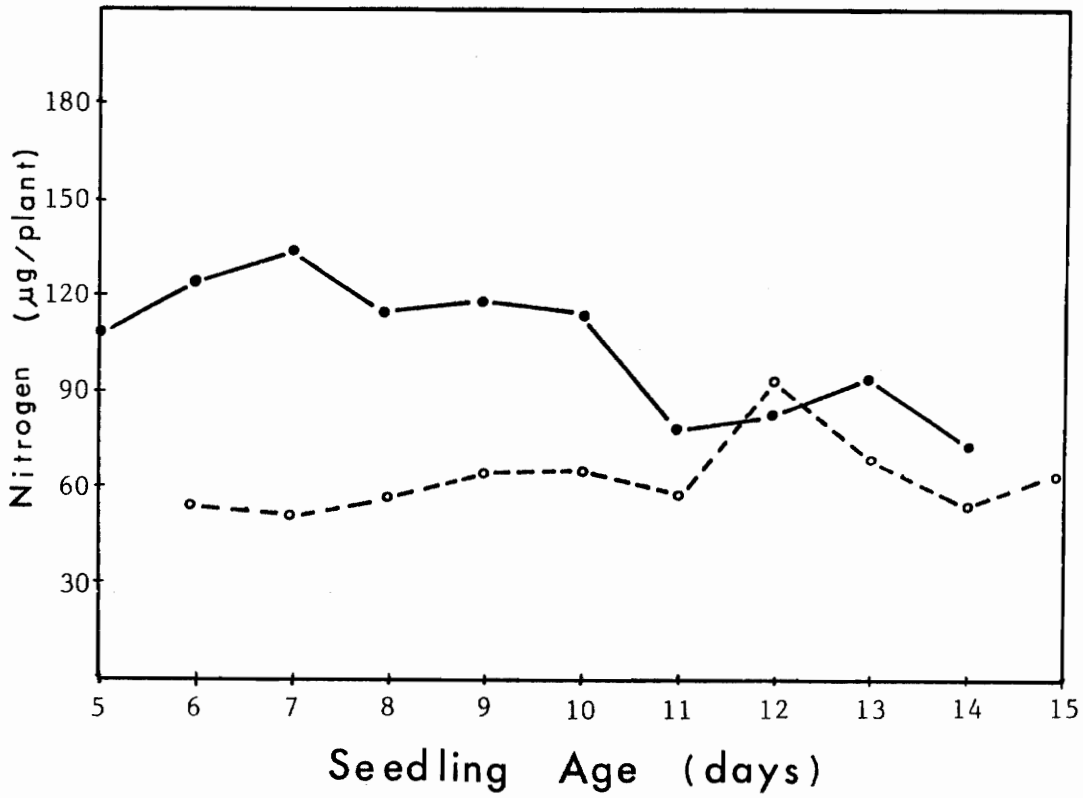

Fig. 7. Changes in total nitrogen content of tall and dwarf tomatoes during development. Tall 'Fireball', (●—●); dwarf 'Md. 412-4', (o---o). Each point represents duplicate analyses of each of 2 samples containing 10 plants/sample. Mean nitrogen content of the tall (104) was significantly greater than the dwarf (64) at the 5% level of probability.

Table III. Total nitrogen (mg/g fresh wt. and µg/plant) of tall and dwarf tomato seedlings during development.[1]

Age	mg/g fr. wt.		µg/plant	
(days)	Tall	Dwarf	Tall	Dwarf
0	58.5a	47.5a	150.5a	130.9a
2	29.1b*	25.2b	123.8a	115.6a
4	25.8bc	23.0bc	125.5a	118.6a
6	15.5cde	17.2bcd	126.6a	104.7a
8	23.0c	18.0bcd	113.8a	121.2a
10	11.6de	11.4bcd	112.4a	93.9a
12	9.0de	9.4cd	128.6a	99.2a
14	7.8de	7.1d	115.0a	104.0
16	6.9de	7.0d	123.9a	122.3a
18	6.1e	6.2d	146.8a	122.2a
Total	19.3ns	17.2	126.7*	113.3
Interaction	ns	ns	ns	ns

[1]March-April 1974 experiments. Values represent duplicate analyses of each of 3 samples containing 10 plants each. Comparable observations within columns not followed by a letter in common are significantly different by Duncan's Multiple Range Test at the 5% level of probability. ns Denotes not significant.

* Denotes significant differences between cultivars at the 5% level of probability.

Table IV. Total nitrogen (mg/g dry wt.) in the organs of tall and dwarf tomatoes during seedling development.[1]

Age	Roots		Hypocotyl		Leaves		Total	
(Days)	Tall	Dwarf	Tall	Dwarf	Tall	Dwarf	Tall	Dwarf
5	86	--	73a	--	60	--	219	--
6	76*	25	70**	33	59*	43	205**	101
7	59	23	71*	35	68**	44	198**	102
8	64	23	53**	28	62*	40	179**	91
9	43	13	48**	20	56	53	147**	86
10	42	16	43	20	50	51	135**	87
11	16	14	13	16	44	47	73	77
12	17	14	21**	52	49	48	87*	114
13	19	16	14**	18	35	46	68	80
14	12	10	11	13	31	30	54	53
15	--	11	--	14	--	36	--	61
Total	43**	17	42**	25	51**	44	137**	85
Inter-action	1%		1%		1%		1%	

[1]April 1974 experiments. Values represent duplicate analyses of each of 2 samples containing 10 plants each. Tall 15 day-old samples and dwarf 5 day-old samples were not analyzed. Dwarf 6 day-old values represent one sample.

(*) and (**) denote significant differences between cultivars at the 5% and 1% levels of probability respectively.

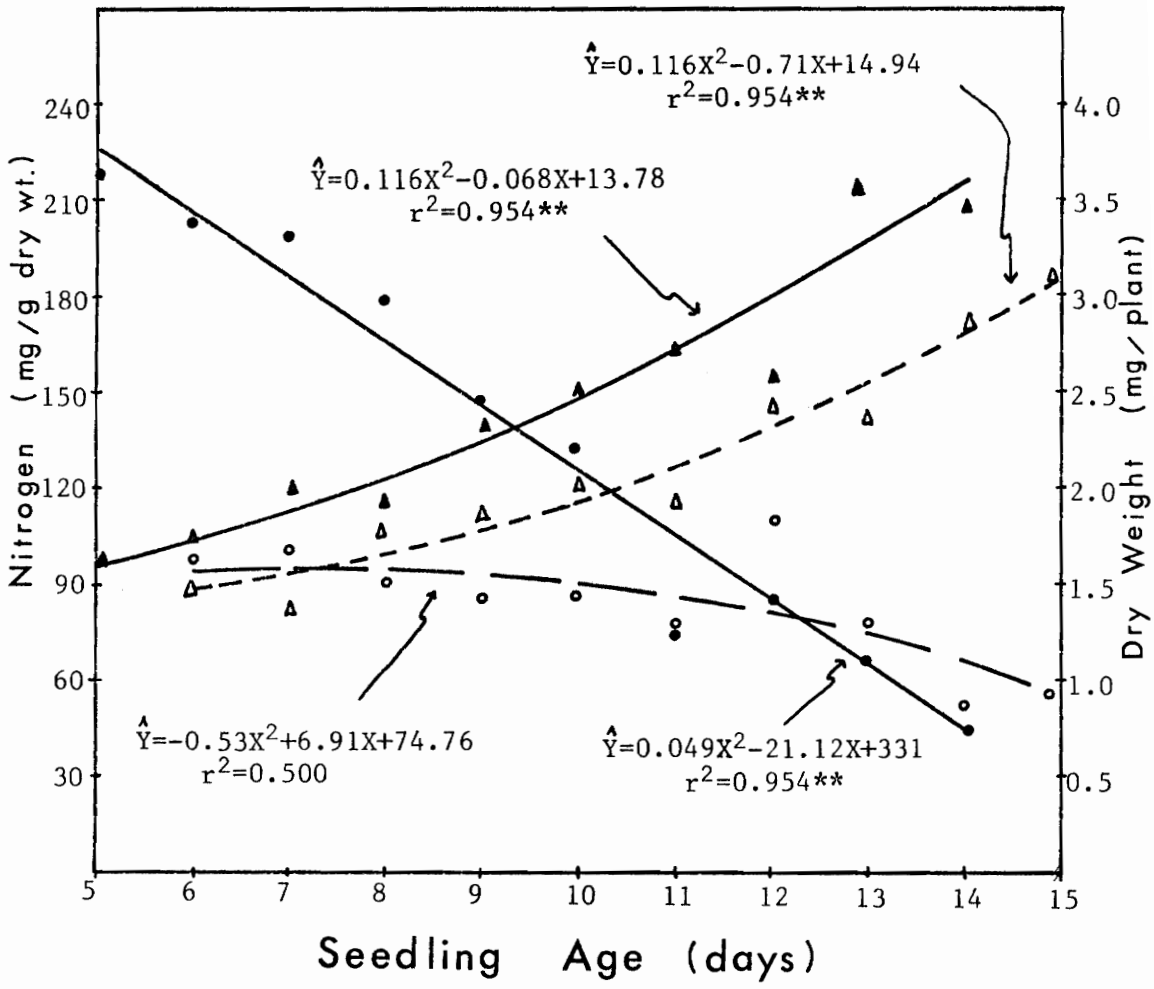

Fig. 8. Changes in total nitrogen and dry weights of tall and dwarf tomatoes during development. Tall 'Fireball' nitrogen, (●—●); dwarf 'Md. 412-4' nitrogen (o---o); tall dry weight (▲—▲); dwarf dry weight, (Δ---Δ). (**) denotes significant correlation at the 1% level of probability.

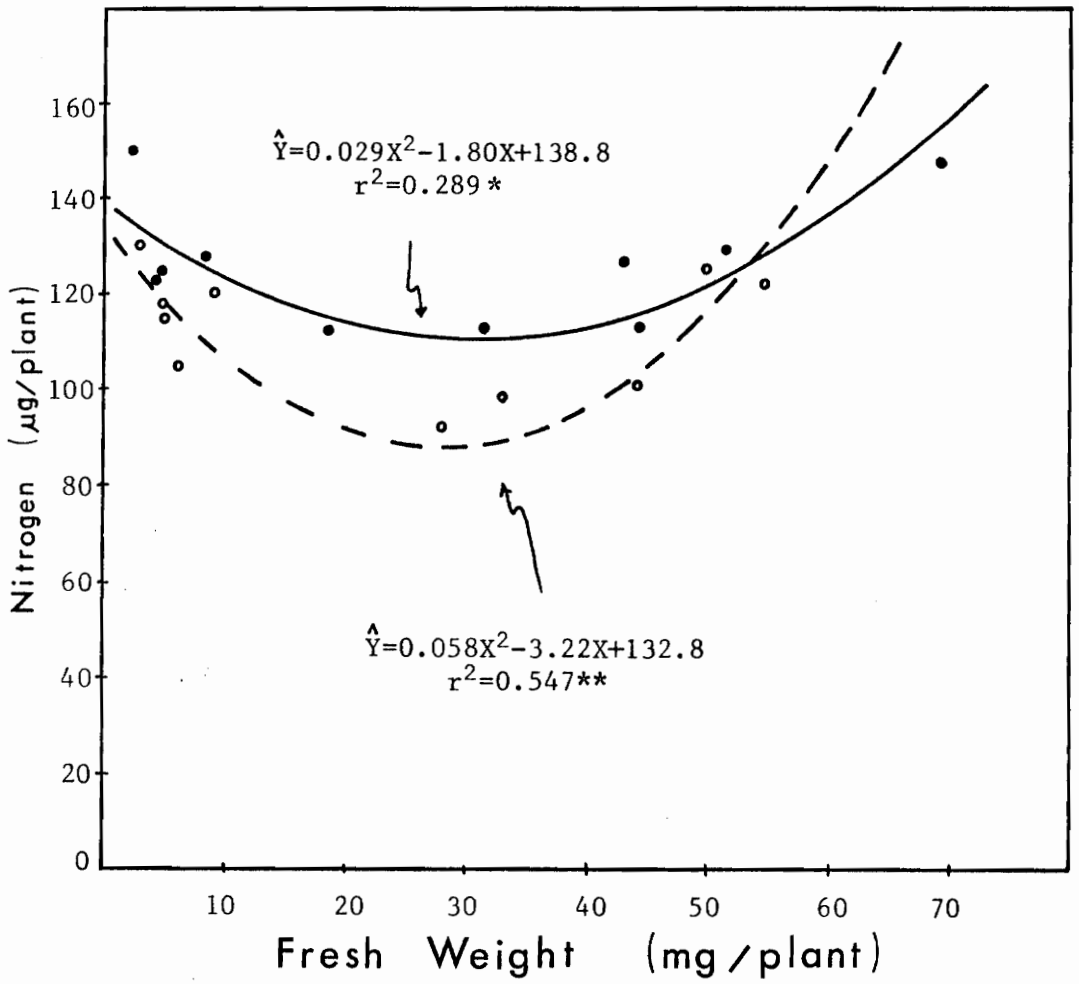

Fig. 9. Utilization of nitrogen in the production of fresh weight in tall and dwarf tomatoes during development. Tall 'Fireball', (●—●); dwarf 'Md. 412-4', (o---o). (**) denotes significant correlation at the 1% level of probability.

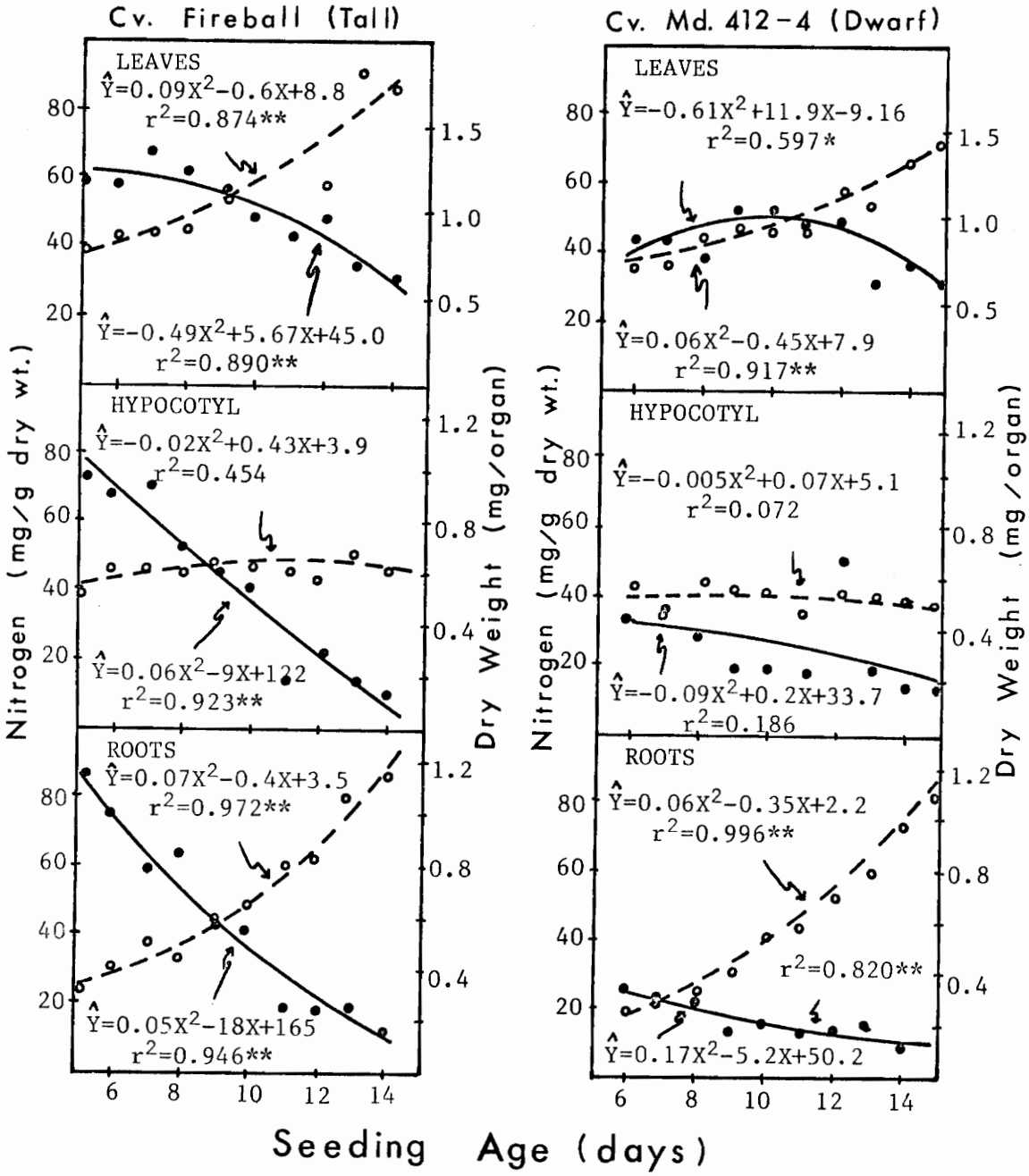

Fig. 10. Changes in nitrogen and dry weights of the roots, hypocotyl and leaves of tall and dwarf tomatoes during development. Nitrogen (●—●); dry weight, (o---o). (*) and (**) denote significant correlation at the 5% and 1% levels of probability, respectively.

Protein- The total Tris-soluble protein per plant of tall seedlings was significantly higher than the content of the dwarf. Furthermore, the protein content of the tall tomato increased significantly during the initial 48 hours of water imbibition, but not in the dwarf.

Protein decreased in both cultivars during development, but the most rapid rate of decline occurred during the first 10 days of development. (Table V; Figs. 11, 12). However, the tall cultivar was more efficient in the utilization of protein for the production of fresh weight than the dwarf (Fig. 13). Leaf protein of the two cultivars did not differ. However, the protein in the hypocotyl and roots of the tall tomato were higher than the corresponding organs of the dwarf (Table VI).

The decrease in protein of the leaves and hypocotyl of the tall cultivar was faster than those of the dwarf. Root protein of the tall cultivar however, declined slower than the dwarf (Fig. 14).

Table V. Total soluble protein (mg/g fresh wt. and µg/plant) of tall and dwarf tomato seedlings during development.[1]

Age (Days)	mg/g fresh wt.		µg/plant	
	Tall	Dwarf	Tall	Dwarf
0	181.7a**	196.9a	553a	596a
2	136.1b**	118.4b	654b**	563a
4	99.3c**	89.4c	502a	474b
6	71.8d**	94.6c	505a*	465b
8	66.8d	53.8d	321c**	492b
10	30.0e	25.2e	245d**	170c
12	15.2f	16.8f	192de*	144c
14	11.1f	9.8f	153e*	119c
16	8.4f	8.0f	146e*	113c
18	8.9f	8.3f	156e*	125c
Total	62.9ns	62.6	343*	326
Interaction	1%		1%	

[1]August 1975 experiments. Values represent duplicate analyses of each of 4 samples containing 10 plants each. Duplicate analyses were not made on samples 12 days and older.

Comparable observations within a column not followed by a letter in common are significantly different by Duncan's Multiple Range Test at the 5% level of probability.

(*) and (**) denote significant differences between cultivars at the 5% and 1% levels of probability, respectively. (ns) indicates not significant.

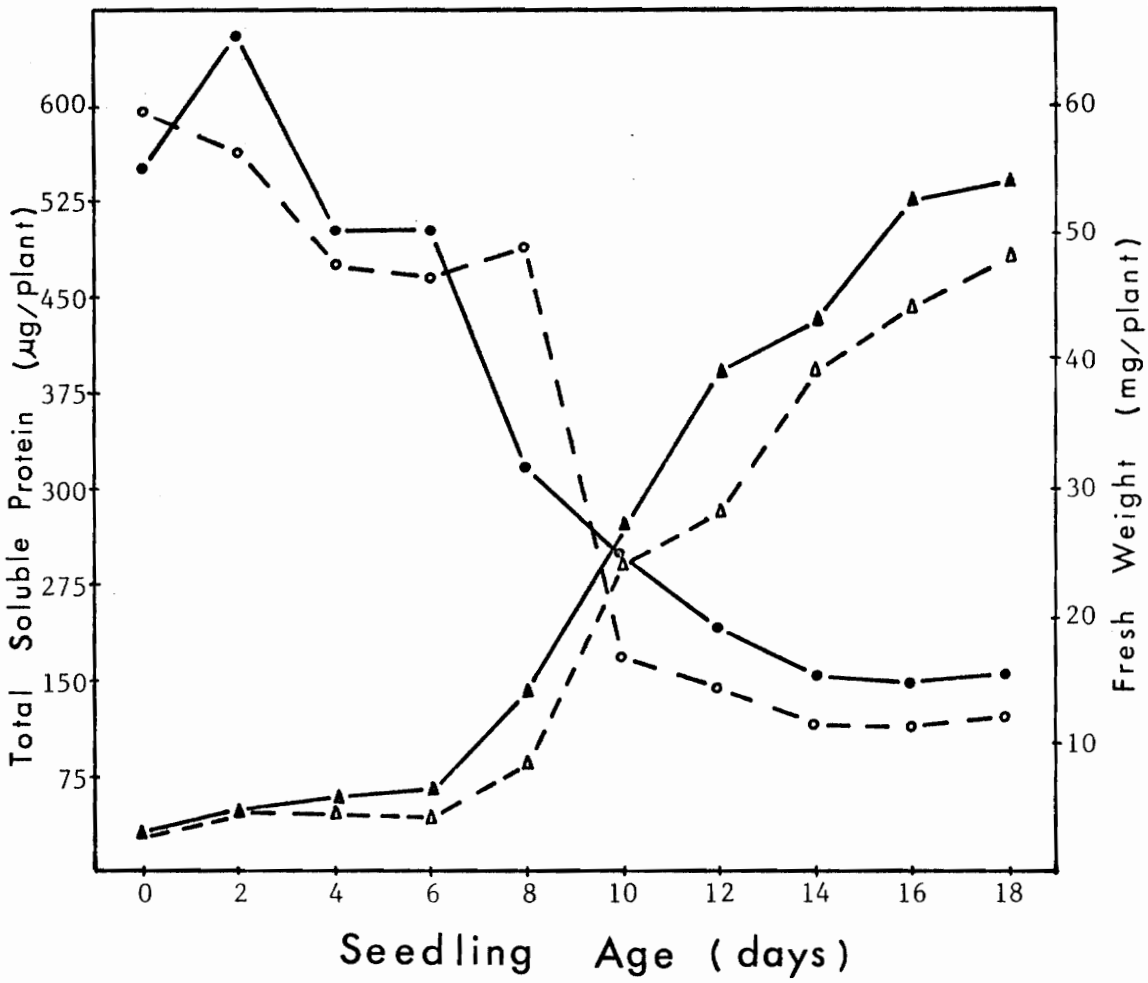

Fig. 11. Changes in soluble protein and fresh weight of tall and dwarf tomatoes during development. Tall 'Fireball' protein, (●—●); dwarf 'Md. 412-4' protein (o---o); tall fresh weight, (▲—▲); dwarf fresh weight, (Δ---Δ). See Table V for sample size and significant differences between cultivars and between ages.

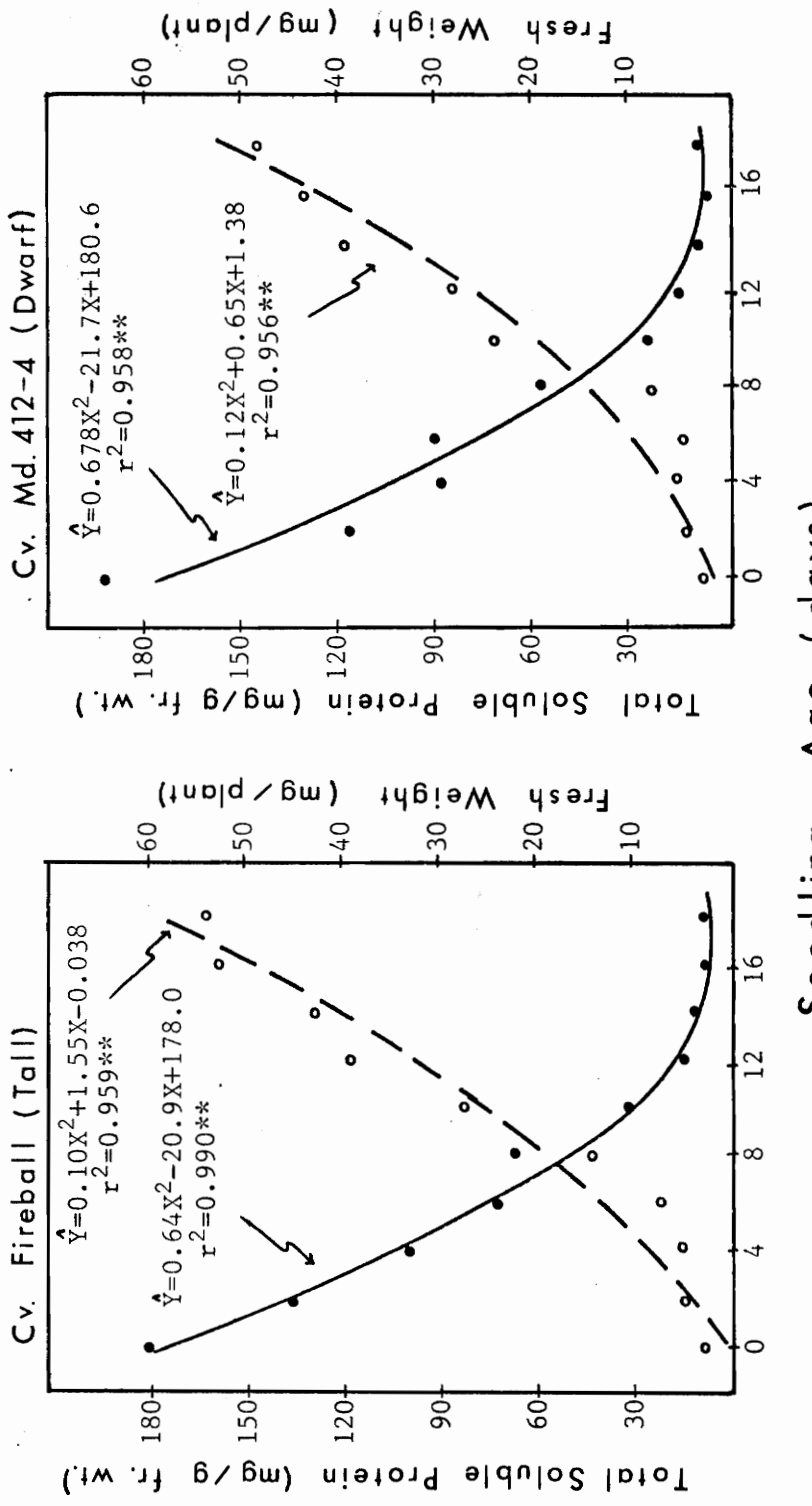

Fig. 12. Changes in total soluble protein and fresh weight of tall and dwarf tomatoes during development. Protein, (●—●). Fresh weight, (o---o). (**) denotes significant correlation at the 1% level of probability.

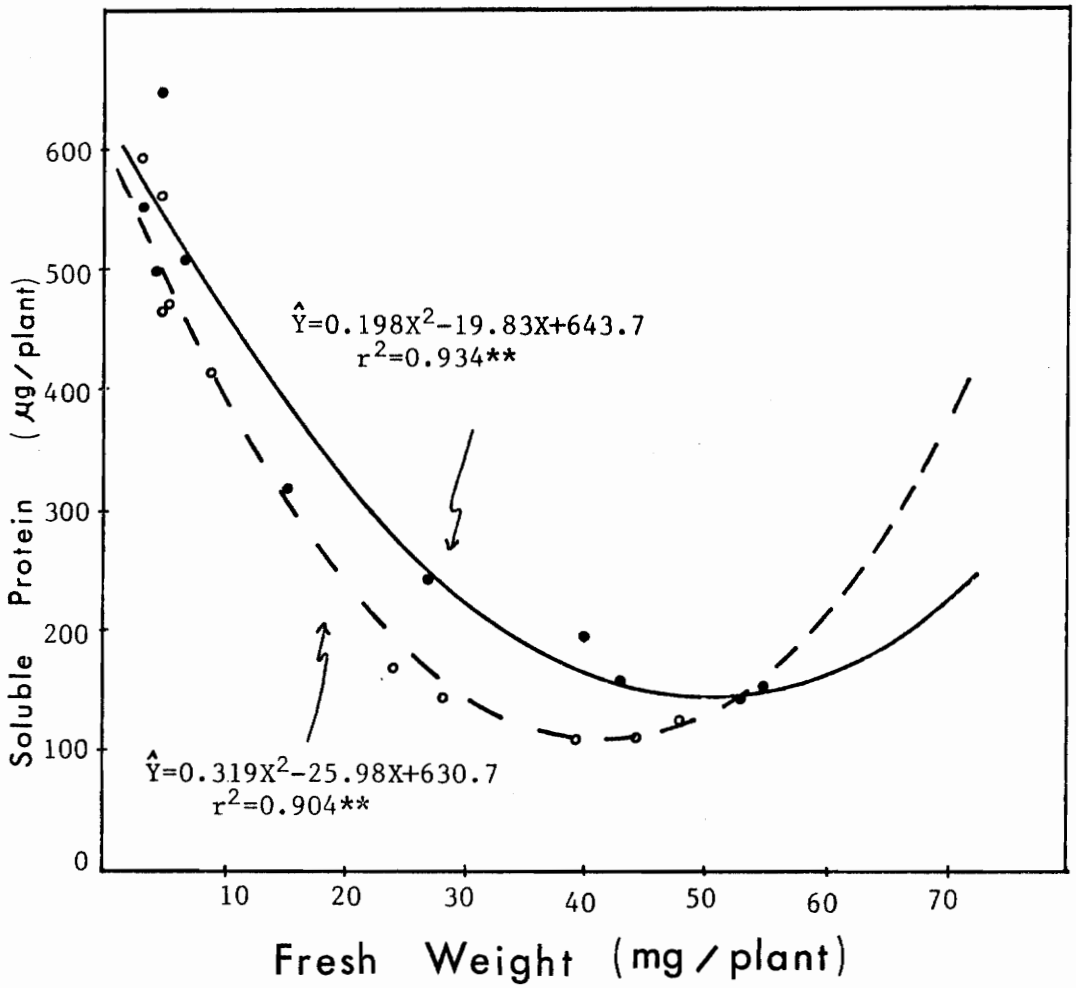

Fig. 13. Utilization of protein in the production of fresh weight in tall and dwarf tomatoes during development. Tall 'Fireball', (●—●); dwarf 'Md. 412-4', (o---o). (**) denotes significant correlation at the 1% level of probability.

Table VI. Soluble protein (mg/g fresh wt.) in the organs of tall and dwarf tomatoes during seedling development.[1]

Cultivar	Developmental Age (Days)					Total	Interaction
	10	12	14	16	18		
			Total				
Fireball	30.0a*	15.1b	11.1b	8.5b	8.9b	14.7	1%
Md. 412-4	25.1	16.8	9.8b	7.9b	8.3b	13.6	
			Leaves				
Fireball	22.8	11.7b	7.9c	5.6c	5.6c	10.7	ns
Md. 412-4	19.4a	13.3b	7.0c	5.5d	5.5d	10.1	
			Hypocotyl				
Fireball	4.1a	2.2b	2.1bc	1.8c*	1.9bc	2.4*	ns
Md. 412-4	3.6a	2.3b	1.9bc	1.2d	1.8bc	2.2	
			Roots				
Fireball	3.1a**	1.2b	1.1b	1.1b	1.4b	1.6**	1%
Md. 412-4	2.1a	1.2b	0.9b	1.2b	1.0b	1.3	

[1]August 1975 experiments. Values represent single analyses of each of 4 samples containing 10 plants each. Values of hypocotyl and leaves of 10-day old plants represent duplicate analyses.

Comparable observations within rows not followed by a letter in common are significantly different by Duncan's Multiple Range Test at the 5% level of probability.

(*) and (**) Denote significant differences between cultivars at the 5% and 1% levels of probability, respectively. (ns) indicates not significant.

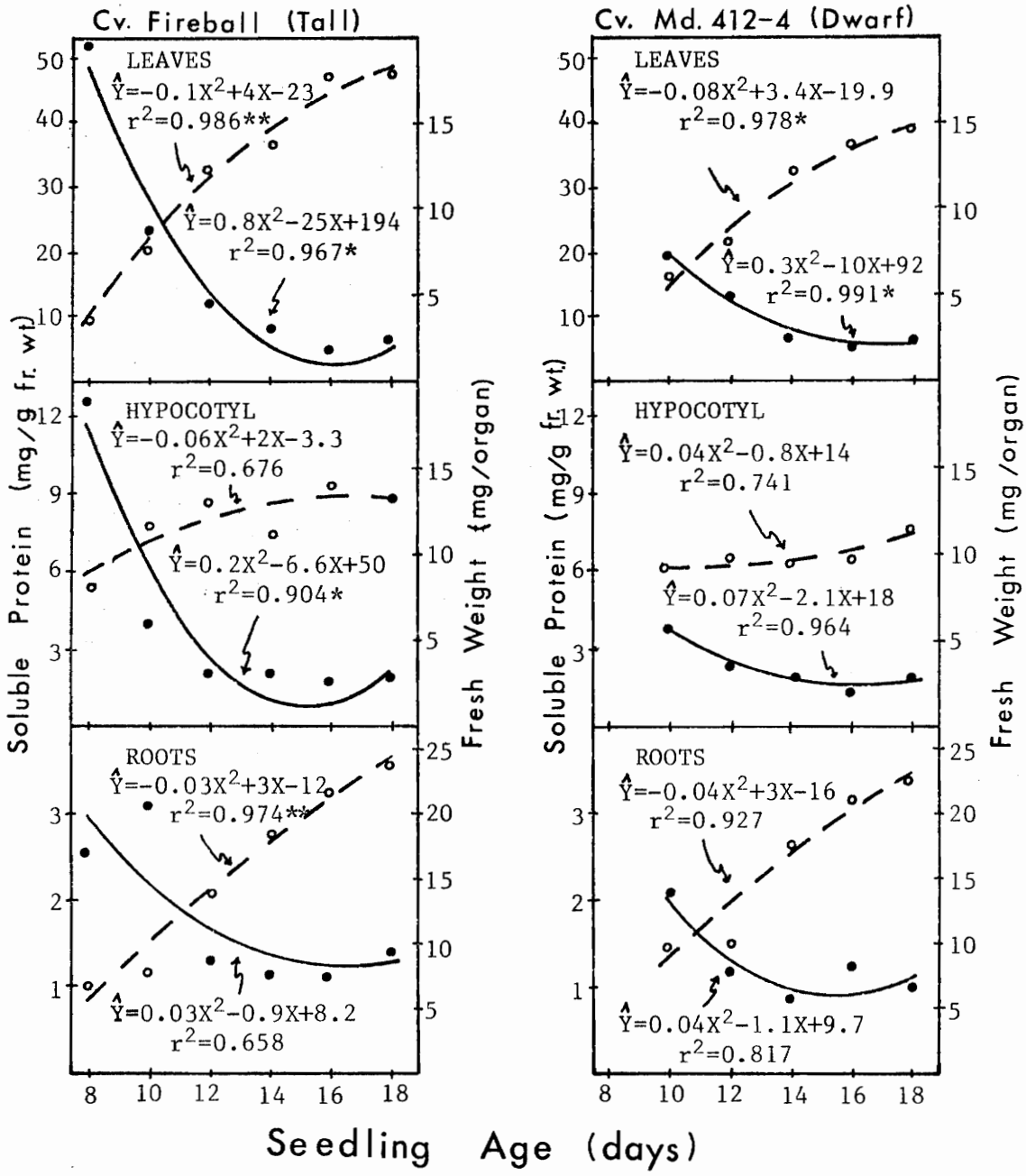

Fig. 14. Changes in soluble protein and fresh weights of roots, hypocotyl and leaves of tall and dwarf tomatoes during development. Protein (•—•); fresh weight, (o---o). (*) and (**) denote significant correlation at the 5% and 1% levels of probability, respectively.

DISCUSSION

This study reveals that the tall tomato differs from the dwarf in several ways: (1) the tall has more total Kjeldahl nitrogen and Tris-soluble protein; (2) it exhibits a significant increase in protein content during the initial 48-hours of seeds imbibition; (3) it has a higher amount of protein in the roots; (4) the rate of decline of total nitrogen of the tall tomato is faster than the dwarf, and (5) the tall tomato is more efficient in the utilization of plant nitrogen and protein.

An increase in the protein content of the tall tomato during early seeds imbibition was observed, but the factors or processes causing the increase are not known. However, similar increases in amino acids and protein content following imbibition of *Pisum sativum* seeds (Virtanen, *et al.*, 1953) and *Arachis hypogaea* seeds (Marcus and Feeley, 1964) have been reported.

The higher protein content of the roots of the tall plant as compared to the dwarf suggests that the root system may have a considerable influence(s) on the growth of the shoot. Studies concerning the nature of size-controlling rootstocks of citrus and apples on top growth lend credence to this idea (Bitters and Batchelor, 1951; Rao and Berry, 1940). Chibnall (1939) observed that roots prevented the decline of bean (*Phaseolus vulgaris*) leaf protein and suggested that root-secreted hormones such as cytokinins slowed the rate of protein degradation.

In the tomato, Nightingale *et al.* (1928) reported that the roots have a high percentage of assimilated nitrogen in the form of protein. MacVicar and Burris (1948) also noted that tomato roots synthesized considerable amounts of amides when $(^{15}NH_4)_2SO_4$ was applied to plants grown in sand culture. Hoff *et al.* (1974) found that the site of amino acids synthesis in the tomato was primarily in the roots under NH_4-N nutrition and in the leaves under NO_3-N nutrition.

In contrast, Evans and Alldridge (1965) showed that the activity of peroxidases in the roots of tall and dwarf plants was essentially the same.

Protein and nitrogen concentrations decreased in the tall and dwarf tomatoes during development (Figs. 5, 8, 12). Lugg and Weller (1941) found a 74% loss of protein nitrogen during the first two weeks of lupine (*Lupinus angustifolius)* seedling growth and in the tomato, Hall and Cocking (1966) have reported decreases in cotyledonary protein content during the second two weeks of development.

It was also observed that as nitrogen and protein were declining at a decreasing rate, growth was occurring at an increasing rate (Figs. 5 and 12). However, it is not clear whether (a) the decline in protein and nitrogen is a resultant of growth, or (b) growth is the resultant of the decline in protein and nitrogen.

Brumback *et al.* (1974) found that the application of GA_3 to dwarf seedlings resulted in increased growth with a concomitant decrease in protein N (N x 6.25). Conversely, the application of

abscisic acid to tall seedlings resulted in suppressed growth and an increase in protein N and soluble protein. Similarly, Humphries (1968) found that when the growth of bean plants (*Phaseolus vulgaris*) was inhibited by CCC or B_9, the decline in total and protein nitrogen of the leaves was delayed. Thus the evidence supports the view that the decline in protein and nitrogen is a resultant of growth.

It appears however, that this view does not hold true for 10-18 day-old seedlings (Figs. 5 and 12) since growth is still rapid while nitrogen and protein are not changing appreciably. During this time, growth may be more dependent on the manner in which these substances are utilized rather than their absolute contents.

The total protein content of a plant at any given time is a resultant balance between the sum of the rate of protein synthesis plus storage protein and utilization or degradation of these proteins (Webster, 1959). Thus, it is conceivable that the higher amount of protein of the tall tomato may be due to (a) a greater rate of protein synthesis over the rate of degradation or (b) a greater rate of protein degradation in the dwarf than the rate of synthesis.

Tall and dwarf tomatoes also differ in the rates of utilization of nitrogen and protein (Figs. 10 and 14). The tall tomato is more efficient and utilizes a lesser amount of nitrogen and protein than the dwarf to produce a comparable amount of fresh weight.

The roots, hypocotyl and leaves of the tall and dwarf utilize

nitrogen and protein differentially in growth. The greater growth of the leaves of the tall plant coincided with a greater rate of decline in nitrogen and protein (Figs. 9 and 12). However, similar relationships between these substances and growth in the hypocotyl and roots as well as those in the leaves may be confounded by differential rates of utilzation and/or translocation to other organs.

Certain studies have been conducted on the nature of the amino acids in the seeds of three tomato cultivars (Raymal *et al.*, 1974) and on the composition of some nitrogenous fractions in actively growing tomato plants (Clark, 1936). Differences in these nitrogenous substances were found between cultivars and with different nutrient treatments. Since these studies were carried out primarily with tall tomato, relatively little is known concerning the nature of these compounds in the dwarf. Preliminary experiments using cellulose acetate electrophoresis have shown that qualitative differences in proteins of the two types may exist (Brumback, unpublished data). Thus, more investigations on the qualitative aspects of nitrogenous compounds should be done on both the tall and dwarf plants in order to fully characterize the differences between them.

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APPENDIX

Table I. Total nitrogen (mg/g fresh wt.) in the organs of tall and dwarf tomatoes during seedling development.[1]

Cultivar	Seedling Age (days)					Total
	10	12	14	16	18	
			Total			
Fireball	4.4*	5.6*	6.1*	5.3	5.0	5.3*
Md. 412-4	7.9	10.3	7.3	4.7	4.5	6.9
			Leaves			
Fireball	2.9*	3.7*	4.1	3.4	3.0	3.4*
Md. 412-4	5.3	7.2	4.9	3.4	3.2	4.8
			Hypocotyl			
Fireball	0.8	0.9*	1.0*	0.9	0.9	0.9*
Md. 412-4	1.7	1.8	1.2	0.6	0.6	1.2
			Roots			
Fireball	0.7	1.0*	1.0*	1.0*	1.1	1.0ns
Md. 412-4	0.9	1.3	1.2	0.7	0.7	1.0

[1]February 1974 experiments. Values represent duplicate analysis of single samples containing 10 organs each. (*) Denotes significant differences between cultivars at the 5% level of probability. (ns) indicates not significant.

Vita

Thomas B. Brumback, Jr., son of Thomas B. Brumback, Sr. and Margaret Mehales Brumback, was born on 3 March, 1952, in Winchester, Virginia. He attended James Wood High School in Winchester where he was graduated in 1970.

In the fall of 1970 he entered Virginia Polytechnic Institute and State University. During his undergraduated education he received scholarships from the Virginia Nurserymen's Association and the Women's Auxiliary of the Virginia State Horticultural Society. He served as Head Resident Advisor for a dormitory housing 1000 students and was president of the Virginia Tech Horticulture Club. He was a member of the national honorary society Phi Kappa Phi and the honorary agricultural society Gamma Sigma Delta. In June 1974, he was awarded a Bachelor of Science (with distinction) in Horticulture.

In September 1974, he was awarded a research assistantship in Horticulture at Virginia Polytechnic Institute and State University and began work toward the Master of Science degree.

He was married to Lisa Pine on 15 June, 1974.

Thomas B. Brumback Jr.

DEVELOPMENTAL CHANGES IN NITROGEN AND PROTEIN OF TALL AND DWARF TOMATO SEEDLINGS *LYCOPERSICON ESCULENTUM* MILL.

by

Thomas B. Brumback, Jr.

(ABSTRACT)

Tris-soluble protein and total nitrogen of tall and dwarf tomato (*Lycopersicon esculentum* Mill.) seedlings were determined by the method of Lowry *et al.* and a modified micro-Kjeldahl direct Nesslerization technique.

The tall tomato 'Fireball' differs from the dwarf 'Md. 412-4' in several ways: (1) the tall has more total nitrogen and protein; (2) it exhibits a significant increase in protein during the initial 48-hours of seeds imbibition; (3) it has a higher amount of protein in the roots; (4) the rate of decline of total nitrogen is faster than the dwarf, and (5) the tall tomato is more efficient in the utilization of plant nitrogen and protein.

The greater growth of the tall resulted in a more rapid decline in nitrogen. However, in 10-18 day-old seedlings, growth was still rapid while nitrogen and protein did not change appreciably. The tall was more efficient and utilized a lesser amount of nitrogen and protein in the production of a comparable amount of fresh weight.

It is suggested that the roots have considerable influence(s)

on the growth of the shoot and that the decline in nitrogen and protein is a resultant of growth. It is indicated that the differences in growth may be more dependent on the manner in which nitrogen and protein are utilized rather than their absolute content.