HIGH TEMPERATURE EFFECTS ON EARLY DEVELOPMENT OF TOMATO (*Lycopersicon esculentum* Mill.)

by

Mei-ling Hshieh

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH MAJOR IN HORTICULTURE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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High-temperature effects on early development of tomato (*Lycopersicon esculentum* Mill.)

Hshieh, Mei-ling, Ph.D.

The University of Arizona, 1990

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GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Mei-ling Hshieh

entitled High temperature effects on early development of tomato (*Lycopersicon esculentum* Mill.)

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

Fred R. Katterman — Date: 30 July 1990

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

D. T. Ray
Dissertation Director

July 27, 1990
Date

STATEMENT BY AUTHOR

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ABSTRACT

In southern states, plantings for fall and winter tomato (Lycopersicon esculentum Mill.) production often have problems in stand establishment. Tomato seed sown in late summer or early fall often encounter soil temperatures greater than 40° C. This exceeds the generally accepted maximum germination temperature of 35° C, and is thought to contribute to poor seedling growth and vigor. Studies were conducted on tomato to evaluate the effects of high temperatures on germination and seedling development, and their physiological and anatomical responses.

At the optimum temperature of 25° C, seed of all tested tomato lines had 85% or higher germination in petri dishes. Germination was reduced dramatically when the temperature was greater than 35° C, and in alternating temperatures of either 40/25 or 40/30℃, seed germination was also low. Seed imbibed for 24 to 48 hours at a constant temperature of 40° C, also showed the deleterious effects of high temperature and had reduced germination. Seed germination did not decrease dramatically if seed were imbibed for more than 24 hours at a constant temperature of 25° C. The time interval of 24 to 48 hours following imbibition was considered to be the most heat sensitive period in tomato seed germination.

There were no differences in the banding pattern of heat

shock proteins (hsps) from emerged seed of either heat sensitive or heat tolerant lines. However, at temperature of 35 and 40℃ the width of hsps bands increased. Testing for electrolyte leakage of emerged seed showed that leakage played a role in influencing the isotope absorption of root radicles.

Normal seedling development was observed at 23/21° C in terms of secondary roots formation, and fresh weight of roots, cotyledons and leaves. Alternating temperatures of either 40/25, or 40/30° C resulted in delayed development of seedlings when seed were imbibed, germinated and grown two days at 23/21° C before heat treatments were applied. Secondary root and leaf primordia could be detected microscopically when seedlings were grown at 23/21° C for either two or three days. Yet, development was delayed at high temperatures, and a reduction in root number, leaf number, and fresh and dry weight of roots, cotyledons and leaves was observed.

CHAPTER 1

INTRODUCTION

The cultivated tomato (Lycopersicon esculentum Mill.) is a relatively recent addition to the world's important food crops, with an annual worldwide production approaching 50 million metric tons (Yamaguchi, 1983). It can be grown outdoors successfully from the equator to as far north as Alaska (Tigchelaar, 1986), although its yield in the tropics is much lower than in temperate regions (Villareal et al., 1978). This reduction in yield generally is attributed to high temperatures which cause a reduction in flowering and fruit set, and/or an increase in pathogen multiplicity (El Hassan, 1978; Villareal, 1978). High temperatures during germination and early seedling development may also be a problem in many regions where tomatoes are grown. In the southern states, tomatoes often are planted for winter production during late summer when soil temperatures may far exceed the optimum. For example, recorded soil surface temperatures in July when tomato is being planted are as high as 55° C (P. Leeper, Texas A&M Univ., personal communication).

High temperature stress may be a major cause of yield loss in crop plants, but relatively little effort has been made towards breeding for tolerance to high temperatures. This is due in part to a limited understanding of the genetic and/or physiological basis for heat tolerance in plants, difficulty in controlling temperature in the natural environment, and little understanding of which stages of plant development would be best used in seletion (Marshall, 1982).

In this study, I have examined the effects of high temperatures on tomato seed germination and early seedling development. The specific objectives were: (a) to determine which characteristics could be used as criteria for separating genotypes; (b) to see if differences in germination could be linked to either the production of heat shock proteins or electrolyte leakage; and (c) to describe the effects of high temperatures on seed germination and seedling development.

CHAPTER 2

LITERATURE REVIEW

Origin and History

Tomato is a tender perennial that is cultivated almost universally as an annual. Numerous wild and cultivated relatives of the tomato still can be found in a narrow, elongated mountainous region of the Andes in Peru, Equador and Bolivia, as well as, in the Galapagos Islands. These relatives of the edible tomato occupy diverse environments based on latitude and altitude (Alcazar-Esquinas, 1981).

The name "tomato" came from the Nahuatl language of Mexico, and variants of this name have followed the tomato in its distribution throughout the world (Heiser, 1969). The first written account documenting the arrival of the tomato in the Old World appeared in 1554, by the Italian herbalist Pier Andrea Mattioli (Tigchelaar, 1986). The tomato was introduced into the United States in the latter part of the eighteen century (Hayward, 1948).

Effects of Temperature Regulation on Early Tomato Development

Germination

Seed where tomatoes are produced in fall and winter are planted during late summer when soil temperatures often are greater than 40° C, or even 50° C, in tropical and subtropical regions (Geiger, 1959; Valdes et al., 1985). These high soil temperatures exceed the generally accepted maximum germination temperature of 35° C for tomato (Lorenz and Maynard, 1980), and contribute to poor stands because of weak germination and emergence (El Hassan, 1978; Van Maren, unpublished data). Coons et al. (1989) found that the optimum temperature for tomato germination in different cultivars was between 26 and 32° C. Brooks (1969) and Wittwer (1960) recommended the optimum germination temperature to be 18.5 to 21 and 24° C, respectively. Thompson (1974) found that the maximum temperature for 50% germination ranged from 35 to 38° C. However, he also noted that many tomato cultivars germinate rapidly and maximally in the temperature range of 26 to 32° C. Jozefowicz (1930) and Lewis (1953a) also stated that temperatures below or above the optimals of 26 to 32° C retarded germination and subsequent plant development, whereas those near the lower or upper extremes were injured by the same processes. However, Onwueme (1974) showed that the degree of high temperature injury depended on when heat stress was applied, and effects were most pronounced when heat stresses were applied 24 hr after the start of imbibition.

Coons et al. (1989) studied the effects of high temperatures on germination and found that cultivars differed widely in germination percentage as temperatures were increased. Germination was greater than 90% in all cultivars at 25° C; at 30° C, germination varied from 60 to 95%; and at 35° C, germination ranged from 5 to 55%. When seed were maintained at 40° C, germination percentages were less than 10% in all tested cultivars. Interestingly, most seed that were held at 40° C still remained viable as indicated by tetrazolium tests and by subsequent germination after the seed were returned to 25° C.

Seedling development

Tomato cotyledons store and assimilate food. With emergence, they develop chloroplasts and increase in size. Calvert (1959) found that cotyledon area increased more at 15.5° C, rather than at either 18.5 or 21° C, but dry matter increased the most at 18 to 20° C. However, Hussey (1965) found that the cotyledons of 4-day-old seedlings expanded more in area and showed a greater dry matter increase at 25° C than at 15° C night temperature.

For shoot growth, Hussey (1965) found that the optimum temperature was 25° C. Aung (1978) studied the effect of temperatures on tomato seedling growth and found that the dry matter in stems accumulated more at 26° C than at 20 or 14° C when plants were grown for 7 weeks at constant temperatures of 26, 20 and 14° C (16hr light and 8hr dark). The seedlings at the 2-leaf stage suffered significant growth reduction when exposed to a brief daily treatment of 30° C night temperatures for 4 to 5 weeks (Bendix and Went, 1956). In older (20 to 40 cm tall) tomato plants, the rate of stem elongation was greater when plants were grown at a fluctuating 26.5° C day (D) and 19 to 20° C night (N) temperature, than at a constant 26.5° C, or a 19 to 20° C D and 26.5° C N temperature (Went, 1945). Saito and Ito (1962) found that 50-day-old tomato plants showed the greatest stem length and diameter at 30/17° C (D/N). On the other hand, Calvert (1964) found that stem length was affected only by day temperature. Went (1944) and Hussey (1965) found that tomato plants grew better at a constant day and night temperature than at fluctuating high day and lower night temperatures.

Aung (1978) concluded that tomato seedlings were most sensitive to temperature soon after cotyledon expansion occurred. He noted that the rate of leaf primordia initiation was hastened, dry matter accumulation was greater and more leaves were formed at a constant 25° C than at a constant 15° C. However, Hussey (1963) found that the main shoot had twice the size and plants initiated flowers sooner at 15° C than at 25° C.

White (1937) found, in aseptic agar culture, that growth of isolated roots was greatly reduced at 5, 8, 10, and 40° C relative to 20 to 33° C. In addition, the roots grew slowly at 15 and 35° C. Byrne et al. (1975) studied adventitious root development in "Fireball" tomatos grown at 26° C D and 20° C N, and found that root primordia originated from the pericyclic and endodermal regions. Root histogenesis was complete 2 days after initiation. They also found that emergence of roots through the cortex occurred 5 days after initiation and the pattern of root initiation in the hypocotyl and primary root of intact seedlings occurred acropetally. Aung (1978) found that root size was significantly modified by night temperature and that roots developed the heaviest when tomatoes were grown at 26.5° C D and 16 to 22° C N.

Physiological Responses to High Temperatures

Temperature plays an important role in controlling germination in many plant species. It not only affects the germination process itself but also is involved in dormancy. Optimal germination temperatures vary from species to species, and even within cultivars of some crop plants. As temperature is elevated, many physiological processes of plants that affect germination and seedling development, may be inhibited. Bewley and Black (1978) concluded that high temperature could cause protein denaturation, disruption of membrane structure and irreversible inhibition of photosyhtnesis.

Germination rate

Bewley and Black (1978) believe that both duration and the degree of temperature will affect processes involved in germination. Germination percentage has been used most often in seed germination studies, but does not take into account the time factor. They emphasized that the determination of germination rate was of greater value in characterizing seed responses to temperature. They stated that populations behaved fairly homogeneously as far as germination capacity was concerned, but populations were heterogeneous with respect to germination rate.

Oxygen and water uptake

Come and Tissaoui (1973) found that germination of non-dormant embryos required more ambient oxygen as temperatures were increased. This was probably due to both the higher metabolic rates in embryos and the lower solubility of oxygen in water as temperatures were increased.

Water potentials in dry seed are extremely low and are as low as -1000 bars (Bewley and Black, 1978). At the beginning of imbibition, water is absorbed purely physical processes. Actual germination is not possible until the water potential of the seed is greater than -15 bars. The rate of water uptake is temperature-dependent, and may be correlated with the lowering of water viscosity that occurs as temperatures are raised (Berrie, 1984).

Proteins

Bewley and Black (1978) stated that temperature altered protein activity by denaturation, and could account for the retarding effect on germination rate at both low and high temperatures. However, the germination process might be promoted by certain conformational changes in proteins. Teeri (1980) showed that changes in protein content induced by temperature might alter protein adaptation, these changes might be directly related to altered protein function at the new temperature.

Possible Indicators of Heat Sensitivity

Heat shock proteins (hsps)

Recently researchers have attempted to see whether hsps are involved in heat related responses of plants. Atkinson and Walden (1985) stated that hsps are formed when plants or animals are exposed to temperatures 8 to 10° C higher than optimum. However, Linquist (1986) said that for organisms which grew over a broad range of temperatures, the maximum response usually was achieved at 10 to 15° C above the optimum growth temperature, and organisms that grow under a more restricted range at 5° C above their optimum.

Two classes of hsps occur in plants: 1) a high molecular weight (MW) class (68 to 110 KDa), similar to those found in many eukaryotes; and 2) a low MW class (15 to 28 KDa), especially abundant in vascular plants. Hsps found in plants are similar over a wide range of species, in all tissues, and at several developmental stages (Atkinson and Walden, 1985; Cheney and Shearn, 1983; Nover et al., 1983; Nover and Scharf, 1984; Scharf and Nover, 1982; Sirotkin and Davidson, 1982).

The induction of hsps is rapid, with temperatures at which maximum production occurred were at 40 to 45° C in corn (Baszczynski et al.,1982; Key et al., 1981) and 36 to 37° C in Drosophila (Lindquist, 1980a). Heat shock messages appeared in the cytoplasm of organisms within a few minutes after temperatures were elevated, and they were immediately translated with very high efficiency (Lindquist, 1980b). As long as cells were maintained at high temperatures, hsps continued to be the primary products of protein synthesis (DiDomenico et al., 1982a and 1982b). In other studies, hsps increased for approximately 3 to 4 hours after which time they began to decline (Altschuler and Mascarenhas, 1985; Atkinson and Walden,1985; Kimpel and Key, 1985a; Sachs and Ho, 1986). When hsps were formed at higher temperatures, a significant reduction in normal protein synthesis occurred (Altschuler and Mascarenhas, 1985; Burke, et.al. 1985). When plants or cells were returned to a more optimum temperature, synthesis of hsps stopped, but the hsps already formed were stable for as long as 20 to 21 hours (Atkinson and Walden, 1985; Kimpel and Key, 1985a). Normal protein snythesis gradually resumed, with the timing a reflection of the severity of the preceding heat shock (DiDomenico et al, 1982a and 1982b). If temperatures were increased gradually rather than abruptly, synthesis of hsps occurred at even higher temperatures in both the laboratory and the field (Altschuler, 1982; Atkinson and Walden, 1985; Burke et al., 1985; Kimpel and Key, 1985a and 1985b).

Specific functions of hsps are very little known. A widely held assumption is that they protect organisms from the detrimental effects of heat and other forms of stress (Lindquist, 1986). Hsps are found in heat pretreated plants but not in those which did not receive the pretreatment (Altschuler, 1982; Atkinson and Walden, 1985; Kee and Nobel, 1985; Kimpel and Key, 1985a; Lin et al., 1984). The addition of cycloheximide, which blocked protein synthesis, prior to the heat shock also blocked the acquisition of thermotolerance (McAlister and Finkelstein, 1980; Plesofsky-Vig and Brambl, 1985). The hsps appeared to associate with cellular organelles during the pretreatment and to disassociate when returned to a lower temperature, and quickly reassociated again when exposed to high temperatures (Lin et al., 1984). This rapid reassociation might give them more protection relative to plants not receiving the pretreatment. Some lines which survived at high temperatures without pretreatment were found to have constitutive hsps (Iida and Yahara, 1985; Mackie and Wilson, 1972).

Ougham and Stoddart (1986) studied the formation of hsps during germination in sorghum seed and found that seed in the early stages of germination were incapable of hsp synthesis, and heat shock delivered at 16 hours after the start of imbibition led to a general reduction in incorporation of labelled amino acid in sorghum embryos. Cultivar differences in the time at which the hsp synthesis first appeared, and the particular susceptibility of early germination processes in some sorghum lines to high temperatures might be related to their inability to synthesize hsps and acquire thermotolerance.

Although most workers believe that hsps contributed to themotolerance at high temperatures, a number of contradictions have been found in the literature. Hall (1983) reported that the addition of cycloheximide to yeast cells did not prevent the induction of thermotolerance. Ferrini et al. (1984, reviewed by Lindquist, 1986) showed that the isolation of two melanoma cell lines with widely different levels of thermotolerance showed no qualitative or quantitative differences in hsp snythesis. The transformation of mouse embryo cells with SV40 also caused them to produce higher levels of hsps both at normal temperatures and after heat shock, while their sensitivity to heat increased (Omar and Lanks, 1984). The implication was that hsps were not providing these cells with thermotolerance.

Membrane leakage

Membranes in resting seed are partially disorganized, so that the seed is not an osmotic system (Meidner and Sheriff, 1976). The membranes presumably become re-established within minutes of imbibition to prevent further leakage, and their changes are physical rather than those of metabolic breakdown and resynthesis. As temperatures rise above minimum, temperature dependent transitions in cell membranes might be important. Raison et al. (1980) stated that the loss of physiological function of membranes at high temperatures can be explained as an imbalance between the relative strengths of hydrophobic and hydrophilic interactions among proteins, lipids, and an aqueous environment. Therefore, leakage measurements could be used for testing membrane integrity.

Initial water uptake by seed during imbibition was accompanied by the release of a large volume of gas and by a rapid leakage of substances (Bewley and Black, 1978). Leaching is temperature dependent. The changes in leakage were correlated with a phase change in the membrane (Berrie, 1984). The very sharp upper cut off point in many seed could be due to changes in membrane fluidity (Bewley and Black, 1978). Evaluation of leakage from seed after 48 hours of imbibition is particularly important, because leakage from seed and roots indicates membrane damage (Parrish and Leopold,

1977) and stimulates pathogen germination and infection (Kraft and Erwin, 1967). Benzioni and Itai (1973) also found that the leakage of tobacco leaves after 2 min at 47.5° C was reversible, indicating that leakiness is an early step in heat injury. Levitt (1980) also suggested that leakage is the first true sign of heat injury.

Cell membrane stability (CMS), which was evaluated as percentage injury calculated from the value of electrical conductivity measured on pieces of leaf before and after they were killed, has been used in evaluation of drought tolerance in sorghum as well as drought and heat tolerance in wheat (Blum and Ebercon, 1976 and 1981). Bewley (1979) considered CMS to be a major component of drought tolerance. An attempt was made in this study to see whether CMS was effective as a screening character for heat tolerance in tomato during germination and young radicle growth.

Anatomical Changes in Plant Tissue

Hammond (1941) and Stephens (1944) found that the shape and size of leaves in *Gossypium* were affected by altering the number of cells. In *Tropaeolum* (Whaley and Whaley, 1942) and *Antirrhinum* (Harte and Meinhard, 1979, reviewed by Chandra Sekhar and Sawhney, 1990), leaf shape differences were attributed to differences in the rates of cell division. In the lanceolate mutant of tomato, differences in shape and size of the leaf were attributed to increased cell size, reduced number of cells, and premature differentiation of meristematic cells in the development of the leaf (Caruso, 1968; Dengler, 1984; Mathan and Cole, 1964; Mathan and Jenkins, 1962). However, in a solanifolia mutant of tomato, differences in shape and size of the leaf might not be related to differences in the size of the shoot apex, but some of the cytological differences were established in the mutant at an early stage of leaf development and significant differences existed in the timing of leaflet initiation (Chandra Sekhar and Sawhney, 1990). Most workers in anatomical research are inclined to believe that differences in shape and size of leaf tissue are controlled genetically.

However, Bandurski et al. (1953) found that leaf tissue of tomato grown at 4° C night temperature was compact and its leaf was also small, fleshy and very dark green. On the other hand, leaf tissue was relatively spongy with increases in cell number and cell surface and lighter leaf color when tomato was grown under 30° C night temperature. In addition, the leaf structure and chloroplast ultrastructure of soybean were also affected by light intensity and temperature (Ballantine and Forde, 1970).

Goss (1977) showed that in barley 50KPa of applied pressure to the root shortened the region of initiation of laterals from a zone extending 30 mm from the apex to a zone extending only 4 mm. Elongation of etiolated soybean hypocotyls was reduced by moisture deficiency because the maximum cortical cell length below the meristem was less in stressed seedlings (Paolillo, 1989). These findings are good lines of evidence for believing that environmental factors and applied presure would also affect the structure and/or components of the tissue at the cell level.

In addition, Wareing and Phillips (1975) indicated that the progressive steps of shoot and root differentiation as the first level, differentiation of organs in the shoot as the second level, and differentiation of the cell and tissue within each organ as the third level. Thus, the chain of developmental events in the plant body took place in a very orderly manner. Therefore, I believe that the environmental requirement for differentiation and development of the tissue is another special topic that needs to be studied through anatomical studies of the tissue.

CHAPTER 3

MATERIALS AND METHODS

Plant Culture

Plant materials

Most tomato seed were supplied by Peto Seed Company (Saticoy, California) including 'Nema1200', 'Peto#2', PSr28693, PSr28793, 'UC-82-L' and 'Walter'. In some studies, seed were obtained from Dr. C.M. Rick, UC Davis (54-718) or increased (54-718, 'Walter' and 'UC-82-L') in the greenhouse from January to June, 1989.

Petri dish culture

Seed germination tests and emerged seed for other studies were supplied from petri dish cultures. Seed of one line were placed in a 9cm glass petri dish containing 2 filter papers (Whatman No. 1) with 4 ml of deionized water as one replication. No additional water was added. Every 18 to 24 dishes were randomly stacked in a covered plastic tub (Rubbermaid Co.) that contained a dish of water to maintain 100% relative humidity or the petri dishes were wrapped individuallt with parafilm. Then the plastic tub or petri dishes were placed in growth chambers.

Vermiculite culture

One or two seed were placed in each plastic cell (4 x 3 x 5.5 cm/cell) of a tray with 72 cells filled with vermiculite (Terra Lite size 3). All cells were covered with Saran wrap (Dow Chemical) before imbibition, placed in a growth chamber, and watered as needed. Half strength modified Hogland solution (Hogland and Arnon, 1938) was used to water daily.

Environmental control

Two growth chambers were used. One was a Percival Model 5006 (Boone, Iowa) with fluorescent lights and a light intensity of 150 $\mu E\ m^{-2}s^{-1}$. The other was personally designed and made by Dr. K. Matsuda with fluorescent lights and a light intensity of 200 $\mu E\ m^{-2}s^{-1}$, and its relative humidity was kept at 50% ± 10% by placing a water tray in the chamber.

Germination

Water uptake

Twenty five seed of each line ('Nema1200', 'Peto#2', PSr28693, PSr28793, 'UC-82-L' and 'Walter') were weighed, and placed in petri dishes and the Percival growth chamber at temperatures of either 25 ± 1 or 40 ± 1° C. After 1, 2, 3, 6, 9, 12, 15, 24, 36, or 48 hours of imbibition, seed were blotted and reweighed. The percentage of water was absorbed determined as follows:

$$\% \text{ of water absorbed} = (W_t - W_o) \times 100 / W_o$$

where W_t and W_o were the weights of imbibed and unimbibed seed, respectively.

Alternating temperatures

Petri dishes. Tomato lines and general procedures were the same as those used in the water uptake study. In one study, seed were imbibed at 25 ± 1° C for 1 to 48 hour(s), and then transferred to 40 ± 1° C where observed at daily intervals until germination percentage no longer increased or for a maximum of 2 weeks. The controls in this study were maintained continuously at 40° C. In a reciprocal experiment, seed were imbibed initially at 40° C for various periods and later transferred to 25 ± 1° C where their germination was observed. Germination was counted when the emerged radicle was 2 to 3 mm in length. Germination percentage and germination rate were calculated. Germination % equaled the number of germinated seed x 100 / number of seed in the dish. Germination rate equaled the sum of (number of seed germinated/days of imbibition when germination was measured).

Vermiculite. Seed of 54-718, 'Walter' and 'UC-82-L' were surface sterlized by immersing for 10 minutes in 2.5% sodium hypochloride, rinsing thoroughly, and soaking in continuously aerated tap water for 2 or 15 hours at 23 ± 1° C. Then seed were sowed in trays with cells containing vermiculite. Fourty-eight seed were used per replication, with 2 replications per line. Trays were placed in the personally designed growth chamber with different temperatures. In one study, seed were imbibed continuously at 23, 35 or 42° C for 5 days and then moved into 23° C. Germination was determined daily for up to 4 weeks.

In another study, seed in each cell were placed in alternating day/night temperatures of 40/30 or 40/25° C (D/N 14/10 hours). Seed germination was observed for 3 weeks.

Detection and Measurement of Heat Shock Proteins

Chemicals used in this section were from Sigma chemical company, except acrylamide which was from Eastman Kodak company. All seed, microcentrifuge tubes, filter papers, micropipette tips, water and chemicals were sterilized. Studies were performed with 2 heat tolerant ('Nema1200', 'UC-82-L') and 2 heat sensitive ('Walter', 'Peto#2') cultivars.

Extraction of proteins

To reduce possible errors due to bacterial contamination, all seed were surface sterlized by immersion in ethyl alcohol for 5 min, then in 2.5% sodium hypocholoride for 5 min, and then rinsed with sterilized deionized water before imbibing in a petri dish under aseptic conditions for 3 days at 25° C. Individual seed with a radicle length of 3 mm were transferred into a 1.5 ml capped microcentrifuge tube (West Coast Scientific, No. 2075) and held in 10μl of 0.025% (v/v) Triton X-100 (Rohm & Hass, Sigma No. T-6878) for 0.5 hour. Then the soluton was discarded and replaced with 10μl of 0.025% Triton x-100 solution that contained 15 μCi of ^{35}S-Methionine (Trans^{35}S-label cat. no. 51006 ICN Radiochemicals) and seed were incubated at 30, 35 or 40^{o} C for 2.5 hours. Caps were perforated to allow for gas expansion and two replicated samples were used. Following incubation, seed were rinsed three times with 0.025% Triton X-100, ground with 200 μl of the extraction buffer (60mM Tris-HCl, pH=8.0, 60 mM dithiothreitol, 2 % lithium dodecyl sulfate, 15% sucrose, 5mM amino-N-caproic acid, 1 mM benzamidine, 0.01% bromophenol blue). The homogenate was placed in a boiling water bath for 2 min, cooled in ice, and centrifuged at 14,000 rpm for 10 min in a Brinkmann model 5415 microfuge, held in the refrigerator at 4^{o} C. The supernatant was transferred into another microcentrifuge tube and 3 batches of 10 μl each were transferred to a sheet of paper (Whatman filter paper) with diameter of 1 cm for determination of total absorbed radioactivity and radioactivity present as proteins. The remaining solution was saved for gel electrophoresis of proteins. The sample used for determining total radioactivity was dried, and placed in 8 ml of cocktail (4l Toluene + 19.9g PPO (2,5-diphenyloxazole) + 1.2g POPO (1,4-bis[2-95-

phenyloxazolel)]benzene), and counted in a Liquid Scintillation Spectrometer (Beckman LS8100). Each of the two samples used for determining radioactivity in proteins was treated with ice cold 10% (w/v) tricholoracetic acid (TCA) for 15 min on an orbital shaker (Lab-line Instruments), then with hot 10% TCA for 5 min on a magnetic stirring hot plate, then with cold 10% TCA for 15 min on the shaker, and finally washed with 95% ethyl alcohol for 1 min and dried before counting.

Polyacrylamide Gel Electrophoresis

The proteins present in homogenates were separated according to MW with a modification of the sodium dodecyl sulfate (SDS) gel electrophoresis procedure developed by Laemmli (1970). The lower separating gel was prepared for a vertical slab gel unit (Model SE 400, the sturdier, Hoefer Scientific Instruments) by mixing H_2O 13.7ml, 20% SDS 150μl, separating gel buffer 6ml (1.875 M Tris base, 0.5 ml concentrated HCl, pH=8.8), 30% (w/v) Acrylamide 10 ml, TEMED 15 μl, and 10% Ammonium persulfate 150 μl. After 2 hours polymerization, the upper stacking gel was prepared by mixing H_2O 11.7 ml, 20% SDS 80 μl, stacking gel buffer 1.5 ml (1.25 M Tris, 1ml concentrated HCl, pH=6.8), 30% Acrylamide 1.5 ml, TEMED 22μl and 10% Ammonium persulfate 220 μl and polymerized for another 2 hours. Aliqouts from the various homogenates were than added such that each contained 50,000 cpm as proteins. Additional extraction buffer was loaded to make 25μl volume in each lane of the gel before adding electrophoresis buffer (25mM Tris base, 14.4g Glycine and 0.1% (w/v) SDS, pH=8.3). Electrophoresis was performed with an EC 452 (E-C Apparatus Corporation) for 4 hours at 25 milliamps. Gels were then stained overnight in 0.25% Coomassie (Brillant Blue R 0.25% (w/v) , 50% Methanol and 10% Acetic acid v:v:v), destained in Destain solution I (50% Methanol and 10% glacial Acetic Acid in glass distilled water) for two hours, destained in Destain solution II (30% Methanol and 10% glacial Acetic Acid in glass distilled water) for 2 hours twice, and finally rinsed with tap water. The gel was mounted between two pieces of Cellophane (untreated Cellophane Type 128, cat. # PUT-76) which were previously soaked with a solution of 20% ethanol and 10% acetic Acid, and dried overnight.

Autoradiography of the gel

Radioautographs of the gels were obtained by placing the gel adjacent to x-ray film (Kodak Diagnostic film X-Omat AR GBX-2) in an X-ray exposure holder (Kodak X-ray Exposure Holder 8x10 in RTS) and stored in the freezer at -80^{o} C for the appropriate time, for example 50,000cpm for 48 hours.

<u>Electrolyte Leakage Test</u>

Leakage tests on germinated seed ('Nema1200', 'Walter', 'Peto#2' and 'UC-82-L') were conducted by an electrolyte leakage procedure that Wallner et al. (1982) used for measuring heat stress damage. Seed were imbibed in petri dishes at 25° C for 3 days. Groups of 10 seed with radicle lengths of 2 to 6 mm were placed in a test tube with 10 ml of deionized water. The tubes were sealed with paraffin film, and incubated at 30, 35 or 40° C for 4, 6, 8, or 12 hours, and then cooled to room temperature (23.5° C). Electrical conductivity (μmhos) with a Altex conductivity bridge (model RC-16C) with Beckman conductivity cell (G01). Then samples were boiled for 15 minutes, cooled to 23.5° C and conductance was remeasured. Percentage of leakage and injury were calculated as follows: % leakage = [ht1 (or ct1)] x 100 / [ht2 (or ct2)]; % of injury = { 1 - [1 - ht1/ht2]/ [1 - ct1/ct2] x 100, where seedlings maintained at 23.5° C were designated as control (ct), and samples exposed to 30 ° C or higher were considered as heat treated (ht), and conductance values (μmhos) measured before and after boiling were 1 and 2, respectively (Martineau et al. 1979).

Seedling development

Duration of high temperature

In one study, seed ('Nema1200', 'Walter', 'Peto#2' and 'UC-82-L', PSr28693, and PSr28793) were imbibed and germinated in petri dishes at 25 ± 1° C for 3 days in the Percival growth chamber. Three emerged seed with radicle lengths between 2 to 5 mm were transferred to fresh petri dishes and exposed to 40 ± 1° C for 4, 6, 8, or 12 hours, and returned to 25 ± 1° C and measured for shoot and root elongation at day 8 after the start of imbibition. As a control (hr0), seed were kept at 25° C. The study was repeated three times. Percentage inhibition in root and shoot growth, the growth rate and relative growth rate of roots were calculated as follows: % inhibition in root or shoot growth= [(length of hr0 -length of treated)/length of hr0] x 100; root growth rate = (length at day 8 - length at day 3)/5; relative growth rate of root = [log(length at day 8) - log(length at day 3)]/5 (Briggs et al., 1920).

In another study, seed were germinated at room temperature (23/21° C) for 3 days. The seedlings with radicle lengths between 2 to 5 mm were transfered into fresh petri dishes or cell pots of vermiculite and then held at 40° C in the personally designed growth chamber for 3 or 6 hrs before being moved back to room temperature. Seedlings were measured for growth on the 8th day. Parameters of growth measured were the number, length, fresh weight (fw) and dry weight (dw) of root, shoot and cotyledons.

Alternating temperatures

The age of seedlings was counted from the time the seed radicle emerged. In the first study, the growth of seedlings up to 15 days post emergence was used to determine the proper age of seedlings for further investigation. Increased tomato seed (54-718, 'UC-82-L', and 'Walter') were used. The seed were surface sterilized as in the germination study in vermiculite. Then seed were sown, germinated and grown at 23/21° C (14/10 hours D/N) in personally designed growth chamber. Growth was measured at days 2, 6, 8, 10, 12, and 15 after emergence. The number of secondary roots were counted in the first one centimter section of the root basipetally. The fresh and dry weight of leaves, cotyledons, stems and roots for each sample were recorded.

The second study was to focus on the effect of temperatures, and day lengths at different developmental stage on the development of seedlings. Germinated tomato seed (cv. Walter) were studied in the combination of temperature (23/21, 40/25, and 40/30° C), stage (emerged seed and 2 day old seedlings) and day length (14/10 and 10/14 hours D/N) to see how these factors affect seedling growth. One set of seed was germinated in petri dishes under room temperature (23/21° C) with a day length of 14/10 hours D/N, transferred into a small cells (one seed per cell) with vermiculite and placed into the personally designed chamber at different temperatures at the same day length. Seedlings were watered with half strength Hogland's solution daily. The same process was repeated for the other day length of 10/14 hours D/N.

Another set of seed was placed under the same conditions, except that the germinated and transferred seed were grown for 2 days at room temperature until their cotyledons were fully expanded. Then the 2-day seedlings were treated with temperatures in the personally designed growth chamber. The number of secondary roots and of leaves as well as fresh and dry weight of stem, root, cotyledon and leaf were measured when seedlings were 10-day-old.

Anatomical Studies

The tomato cultivar 'Walter' was used. Seed were imbibed and germinated in a petri dish. Emerged seed were transferred into another petri dish or vermiculite for temperature treatments and the samples were collected at the given day for anatomical analyses of the stems, roots, shoot apex, cotyledons, and true leaves. Samples taken are shown in Table 3.1.

Sections of tissues were killed and fixed according to Feder and O'Brien (1968). One cm lengths of tissue were placed in small vials containing 3% (v/v) glutaraldehyde in 0.07 M Sorensen's phosphate buffer with pH 6.8. The segments were vacuum infiltrated for 20 to 30 min, and then held in a fixative solution for 48 hours at 4° C. Then samples were rinsed twice in cold Sorensen buffer for 10 min each time. The samples were dehydrated using a series of dehydration materials with two changes in 4 to 24 hours of each in methyl cellosolve, absolute ethanol, N-propanol and N-butanol. In preparing samples for embedding in paraffin, the samples were transferred from N-butanol into paraffin/xylene mixture 50% (v/v) for two hours, and then were changed 2 to 3 times with pure paraplast in the oven at 56° C. The samples were finally embedded in plastic trays filled with paraplast and sectioned to 12 μm thickness with a rotary microtome (Spencer Lens Co., Buffalo, N. Y.) using single edge disposable razor blades. The samples were mounted on microscopic slides.

The staining process was followed as in Sakai (1973). The slides with the samples were placed in 0.05% toluidine blue O for 2 to 5 min. Then the slides were rinsed in distilled water for 1 min and air dried. The paraffin was removed with two changes of xylene and a coverslip was mounted with a synthetic resin.

Statistical Analysis

Analysis of variance (ANOVA) for a two or three factors Factorial Design was used to analyze all data obtained from the studies. The Duncan's multiple range test at the 5% level was used to compare the means for treatments and tomato lines tested in the experiments. The percentage data were transformed by square root and arcsin prior to the ANOVA procedure, as suggested by Gomez and Gomez (1983).

Table 3.1 Tomato samples which were treated and used for anatomical analysis

Temp (℃)	stage[z]	day	medium	parts collected
23/21	G	2	vermiculite	shoot apex root stem cotyledon
	G	3	petri dish	cotyledon root stem
	S	8	vermiculite	shoot apex root stem cotyledon leaf
40/25	G	2	vermiculite	root stem
	G	8	vermiculite	shoot apex root stem cotyledon leaf
	S	8	vermiculite	shoot apex root stem cotyledon leaf
40/30	G	2	vermiculite	root stem
	G	8	vermiculite	shoot apex root stem cotyledon leaf
	S	8	vermiculite	shoot apex root stem cotyledon leaf

z: emerged seed (G) or 2 day old seedlings (S) were treated with the given temperatures for 2, 3, or 8 days.

CHAPTER 4

RESULTS AND DISCUSSION

Water Uptake of Seed During Germination

Comparisons of water uptake showed that seed from 6 lines imbibed their major fraction of water during the first 12 to 15 hours. Water uptake rates in this interval were higher at 40° C than at 25° C. Water absorption by the seed could be detected within 1 hour at both temperatures (Fig 4.1). These phenomena could be because seed with a high negative water potential absorb water physically at the start of imbibition (Bewley and Black 1978). Water viscosity was lower when temperature rose (Berrie, 1984). Therefore, seed imbibed at 40° C absorbed much more water than seed imbibed at 25° C for the first 12 hours of imbibition. The water potential of seed increases during imbibition, thus decreasing its absorbing ability at later stages of imbibition. However, the total amount of water absorbed among lines was the same during the imbibition process.

Effect of High Temperature During Imbibition on Tomato Seed Germination

In petri dishes

Seed reached their maximum percentage germination

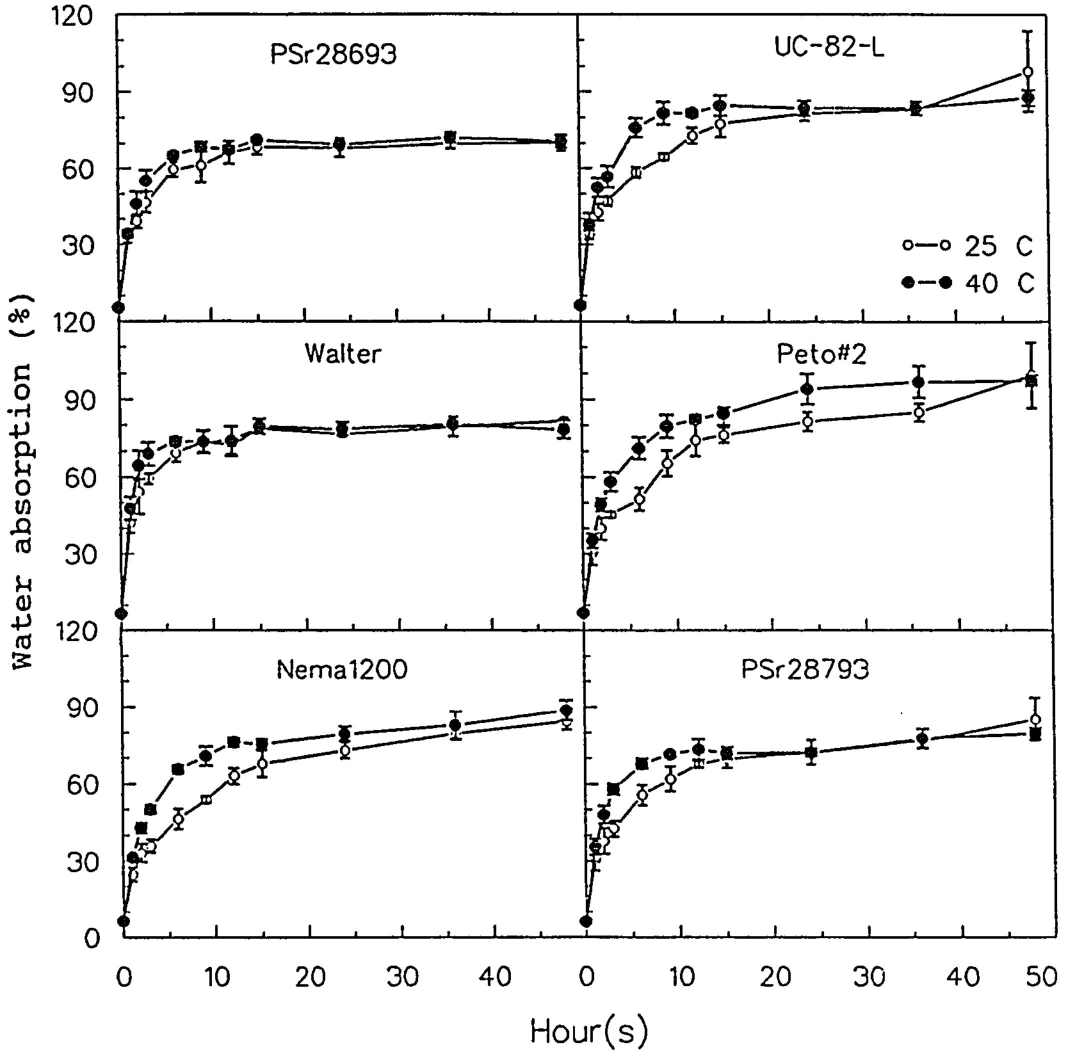


Fig.4.1 Water absorption (%) in six lines of tomato seed imbibed at constant 25 or 40° C.

about 3 to 4 days after the start of imbibition, with 85% or higher germination in all lines when seed were imbibed and germinated at a constant 25° C (see hr 0, Table 4.1). All the tested lines had less than 4% germination when their seed were imbibed and germinated at 40° C continuously (see hr 0, Table 4.2).

In an effort to identify the period of high sensitivity to heat stress, seed were imbibed at 25° C for periods up to 48 hours and then were transferred to 40° C and held for a total of 2 weeks to examine germination (Table 4.2). In this case, germination percentages in all lines were no different than in treatments given continuous 40° C from imbibition if seed were imbibed for less than 15 hours at 25° C. However, all lines showed marked increases in germination percentage if the 40° C treatment was applied after 24 hours of imbibition at 25° C. These results indicated that the time interval of 24 to 36 hours following imbibition at 25° C was when the seed had pronounced changes in response to subsequent high temperatures.

PSr28793 and 'UC-82-L', approached the same germination percentage under continuous 25° C, as when they were treated with 40° C after 48 hour imbibition at 25° C. But PSr28693 had only 25% and 'Walter' only 60% of their germination percentage obtained under 25° C conditions. Such data suggested that PSr28793 and 'UC-82-L' might be developed

Table 4.1. Germination percentage of six lines of tomato seed imbibed at 40° C for the given hour(s) and then transferred to 25° C.

	Lines						
Hour(s) at 40° C	Nema 1200	Walter	PSr 28693	PSr 28793	Peto #2	UC-82-L	Mean
0	96	90	98	97	87	92	93 a[z]
1	94	88	98	96	89	93	93 a
2	97	91	98	99	90	95	95 a
3	98	87	73	97	91	97	91 a
6	96	88	100	96	85	94	93 a
9	97	84	97	98	85	94	93 a
12	92	88	99	97	82	94	92 a
15	95	89	97	99	83	95	93 a
24	95	84	97	93	80	93	90 a
36	95	60	80	94	71	82	80 b
48	53	21	69	91	62	85	64 c
Mean	92 ab	79 c	92 ab	96 a	82 c	92 ab	

z: No significant interaction between lines and hours, so means lumped for comparision; Mean separation based on Duncan's multiple range at 5% level; analysis on arcsin transformed data.

Table 4.2. Germination percentage of six lines of tomato seed imbibed at 25° C for the given hour(s) and then transferred to 40° C.

Hour(s) at 25° C	Lines: Nema 1200	Walter	PSr 29693	PSr 28793	Peto #2	UC-82-L	Mean
0	1	0	0	1	3	2	1 [z]
1	1	0	0	0	2	0	1
2	2	0	0	1	0	0	1
3	4	1	0	0	0	0	1
6	1	2	0	0	1	2	1
9	2	0	0	0	0	0	1
12	3	0	0	1	3	0	1
15	2	1	0	0	0	1	1
24	2	1	0	18	12	11	7
36	40	20	11	41	38	67	36
48	71	53	24	84	67	88	64
Mean	11	7	3	13	11	15	

z: Interaction between lines and hours was significant so no mean separations on average values.

sufficiently in their physiological events after 2 days of imbibition at 25° C to resist the deleterious effects at 40° C, but PSr28693 and 'Walter' were not.

This possibility was also supported in the germination rate studies (Table 4.3) which showed that 'UC-82-L' and PSr28793 had the highest germination rates, whereas PSr28693 and 'Walter' had the lowest under the same conditions. These data were in partial accord with Coons et al. (1989) view that 'UC-82-L' and 'Nema 1200' were resistant and 'Walter' and 'Peto#2' were sensitive to high temperature during germination.

The reciprocal study in which seed were imbibed initially at 40° C and then transferred to 25° C showed that seed of all lines could recover from any deleterious effects of high temperature if the duration of high temperature was 24 hours or less (Table 4.4). Since some lines showed marked reductions in germination percentage (Table 4.1) when seed imbibed at 40° C for 24 hours or more and all lines showed decreased germination rates after 24 hours (Table 4.4), it indicated that the period of 24 to 48 hours was considered critical for the development of factors that permitted tomato seed to recover from high temperature stress.

It was likely that PSr28793 and 'UC-82-L' could recover much quicker than Walter after the heat stress ended, because germination percentage of PSr28793 and 'UC-82-L' were

Table 4.3. Germination rate[z] of six lines of tomato seed imbibed at 25° C for the given hour(s) and then transferred to 40° C.

	Lines						
Hour(s) at 25° C	Nema 1200	Walter	PSr 28693	PSr 28793	Peto #2	UC-82-L	Mean
0	0	0	0	0	0	0	0[y]
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
6	0	1	0	0	0	0	0
9	1	0	0	0	0	0	0
12	1	0	0	0	0	0	0
15	0	0	0	0	0	0	0
24	0	0	0	2	1	1	1
36	4	2	1	4	4	8	4
48	8	6	2	9	7	10	7
Mean	2	1	0	1	1	2	

z: Germination rate equaled the sum of (number of seed germinated/days of imbibition when germination was measured).
y: Interaction between lines and hours was significant so no mean separations on average values.

Table 4.4. Germination rate[z] of six lines of tomato seed imbibed at 40° C for the given hour(s) and then transferred to 25° C.

Hour(s) at 40° C	Nema 1200	Walter	PSr 28693	PSr 28793	Peto #2	UC-82-L	Mean
0	10	9	10	10	9	10	10 a[y]
1	10	9	9	10	9	11	10 a
2	10	9	10	11	9	12	10 a
3	10	8	7	10	9	12	9 ab
6	9	8	11	9	8	11	9 ab
9	8	7	8	10	9	10	9 ab
12	9	7	10	8	7	9	8 b
15	8	7	8	9	8	10	8 b
24	6	6	8	7	6	7	7 c
36	5	4	5	5	4	6	5 d
48	4	2	4	6	4	5	4 d
Mean	8 bc[y]	7 c	8 bc	9 ab	8 bc	10 a	

z: Germination rate equaled the sum of (number of seed germinated/days of imbibition when germination was measured).
y: No significant interaction between lines and hours, so means lumped for comparisons; Mean separation based on Duncan's multiple range at 5% level.

not reduced when seed were initially exposed to 40° C for 48 hours. However, germination percentage of 'Walter' reduced pronouncedly. Similarly, germination rates of PSr28793 and 'UC-82-L' were almost twice that of 'Walter'. The germination rate of all lines was reduced to one half or even one third of the value obtained from seed imbibed and germinated at a constant 25° C. This indicated that germination rate was more affected than germination percentage by high temperture.

The efforts to categorize tomato lines as heat tolerant or heat sensitive at germination were based on the germination percentage and germination rate of seed treated with constant temperature in our studies. It was partially in accord with the experiment conducted by Ellis' group. Ellis et al. (1987) stated that the response of the seed germination rate defined and calculated through probit analysis would be a reliable new approach to be used in germplasm screening programs when seed were treated with constant temperatures for a period of time. Their new approach has been tested in chickpea, lentil, soybean, cowpea, and both dormant and aged barley seed. For testing Ellis' approach, more lines in addition to PSr28793, 'Walter', 'Peto#2' and 'UC-82-L' and more constant temperatures (15, 20, 25, 30, 35, 40° C) were used. And all lines were grown in the greenhouse in order to increase the seed under the same environment.

Unfortunately, none of the tested lines germinated at 40° C. It was impossible to test the Ellis' hypothesis due to lack of germination data under high temperature. However, germination percentages of all lines ranged from 56 to 100% at 15° C, 80 to 100% at 20 and 25° C, and 84 to 100% at 30° C. Tomato lines had a higher germination percentage and rate at 30° C which was not in accord with the result of Coons et al. (1989) which showed that 25° C was optimal. It was likely that the environment under which the seed are developed would affect their germination.

In vermiculite

Studies were performed to determine if germination percentage could be improved by imbibing seed in vermiculite rather than in petri dishes, as suggested by Dr. A. Benzoini (Div. of Life Sciences, Negev Institute for Arid Zone Research, Israel, personal communication), and to establish criteria for distinguishing heat sensitive from heat tolerant tomato lines at germination. Also, alternate temperatures were evaluated for effect on germination. Germination percentages of both 54-718 and 'Walter' (increased seed) were improved considerably, and were close to those obtained by seed maintained at room temperature (23° C), when seed were imbibed in vermiculite for 5 days at high temperatures, and then transferred to room temperature (Fig 4.2, bottom). Such

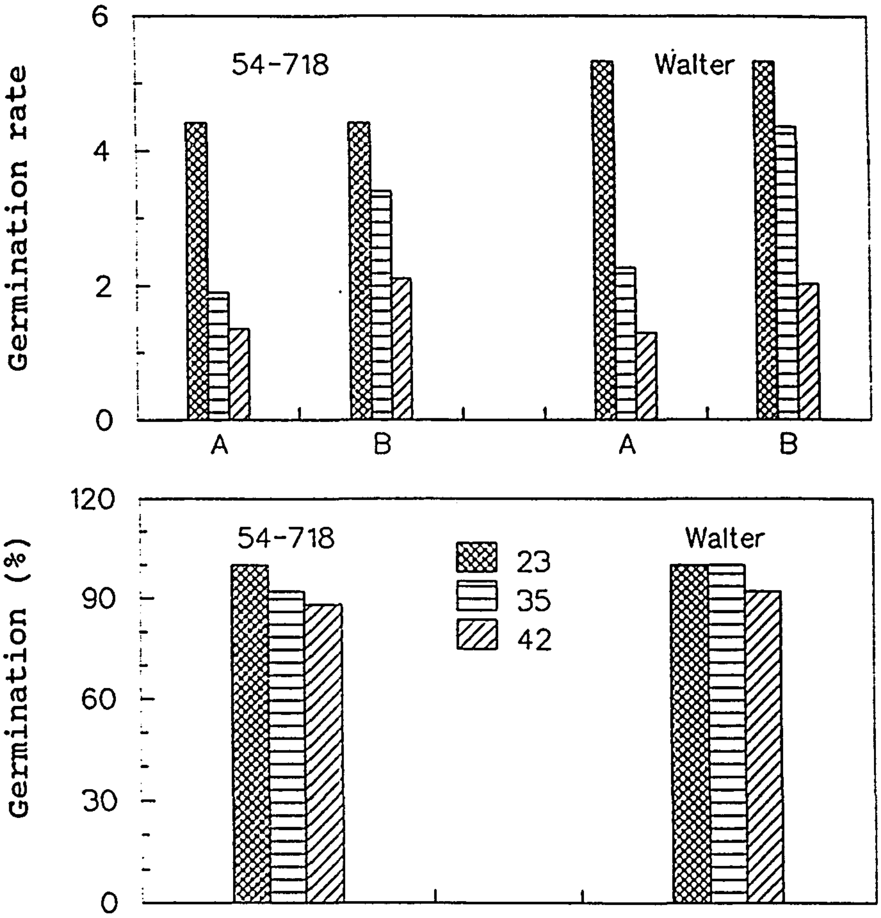


Fig.4.2 Germination rate and germination percentage of two lines of tomato seed imbibed at constant 23, 35, 42° C for 5 days and then moved into 23° C for 4 weeks.

A: germination rate calculated from the start of seed imbibition.

B: germination rate calculated from seed been moved back to 23° C data suggest that high temperature during the early imbibition might not be responsible for the lack of germination that occurred in petri dishes (Table 4.4). Possibly, high temperature caused reduction in oxygen solubility in water and it might be the major contributor to poor germination. Come and Tissaoui (1973) stated that embryos had potentially higher metabolic rates and required more oxygen at higher temperatures.

Germination rate, calculated from the beginning of imbibition, was considerably reduced (Fig 4.2, above A) and was highly significantly different ($P < 0.001$) among temperature treatments. The germination rate of the seed was also calculated when the seed were moved into room temperature after the heat treatment. There was some reduction at 35° C and more than a 50% reduction at 42° C (Fig 4.2, above B). This indicated that seed recovered slower when imbibed at 42° C for 5 days. In addition, germinaiton rate of 'Walter' at 23° C was much lower than what at 25° C (see hr 0 Table 4.4 & Fig 4.2, above A). The difference could be contributed to the method of counting - observing the extrusion of the epicotyle from soil surface which could be one or two days longer than the time of radicle emergence.

It was hypothesized that heat treated seed during imbibition would cease or inhibit some basic physiological changes in their development. These processes would resume after the release of heat eventhough the seed have been treated for 5 days. The seed were still viable as what Coons et al. (1989) found. Three conclusions can be made: 1> Temperature as high as 42° C with 5 days duration was not deleterious to imbibed tomato seed. 2> Germination rate was more sentitive than germination to test temperature response, as in agreement with Bewley and Black (1978). 3> The reduction in germination rate after a period of high temperature, might be best explained as being due to conformational changes in protein, which inactivate enzymes (Bewley and Black 1978) and/or alter the content and function of proteins (Teeri 1980), rather than due to serious breakdown, denaturation or aggregation of proteins. A breakdown of proteins would not be recovered, and proteins would resume their activities and function easier if they just went through conformational changes or activation of the molecules after the release of heat.

In establishing the criteria for heat stress injury on any crop at any developmental stage, the characteristic chosen should be representative and relieable in showing its different response to stress treatment. In the above germination study, it was indicated that a 5 day heat treatment was not long enough to reduce the germination percentage by 50%, but the germination rate did show more than 50% injury when seed were treated with heat for 5 days (Fig

4.2, above A). Thus, the effort to establish the criteria for distinguishing heat sensitive and heat tolerant tomato lines by germination alone was difficult to accomplish.

Tomato lines (54-718, 'Walter' and 'UC-82-L') were used in the preliminary study on the effect of alternate temperatures (40/30 and 40/25° C 14/10 hours D/N) on seed germination. The germination percentage and germination rate were 91.7, 95.8 and 95.8; 1.75, 2.08 and 1.05 for 54-718, 'Walter' and 'UC-82-L', respectively when seed were imbibed and germinated at 40/30°C in vermiculite; and they were 87.5, 20.8 and 70.8; 1.78, 0.24 and 1.89 when seed were at 40/25° C. Germination reduced dramatically in 'Walter' when seed were at 40/25° C. In addition, young seedlings of all lines grew poorly at these temperature combinations. It indicated that high temperature had significant effects on tomato embryo development at late germination and very early seedling development. Therefore, studies to observe the effects of temperature on seedling development were conducted by allowing seed to emerge in petri dishes at room temperature, before being transferred to vermiculite and subjected to high temperatures.

Detection and Measurement of Heat Shock Proteins (hsps)

High temperature effects on germination were found most critical during the period of 24 to 48 hours from the start of imbibition in the studies above. Initial attempts to measure hsps synthesis were performed on seed that were imbibed at 25° C for 24 to 36 hours. The studies showed that only a very small fraction of the radioactivity was absorbed by the seed and various attempts were made to increase absorption. Since slitting the seed, treating with bleach or scarifying with concentrated sulfuric acid did not increase uptake of labelled amino acid, all further attempts to study hsp synthesis in ungerminated seeds were abandoned. However, seed imbibed for 3 days, which had radicles of 2 to 3 mm, did absorb considerable label that was incorporated into proteins.

The ^{35}S-Methionine isotope with concentration of 5μCi per 10μl and two to three hours incubation were used for hsps studies. For determining the isotope concentration used in emerged tomato seed, the concentrations and duration of isotope incorporation were tested under different temperatures (Table A.3). The quantity of 15μCi/10μl of radioisotope was used for this part of the study.

No differences were found in the pattern of the hsps between heat tolerant ('Nema1200' and 'UC-82-L') and heat sensitive ('Walter' and 'Peto#2') tomato cultivars at different temperatures (Fig 4.3). But the 40° C treatment had thicker bands at 110, 87, 72, 17, 13, and 12.6 KD, which indicated that more hsps had been synthesized relative to the lower temperatures. It could be assumed that the synthesis

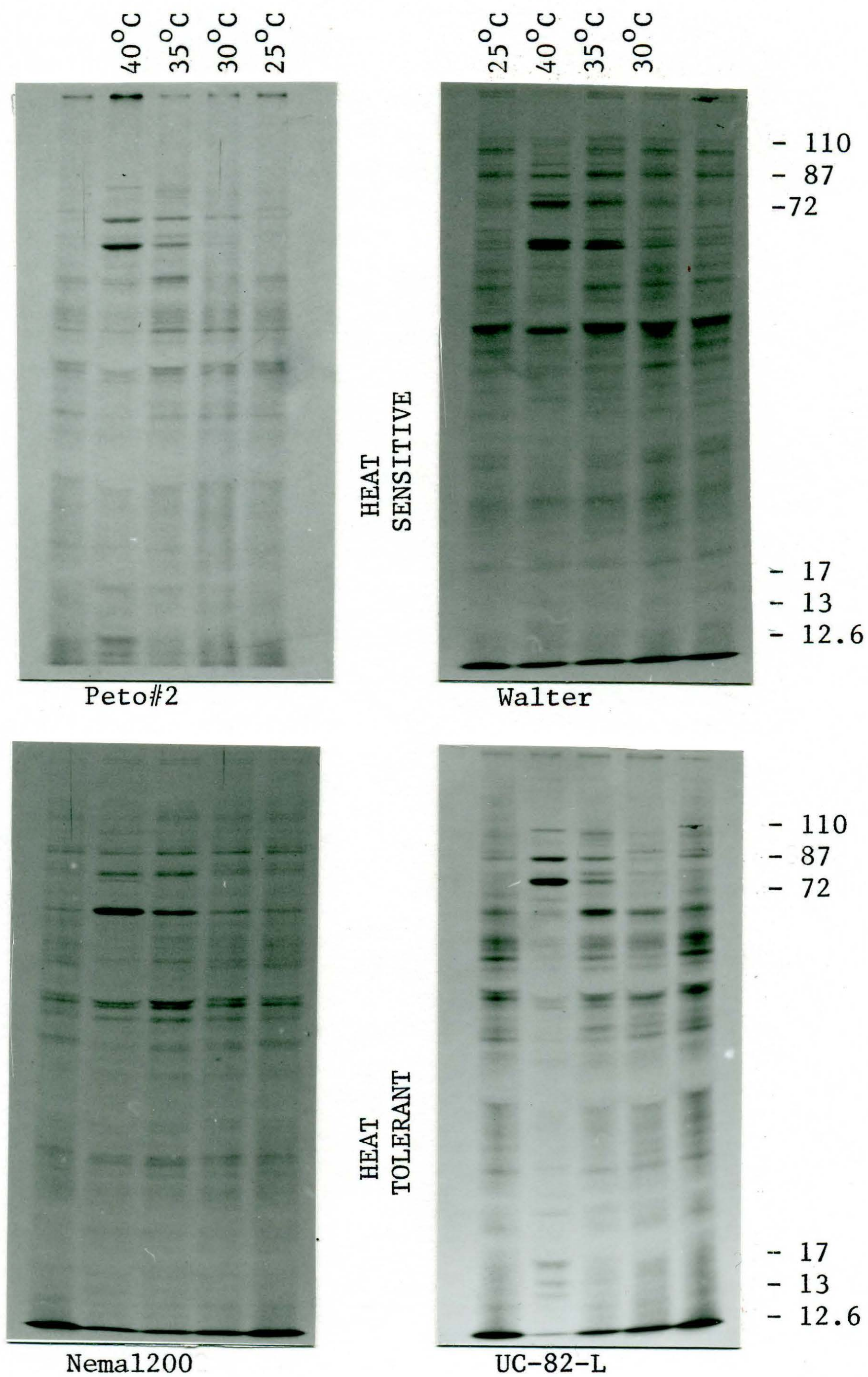


Fig.4.3 Heat shock proteins synthesized in four tomato lines in emerged seed at four temperatures.

of hsps in the emerged tomato seed was at the expense of other proteins being synthesized under high temperature. The labelled proteins were only half or even one third in the 40°C treatment, when compared to the 25° C treatment (Table 4.5). Altschuler and Mascarenhas (1985) and Burke, et al. (1985) had the same conclusion.

Some differences were found among cultivars in total isotope incorporation, labelled proteins and percentage of label into proteins, i.e. 'Nema1200' and 'Walter' had higher values than 'Peto#2' and 'UC-82-L' (Table 4.5). It seemed that quantitative changes in these measurements (total isotope incorporation and labelled proteins) did not parallel with whether the seed sources were heat tolerant or heat sensitive at germination. The hsp bands appeared at all temperatures, with the only differences in being thickness of the bands (Fig 4.3). There appeared to be no significant differences in heat response among the tested tomato cultivars in terms of genes being turned on and off in hsp synthesis. In general, the total isotope incorporation, labelled protein synthesis, and the percentage of label into proteins in emerged tomato seed among cultivars were decreased at higher temperatures (Table 4.5 & Table A.3). Even though it was not shown to be consistently a significant temperature difference in labelled protein synthesis and the percentage of label into proteins, there was a decrease in both when temperature reached 35° C.

Table 4.5. Total radioisotope incorporation, labelled proteins(cpm)[z], and percentage of label into proteins of emerged seed of four tomato cultivars at four temperatures.

	Cultivars				
Temp(℃)	Nema1200	Walter	Peto2	UC-82-L	Mean
-----	Total isotope incorporation (cpm)	----			
25	438316	337841	288875	274736	334942 a[y]
30	506030	300146	280623	186130	318232 a
35	326066	316769	192410	169887	251283 ab
40	185647	209318	149486	126031	167620 b
mean	364014 a[y]	291019 ab	227848 b	189196 b	
------------	Labeled proteins (cpm)	----------			
25	89291	83383	40627	48325	65406 a
30	144024	73264	34309	19554	67788 a
35	91107	94754	24279	22667	58202 ab
40	38668	49467	14153	14092	29095 b
mean	90772 a	75217 a	28342 b	26159 b	
----	Percentage of label into proteins (%)	----			
25	17.0	24.5	14.0	17.5	18.3
30	28.5	24.5	10.5	10.5	18.5
35	27.5	29.0	11.0	12.5	20.0
40	20.5	22.5	9.0	11.0	15.8 ns
Mean	23.4 a	25.1 a	11.1 b	12.9 b	

z: counts per minute
y: No significant interaction between lines and hours, so means lumped for comparisions; Mean separation within parameter based on Duncan's multiple range at 5% level; analysis on arcsin transformed data as data were in percentage.

The decrease started to show dramatically at 35° C for 'Peto#2' and 'UC-82-L' and at 40° C for 'Nema1200' and 'Walter'.

Electrolyte Leakage Test

Germinated tomato seed were treated with different temperatures for different lengths of time to test the leakage and injury of seed and to see whether injury could be used as criterium for selection of heat tolerance in tomato. At the same time, root and shoot growth were investigated.

Leakage of the root radicle in response to temperature indicated that the higher the temperature and the longer the exposure; the more leakage and injury (Table 4.6 & Table 4.7). The leakage and injury of the tomato root did not differ much for the sample at 25 and 30° C, and at 4 and 6 hour treatment. There was a significant effect on root damage when the temperature was higher than 35° C. When root radicles were treated at 40° C for 12 hours (Table 4.6 & Table 4.7), the leakage and injury of the emerged roots nearly reached 50% in all cultivars. The leakage of the root radicel among cultivars was closely related to the incoporation of labeled proteins into emerged seed (Table 4.5 & Table 4.6). This might explain why 'Peto#2' and 'UC-82-L' had less incorporation of the isotope, that is because their root radicles were damaged and leaked more when they were treated

Table 4.6. Electrolyte leakage (%)[z] of emerged seed for four tomato cultivars, temperatures and durations of temperature treatments.

Temp (℃)	hr	Cultivars				Mean
		Nema1200	Walter	Peto#2	UC-82-L	
40	12	54.8	58.2	67.5	66.1	
	8	51.7	45.9	52.4	53.0	
	6	41.6	38.3	45.3	40.9	
	4	25.0	36.8	32.0	35.5	44.3[y]
35	12	50.0	41.4	59.0	53.9	
	8	31.0	32.5	35.7	30.4	
	6	30.4	27.4	36.2	28.6	
	4	24.3	22.9	23.6	23.2	34.4
30	12	34.6	25.1	41.9	37.1	
	8	23.2	22.6	31.3	28.2	
	6	23.8	22.3	27.2	27.4	
	4	15.3	20.0	19.1	20.2	26.2
25	12	25.7	19.0	30.0	24.2	
	8	18.5	19.1	25.4	23.8	
	6	22.4	20.9	19.4	22.5	
	4	21.7	16.3	18.9	19.8	21.7
Mean		30.9[y]	29.3	35.3	39.0	

z: Electrolyte leakage (%) was calculated by the formula shown in the materials and methods.
y: Interactions were significant, so mean separations were not done.

Table 4.7. Injury (%)[z] of emerged seed for four tomato cultivars, temperatures and durations of temperature treatments.

Temp (℃)	Hr	Cultivars Nema1200	Walter	Peto#2	UC-82-L	Mean
40	12	45.5	48.6	53.5	53.4	
	8	39.9	33.2	36.5	38.2	
	6	25.1	21.9	32.5	23.2	
	4	3.8	24.8	16.1	19.9	32.3 a[y]
35	12	32.9	27.9	41.4	39.3	
	8	14.8	16.7	14.3	8.4	
	6	11.3	8.1	21.3	7.2	
	4	3.0	8.3	5.6	4.6	16.6 b
30	12	12.2	14.1	18.9	17.2	
	8	5.8	5.8	8.4	4.9	
	6	2.3	3.4	10.1	5.8	
	4	0.1	4.7	0.6	1.1	6.9 c
Mean		16.4	18.1	21.6	18.6	

z: Injury (%) was calculated by the formula shown in the materials and methods.

y: Interaction between cultivars and hours was significant, so means for temperatures lumped for comparisions; Mean separation based on Duncan's multiple range at 5% level; analysis on arcsin transformed data.

with higher temperatures.

There was significant interaction between cultivars and the durations of the temperature treatments to both leakage and injury, and highly significant interaction between temperatures and their durations for leakage (Table A.5). Thus, leakage is a better indicator of heat damage to tomato root radicles. 'Nema1200' had the least injury and is considered a heat tolerant line. But it did not apply to 'UC-82-L' as heat tolerant and 'Walter' as heat sensitive, which were screened in germination stage.

The reduction in tap root length was observed after any treatment at 40° C, except PSr28793 (Table 4.8). 'Walter' and 'UC-82-L' showed the most sensitive response to temperature, with the shortest root lengths and least root growth (Table 4.8 & Table 4.9), even though there was no significant difference among tomato lines (Table A.6) in root length. The tap root radicle, which was exposed directly to the high temperature, showed a visible browning at the tip, and had more significant inhibition (60 to 90 %) in growth when emerged seed were treated at 40° C for 12 hours.

There was highly significant interactions between lines and durations of temperatures in shoot length (Table A.6). High temperatures had an effect on shoot growth when emerged seed were treated for more than 8 hours (Table 4.10). However, the inhibition of shoot growth was about 50% on

Table 4.8. Length[z] and inhibition (%) of seed radicles of six lines of 8-day-old tomato seedlings grown in petri dishes at 25° C after heat treatment (40° C) to emerged seed for 4 to 12 hours.

Hour(s) at 40° C	Lines: Nema 1200	Walter	PSr 28693	PSr 28793	Peto #2	UC-82-L	Mean
	------ Radicle length(cm)[z] ---------						
0	11.2	11.4	7.6	9.1	12.4	10.2	10.1 a[y]
4	8.2	4.7	7.7	10.4	11.2	8.3	8.2 b
6	6.9	3.9	8.8	7.4	10.7	7.3	7.0 b
8	6.9	4.4	7.4	5.2	7.3	2.6	5.5 c
12	3.1	1.3	0.8	2.6	4.2	0.8	1.7 d
Mean	7.2	5.2	6.5	7.0	7.5	5.9 ns	
	----- Inhibition (%)[x] -----						
0	0.0	0.0	0.0	0.0	0.0	0.0	
4	26.9	59.1	0.0	0.0	9.7	19.2	
6	38.5	65.9	0.0	18.7	13.7	28.4	
8	38.5	38.5	2.6	42.5	41.1	74.3	
12	72.5	88.4	90.0	71.1	66.1	92.2	

z: Radicle length of seedlings were measured as day 8 minus day 3.
y: No interaction between lines and hours, so means lumped for comparisions; Mean separation based on Duncan's multiple range at 5% level.
x: Inhibition (%) = {(length of hr0 seed - length of treated seed)/length of hr0 seed)} x 100.

Table 4.9. Radicle growth rate[z] of six lines of 8-day-old tomato seedlings grown in petri dishes at 25° C after heat treatment (40° C) to emerged seed for 4 to 12 hours.

Hour(s) at 40° C	Lines (cm/day) Nema 1200	Walter	PSr 28693	PSr 28793	Peto #2	UC-82-L	Mean
0	2.2	2.2	1.7	1.8	2.6	2.0	2.1 a[y]
4	1.6	0.9	1.5	2.0	2.5	1.6	1.7 b
6	1.3	0.7	1.7	1.4	1.5	1.4	1.3 c
8	1.3	0.8	1.4	1.0	1.2	0.5	1.0 c
12	0.5	0.2	0.1	0.4	0.3	0.0	0.3 d
Mean	1.4	0.9	1.3	1.3	1.6	1.1 ns	

z: Difference of root length of seedling was measured as day 8 minus day 3 and devided by 5 days.

y: No significant interaction between lines and hours, so means lumped for comparisions; Mean separation based on Duncan's multiple range at 5% level.

Table 4.10. Shoot length[z] and inhibition (%) of six lines of 8-day-old tomato seedlings grown in petri dishes at 25° C after heat treatment (40° C) to emerged seed for 4 to 12 hours.

Hour(s) at 40° C	Lines						Mean
	Nema 1200	Walter	PSr 28693	PSr 28793	Peto #2	UC-82-L	
	------ Shoot length(cm)[z] ---------						
0	2.7	2.7	1.8	1.4	2.6	2.9	2.3[y]
4	2.0	1.5	1.3	1.6	2.0	2.0	1.7
6	1.8	1.4	1.4	1.6	1.7	1.5	1.5
8	1.7	1.5	1.5	1.3	1.4	1.5	1.5
12	1.4	0.9	1.2	1.1	1.2	1.5	1.2
mean	1.9	1.6	1.5	1.4	1.6	1.9	
	----- Inhibition (%)[x] -----						
0	0.0	0.0	0.0	0.0	0.0	0.0	
4	26.2	42.7	24.9	0.0	23.1	30.7	
6	34.1	48.3	19.2	0.0	34.6	48.8	
8	35.2	43.8	15.3	5.0	46.2	47.8	
12	48.7	65.2	30.5	19.3	53.9	47.8	

z: Shoot length of seedlings were measured at day 8.
y: Interaction between lines and hours was significant, so mean separations were not done.
x: Inhibition (%) = {(length of hr0 seedling - length of treated seedling)/length of hr0 seedling)} x 100.

average, less than what was observed in root growth (Table 4.8). In addition, the ratio of shoot and root increased as the duration of the temperature treatment increased (Table 4.11). There was some secondary roots appearing at day 3 of treatment. The shoot recovered better than roots, and the meristematic region of tap root was damaged and the formation of secondary root was initiated.

In general, radicles had some damage and reduced its growth at 4 hours of heat treatments, with a greater effect at 6 hours, and the most injury from the 12 hour treatment (Table 4.8). However, the root started to show differences in response to high temperatures at the 8 hour treatment, and significant differences for relative growth rate at the 12 hour treatment (Table 4.12). But the emerged tomato seed only showed a significant reduction in the 12 hour heat treatment if the ratio of shoot and root was considered (Table 4.11).

It seemed that there is no characteristics to consistently separate heat tolerant from heat sensitive cultivars. The 3 hour temperature treatment to radicle and the subsequent hsp analysis would not cause serious damage to the radicle. On the other hand, it is not promising to study the synthesis of hsps as a function of time because there was poor isotop incorporation after a short time of isotope incubation (less than 3 hours) and there was serious damage

Table 4.11. Ratio of shoot to root length[z] of six lines of 8-day-old tomato seedlings grown in petri dishes at 25° C after heat treatment (40° C) to emerged seed for 4 to 12 hours.

Hour(s) at 40° C	Lines						Mean
	Nema 1200	Walter	PSr 28693	PSr 28793	Peto #2	UC-82-L	
0	0.26	0.24	0.23	0.16	0.21	0.29	0.23[y]
4	0.25	0.50	0.18	0.16	0.18	0.27	0.26
6	0.33	0.46	0.16	0.22	0.19	0.25	0.27
8	0.37	0.42	0.20	0.26	0.23	1.00	0.41
12	0.78	0.72	1.97	0.61	1.36	1.98	1.24
Mean	0.40	0.47	0.55	0.28	0.43	0.76	

z: Ratio = shoot length / root length.
y: Interaction was significant so mean separations were not done.

Table 4.12. Relative growth rate of root[z] of six lines of 8-day-old tomato seedlings grown in petri dishes at 25° C after heat treatment (40° C) to emerged seed for 4 to 12 hours.

Hour(s) at 40° C	Lines						Mean
	Nema 1200	Walter	PSr 28693	PSr 28793	Peto #2	UC-82-L	
0	0.39	0.28	0.42	0.28	0.30	0.37	0.28ab[y]
4	0.31	0.37	0.35	0.39	0.50	0.35	0.30 a
6	0.34	0.27	0.35	0.24	0.33	0.31	0.25ab
8	0.36	0.31	0.29	0.25	0.34	0.15	0.23 b
12	0.23	0.14	0.08	0.22	0.29	0.01	0.08 c
Mean	0.24	0.20	0.25	0.23	0.28	0.20 ns	

z: Relative growth rate of root = {log(root length at day 8) - log(root length at day 3)}/5.

y: No significant interaction between lines and hours, so means lumped for comparisions; Mean separation based on Duncan's multiple range at 5% level.

to the root with longer exposure (longer than 6 hours) under high temperature.

Seedling Development

Growth development of 15 day tomato seedling

The study was done in order to determine the age of toamto plants to be used in studies of the effect of temperature on seedling development. Three lines, 54-718, 'Walter' and 'UC-82-L' (increased seed), were used.

There were significant differences for stem length and its fresh and dry weight among lines (Table A.7). This showed that stem growth in 'Walter' and 'UC-82-L' was faster than in 54-718, especially for the first 10 days when they were grown at 23/21^{o} C (Tables A.8, A.9 & A.10). Fresh weight of their stems increased much more than 54-718. Stem growth characteristics in tomato seedling could be as a good criterium for distinguishing difference between cultivars or lines. The total fresh weight of the plant in 'Walter' and 'UC-82-L' was also higher than 54-718.

The proportion of root and stem fresh weight in seedlings was fairly constant as the plant grew (Fig 4.4). The root number of the first one centimter of the root basipetally increased dramatically at day 10 (Table 4.13). The fresh and dry weight in root, stem, cotyledon and leaf also increased considerably at day 10. This indicated that

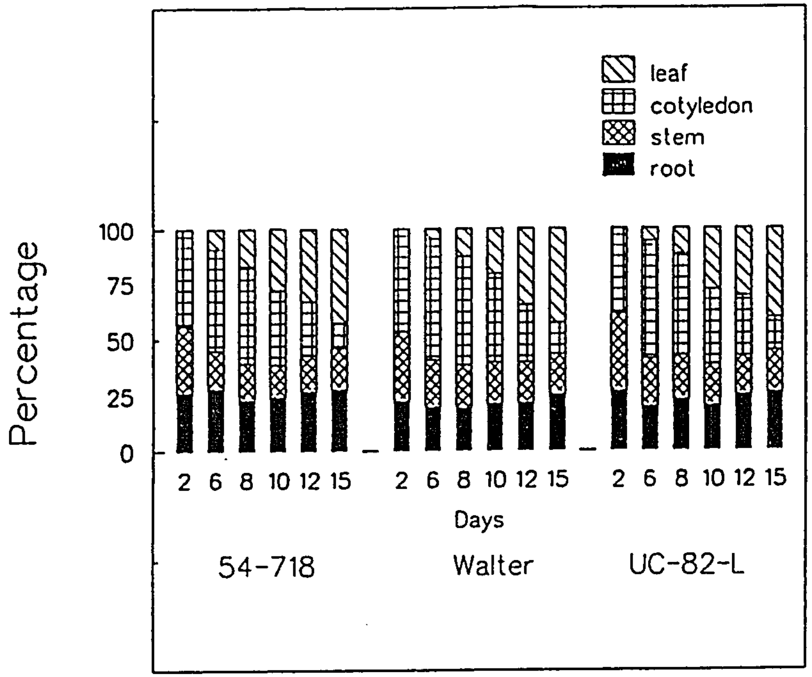


Fig.4.4 Changes in the proportion of tissues in fresh weight in 3 lines of tomato seedlings grown for 15 days under 23/21° C 14/10 (D/N).

Table 4.13. Growth characteristics of 2- to 15-day-old tomato seedlings (lines 54-718, Walter and UC-82-L) grown at 23/21° C 14/10 hours D/N.

Day	Stem length	Stem fw (mg)	Stem dw (mg)	Root #	Root length	Root fw (mg)
2	2.29 e	125.3 f	6.25 e	3.75 d	5.85 d	98.4 e[z]
6	3.00 d	262.4 e	14.55 d	5.18 c	9.72 c	315.8 d
8	3.21 cd	347.2 d	16.08 d	5.92 c	10.76 bc	427.2 d
10	3.39 cb	476.1 c	22.73 c	8.82 b	10.89 bc	644.7 c
12	3.66 b	849.1 b	46.82 b	11.09 b	12.96 ab	1274.5 b
15	5.25 a	1974.0 a	112.08 a	16.36 a	14.82 a	2948.0 a

Day	Root dw (mg)	Cotyl fw (mg)	Cotyl dw (mg)	Leaf #	Leaf fw (mg)	Leaf dw (mg)
2	5.55 e	168.92 e	12.99 d	0.00 e	0.0 f	0.00 f
6	15.91 d	677.36 d	55.27 c	2.09 d	94.9 e	9.09 e
8	20.33 d	868.17 c	66.75 bc	2.67 c	266.7 d	24.67 d
10	34.00 c	988.09 c	83.09 b	3.00 c	750.6 c	79.18 c
12	71.09 b	1241.00 b	113.45 a	3.73 b	1679.9 b	175.00 b
15	175.17 a	1492.17 a	132.17 a	4.58 a	4628.8 a	505.75 a

z: No significant interaction between lines and days, so means lumped for comparisions; Mean separation based on Duncan's multiple range at 5% level.

the cell pot size might be a limitation to tap root growth, thus generating secondary roots in order to keep the plant growing vigorously. Therefore, the rest of the experiments in this section were conducted by using 8-day-old plants in order to study the effect of temperatue on root growth and secondary root initiation.

The cotyledons were actively growing for one week to ten days, in terms of the percentage of fresh weight in whole plant (Fig 4.4). It had significant difference in fresh weight (also the indication of size) among lines (Table A.7). This might be also an good illustration of characteristic of individual line.

The first true leaves were usually visible 3 to 4 days after emergence. However, they were too small to be weighted. They grew vigorously and accounted for about 42% of fresh weight (Fig 4.5) and 55% of dry weight (Tables A.8, A.9 & A.10) in 15-day-old plants. The young seedlings had 2 to 3 leaves and 4 to 5 leaves when the plant was 8- and 15-day-old respectively. There were no differences among lines. Leaves had less water content than roots, shoots and cotyledons.

Seedling development in petri dishes vs vermiculite

'Walter' (Peto Seed Company) was used to test the effects of high temperature on the development of tomato seedlings. Walter was chosen because it appeared to be a high

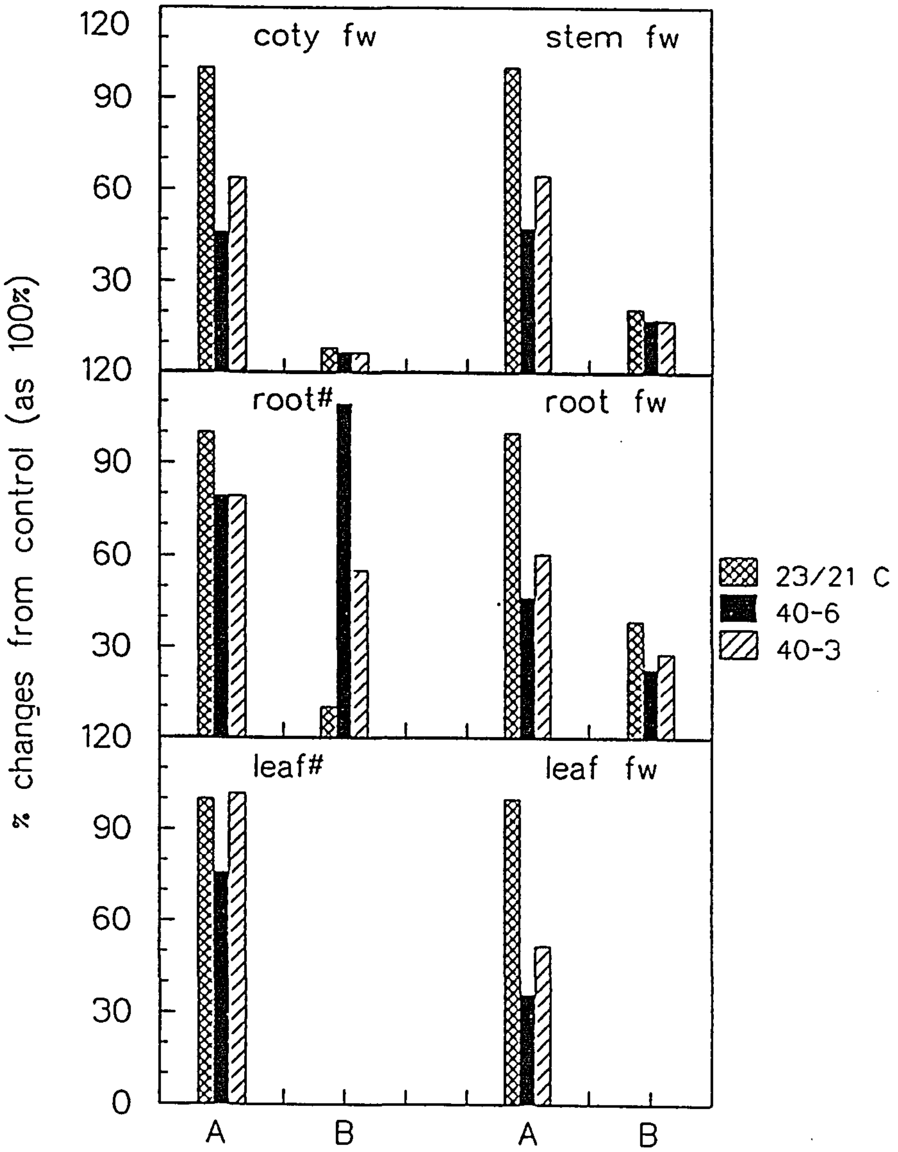


Fig.4.5 Percentage changes from control (23/21° C) of growth characteristics of tomato seedlings (cv. Walter) grown at 23/21° C after heat treatment (40°C) for 3 or 6 hours.
A: in vermiculite with nutrients.
B: in petri dishes without nutrients.

temperature sensitive cultivar in the germination tests.

Emerged seed, imbibed and germinated at room temperature, were transferred into petri dishes with no nutrient supply or into vermiculite with half strength Hogland's solution and treated at 40° C for 3 or 6 hours. The seed were then moved into room temperature to study the seedling development.

Stem growth of tomato seedlings in petri dishes was restricted by almost 50% in length (Table A.11) and by 80% in fresh weight in all temperature treatments and it showed highly significant differences between nutrient and non-nutrient treatments (Fig 4.5, top right and Table A.12). There was less than 30% reduction in stem length and 40 to 50% reduction in fresh weight of seedlings when the emerged seed in vermiculite and nutrients were treated with a period of heat.

In petri dishes and at room temperature, roots grew straight and the length was longer than those grown in vermiculite with nutrients (Table A.11), even though there was no nutrients added to the petri dishes. On the other hand, roots of 8-day-old plants grown in petri dishes and at room temperature had the least number of secondary root and lower fresh weight (Fig 4.5, middle). Root number was considerabley increased by a short period of heat when compared to the ones grown at room temperature. The greatest increase was at 40℃

for 6 hours in the petri dish (Fig 4.5, middle left). Root fresh weight was reduced significantly at all temperature treatments when the seedlings were grown in petri dishes (Fig 4.5, middle right and Table A.12). The differences between two samples of the emerged seed in petri dishes and vermiculite could be explained as: 1> The root radicles, including the meristem, in the petri dish with 6 hour heat treatment were injured resulting in no new tap root growth. Inhibiting tap root growth enhanced the initiation of secondary roots and the secondary roots developed faster at room temperature ($23/21^{\circ}$ C). 2> Serious damage to the tap root in the petri dishes might be due to the reduction in oxygen content in the water as the temperature rose. Oxygen becomes less soluble in water as temperature rises (Bewley and Black, 1978). 3> The temperature in the petri dish might cool slower when the dish was moved back to room temperature. On the other hand, the temperature of the vermiculite with water might have taken longer to rise to 40° C. The root radicles in this treatment may actually not have been heated for 6 hours. However, it would be similar to when tomatoes were grown in fields with irrigation.

There was an interaction of the fresh and dry weight of the cotyledons and leaves (Table A.12). Also, there was a significant difference leaf number due to nutrient treatment. Cotyledons and leaf development were affected by temperature and nutrient treatments. No leaf growth and less cotyledon growth could be found in the samples grown in petri dishes at all treatments (Fig 4.5, top right B). Leaf differentiation, indicated as leaf number, and cotyledon growth, as fresh weight, seemed not to be affected by 3 hour heat treatment. Leaf and cotyledon development, indicated as fresh weight, was affected severely by the treatment of 6 hours at 40^{o} C in the petri dish.

Conclusions were: 1> Nutrients are very important for tomato seedlings to grow. More serious heat damage to developing seedlings, especially in cotyledons and leaf development, would result if there was no nutrient in the growing medium. The study of the effect of temperature on young seedling growth was not recommended to be conducted in the petri dish. 2> Any length of heat treatment to the germinated tomato seed grown in vermiculite with nutrient was considered to have some restriction to the earlier seedling growth, especially after 6 hours of heat treatment (Fig 4.5).

Effects of temperature, stage, and day length on the development of tomato seedling

There were significant interactions for all tested characteristics except the characteristic of root number. It had the most interaction between day length and temperature (Table A.13). The result could be explained as follow:

The stem growth (length) was more when emerged tomato seed were grown under 40/30 and 40/25° C than seed grown at 23/21° C, 14/10 D/N. Stem fresh and dry weights were significantly more in seedlings grown at 23/21° C, 14/10 D/N (Fig 4.6 & 4.7).

Young plants (10-day-old) had more secondary roots, fresh and dry weight of root system when they were treated with temperature combinations at stage of 2-day-old than at stage of emerged seed (Fig 4.7 & Table A.14 & A.15). Emerged seed were more sensitive to high temperatures and reduced their further seedling development. The initiation and the earlier development of the secondary roots could be completed when emerged seed were grown at 23/21° C for 2 days before they were moved to high temperature. The growth and development of roots as fresh and dry weight was vigorous at 23/21° C which was in accord with what White (1937) found.

It seemed that heat affected the germinated tomato seed more than 2-day-old seedlings, especially in leaf development. It was highly possible that the first few true leaves were initiated when seed were emerged. There was only half leaf or 2 to 3 leaves developed in 8- or 10-day-old seedlings when germinated seed or 2-day-old seedlings were grown under high temperature combinations with 14/10 hours D/N repectively (Table A.14). However, leaf development was enhanced by increasing leaf number and its fresh weight when seedlings were grown at high temperature combinations with

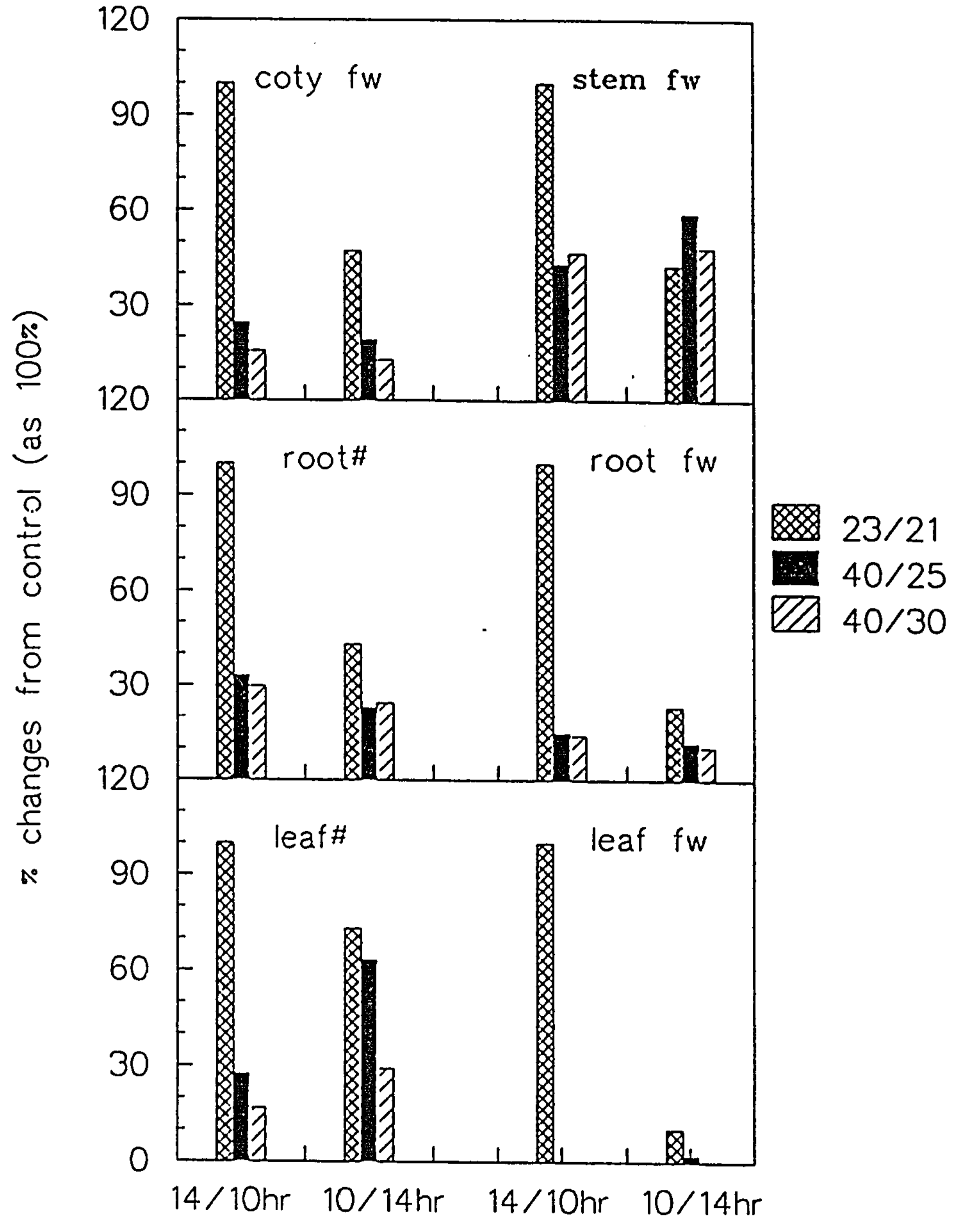


Fig.4.6 Percentage changes from control (23/21° C) of growth characteristics of tomato seedlings (cv. Walter) treated with temperatures and photoperiods to germinated seed.

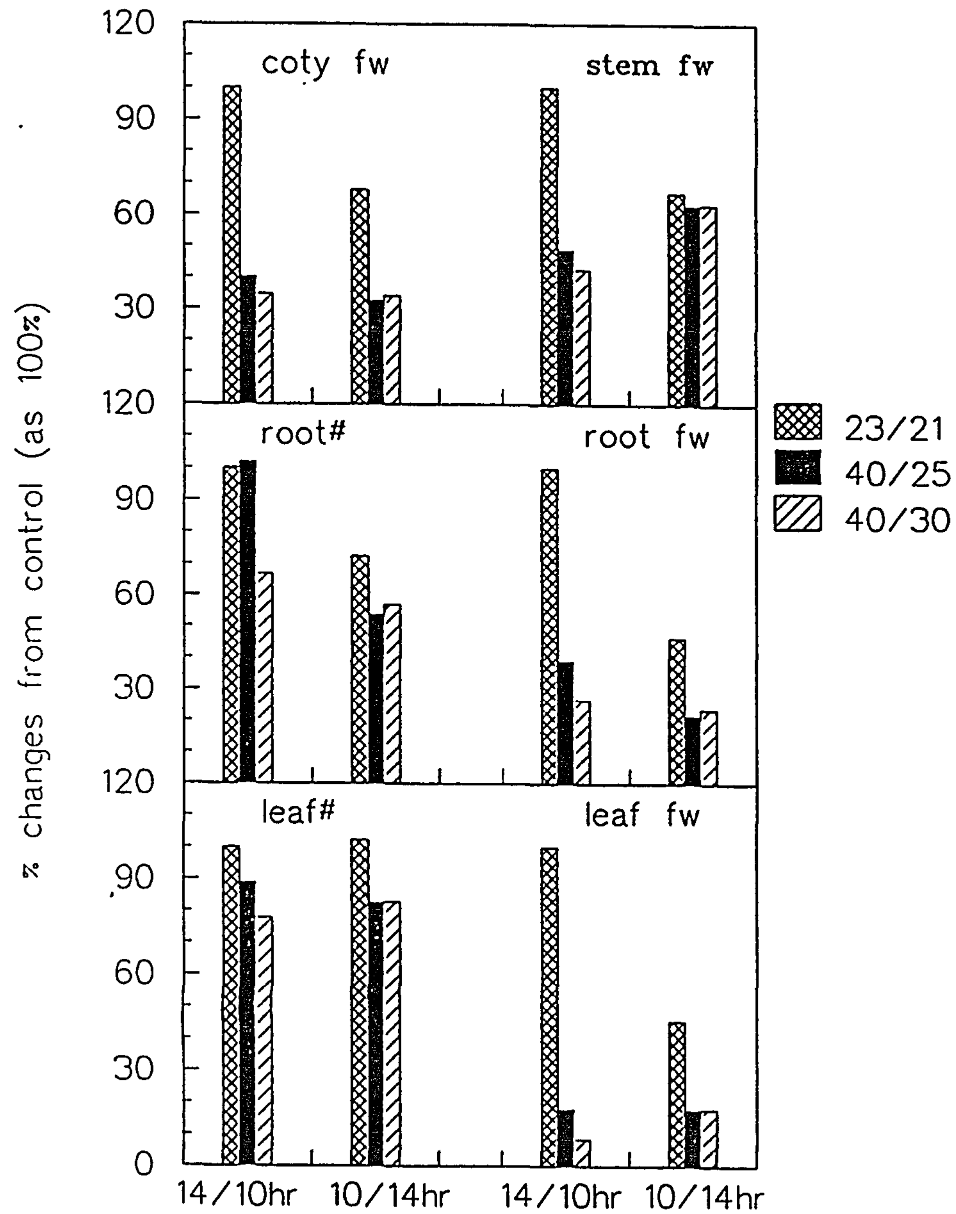


Fig.4.7 Percentage changes from control (23/21° C) of growth characteristics of tomato seedlings (cv. Walter) treated with temperatures and photoperiods to 2-day-old seedlings.

10/14 hours (D/N) (Fig 4.6 & Table A.15). It seemed that leaf development needed lower night temperatures or a shorter heat duration. Both of the temperature combinations of 40/25 and 40/30° C had the greatest effect on leaf development and the least on stem growth.

There was significantly more fresh and dry weight of roots, cotyledons and leaves and the dry weight of stems in seedlings which were grown at 14/10 hours D/N (Table A.7), especially at 23/21° C. Tomato seedlings had more fresh and dry weight in the roots and cotyledons when seedlings were grown at 14/10 hr with higher temperature combinations. On the contrary, it seemed that seedlings grown at 40/30 and 40/25° C and 10/14 hours would have more stem growth in fresh weight, but not in length. They also had more leaf growth in fresh weight and in leaf number, especially if seedlings were treated with a high temperature combination at the 2-day-old stage (Fig 4.7 and Table A.14 & A.15).

Anatomical studies

The number of cells per unit length in a longitudinal section of the stem of 8-day-old tomato seedlings were less when emerged tomato seed were treated with high temperatures (Table 4.14). This indicated that the longer stems resulting from heat treatment on emerged seed (Table A.14), was due to cell elongation in the stem tissue. In addition, stem

Table 4.14. Shoot anatomy characteristics of tomato seedlings (cv. Walter) grown in petri dishes (*) or vermiculite at different temperatures for 2 or 8 days.

Temp (° C)	St[z]	Day	Shoot dia(mm)	Stele dia(mm)	Cell#/ width	Cell#/ length	Stele/ shoot area
23/21	G*	3	0.28	0.11	10.00	3.15	0.159
23/21	G	2	0.25	0.08	12.05	8.00	0.118
40/25	G	2	0.37	0.10	--	--	--
40/30	G	2	--	--	9.00	2.90	--
40/25	G	8	0.58	0.14	8.50	1.20	0.058
40/30	G	8	0.58	0.15	6.00	1.65	0.051
23/21	S	8	0.52	0.15	5.70	3.00	0.088
40/25	S	8	0.53	0.16	6.00	2.80	0.082
40/30	S	8	0.43	0.14	10.00	2.50	0.104

z: Emerged tomato seed (G) or 2-day seedlings (S) were treated with temperatures of 23/21, 40/25 or 40/30° C.

diameter in the same treatment was larger and its ratio of stele to shoot area was smaller (Table 4.14).

It seemed that root diameter of 2- or 8-day-old seedlings was not affected by temperatures. But the ratio of stele to root area was decreased as the seedlings were treated with high temperatures (Table 4.15). The cross section of root which was grown in the petri dish for 3 days after emergence of seed at $23/21^{\circ}$ C already had the secondary root formation (Fig 4.8). This indicated that secondary roots could be initiated and developed naturally. Two conclusions are possible: 1> Heat was not a necessary induction factor for secondary root formation. Heat was more likely an enhancing factor as Batten and Mullins (1978) stated that ethylene was an enhancing factor instead of inducing factor in root formation. 2> Secondary root development was slower under $23/21^{\circ}$ C and it would not be visible till day 8 if emerged seed were in the petri dish. It might indicate that root development was controlled not only by temperature but also by pressure and/or ethylene which would induce or be produced when the root became in contact with the soil.

Seedling cotyledons developed well under $23/21^{\circ}$ C with the largest size and thickest of the palisade layer (Table 4.16). Their development were reduced if the emerged seed or young seedlings were grown under high temperature.

Two-day-old seedlings or 3-day-old seedlings with no

Table 4.15. Root anatomy characteristics of tomato seedlings (cv. Walter) grown in petri dishes (*) or vermiculite at different temperatures for 2 or 8 days.

Temp (° C)	St[z]	Day	Root dia(mm)	Stele dia(mm)	2nd root length(mm)	Stele area/ root area
23/21	G*	3	0.36	0.08	0.05	0.054
23/21	G	2	0.31	0.07	--	0.052
40/25	G	2	0.43	0.10	--	0.052
40/30	G	2	0.38	0.08	0.04	0.050
40/25	G	8	0.64	0.17	0.07	0.071
40/30	G	8	0.62	0.18	0.49	0.086
23/21	S	8	0.55	0.17	0.48	0.160
40/25	S	8	0.39	0.12	0.42	0.095
40/30	S	8	0.57	0.16	0.42	0.080

z: Emerged tomato seed (G) or 2-day seedlings (S) were treated with temperatures of 23/21, 40/25, or 40/30 ° C.

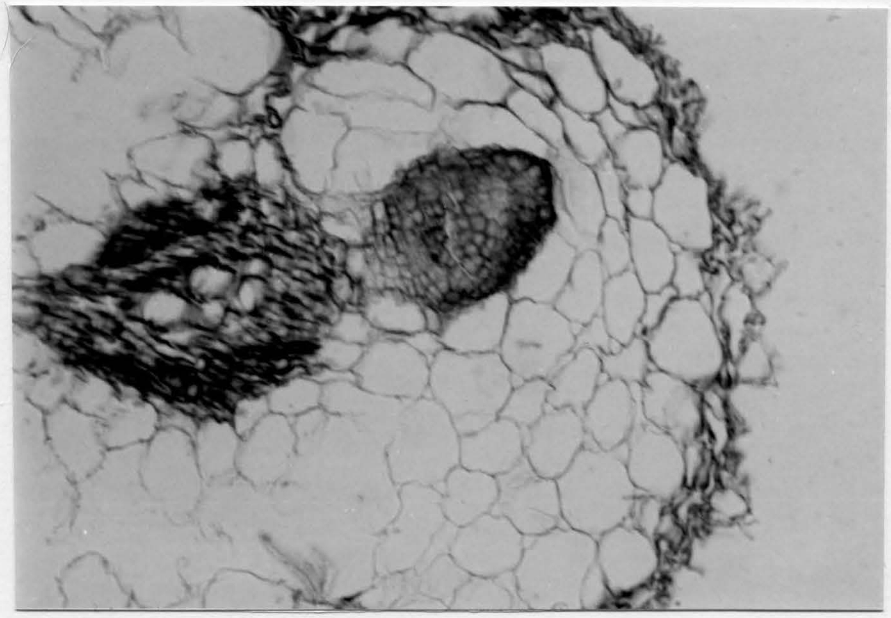


Fig.4.8 Cross section of secondary root in a 3-day-old tomato seedling (cv. Walter) grown at 23/21^{o} C in petri dish.

Table 4.16. Anatomy characteristics of cotyledons in tomato seedlings (cv. Walter) grown in petri dishes (*) or vermiculite at different temperatures for 2 or 8 days.

Temp (° C)	St[z]	Day	Thick-ness(mm)	Width (mm)	Palisade (mm)	Spong cell layers
23/21	G*	3	0.12	0.75	0.03	7.00
23/21	G	2	0.07	1.12	0.03	5.00
40/25	G	2	--	--	--	--
40/30	G	2	--	--	--	--
40/25	G	8	0.15	1.79	0.05	--
40/30	G	8	0.15	1.73	0.05	--
23/21	S	8	0.20	2.84	0.07	lot air space
40/25	S	8	0.11	1.97	0.04	2.00
40/30	S	8	0.15	1.67	0.06	6.00

z: Emerged tomato seed (G) or 2-day seedlings (S) were treated with temperatures of 23/21, 40/25, or 40/30° C.

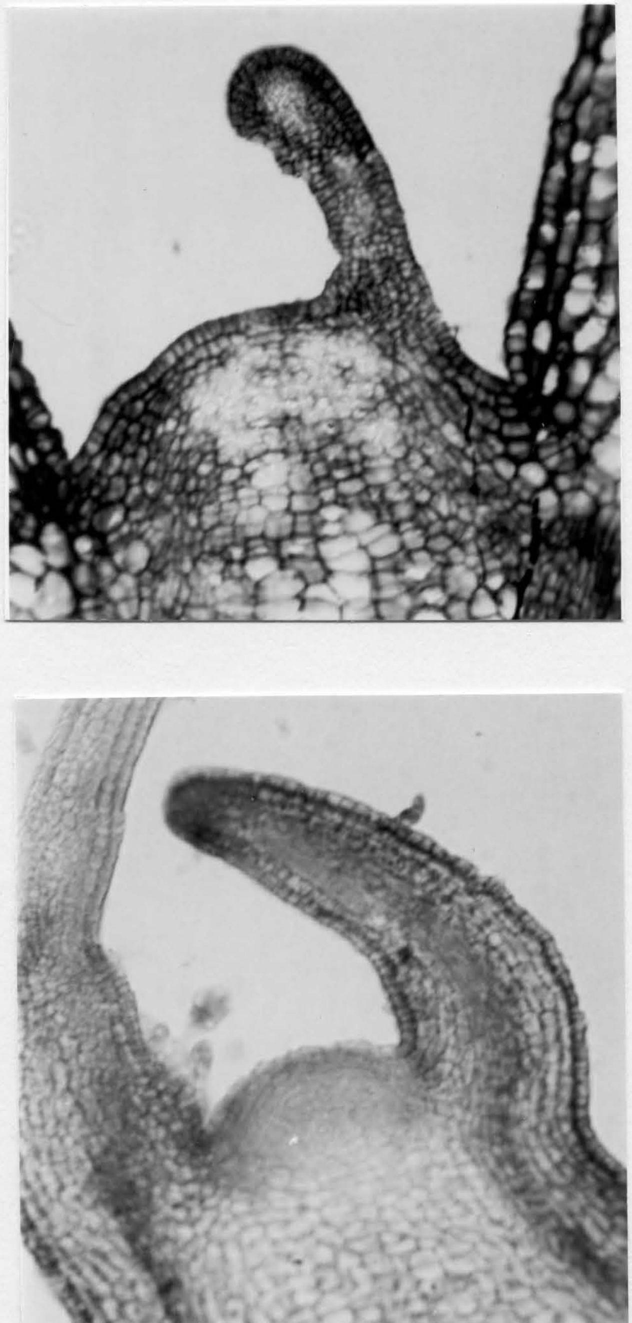
nutrients usually did not have visible leaves. Leaves grew vigorously at 23/21° C as indicated by the leaf width and leaf size. Leaf growth was affected by high temperatures by reducing its size. It seemed that the palisade layer was more affected by the 40/30° C temperature (Table 4.17).

The section of the shoot apex illustrated clearly that 2-day-old seedlings had leaf primordia even though no leaf trace could be visible till 2 or 3 days latter (Fig 4.9 A). This demonstrated that the differentiation of leaf primordium could be even earlier. The 8-day-old seedlings had two well developed leaf primordia, one much longer than the other (Fig 4.9 B). The early growth of young leaves also showed the first one was much bigger than the second one in size. However, the shoot apex of the same age showed much shorter leaf primordia if the seedlings were grown under 40/30 and 40/25° C (Fig 4.10 & 4.11). The shoot apex was also wider in diameter, shorter in length, and more pointed when seedlings were grown under high temperature combinations. In addition, two leaf primordia with almost the same length could be seen. It strongly proved that leaves developed much slower under high temperatures and leaf differentiation was not affected by high temperatures, at least for early seedling growth.

Table 4.17. Leaf antomy characteristics of tomato seedlings (cv. Walter) grown in petri dishes (*) or vermiculite at different temperatures for 2 or 8 days.

Temp (° C)	St[z]	Day	Thick-ness(µm)	Width (mm)	Palisade (mm)	Spong cell layers
23/21	G*	3	--	--	--	--
23/21	G	2	--	--	--	--
40/25	G	2	--	--	--	--
40/30	G	2	--	--	--	--
40/25	G	8	0.05	0.64	0.03	--
40/30	G	8	0.05	0.74	0.02	--
23/21	S	8	0.07	3.42	0.04	lot air space
40/25	S	8	0.07	1.30	0.04	--
40/30	S	8	0.06	0.56	0.02	--

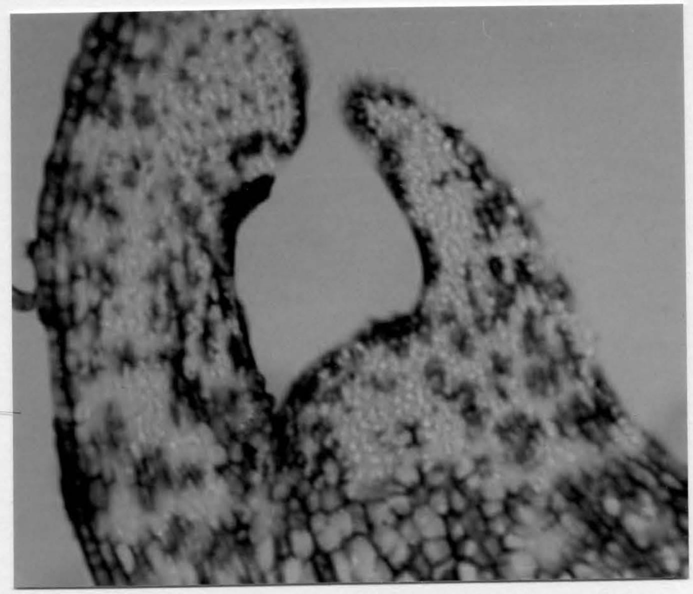
z: Emerged tomato seed (G) or 2-day-old seedlings (S) were treated with temperature of 23/21, 40/25, or 40/30° C.



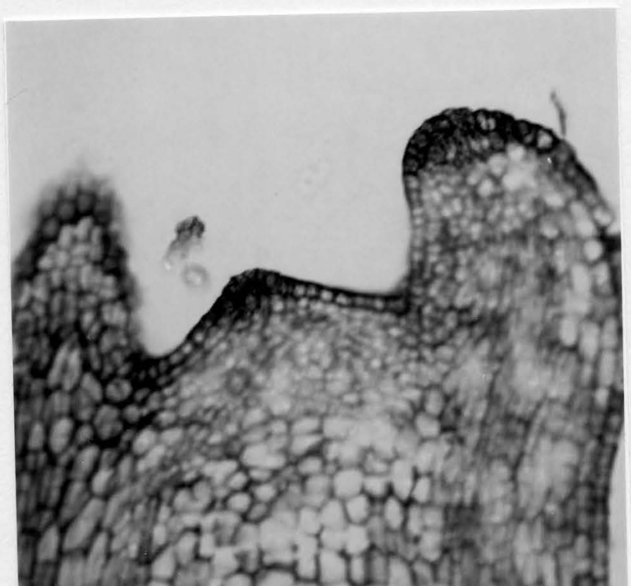
A

B

Fig.4.9 Leaf primordium (a) in shoot apex of 2-day- (A) and 8-day-old (B) tomato seedling (cv. Walter) grown at 23/21^{o} C.

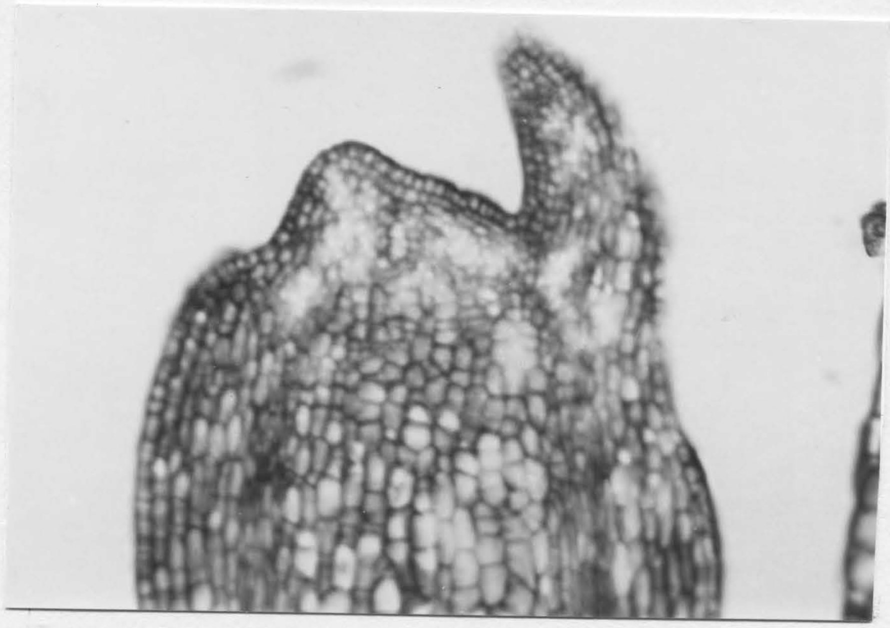


A

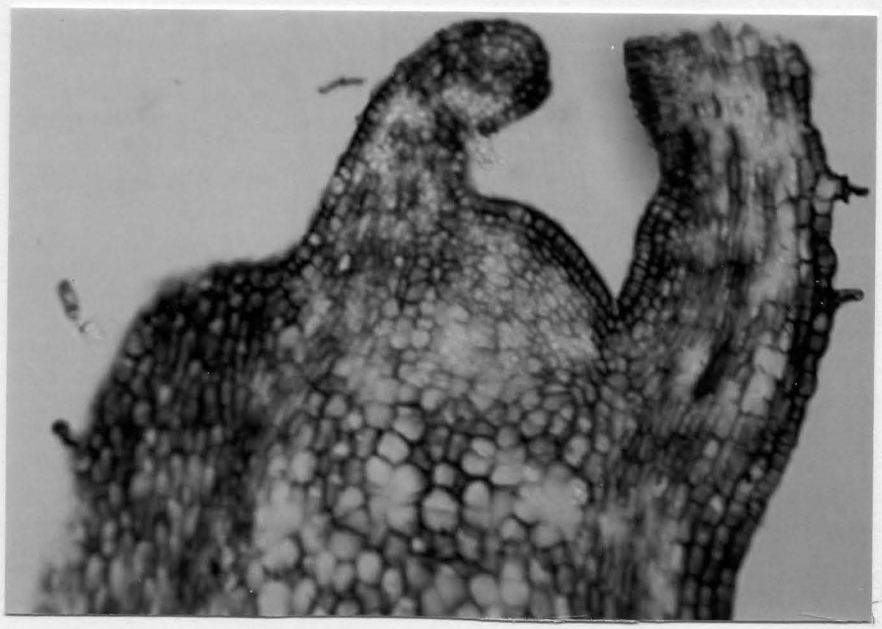


B

Fig. 4.10 Leaf primordium (a) in shoot apex of 8-day tomato seedling (cv. Walter) grown at 40/30 (A) and 40/25^{o} C (B) from emerged seed.



A



B

Fig.4.11 Leaf primordium (a) in shoot apex of 10-day tomato seedling (cv. Walter) grown at 40/30 (A) and 40/25^{o} C (B) from 2-day-old seedling.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The effect of heat stress, including high temperatures as well as the duration of high temperatures, on tomato germination and seedling development was investigated. The evaluation of the experimental results was accomplished through the consideration of germination percentage, germination rate, heat shock proteins, organ number, fresh and dry weight of seedlings, and anatomical studies of tissues.

The results showed that tomato was most sensitive to heat from one day of imbibition to two days after the radicle emerged during germination. Germination of tomato seeds decreased dramatically as temperature reached a constant 35°C. Seedling development was delayed if the emerged seed were treated with 40/25 or 40/30° C for 14/10 hours D/N, especially in the fresh and dry weights of cotyledons and leaves , and widths of the organs and leaf primordia in the shoot apex. Seedlings had many secondary roots if the emerged seed were treated with 40° C for 6 hr. It was unlikely that heat was the inducing factor because secondary roots were detected when seedlings were only 3-day-old and grown in petri dish without any restriction from the soil medium.

Heat shock proteins of emerged tomato seed appeared at all tested temperatures, but showed thicker bands from the samples treated at 35 and 40° C. It was difficult to use hsps as a criteria to distinquish heat tolerant from heat sensitive lines, even though hsps have been used as indictors for heat stress selection programs in other crops, such as sorghum.

In heat stress studies, we should consider that temperature is changing diurnally as in the field. It is hard , however, to manipulate this exact situation in the laboratory. In addition, heat stress in plants is often confounded by water stress or draught stress.

APPENDIX A

EXPERIMENTAL RESULTS

Table A.1. Water absorption (%)[z] in six lines of tomato seed imbibed at constant 25 and 40° C.

Temp (°C)	Hr	Nema 1200	Walter	PSr 28693	PSr 28793	Peto #2	UC-82 -L	Mean
		Lines						
25	0	7	7	5	6	6	6	
	1	25	42	32	31	30	34	
	2	33	54	39	38	40	43	
	3	36	59	47	43	45	47	
	6	46	69	60	56	52	58	
	9	54	74	61	62	65	65	
	12	63	72	66	68	75	73	
	15	68	79	68	70	76	78	
	24	73	77	68	72	82	82	
	36	80	79	70	77	85	83	
	48	84	82	70	85	94	92	57
40	0	7	7	5	6	7	6	
	1	31	48	34	36	35	54	
	2	43	65	46	48	49	53	
	3	50	69	55	58	58	57	
	6	66	74	65	68	71	76	
	9	71	74	68	71	73	82	
	12	76	74	67	73	83	82	
	15	75	80	71	72	85	85	
	24	79	79	70	72	94	84	
	36	83	80	72	78	97	84	
	48	89	78	71	80	97	88	63
Mean		56	65	55	58	64	64	

z: % of water absorbed = $(W_t - W_o) \times 100 / W_o$, where W_t and W_o were the weights of imbibed and unimbibed seed, respectively.

Weight (mg) of 25 seed before imbibition:

Nema1200	Walter	PSr28693
(100.84±2.74)	(55.09±2.49)	(63.02±2.46)
PSr28793	Peto#2	UC-82-L
(70.61±3.32)	(90.86±2.95)	(84.56±3.15)

Table A.2. Significance of germination percentage and rate of six lines of tomato seed imbibed at 25 or 40° C for 1 to 48 hour(s) and then transferred to 40 or 25° C respectively.

Characteris -tics	Cultivar	Hour(s)	Interaction
25 to 40° C			
Germination %	***	***	***
Germination rate	***	***	***
40 to 25° C			
Germination %	***	***	ns
Germination rate	***	***	ns

Lines: Nema1200, Walter, PSr29793, PSr28693, Peto#2, and UC-82-L.

Table A.3. Significance of total isotope incorporation, labelled proteins and % of label into proteins of seed in four tomato cultivars and temperature treatments.

Characteris -tics	Cultivars	Temperature	Interaction
Total isotope incorporation	*	*	ns
Labelled proteins	*	*	ns
% of label into proteins	***	ns	ns

Cultivars: Nema 1200, Walter, Peto#2 and UC-82-L.
Temperatures: 25, 30, 35 and 40° C.
* : significant at 5% level; **: significant at 1% level;
*** : significant at 0.1% level; ns: non-significant for statistical analysis in all tables in the appendix section.

Table A.4. Total isotope incorporation, labelled protein (cpm)[z], and percentage of label into proteins of emerged seed of four temperatures, three isotop concentrations, and incubation durations.

Hr	Temp(℃)	Concentration 1x	3x	4x	5x	Mean
-------	Total isotope incorporation (cpm) ------					
1	25	308605	-	-	-	
	30	20120	137490	222878	-	
	35	15911	-	-	-	
	40	14265	97271	209376	247034	110579
2	25	-	-	-	-	
	30	126281	-	-	-	
	35	-	210603	-	-	
	40	70737	178971	214345	376082	196170
3	25	98040	209458	-	-	
	30	100300	245896	-	-	
	35	-	295262	-	-	
	40	61821	216581	259454	334164	202331
	Mean	59815	198942	226513	319093	
-------	Labelled proteins (cpm) -------					
1	25	5480	-	-	-	
	30	2059	23102	37103	-	
	35	708	-	-	-	
	40	763	11271	24532	37658	15853
2	25	-	-	-	-	
	30	27526	-	-	-	
	35	-	21401	-	-	
	40	5164	17584	9390	25009	17679
3	25	17485	49056	-	-	
	30	16947	55816	-	-	
	35	-	32847	-	-	
	40	3580	13043	27235	32906	27657
	Mean	8857	28015	24565	31858	

Table A.4. continued.

Hr	Temp(°C)	Concentration 1x	3x	4x	5x	Mean
	------	Percentage of label into proteins (%) -----				
1	25	16.0	-	-	-	
	30	10.0	15.0	16.0	-	
	35	4.0	-	-	-	
	40	5.0	11.0	12.0	16.0	11.7
2	25	-	-	-	-	
	30	21.0	-	-	-	
	35	-	10.0	-	-	
	40	8.0	10.0	4.0	7.0	10.0
3	25	18.0	23.0	-	-	
	30	16.0	23.0	-	-	
	35	-	11.0	-	-	
	40	6.0	6.0	10.0	9.0	13.56
	Mean	11.56	13.63	10.5	10.67	

z: counts per minute.
Concentration of 1x = 5μCi of ^{35}S-methionin/10μl.
Cultivar used: Walter.

Table A.5. Significance of percentage leakage and injury of emerged seed in four tomato cultivars, temperatures and durations of temperature treatments

	Hr	Temp	Cultivar	Interactions h*t	h*c	t*c	h*c*t
Leakage	***	***	***	***	*	ns	ns
Injury	***	***	*	ns	*	ns	ns

Cultivar: Nema1200, UC-82-L, Peto#2, and Walter.
Temp: 25, 30, 35, and 40° C.
Hr: 4, 6, 8, 12 hours.

Table A.6. Significance of characteristics of 8-day-old tomato seedlings grown at 25℃ in six lines after heat treatment (40℃) to emerged seed for 4 to 12 hours.

Characteris -tics	Lines	Hour(s)	Interaction
Root length (cm)	ns	***	ns
Shoot length (cm)	***	***	***
Root growth rate	ns	***	ns
Reletive growth rate of root	ns	***	ns
Shoot/root	*	***	*

Lines: Nema1200, Walter, PSr28793, PSr28693, Peto#2 UC-82-L.

Table A.7. Significance of characteristics of tissues of 2- to 15-day-old tomato seedlings grown in vermiculite at 23/21℃ D/N in three lines.

Characteristics	Days	Lines	Interaction
Stem length	***	***	ns
Stem fw (mg)	***	***	ns
Stem dw (mg)	***	**	ns
Root #	***	ns	ns
Root length	***	ns	ns
Root fw (mg)	***	ns	ns
Root dw (mg)	***	ns	ns
Cotylesons fw	***	***	ns
Cotyledons dw	***	ns	ns
Leaf #	***	ns	ns
Leaf fw (mg)	***	ns	ns
Leaf dw (mg)	***	ns	ns

Lines: 54-718, Walter and UC-82-L.
Days: 2, 6, 8, 10, 12, 15 day.

Table A.8. Growth characteristics of 2- to 15-day-old tomato seedlings (Line 54-718) grown at 23/21°C 14/10 (D/N).

Day	Stem length (cm)	Root length (cm)	Leaf #	Root #	St/rt ratio	Stem fw (mg)	Stem dw (mg)	Root fw (mg)	Root dw (mg)	Cotyledon fw (mg)
2	1.93	5.18	0.00	3.75	0.37	8.30	0.48	7.45	0.44	12.05
6	2.87	6.70	2.25	5.50	0.36	24.00	1.43	40.33	2.08	64.88
8	2.90	8.08	3.00	6.25	0.36	25.38	1.13	37.68	1.68	67.80
10	3.07	11.43	3.00	10.00	0.27	36.10	1.80	62.50	3.17	81.20
12	3.53	11.67	3.50	11.75	0.30	74.68	4.15	127.95	6.85	104.78
15	5.13	14.20	4.50	18.33	0.36	164.53	9.93	252.50	14.90	116.67

	Cotyledon dw (mg)	Leaf fw (mg)	Leaf dw (mg)	Total fw (mg)	Total dw (mg)	Water content (%)	Stem water (%)	Root water (%)	Cotyledon water (%)	Leaf water (%)
2	1.05	0.00	0.00	27.80	1.96	92.89	94.28	93.99	91.31	--
6	6.10	13.65	1.53	142.85	11.13	92.28	93.97	94.92	90.70	88.94
8	5.68	27.75	2.65	158.60	11.13	93.02	95.56	95.65	91.62	90.76
10	7.40	71.90	7.50	251.70	19.87	92.16	95.02	94.97	90.95	89.66
12	10.13	158.08	16.75	465.48	37.88	92.02	94.58	94.70	90.51	89.61
15	11.80	376.90	44.38	881.43	81.00	90.87	93.93	93.93	89.97	88.34

Table A.9. Growth characteristics of 2- to 15-day-old tomato seedlings (cv. Walter) grown at 23/21°C 14/10 (D/N).

Day	Stem length (cm)	Root length (cm)	Leaf #	Root #	St/rt ratio	Stem fw (mg)	Stem dw (mg)	Root fw (mg)	Root dw (mg)	Cotyledon fw (mg)
2	2.70	6.10	0.00	4.75	0.44	15.20	0.68	11.03	0.63	22.65
6	3.03	8.88	2.00	4.50	0.34	25.00	1.45	24.45	1.18	63.38
8	3.40	10.30	2.25	5.75	0.33	36.93	1.60	39.68	2.05	91.90
10	3.43	10.20	3.00	7.52	0.34	47.03	2.25	61.58	3.50	105.50
12	3.77	12.63	4.00	11.33	0.30	96.63	5.03	115.90	6.70	131.67
15	5.33	15.13	4.75	14.75	0.35	218.00	11.73	317.20	18.70	167.85

Day	Cotyledon dw (mg)	Leaf fw (mg)	Leaf dw (mg)	Total fw (mg)	Total dw (mg)	Water content (%)	Stem water (%)	Root water (%)	Cotyledon water (%)	Leaf water (%)
2	1.65	0.00	0.00	48.88	2.95	93.97	95.56	94.33	92.72	--
6	4.90	5.98	0.38	118.80	7.90	93.33	94.04	95.01	92.31	94.09
8	6.38	25.85	2.35	194.35	12.38	93.74	95.69	94.98	93.17	91.26
10	8.58	56.70	6.08	270.80	20.40	92.57	95.21	94.44	92.00	89.35
12	10.93	185.57	19.03	529.77	41.70	92.14	94.85	94.20	91.80	89.72
15	13.80	528.15	53.58	1231.20	97.80	92.15	94.70	94.14	91.89	89.97

Table A.10. Growth characteristics of 2- to 15-day-old tomato seedlings (cv. UC-82-L) grown at 23/21°C 14/10 (D/N).

Day	Stem length (cm)	Root length (cm)	Leaf #	Root #	St/rt ratio	Stem fw (mg)	Stem dw (mg)	Root fw (mg)	Root dw (mg)	Cotyledon fw (mg)
2	2.25	6.28	0.00	2.75	0.36	14.10	0.73	11.05	0.60	15.98
6	3.13	11.20	2.00	5.67	0.28	30.87	1.50	29.43	1.50	77.37
8	3.33	10.78	2.75	5.75	0.31	41.85	2.10	50.80	2.38	100.75
10	3.60	10.83	3.00	9.50	0.33	56.83	2.65	68.85	3.48	105.33
12	3.73	14.48	3.75	10.25	0.26	86.35	4.95	135.60	7.68	137.75
15	5.30	15.13	4.50	16.50	0.35	209.65	11.98	314.70	18.95	162.13

	Cotyledon dw (mg)	Leaf fw (mg)	Leaf dw (mg)	Total fw (mg)	Total dw (mg)	Water content (%)	Stem water (%)	Root water (%)	Cotyledon water (%)	Leaf water (%)
2	1.20	0.00	0.00	41.13	2.53	93.89	94.88	94.56	92.55	--
6	5.60	8.63	0.80	146.30	9.40	93.59	95.13	94.85	92.82	90.96
8	7.98	26.40	2.40	219.80	14.85	93.29	94.98	95.39	92.08	90.94
10	8.73	95.80	10.00	326.80	24.93	92.44	95.35	95.21	91.82	89.29
12	12.88	164.73	17.10	524.43	42.60	91.95	94.34	94.25	90.90	89.60
15	14.05	483.58	53.78	1170.08	98.75	91.69	94.37	94.09	91.58	88.92

Table A.12. Significance of characteristics of tissues of 8-day-old tomato seedlings (cv. Walter) grown at 23/21℃ D/N after the emerged seed were treated with 40℃ for 3 or 6 hr in petri dishes or vermiculite.

Characteristics	Treatments	Nutrient	Interaction
Stem length	***	***	ns
Stem fw (mg)	*	***	*
Stem dw (mg)	**	***	ns
Root #	*	*	***
Root length	**	ns	ns
Root fw (mg)	*	***	ns
Root dw (mg)	*	**	ns
Cotylesons fw	***	***	***
Cotyledons dw	**	***	**
Leaf #	ns	***	ns
Leaf fw (mg)	**	***	**
Leaf dw (mg)	**	***	*

Table A.11. Characteristics of 8-day-old tomato seedlings (cv. Walter) grown at 23/21° C after heat treatement (40° C) for 3 or 6 hours to germinated seed.

Temp	Nutr	Stem length (cm)	Root length (cm)	Leaf #	Root #	St/rt ratio	Stem fw (mg)	Stem dw (mg)	Root fw (mg)	Root dw (mg)	Cotyledon fw (mg)
23/21	O	1.74	14.76	0.00	0.57	0.12	7.81	0.49	15.36	0.83	7.49
	N	3.40	10.30	2.25	5.75	0.81	36.93	1.60	39.68	2.05	91.90
40-6	O	1.16	6.54	0.00	6.29	0.20	6.61	0.30	9.06	0.70	6.26
	N	2.36	8.51	1.71	4.57	0.33	17.59	0.83	18.47	0.90	42.39
40-3	O	1.45	10.15	0.00	3.17	0.19	6.47	0.40	11.05	0.78	6.02
	N	2.51	7.94	2.29	4.57	0.36	23.89	1.17	24.03	1.20	58.73

Temp	Nutr	Cotyledon dw (mg)	Leaf fw (mg)	Leaf dw (mg)	Total fw (mg)	Total dw (mg)	Water content (%)	Stem water (%)	Root water (%)	Cotyledon water (%)	Leaf water (%)
23/21	O	0.67	0.00	0.00	30.66	1.99	93.51	93.07	94.66	91.06	--
	N	6.38	25.85	2.35	194.35	12.38	93.74	95.69	94.48	93.17	91.26
40-6	O	0.64	0.00	0.00	21.93	1.64	92.50	95.25	92.33	89.83	--
	N	2.73	9.33	0.90	78.61	5.36	93.81	95.19	94.97	93.21	90.23
40-3	O	0.62	0.00	0.00	23.53	1.80	92.27	93.92	95.21	89.43	--
	N	4.31	13.34	1.27	122.26	7.96	93.49	95.21	95.00	92.84	90.41

Nutr: O: without nutrient; N: with nutrient.
Temp: 23/21°C with 14/10 hr (D/N).
40-3, 40-6:emerged seed were treated with 40°C for 3 or 6 hours respectively and moved into 23/21° C.

Table A.13. Significance of characteristics of 8- or 10-day -old tomato seedlings (cv. Walter) grown at 23/21, 40/25 or 40/30° C with 14/10 or 10/14 hr (D/N) from emerged seed or 2-day-old seedlings at 23/21° C.

Characteris -tics	Hr	Temp	Stage	Iinteractions h*t	h*s	t*s	h*s*t
Stem length	***	**	***	***	ns	ns	**
Stem fw (mg)	ns	***	ns	***	*	ns	ns
Stem dw (mg)	***	***	***	***	ns	ns	ns
Root #	***	***	***	ns	ns	ns	ns
Root length	ns	***	***	***	ns	ns	ns
Root fw (mg)	***	***	***	***	ns	ns	ns
Root dw (mg)	***	***	***	***	ns	ns	ns
Cotylesons fw	***	***	**	***	*	**	**
Cotyledons dw	***	***	***	***	ns	ns	*
Leaf #	ns	***	***	**	ns	***	***
Leaf fw (mg)	***	***	***	***	*	ns	*
Leaf dw (mg)	***	***	***	***	ns	ns	*

Hr: 14/10 and 10/14 (D/N).
Temp: 23/21, 40/25, 40/30° C.
Stage: emerged seed or 2-day-old seedlings were treated with temperatures.

Table A.14. Growth characteristics of 8- or 10-day-old tomato seedlings (cv. Walter) grown at 23/21, 40/25, 40/30°C 14/10 D/N from emerged seed (G) or 2-day-old seedling (S) respectively.

Temp (°C)		Stem length (cm)	Root length (cm)	Leaf #	Root #	St/rt ratio	Stem fw (mg)	Stem dw (mg)	Root fw (mg)	Root dw (mg)	Cotyledon fw (mg)
23/21	G	3.11	13.09	2.57	8.14	0.25	44.91	2.39	66.84	3.63	81.23
	S	3.43	10.20	3.00	7.25	0.34	47.03	2.25	61.58	3.50	105.50
40/30	G	3.67	4.67	0.43	2.43	0.81	20.90	1.04	9.46	0.56	12.67
	S	2.50	5.96	2.00	5.43	0.43	19.00	1.33	17.77	1.39	28.00
40/25	G	2.91	4.74	0.71	2.71	0.67	19.41	1.00	9.87	0.65	19.90
	S	2.80	6.13	2.29	8.29	0.47	21.89	1.39	26.09	1.67	32.57

		Cotyledon dw (mg)	Leaf fw (mg)	Leaf dw (mg)	Total fw (mg)	Total dw (mg)	Water content (%)	Stem water (%)	Root water (%)	Cotyledon water (%)	Leaf water (%)
23/21	G	7.49	58.14	6.29	251.13	19.79	92.12	94.65	94.65	90.88	89.23
	S	8.58	56.70	6.08	270.80	20.40	92.57	95.21	94.44	92.00	89.35
40/30	G	1.04	0.00	0.00	43.03	2.64	93.85	94.96	94.13	91.81	--
	S	2.96	4.93	0.69	69.69	6.36	91.03	93.11	92.16	89.72	85.72
40/25	G	1.75	--	--	46.34	3.16	93.33	94.98	92.92	91.23	--
	S	3.16	10.36	1.34	90.90	7.56	91.74	93.65	93.66	90.30	87.22

Table A.15. Growth characteristics of 8- or 10-day-old tomato seedlings (cv. Walter) grown at 23/21, 40/25, 40/30°C 10/14 D/N from emerged seed (G) or 2-day-old seedlings (S) respectively.

Temp (°C)		Stem length (cm)	Root length (cm)	Leaf #	Root #	St/rt ratio	Stem fw (mg)	Stem dw (mg)	Root fw (mg)	Root dw (mg)	Cotyledon fw (mg)
23/21	G	2.90	7.28	1.88	3.50	0.45	19.09	0.86	15.59	0.70	38.36
	S	3.16	9.96	2.63	5.88	0.36	29.94	1.44	30.98	1.71	55.03
40/30	G	4.28	5.73	0.75	2.00	0.77	21.64	0.91	6.69	0.43	10.30
	S	4.11	6.89	2.13	4.63	0.62	28.38	1.30	15.78	0.73	27.51
40/25	G	4.99	4.99	1.63	1.88	1.02	26.49	0.95	8.00	0.43	15.59
	S	3.98	6.94	2.13	4.38	0.59	28.30	1.26	14.46	0.76	26.34

Temp (°C)		Cotyledon dw (mg)	Leaf fw (mg)	Leaf dw (mg)	Total fw (mg)	Total dw (mg)	Water content (%)	Stem water (%)	Root water (%)	Cotyledon water (%)	Leaf water (%)
23/21	G	2.38	5.93	0.49	78.96	4.43	94.46	95.54	95.35	93.91	91.55
	S	4.50	26.39	2.94	142.33	10.59	92.73	95.17	94.78	92.23	87.92
40/30	G	0.83	0.00	0.00	38.90	2.17	94.41	95.70	93.98	91.79	--
	S	0.77	10.35	1.13	82.01	5.25	93.69	95.47	95.23	92.42	89.09
40/25	G	1.05	1.15	0.18	51.23	2.60	95.03	96.46	94.80	93.40	93.04
	S	1.81	10.21	1.06	79.31	4.90	93.81	95.52	94.79	92.95	89.68

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