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Physiological Genetics of Cold Tolerance in the Tomato

**J. Rudich, M.A. Stevens, D. Zamir, O. Miltao,
D. Yakir, S. Wolf, B.A. Bravdo**

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Principal Investigator: Jehoshua Rudich

Cooperating Investigator(s): M.A. Stevens, D. Zamir, O. Miltao, D. Yakir
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Name and Address of Affiliated Institutions:

Principal Institution: Hebrew University of Jerusalem

Cooperating Institution(s): University of California, Davis

Project's starting date: April 1980

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Signatures:

Principal Investigator

Jehoshua Rudich

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Institution's Authorizing Official

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Abstract

During the last three years our research efforts have centered on the evaluation of several techniques in an effort to develop reliable screening procedures to select cold tolerance in individuals of segregating populations. The methods include "plastochron index", "germination", "emergence", "chlorophyll fluorescence", "electrolyte leakage", "pollen germination" and "reduction of TTC". (2,3,5 triphenyl-tetrasolium chloride).

Possible application of P.I technique has been extensively explored using controlled environment chambers. The technique was found to be simple and accurate and can be used for large scale screening of genotypes. Our study shows good correlation between the plastochron index and time, dry weight and leaf area, and is used in segregating generations to characterize temperature effects.

During the first day of chilling, there is a rapid reduction in variable fluorescence in leaf samples of L. esculentum. Reduction in fluorescence of L. hirsutum, L. chilense and S. lycopersicoides was much less. The method is simple and is practical screening procedure. Measuring electrolyte leakage after cold treatments also differentiated between wild species and the tomato cultivar.

Crosses between the tomato cultivar U.C. 82 and five accessions from wild species were made, the F_1 F_2 BC_1 BC_2 BC_3 were screened with the methods mentioned above. In each population there were plants that showed cold resistance similar to the wild parent, suggesting introgression of cold tolerance to the cultivar. As reliable screening procedures have been developed, it became possible to speed efforts to introgress chilling tolerance from wild accessions, presently BC_3 and BC_5 of L. chilense, BC_4 and BC_3 of L. hirsutum are being completed. All the backcrosses are made to the variety UC 82. In the breeding program with low temperature germination BC_4 has been produced.

Crosses of high temperature tolerant lines with advanced lines from this low temperature program will be used for the breeding program after termination of this project.

COLD TEMPERATURE TOLERANCE OF WILD TOMATO SPECIES¹

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Abstract. Several wild species of tomato, Lycopersicon hirsutum (LA 1363 and LA 1777), L. chilense (LA 1969 and LA 1971), and Solanum lycopersicoides (LA 1964) were examined for cold tolerance and compared to a fast germination genotype of L. esculentum (PI 341988) and to the cultivar UC 82B. These wild species grow naturally above 3000 m and are presumed to be cold tolerance because of natural habitat.

A number of characteristics including germination, emergence, chlorophyll fluorescence, electrolyte leakage and plastochron index have been used to evaluate chilling resistance. PI 341988 germinated faster at temperature above 10°C but the wild species have lower base temperature for germination. Growth rates at 12/6°C (day/night) were compared to 24/18°C and were found to be higher in the wild species than UC 82B. Measuring physiological responses by chlorophyll fluorescence and electrolyte leakage during cold treatments also differentiated between the wild species and the cultivar. Crosses between the cultivar and the wild species were made and segregating populations were screened using the methods mentioned above. In each population there were plants that showed cold resistance similar to the wild parent, suggesting a possibility of introducing cold tolerance to the cultivar.

Tolerance of tomatoes to chilling temperatures is of considerable practical importance. Chilling resistant genotypes could be earlier, more adaptable, use less water, have higher yields of better quality fruits when temperatures are too low for optimum production. Energy costs for greenhouse production could be lower for a chilling-resistant cultivar. For several reasons earliness is a much sought after characteristic in tomato breeding programs. It extends the harvest season, allowing for more efficient use of available processing facilities. It results in water savings in areas with a Mediterranean climate because early plant growth makes better use of late season rains and water available in the root zone. Production is usually better in early varieties because they avoid high temperature problems which can reduce fruit set during mid-summer.

Temperatures less than 10°C injure many tropical and subtropical crops, including tomatoes. This injury, which occurs at temperatures higher than 0°, has frequently been called chilling injury and was reviewed by Lyons (1973). There have been several reports on the temperature response of tomato (14, 15). Chilling injury in tomato occurs at 10-12°C (2, 9). Data are available which indicate cultivar and species differences for response to cold temperatures in many tropical crops, including tomato, as well as differences in sensitivity of various plant tissues and developmental stages (13).

The time required for germination of tomato (~~Lycopersicon esculentum Mill~~) seeds is influenced by soil temperature, soil water content, and soil texture. Soil temperature from 10 to 25°C affect the time to germination, while temperatures less than 10°C inhibit germination in most cultivars. However, differences among cultivars have been demonstrated (7). Germination

under cold temperatures can be improved by selecting from segregating seedling populations individuals which require a short germination period (3). It has been demonstrated that rate of emergence under low and high temperature conditions is heritable (15). The cool temperature germination capability of PI341985 appears to be controlled by a single gene in certain crosses (3).

An increase in temperatures from 12 to 24°C has a positive effect on plant growth. Low night temperatures (10-16°C) during the vegetative stage, after full cotyledon expansion, slowed vegetative growth and advanced reproductive growth by early initiation of the first inflouescences (14, 15). Temperatures of less than 10°C inhibit plant growth and flowering.

In most tomato genotypes photosynthesis is maximum at about 25°C (1). The evidence indicates that at 10°C net photosynthesis becomes negative in most genotypes.

It has been proposed that the single response for chilling injury is found in the evidence that cellular membranes in sensitive plants undergo a physical phase transition from a normal flexible liquid crystalline to a solid gel structure at the temperature critical for chilling injury (6). There is evidence that this response in chilling sensitive plants is mediated by a higher proportion of saturated fatty acids in the membrane than in chilling resistant plants.

It has recently been found that during chilling at 0°C, variable fluorescence in dark-adapted leaves decreased in cold sensitive plants but not in cold resistant plants (11). The intensity of this chlorophyll fluorescence is correlated with photosynthetic electron transfer activity and is frequently employed as a means of studying photosynthetic mechanisms. Currently, in vivo chlorophyll fluorescence is also being used to assess the effects of genetic, environmental and other stresses on plant cells.

To develop a high yielding, adaptable, cold tolerant cultivar, several characteristics are needed: 1) seed germination at less than 10°C, 2) seedling vigor and growth under cool temperature conditions, 3) a genotype with early flowering and fruit set capability is desirable. Germplasm for cold tolerance exists in several cultivated and wild specie accessions (8). The potential of this material has not been realized because of a lack of understanding of the physiology of the cold tolerance and a lack of effort introgressing this potential into cultivars.

The purpose of this research was to characterize the cold tolerance status of several genotypes with proven or presumed (because of natural habitat) cold tolerance.

MATERIALS AND METHODS

Plant material used in the study included: 1. Lycopersicon esculentum, UC 82B, PI 341988. 2. L. hirsutum, LA 1363 and LA 1777. 3. L. chilense, LA 1969 and LA 1971. 4. Solanum lycopersicoides, LA1964. Seeds of the wild species accessions were kindly provided by Dr. C. M. Rick, Department of Vegetable Crops, U C Davis.

Germination. Seeds of all genotypes used in these studies were extracted and processed by a standard procedure involving treatment with 6% hydrochloric acid for 4 hours. Seeds were incubated in petri dishes on moisture filter paper at 12/6°C (12 hours at each temperature) in the dark for 10 days. Germination seeds were counted when the radicle was 1 mm long. For germination on a thermogradient plate, filter paper were placed directly on the plates at various temperatures for 20 days.

Emergence. Seeds were planted in UC Mix sterilize soil at a depth of 1.5 cm, with a constant soil temperature of 9°C. Emergence was measured as the time required for appearance of the cotyledons.

Plastochron Index. The plastochron was defined as "the time interval between initiation of two successive leaves." It was proposed that if successive plastochrons are equal in duration, it could be used as a unit of development scale (5). Possible application of plastochron index (P.I.) for selecting cold tolerance was explored using a controlled environment chamber. Plants were grown for 20 days at 24/18°C (day/night) with a 15 hour photoperiod, followed by a growth period of 20 days at 12/6°C (day/night). The growth rate in each temperature regime was measured using P. I. (5). The P.I. at low temperature relative to that at high temperature was calculated and a higher relative rate was assumed to be related to greater resistance to chilling.

Chlorophyll Fluorescence. When photosynthetic organisms absorb photons via chlorophyll, a portion of the acquired energy, usually less than one percent, is dissipated by emission. To measure leaf chlorophyll fluorescence, discs (7 mm diameter) were cut from the center of the left hand side of a leaflet of the first fully expanded leaf, and placed abaxial surface up on moist filter paper. The leaflet was covered with a filter paper with a hole in the center. After dark adaptation for 1 hour, chlorophyll fluorescence was recorded using a portable fluorometer, (Richard Brancher Research Ltd, Ottawa, Model SF-10).

An experimental ^paparatus was constructed in which 104 leaf discs can be simultaneously treated with low temperature and fluorescence measurements of each sample taken (in situ) at the low temperature.

Electrolyte leakage. Leaflets from young fully expanded leaves were cut with a razor and washed 3 times with deionized water. The leaflets were suspended in 10 ml of deionized water and chilled for the duration of the experiment. The conductivity of the bathing solution ^{was} measured with a Selectro Mark Analyzer (Markson Science, Inc.) conductivity meter. The tissue was kept in the dark, except for brief daily exposure to room fluorescent lighting during the readings of conductivity. At the end of the chilling period, the tubes were capped and autoclaved for 20 minutes. After a further 4 hours the conductivity of the bathing solution was measured. This measurement was assumed to represent the total potential leakage of the cells and the measurements taken during the chilling period were expressed as a percentage of electrolyte lost from the autoclaved tissue.

RESULTS

Germination. After 30 days at 12/6°C (12 hours at each temperature), germination percent was greatest in PI 341988. LA 1777 (L. hirsutum), LA 1969 (L. chilense), and LA 1964 (S. lycopersicoides) also germinated at these low temperatures (Table 1). The germination percentage in LA 1964 was very low at all temperatures; however, the germination at 12/6°C was more than 80% of the germination at 25°C. It is difficult to obtain seeds of S. lycopersicoides which germinate well. Studies with the thermogradient plate showed that the germination percent of PI 341988 was good at 11°C but fell off rapidly as the temperature approached 10°C. On the other hand, there was a more gradual decrease in the germination percentage of LA 1969 and LA 1777 showing that the base temperature for germination of these lines is lower (Fig. 1).

Emergence. Emergence of the wild accessions is delayed and variable at normal temperatures as well as at low temperature. Progeny of the early backcrosses also emerge slowly at normal temperature, with a high degree of variability, making emergence a poor criterion for cold tolerance selection in the wild species and early backcrosses. However, after the third backcross, emergence becomes more uniform and selection of progeny which emerge rapidly under low temperatures becomes practical. The third and fourth backcrosses of UC 82B x LA 1777 and UC 82B x LA 1969, were examined for emergence. In each case there were individual selections with a higher percent emergence at low temperature than either parent.

Six plants were chosen from the BC₃ of UC 82B x LA 1777 population and their F₂ seeds were checked again for emergence (Table 2). Percent emergence of F₂ plants was higher in the lines that emerged more rapidly than the lines that emerge only after the end of the cold treatment indicating that selection was effective. F₂ progeny of plants which emerged more rapidly tended to have a higher relative plastochron index at low temperatures (Fig. 2).

Plastochron Index (P. I.). The accessions used in this study had successive plastochrons which were equal in duration (Fig. 3). It was found that P. I. is linear with time between the second and the twelfth leaf stages. In later stages irregularities in growth rate increased, possibly due to variation in initiation of the reproductive stage.

The growth rate of high altitude accessions of LA 1363 and LA 1777, (L. hirsutum), LA 1969 and LA 1971 (L. chilense), and LA 1964 (S. lycopersicoides) were significantly less affected by low temperature than that of UC 82B. PI 341988 had an intermediate growth rate at this temperature (Fig. 4).

Chlorophyll Fluorescence. During the first day of chilling there is a rapid reduction in variable fluorescence in leaf samples of UC 82B, while there was no reduction in the chilling resistance accessions (Fig. 5). After 3 days of chilling the variable fluorescence of UC 82B was almost zero. Reduction in the fluorescence of the other accessions was much less. To ascertain whether the differences between UC 82B and the wild accessions is due to the cold treatment, we checked the variable fluorescence at a higher temperature (16°). There was very little reduction of variable fluorescence after three days in any of the lines, furthermore, there was no significant difference in amount of reduction among the lines. UC 82B had little reduction in fluorescence after five days and its fluorescence, as a percent of the original value, was higher than any of the wild species (Fig. 6).

Simplicity and speed are important in a practical screening procedure. With the present fluorescence apparatus 104 plants can be simultaneously tested at any desired temperature. Three fluorescence measurements are taken during the 48 hours of each experiment. A seven second light pulse was found to be suitable to induce the fluorescence rise and 104 samples can be measured in 20 minutes.

Electrolyte leakage. Study of electrolyte leakage at 0°C did not differentiate between UC 82B and the other Lycopersicon accessions, however, LA 1964 (S. lycopersicoides) showed less leakage (Fig. 7). Exposure of the leaves to 1°C resulted in more severe electrolyte leakage from LA 1777 (L. hirsutum) and UC 82B than from LA 1969 (L. chilense) and LA 1964 (S. lycopersicoides) (Fig. 8). There was severe leakage from L. hirsutum leaves even after exposure of the leaves to 15°C for 72 hours.

Measurement of electrolyte leakage during cold treatment is probably not a sufficient means of differentiating genotypic cold tolerance. However, when electrolyte leakage, measured as a percent of leakage from autoclaved tissue, was expressed as a ratio between values obtained at 1.5° and 15°C, there was a difference between UC 82B and the wild accessions (Table 3).

Interspecific Crosses. One of the most promising sources of chilling resistance is S. lycopersicoides, a perennial, native to the south coast of Peru. It shows a unique resemblance to the tomatoes in such characteristics as its yellow flowers, nature of leaf dissection, and lack of tubers. LA 1964 (S. lycopersicoides) was found at a higher altitude and at a more southern latitude than the other wild species studies, indicating that it withstood lower temperatures.

Results reported here indicate that S. Lycopersicoides may have some degree of freezing resistance and therefore it is considered an extremely important plant material for chilling tolerance.

The F₁ hybrids between L. esculentum and S. lycopersicoides were obtained with difficulty. The former was used as the pistillate parent, and after hundreds of crosses a few fruits were set containing a very few small seeds. Since the seeds did not germinate, fruits were opened under sterile conditions, the ovules dissected, and the endosperm-embryo contents placed in sterile culture (12). In these cultures embryos grew slowly at first, many being deformed, but after the initial period of slow growth, they developed very rapidly. The hybrids grew with much greater vigor than either parent. From chlorophyll fluorescence measurements it seems that the cold tolerance character was present in the hybrid (Fig. 9). Unfortunately, the fertility of

the hybrids is extremely low, and efforts to produce backcross progeny have not been successful. The relatively low degree of chromosome pairing in the hybrid suggests that some degree of fertility might be restored in amphidiploids (10). In an effort to overcome the sterility barrier, the F_1 plants were treated with colchicine to double the chromosome number. At the same time the cultivated tomato (UC 82B) was treated using the same procedure, and a cross between the two polyploid accessions will be attempted.

Discussion

Two related species of tomato with green fruits, Lycopersicon hirsutum (LA 1363, LA 1777) and Lycopersicon chilense (LA 1969, LA 1971) which grow naturally above 3000 m are more resistant to low temperature than the cultivar UC 82B. The genetic potential for cold tolerance of these wild species was demonstrated by several methods used in this study.

It was reported that L. esculentum PI 341985 can germinate considerably faster than other cultivated varieties at a constant temperature of 10°C (7). Studies with a thermogradient plate showed 100% germination of PI 341988 after 15 days at 12°C but no germination at 10°C. LA 1777 (L. hirsutum) and LA 1969 (L. chilense) had 25% and 40% germination, respectively, after 15 days at 10°C, showing lower base temperature for germination than the L. esculentum lines.

The differing abilities to grow at low temperature were expressed by the P.I. method. The ratio between plastochron index at low temperatures ($12^{\circ}/6^{\circ}\text{C}$) and at normal temperatures ($24/18^{\circ}\text{C}$) was significantly higher for all of the wild species which were examined in this study.

An assay for use in a breeding program should be rapid and non-destructive to the whole plant. The P.I. method was found to be simple and accurate and can be used for large scale screening of genotypes. This method reduces variability in a population by eliminating variation among plants of the same chronological age but different stage of development.

Two other methods for measuring physiological responses also differentiated between the wild species and the cultivar. Chlorophyll fluorescence was found to be simple and a rapid method for screening large populations. Forty-eight hours of cold treatment was adequate to distinguish between the genotypes. Electrolyte leakage as a method for screening is of limited value. Differences between the plants can be seen only after a long exposure to low temperature. After a long treatment factors other than chilling injury, such as senescence and microbial growth, may become important. This makes it a questionable, as well as a complicated and tedious tool for selection.

Most of the work of breeding for cold tolerance has been done by selection for seed germination at low temperature (3, 4, 7). Very little has been done for selection of tomato cultivars that are tolerant to low temperature during other stages of development. In none of the earlier work were wild species used as a source of cold tolerance.

In our breeding program, several backcrosses to the commercial variety UC 82B were made and the segregating population after each backcross was examined by the methods mentioned. In each population there were plants that showed similar cold resistance to the wild parent. Evidence of cold tolerance by these parameters was found following backcross 5.

The control of cold tolerance is a complicated mechanism which involves many genes. Our results support the assumption that some of the genes are dominant. Not much is known about the physiological basis of cold tolerance in tomatoes. Understanding of the physiology and the genetic control of cold tolerance will help in using other method of screening and more efficiency introducing of cold tolerance to cultivars.

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Table 1. Germination percent of seeds of UC 82B, PI 341988 and several high altitude accession of Lycopersicon and Solanum at 12°C day/6° night after 15 and 30 days.

			Control
	<u>15 Days</u>	<u>30 Days</u>	<u>10 Days at 25°C</u>
UC 82B (<u>L. esculentum</u>)	0	0	84
PI 341988 (<u>L. esculentum</u>)	6	87	96
LA 1777 (<u>L. hirsutum</u>)	18	21	94
LA 1964 (<u>S. lycopersicoides</u>)	0	8	11
LA 1969 (<u>L. chilense</u>)	8	9	100
LA 1971 (<u>L. chilense</u>)	0	2	96
LA 1363 (<u>L. hirsutum</u>)	0	0	90

Table 2. Emergence data for several BC₃ plants of UC 82B x LA 1777 and their F₂ progeny.

Plant No.	Days for emergence	% emergence in F ₂
	of F ₁ at 9°C	Progeny after 42 Days at 9°C
21-6-1	24	2
23-3-6	42 ^z	0
28-6-10	43 ^z	0
37-5-1	21	15
37-5-3	25	20
92-1-8	42 ^z	0
LA 1777		28
UC 82B		0

^z Emergence occurred after termination the cold treatment (40 days).

Table 3. Ratios between electrolyte leakage values obtained at 1.5°C and 15°C after 5 and 7 days of treatment. Values expressed as the ratio: (Leakage at 1.5°C, % of autoclaved tissue)/(Leakage at 15°C, % of autoclaved tissue).

	<u>5 Days</u>	<u>7 Days</u>
LA 1969 (<u>L. chilense</u>)	0.68 ± 0.19 ^z	0.64 ± 0.21
LA 1971 (<u>L. chilense</u>)	0.71 ± 0.18	0.64 ± 0.15
LA 1363 (<u>L. hirsutum</u>)	0.33 ± 0.09	0.52 ± 0.16
LA 1777 (<u>L. hirsutum</u>)	0.28 ± 0.07	0.45 ± 0.06
UC 82B (<u>L. esculentum</u>)	1.02 ± 0.39	1.12 ± 0.33

^z Mean ± standard deviation for 6 replications.

FIGURE LEGENDS

Figure 1. Percent germination of UC 82B, LA 1777, (L. hirsutum) and LA 1969 (L. chilense) after 15 days at temperature from 8 to 15°C.

Figure 2. Mean relative plastochron index of F₂ progeny related to the number of days to emergence at 9°C of their respective parents, BC₃ UC 82B x LA 1777. Each number is a mean relative P.I. of 10 F₂ plants. * Regression significant at 5% level.

Figure 3. Growth rate as measured by plastochron index during early vegetative growth of tomato plants of several genotypes.

Figure 4. The ratio of the plastochron index (P.I.) at 12/6°C (day/night) to P.I. at 24/18°C (day/night) in plants of UC 82B and PI 341988 (L. esculentum), LA 1363 and LA 1777 (L. hirsutum), LA 1969 and LA 1971 (L. chilense) and LA 1964 (S. lycopersicoides). Standard error of the mean indicated by vertical bars (N=8).

Figure 5. The effect of chilling at 0.5°C on the variable fluorescence in leaf discs of tomato plants of various genotypes.

Figure 6. The effect of warm temperature (16°C) on the variable fluorescence in leaf discs of tomato plants of various genotypes.

Figure 7. Electrolyte leakage at 0°C of UC 82B, and several high altitude accessions.

Figure 8. Electrolyte leakage at 1°C of UC 82B and several high altitude accessions.

Figure 9. The effect of chilling (0.5°C) on variable fluorescence in leaf discs of UC 82B, LA 1964 (S. lycopersicoides) and the F₁ progeny of these parents.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

PLASTOCHRON INDEX: A SELECTION CRITERION FOR GROWTH OF TOMATO PLANTS
AT LOW TEMPERATURES

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Abstract

The effect of low temperature on growth and development of two high altitude accessions of L. hirsutum and L. chilense and varieties of the cultivated tomato L. esculentum was studied. The development of the plants was characterized using as parameters plastochron index, dry weight and leaf area. A linear relationship between plastochron index and time, and between plastochron index and each of the vegetative parameters was found. These results suggest that plastochron index can be used to evaluate development of plants of the three Lycopersicon species. Significant genotypic differences in vegetative growth ability under low temperature conditions were found. Plastochron index rate, dry weight accumulation and leaf area increase were less affected by the lower temperature in the wild species than in the cultivated tomato.

Key- Words: Lycopersicon esculentum; L. hirsutum; L. chilense; chilling stress; breeding; plastochron index; dry weight and leaf area.

Introduction

In the genus Lycopersicon the two species L.hirsutum and L.chilense complete their life cycles at elevations of up to 3000 m in the Peruvian Andes (Rick, 1978; Vallejos, 1979). Accessions of L.hirsutum have been shown to be chilling tolerant with respect to several physiological responses: seed germination, seedling survival,

chlorophyll development, rate of protoplasmic streaming, electrolyte leakage (Paull, Patterson & Graham, 1979), rate of plant growth (Patterson & Payne, 1983), chlorophyll fluorescence (Smillie, 1979) and rate of pollen germination (Zamir, Tanksley & Jones, 1981). L.chilense has not yet been characterized with respect to its low temperature responses . The two wild species can be hybridized with the cultivated tomato and therefore constitute a potential source of tolerance to cold environments (Rick, 1978). A breeding program aimed at utilizing this potential requires the development of selection criteria which could quantify efficiently the cold stress. Plant growth measures such as dry weight accumulation and leaf area changes are generally not suitable because they are destructive.

A possible criterion for plant growth rate is the plastochron index which measures the time between the appearance of consecutive leaf primordia. Erickson and Michelini (1957) who first suggested this measurement technique showed that in Xanthium the plastochron duration is constant and depends on temperature. In the tomato the plastochron index is constant in the range of 3-10 true leaves. Beyond this range flowering begins and the plastochron index is no longer constant (Coleman & Greyson, 1976; Vallejos & Tanksley, 1983). In this study we compared the effects of low and normal temperatures on growth and development of tomato plants as expressed by plastochron index, dry weight and leaf area. The correlations between these parameters were evaluated for wild and cultivated tomato species.

Materials and Methods

The plant material included two varieties of L.esculentum : the first a determinant processing tomato variety ,M82-1-8, and the second a fresh market large fruited breeding line, T5. L.hirsutum accession LA-1777 (Rick, Fobes & Tanksley, 1979) and L.chilense accession LA-1969 (Rick, 1973) both originating from elevations of 3000 m in Peru, were examined.

The plants were germinated in speedling trays (3.7*3.7cm) and transplanted to 3/4 1 pots filled with peat and tuff mixture (1:3) at the 2 true leaf stage. At the age of 3-4 true leaves plants were irrigated to saturation with 0.03% N:P:K (20:20:20) and transferred to two growth chambers: in the control chamber day temperature was 25 C and night 17 C while in the cold chamber day temperature was 12 C and night 6 C. Day length in both chambers was 12 hr and light intensity was 250 E sec. m. The plants were irrigated every two days in the normal temperature treatment, and twice a week in the cold treatment.

Plastochron index was measured every 3 days for the normal temperature and every 7 days for the cold temperature regime. In both cases a total of 4 measurements were taken. The plastochron index was computed according to the following formula: (Erickson & Michelini, 1957), where r is the arbitrary reference length, n the serial number of the leaf just longer than an arbitrary reference length and $n+1$ the serial number of the leaf just shorter than an arbitrary reference length. (Logarithms are to the base e throughout this paper.) In our work the reference length was 10 mm. After each measurement of plastochron index 10 plants were randomly selected from each line and leaf area

(measured by automatic area meter, Hayashi Denko co.) and dry weight after 96 h at 65 C were measured. The correlation coefficients (r) between plastochron index and leaf area and plastochron index and dry weight were calculated and the regression slopes were compared using the multiple range test (Student).

Results

Significant differences were found between the species in their ability to accumulate dry weight under the normal and low temperature treatments. At 25/17 C the rate of dry weight increase of the L. esculentum cultivars was higher than that of L. hirsutum and L. chilense, the latter showing the lowest rate of increase (Fig. 1). Significantly lower rates of dry weight accumulation were observed for all the genotypes under the low temperature treatment regimes (12/6 C); this time however L. hirsutum showed the highest rates of increase followed by the cultivated varieties and the L. chilense accession (Fig. 2). Measurements of leaf area changes revealed a trend similar to that observed for dry weight (Fig. 3, 4). At normal temperatures the determinant processing variety M82-1-8 showed the highest rate of area increase followed by L. hirsutum and the cultivar T5 with L. chilense lagging far behind them. Under the 12/6 C treatment L. hirsutum had a markedly higher rate of leaf area increase followed by M82-1-8, T5 and L. chilense.

In order to examine possible relationships between plastochron index, dry weight and leaf area it was necessary to first establish the suitability of the plastochron index for evaluating the growth responses

of the tomato species under the low and normal temperature regimes. This was essential considering the different growth habits of the tested genotypes: M82-1-8 is a processing tomato variety with a determinant growth habit, T5 and the two wild species are indeterminantal though they differ in the architecture of the vine, T5 and L. hirsutum have heavy and robust branches while L. chilense is more slender and dainty. Under the normal temperature treatment (25/17 C) significant correlation coefficients ($p < 0.001$) were found between plastochron index and time for all the genotypes (Tab. 1). At 12/6 C the correlation coefficients were also highly significant though slightly reduced. These results demonstrate that the plastochron index is an adequate scale for measuring the development of the tomato genotypes under the two temperature regimes. The slopes of the regression lines of plastochron index over time are compared for the genotypes at both temperatures (Fig 5). At normal temperatures the rate of leaf initiation and early elongation is lowest for the determinant L. esculentum variety followed by T5, L. chilense and L. hirsutum. This hierarchy of development among the genotypes is maintained at low temperatures. The ratio between the slope of the regression line at 12/6 C and that at normal temperatures for each genotype was used to compare the accessions since it represents the relative reduction in the rate of leaf initiation and early elongation due to the cold stress. In L. chilense, which using this parameter is the least affected by the low temperature, we observed a reduction of 68% in the plastochron rate. In L. hirsutum the reduction was 75% and in the cultivated varieties 82%.

The correlation coefficients between plastochron index, dry weight and leaf area were highly significant for all the genotypes at both

temperatures (Tab. 1). In order to compare the influence of the cold environment on growth of the tomato plants we analyzed the changes in the slopes of the regression lines of plastochron index over dry weight and leaf area. These regression lines represent the change in dry weight or leaf area associated with a change in the independent variable the plastochron index. For the control temperature the increase in the vegetative parameters associated with a change in a plastochron unit was higher for the cultivated varieties than for the wild species (Fig 6,7). It is interesting to compare the growth characteristics at normal temperatures of M82-1-8 which was bred for fast growth and L. chilense ; for every plastochron unit change the cultivated variety produces 5 times more dry weight and leaf area. The effects of low temperatures on dry weight accumulation differ for the cultivated varieties and the wild species: plants of L. esculentum produce less dry weight at low temperatures than the control. Productivity per plastochron unit for L. hirsutum and L. chilense in the cold is higher than for the control. The changes in leaf area per plastochron unit differ between the wild and cultivated species; leaf area is diminished for all the entries although the change in leaf area per plastochron unit is much less affected by the low temperature in the wild species than in the cultivated varieties.

Discussion

In breeding programs aimed at improving the ability of tomato plants to tolerate low temperatures there is need for nondestructive selection criteria. Therefore growth parameters such as dry weight accumulation and

leaf area increase are not suitable. Previous studies have shown a good correlation between the plastochron index of plants and their development in a fixed environment (Michelini, 1958). Silk (1980) used the plastochron index to describe growth of cantaloupe under diminished water supply conditions. Her study concluded that the plastochron index is a better parameter than chronological time for describing the effects of water stress on leaf and branch growth. The plastochron index was recently used by Vallejos and Tanksley (1983) to describe the cold response of an interspecific backcross between L. hirsutum and L. esculentum. Using genetic markers they identified genes that affect the cold tolerance of the backcross populations.

Our study shows that there is a good correlation between the plastochron index and chronological time both at normal and low temperatures for all the species examined. The linear relationship between plastochron index and the destructive parameters dry weight and leaf area demonstrates that it is possible to use the plastochron index to characterize growth of the tomato species in the different temperature environments. Regression analysis of our data indicates that the correlation between the plastochron index, leaf area and dry weight in tomato is not constant, rather it depends on the environment and the genotype. Tolerance of the wild species to cold stress is expressed in the lower rate of change of the plastochron index per unit of time compared to the cultivated tomato varieties. This nondestructive parameter is being used in our laboratory to characterize the effects of different temperature regimes on growth of segregating F2 generations between the cultivated tomato and the wild species described in this study.

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Figure legends

Figure 1 . Dry weight accumulation at 25/17 C (day/night) of 3 Lycopersicon species: L.esculentum (M82-1-8 and T5), L.hirsutum (LA 1777), L.chilense (LA 1969). Each different letter indicates significance at the 0.01 level.

Figure 2 . Dry weight accumulation at 12/6 C (day/night) of 3 Lycopersicon species: L. esculentum (M82-1-8 and T5), L. hirsutum (LA 1777), L.chilense (LA 1969). Each different letter indicates significance at the 0.01 level.

Figure 3 . Leaf area increase at 25/17 C (day/night) of 3 Lycopersicon species: L. esculentum (M82-1-8 and T5), L. hirsutum (LA 1777), L. chilense (LA 1969). Each different letter indicates significance at the 0.01 level.

Figure 4 . Leaf area increase at 25/17 C (day/night) of 3 Lycopersicon species: L. esculentum (M82-1-8 and T5), L.hirsutum (LA 17777), L. chilense (LA 1969). Each different letter indicates significance at the 0.01 level.

Figure 5 . The slope of the regression lines of plastochron index over time, under low and normal temperature for the 3 Lycopersicon species. Each different letter indicates significance at the 0.01 level.

Figure 6 . The slope of the regression lines of plastochron index over dry weight, under low and normal temperature for 3 Lycopersicon species. Each different letter indicate significance at the 0.01 level.

Figure 7 . The slope of the regression lines of plastochron index over leaf area, under low and normal temperature for 3 Lycopersicon species. Each different letter indicate significance at the 0.01 level.

Table-1. The correlation coefficients (r) between plant height (cm) and chronological time, dry wt and leaf area.

Accession	25/17 °C			12/6 °C		
	Time	Dry Weight	Leaf Area	Time	Dry Weight	Leaf Area
M-82-1-8	.948*	.909*	.932*	.850*	.839*	.725*
75	.952*	.941*	.952*	.910*	.796*	.739*
LA-1777	.992*	.931*	.956*	.967*	.895*	.902*
LA-1969	.961*	.907*	.928*	.951*	.790*	.744*

* Significant at the level of 0.001

Fig 1

Fig 2

Fig 3.

Fig 4

Fig 5

Fig 6

F₁ & 7

CHILLING-INDUCED CHANGES IN NET PHOTOSYNTHESIS AS REFLECTED

BY IN VIVO CHLOROPHYLL FLUORESCENCE

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RUNNING TITLE: Changes in photosynthesis reflected by chlorophyll fluorescence

Abbreviations: DCMU=3-(3',4'-dichlorophenyl)-1,1-dimethylurea ;

F_I, F_P, F_T =Fluorescence yield at different peaks according to

Papageorgiou(1975) ; PFD=Photon flux density ; PSII=Photosystem II

Chilling-induced changes in net photosynthesis as reflected by in vivo
chlorophyll fluorescence

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Abstract

The relationship between in vivo chlorophyll fluorescence measurements and CO₂ uptake determined in an open gas exchange system was studied using tomato plants. In both cases changes in quantum yield as a result of various chilling treatments were followed and a strong correlation was found between the results obtained by the two techniques. As expected, treatment with 3-(3',4'-dichlorophenyl)-1,1-dimethylurea caused a significant reduction in quantum yield of CO₂ uptake. This was accompanied by an increase in quantum yield of chlorophyll fluorescence. Similar antiparallel changes in the rate of CO₂ uptake and in fluorescence occurred when the leaf temperature was lowered from 30 °C to 5 °C. Chilling at high photon flux density for 5 h caused a marked reduction in quantum yields of both fluorescence and CO₂ uptake in subsequent measurements at room temperature. However, chilling in the dark for 20 h caused no change in subsequent quantum yield measurements of either chlorophyll fluorescence or CO₂ uptake. Possible changes in the photosynthetic process which may be involved in the observed changes in quantum yield are discussed.

Key words: Chilling - Chlorophyll fluorescence - Photosynthesis -
Lycopersicon esculentum

Introduction

Chlorophyll fluorescence is a powerful tool in the investigation of the photosynthetic apparatus (Lavorel and Etienne 1977). Recently, portable solid state instruments for the measurement of chlorophyll fluorescence directly from the leaf surface have been developed (Schreiber et al. 1975; Melcarek and Cernohlavek 1977). Techniques in which the in vivo changes in the photosynthetic process can be followed in a nondestructive manner offer great potential for studying the effect of environmental stresses on plants and for breeding for resistance to such stresses. The range of possible applications of this new approach has recently been demonstrated (Smillie and Hetherington 1983).

Several workers have described the effect of chilling on chlorophyll fluorescence (Smillie and Hetherington 1983; Melcarek and Brown 1977a, 1977b; Baker et al. 1983; Murata 1975). In these investigations the chlorophyll fluorescence induction curve following a dark period was studied. The characteristic kinetics of chlorophyll fluorescence during the first two or three minutes of illumination (the Kautsky effect; for a review see Papageorgiou 1975) are complex and are affected by many external factors such as temperature (Murata 1975), CO₂ concentration (Slovacek and Bannister 1973) and dark adaptation (Walker 1981). Correlating changes in the kinetics of chlorophyll fluorescence with changes in the photosynthetic process has proved to be difficult (Schreiber and Berry 1977; Walker 1981; see also Lavorel and Etienne 1977). As a result, the significance of changes in the chlorophyll fluorescence kinetics at room temperature of leaves exposed to chilling are not fully understood. Reduction in the rate of

fluorescence during chilling at 1°C in the dark was interpreted in one case in terms of damage to the oxidizing side of photosystem II (PSII)(Smillie 1979). However, in a study employing conventional gas exchange technique no change in quantum yield was found at room temperature after chilling at 2 °C in the dark for 16 h (Martin and Ort 1982); the investigators interpreted these results as an indication of undamaged PSII.

When an individual leaf of a chilling-sensitive plant is exposed to a combination of low temperature and high photon flux density (PFD) there is a marked decrease in its quantum yield of CO₂ uptake, presumably as a result of photoinhibition (Poweles et al. 1983; Baker et al. 1983). Similar observations were recorded at nonchilling temperature in leaves subjected to excessively high PFD (Poweles and Bjorkman 1982); these authors also recorded a reduction in chlorophyll fluorescence yield at low temperature (77 K) as a result of photoinhibition. These findings were interpreted as indications of damage to the reaction center complex of PSII.

When the electron transfer of photosynthesis is interrupted by 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU), a similar reduction occurs in quantum yield of CO₂ uptake (Martin and Ort 1982). In contrast to the reported effects of either chilling or photoinhibition, DCMU treatment causes a dramatic increase in both rate and yield of chlorophyll fluorescence at room temperature (Walker 1981). This increase in emission might be explained by an increase in the relative concentration of Q (the primary electron acceptor of PSII) in its reduced form, i.e. the form in which Q does not quench chlorophyll fluorescence (Bradbury and Baker 1981).

A knowledge of the relationship between changes in chlorophyll

fluorescence and changes in the photosynthetic process as measured by conventional means such as gas exchange may establish the validity of simple and nondestructive technique such as in vivo chlorophyll fluorescence measurement. The aim of the present work is to study changes in the quantum yield of chlorophyll fluorescence, rather than changes in maximum yield and kinetics, in response to chilling. Such approach, if found valid, would make it simpler to determine the nature of damage responsible for the observed changes.

Materials and methods

Plant material : Plants of Lycopersicon esculentum Mill.(cv. M82-1-8) were transplanted three weeks after sowing into one liter pots containing a mixture of peat,vermiculite and tuff(1:1:2). The pots were placed in a greenhouse where the average temperatures were 24/18°C(day/night). Plants were used for experiments 3 weeks after transplanting. All measurements were conducted on the first fully expanded leaf (leaf 5-6).

Gas exchange measurements : CO₂ uptake (net photosynthesis) and water vapor exchange were determined in an open gas exchange system. Air mixture was prepared with three mixing pumps (Wösthoff) connected in series so that 350 µl l⁻¹ CO₂ was maintained in the leaf chamber. The temperature of the sample was regulated by enveloping the leaf chamber in a water jacket and was recorded using copper/constantan thermocouples connected to an electronic thermometer (Comark, 1064a). Relative humidity was kept at 80+/-5%(unless otherwise stated) by bubbling the air prior to its entering the chamber through a temperature regulated water flask and a flask containing glass beads. The concentration of CO₂ was measured using an infrared gas analyzer (Maihak, UNOR2.). Transpiration was determined with a dew point hygrometer (E G&G). Light was provided by a 400 W H P L R bulb (Phillips) and was regulated by layers of miracloth. Light was passed through a 5 cm water filter before reaching the leaf chamber. The PFD in the 400 to 700 nm range was measured with a quantum photometer (Li Cor,185B).

Chilling treatments : Chilling in the dark was carried out with whole plants. After determination of CO₂ uptake and fluorescence at room

temperature plants were placed in an incubator enclosed in a plastic chamber to prevent dehydration. The temperature was kept at $2 \pm 0.5^{\circ}\text{C}$ for 20 h. The pot, still in the plastic chamber, was then transferred to a dark room at 25°C for one hour. During the following two hours CO_2 uptake and fluorescence were again determined.

Chilling at high PFD was carried out in the gas exchange leaf chamber. An attached leaflet was sealed in the chamber and its CO_2 uptake determined at 25°C . The temperature of the leaf was then lowered to 5°C over a period of 20 min at a PFD of $300 \mu\text{mol m}^{-2} \text{sec}^{-1}$, and the leaflet was maintained at this temperature and at light intensity of $2000 \mu\text{mol m}^{-2} \text{sec}^{-1}$ for 5 h. Following this treatment the temperature was raised back to 25°C over a 20 min time period at a PFD of $300 \mu\text{mol m}^{-2} \text{sec}^{-1}$. These conditions were maintained for one hour to allow equilibration, after which CO_2 uptake was determined. The CO_2 concentration in the chamber was kept at $350 \mu\text{l l}^{-1}$ and the relative humidity at $85 \pm 5\%$ throughout the experiment. The treated leaflet and the opposite untreated one were removed for fluorescence measurement.

DCMU treatments : Whole attached leaves were immersed in an aqueous solution of DCMU ($8.5 \times 10^{-5} \text{ M DCMU}$) containing surfactant for 5 min in the light. The plant was then left overnight for 10 h in the dark at 25°C before CO_2 uptake and fluorescence were determined.

Fluorescence measurements : Fluorescence was measured using a portable plant productivity fluorometer model SF-10 (Richard Banker Research Ltd., Ottawa). Four freshly cut leaf disks, each 10 mm in diameter, were taken from a leaflet and placed between two disks of wet filter paper under a 2x30 mm stainless washer. The samples were then placed in 30-mm

diameter fitted cells on two properly insulated aluminum plates with a temperature-regulated water stream between them. Upon illumination after an appropriate dark period, this arrangement enables the measurement of variable fluorescence of up to 80 samples in about 15 min at any uniformly maintained temperature above -5°C . Good reproducibility was achieved, as the fluorometer probe and the cells had the same diameter so that successive measurements could be taken from the same point on the leaf surface. No difference was found between fluorescence measurements taken from the surface of a whole attached leaf and measurements of freshly cut disks prepared as described above. Two opposite leaflets were always used, with the untreated one serving as a control. The leaflets were removed at the same time, except when whole plants were chilled in the dark, in which case the control sample was cut and measured before the beginning of the treatment. The fluorescence induction curve was recorded by means of a standard strip chart recorder.

Results and Discussion

Upon illumination of dark-adapted leaves, the typical induction curve of chlorophyll fluorescence was obtained (Fig. 1a; cf. Papageorgiou 1975). Lowering the temperature of the sample from 25 °C to 5 °C resulted in a marked increase in fluorescence yield, although the kinetics of the induction curve remained essentially the same (Fig. 1b). Pretreatment of the leaf with DCMU, however, resulted in both a large increase in yield and a change in the kinetics of the induction response (Fig. 1c). When the leaf was chilled while being exposed to high PFD ($2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 5 h at 5 °C) a marked reduction in yield and a change in kinetics were observed (Fig. 1d). Chilling in the dark did not result in a significant change in the kinetics, but the maximum fluorescence yield reached (F_p) was lower than in the pretreated sample (not shown).

In subsequent experiments only F_p was recorded, and was used as a measure of the rate of fluorescence emanating from the antenna chlorophyll of PSII. This was then used to follow changes in the apparent quantum yield of the fluorescence obtained by plotting fluorescence rate versus rate of the incident PFD. It is important to note that although F_p is a transient stage in the induction of photosynthesis it responded to changes in temperature or in light intensity in the same fashion as F_T (the steady state fluorescence). For practical reasons such as speed and maintaining accuracy with simple instrumentation, which are requisite for the application of this method, the F_p was chosen as the parameter which to follow. The results obtained in this way showed that treatment of leaves with DCMU causes an increase in the quantum yield of chlorophyll fluorescence. This corresponded to the expected decrease in the quantum yield of CO_2

uptake measured in the same leaves (Fig 2). The antiparallel trend was also evident when relative changes in fluorescence and CO_2 uptake in response to temperature lowering are recorded simultaneously (fig 3).

The observed trend is not unexpected since photosynthetic CO_2 fixation and fluorescence are competing processes. The fact that changes in net CO_2 uptake are larger than changes in fluorescence can be explained by an increase in the dissipation of de-excitation energy through other channels, such as heat. However, it should be noted that in the results presented in fig 3, CO_2 uptake was determined at saturating light conditions while fluorescence was determined at limiting light even at 5°C . It is also noteworthy that throughout the above experiment conducted in the open gas exchange system, there was practically no change in intercellular CO_2 concentration; increased stomatal resistance could thus be excluded as a cause of reduced CO_2 uptake at the low temperatures.

Exposure of tomato leaves to a combination of high PFD and low temperature resulted in a pronounced reduction in quantum yield of CO_2 uptake (Fig 4B). This is in agreement with previous reports in which either C_3 (Poweles et al. 1983) or C_4 plants (Baker et al. 1983) were used. In the present study the changes in CO_2 uptake were positively correlated with the changes in apparent quantum yield of chlorophyll fluorescence (Fig 4A). Unlike the increase in apparent quantum yield of chlorophyll fluorescence measured in DCMU-treated leaves, here the fluorescence quantum yield was substantially decreased. Poweles and Bjorkman (1982) outlined a hypothesis which may be applicable to such changes in quantum yields of CO_2 uptake and chlorophyll fluorescence. These authors suggest that a modification of

the PSII reaction center complex, leading to an inactivation of PSII photochemistry and an increased energy dissipation via nonradiative decay processes are likely to occur under conditions manifesting photoinhibition.

It has more specifically been suggested (Smillie 1979) that the water splitting mechanism is selectively damaged. As a result there is an increase in the relative concentration of Q, the primary electron acceptor of PSII in its oxidized form in which it is a strong quencher of fluorescence (Bradbury and Baker 1981); on the other hand DCMU treatment would have caused an increase in the relative concentration of Q in its reduced (unquenching) form, thus increasing chlorophyll fluorescence (Fig 1C). It may also be possible that the changes in PSII complex involve an increase in recombination between the primary donor and acceptor of the reaction center (Z^+Q^- ; see also Lavorel and Etienne 1977). In any case, it is important to note that fluorescence measurements enable us to distinguish between two types or rather sites of damage to the photosynthetic apparatus: the first is caused by DCMU, and the second by chilling at high PFD. These two effects cannot be distinguished by the CO_2 uptake determinations.

Chilling ($2^\circ C$, 20 h) in the dark at room temperature was found to have no effect on the quantum yields of fluorescence and CO_2 uptake (Fig 5). These results are in agreement with those obtained by Martin and Ort (1982), who argue that an unchanged quantum yield of CO_2 uptake indicates that the PSII cannot be the primary site of damage by chilling in the dark. Their contention is further supported by the present results obtained from fluorescence measurements (Fig. 5A): if the PSII was the primary site of damage, it might be expected that any damage to the PSII complex would be reflected in the quantum yield

of the fluorescence originating mainly from this site, but no such change in quantum yield was recorded.

Conclusion

The measurement of changes in chlorophyll fluorescence induced by chilling stress at room temperature is a simple and nondestructive method which can conveniently be used on large plant populations in breeding and in studying the effect of chilling stress on plants. It is possible for these purposes to establish a close and meaningful correlation between the plant response to stress and the data obtained by this technique if changes in the apparent quantum yield are followed. The determination of quantum yield of CO_2 uptake by means of gas exchange studies has been known for many years as a powerful tool in assessing damage to the photosynthetic apparatus. In this study a close correlation was found to exist between these measurements and those obtained by the relatively new fluorescence technique. Using a portable fluorometer like the one used here it is possible to obtain an estimation of quantum yield of at least 50 samples in an hour.

The results presented in this paper confirm previous results with tomato and other species of higher plants (Martin and Ort 1982; Poweles et al. 1983; Baker et al. 1983) which indicate that the primary site of damage by chilling in the dark is unlikely to be in the PSII complex. However chilling at high PFD induces more severe damage, which is presumably the result of photoinhibition and is likely to induce a modification in the PSII complex.

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Figure legends

Fig. 1. Typical chlorophyll fluorescence induction curve of dark-adapted (30 min) tomato leaves: A. At room temperature (25 °C). B. The same sample after a period of 30 min in the dark, during which time the temperature of the sample was lowered to 10 °C. C. Leaf disk sample taken from attached leaf treated with DCMU (8.5×10^{-5} M) for 5 min and left overnight in the dark. D. Leaf disk sample taken from attached leaf treated at 5 °C and PFD of $2000 \mu\text{mol m}^{-2} \text{sec}^{-1}$ for 5 h. Open arrow indicate the onset of a 3 min period of illumination with red light (670 nm, $12 \mu\text{E m}^{-2} \text{sec}^{-1}$). Fluorescence (>710 nm) was recorded on a strip chart recorder. The fast transient F_I was resolved by recording separately the first 10 sec of the induction curve at high chart speed.

Fig. 2. Effect of DCMU (8.5×10^{-5} M) on the quantum yield of: A. Chlorophyll fluorescence (>710nm) in tomato leaf disks (induced by illumination for 10 sec with red light(670 nm) following a dark period of 10 min). B. CO_2 uptake in attached tomato leaves (determined in an open gas exchange system with $350 \mu\text{l l}^{-1} \text{CO}_2$ in air, 85% relative humidity). Each point represents the mean of 4 replicates. The largest standard error of the mean is indicated.

Fig. 3. Relative change in chlorophyll fluorescence and CO_2 uptake in tomato leaves. The temperature of the sample was lowered by 5 °C intervals from 30 to 5 °C. Fluorescence(>710nm, induced by 670 nm red light with a PFD of $12 \mu\text{mol m}^{-2} \text{sec}^{-1}$) was recorded from leaf disks, placed on a temperature regulated plate, after a dark period

of 30 min of which 20 min were required in order to reach the desired temperature. CO_2 uptake was determined in an attached leaf enclosed in an open gas exchange system ($350 \mu\text{l l}^{-1} \text{CO}_2$ in air, 85% relative humidity, $\text{PFD } 1500 \mu\text{mol m}^{-2} \text{sec}^{-1}$). Average CO_2 uptake at 30°C was $18 \mu\text{mol CO}_2 \text{m}^{-2} \text{sec}^{-1}$.Each point represent the mean of 4 and 5 replicates for fluorescence and CO_2 uptake measurements respectively.

Fig. 4. Effect of chilling (5°C) at high PFD ($2000 \mu\text{mol m}^{-2} \text{sec}^{-1}$) for 5 h at a relative humidity of 90% on the quantum yields of: A. Chlorophyll fluorescence($> 710\text{nm}$) in tomato leaf disks (induced by illumination for 10 sec with red light (670nm) following a dark period of 10 min). B. CO_2 uptake in attached tomato leaves (determined in an open gas exchange system with $350 \mu\text{l l}^{-1} \text{CO}_2$ in air, 85% relative humidity). Each point represents the mean of 4 replicates. The largest standard error of the mean is indicated.

Fig. 5. Effect of chilling in the dark (2°C , 20 h) on the quantum yield of: A. Chlorophyll fluorescence($> 710\text{nm}$) in tomato leaf disks (induced by illumination for 10 sec with red light(670 nm) following a dark period of 10 min). B. CO_2 uptake in attached tomato leaves (determined in an open gas exchange system with $350 \mu\text{l l}^{-1} \text{CO}_2$ in air, 85% relative humidity). Each point represent the mean of 4 replicates. The largest standard error of the mean is indicated.

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5