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CORRELATION OF SUBJECTIVE AND QUANTIATIVE TECHNIQUES TO MEASURE CHILLING INJURY OF SELECTED LYCOPERSICON SPECIES AND SOLANUM LYCOPERSICOIDES

presented by

Terry L. Kamps

has been accepted towards fulfillment
of the requirements for

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Major professor

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CORRELATION OF SUBJECTIVE AND QUANTITATIVE TECHNIQUES TO MEASURE CHILLING INJURY OF SELECTED *LYCOPERSICON* SPECIES AND *SOLANUM LYCOPERSICOIDES*.

By

Terry L. Kamps

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ABSTRACT

CORRELATION OF SUBJECTIVE AND QUANTITATIVE TECHNIQUES TO MEASURE CHILLING INJURY OF SELECTED LYCOPERSICON SPECIES AND SOLANUM LYCOPERSICOIDES.

By

Terry L. Kamps

Intact plants of Solanum lycopersicoides and Lycopersicon species ecotypes between the four and eleven leaf developmental stage were subjected to 20°C or a chilling stress of 2.5°C for 72 hours. Chilling injury was assayed sequentially on each plant by visually rating damage of specified leaflets (VRL), chlorophyll fluorescence (CF), electrolyte leakage (EL), and visually rating entire plants (VRP). Correlation estimates of genotypic effects were significant between CF and VRL, CF and VRP, VRL and VRP, and VRP and EL. Correlation estimates of the interaction of temperature by genotypic effects were highly significant between CF and VRL, CF and VRP, and VRL and VRP. A relationship between chilling tolerance and collection altitude of wild species ecotypes was apparent. The chilling resistant intergeneric hybrid of Lycopersicon esculentum Mill. cv. Sub-Arctic Maxi x Solanum lycopersicoides suggested dominant nuclear gene control. Temperature by genotype effects corresponded with the terminal or either near proximal leaflet position.

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Introduction

Temperature is a major environmental stress factor limiting crop distribution and production (Sutcliffe, 1977; Thompson, 1970). Genetic manipulation and cultural technology have permitted expanded production of many crops to more diverse environments. However, marginally adapted crops remain subject to reductions in vigor and yield as a result of environmental conditions experienced during the growing season. The length of a growing season may be defined by the temperature requirements of the crop. For example, low temperatures in late spring and early fall shorten the growing season of crops which may be injured if exposed to these temperatures.

The deleterious effects of low, nonfreezing temperatures on several plant species were reported by Bierkander in 1778 (Levitt, 1980). Researchers have continued to investigate plant responses to nonfreezing temperatures, but it was not until 1897 that Molisch (Lyons, 1973) suggested that the injury caused by low temperatures (above $0^{o}C$) be referred to as "chilling injury" (Erkältung); thereby, differentiating it from freezing (below $0^{o}C$) injury (Erfrieren). Currently, chilling injury is considered a physiological dysfunction resulting from a low temperature exposure (Lyons, 1973).

Chilling sensitive species are categorized as those which sustain physiological injury when exposed to temperatures within the 0 - $12^{o}C$ range (Levitt, 1980; Lyons, 1973). Sensitivity and symptom expression vary with a number of factors including 1) center of origin of the species, 2) genetic differences, 3) physiological age, 4) tissue or

cell type, 5) environmental conditions prior to and during exposure to chilling temperatures, and 6) the interaction of time and temperature (Lyons, 1973). Direct injury may result from an irreversible, qualitative physical change; whereas, indirect injury may be due to slower quantitative changes in metabolism. Chilling sensitive crops, including tomato, primarily originated from tropical and sub-tropical lowlands.

The cultivated tomato (Lycopersicon esculentum Mill.) and related species are native to the western slopes of the Andes, a region extending from northern Chile through western Bolivia and Peru to Ecuador (Luckwill, 1943). The tomato has become widely accepted as an edible "vegetable" and has gained worldwide importance as a horticultural crop (Herner and Kamps, 1983). The popularity of the tomato can be attributed to the fruit's attractive color, flavor, and versatility (Rick, 1978). Tomatoes are grown as a fresh market commodity and for many processed products. In the United States both categories rank among the top five vegetable crops grown based on acreage planted, harvested, and economic value (U.S.D.A. Crop Reporting Board, 1984). Sensitivity to chilling injury resulting in shorter growing seasons limits tomato production in the more northern, temperate climates. All stages of development, from seed to harvested fruit, are susceptible to injury induced by low temperatures (Kemp 1968).

Seed Germination and Emergence

The use of transplants in tomato production is a common cultural technique employed to avoid the erratic and reduced germination and emergence of tomato seeds associated with cold soil temperatures below

8 - 10°C. Direct seeding is an economical alternative to the use of transplants for establishment of tomato fields (Oyer and Koehler, 1966; Sullivan and Wilcox, 1971). Sullivan and Wilcox (1971) reported that in addition to reduced costs at planting, direct seeding offered a number of other advantages. Among these are: 1) increased probability of more vigorous and disease-free seedlings, 2) elimination of scheduling problems during critical planting seasons, 3) greater production flexibility, 4) earlier completion of planting operations, 5) increased yield potentials, and 6) the ability to establish high density populations necessary for an economical mechanical harvest. This technology has been widely used in California since 1966 (DeVos et al., 1981). Nearly 100% of the California acreage planted in processing tomatoes is direct seeded (El Sayed and John, 1973; Smith and Millett, 1964; Sullivan and Wilcox, 1971).

In contrast, the short growing season and cold, wet soil conditions in early spring limit the use of direct tomato seeding in the midwestern and eastern United States. Unlike California, direct seeding has not become a standard practice in these areas (DeVos et al., 1981; Sullivan and Wilcox, 1971). The short growing season in these northern areas necessitates early spring seeding to allow sufficient time for the crop to mature (Herner and Kamps, 1983). However, soil temperatures below 10 - 15°C are considered sub-optimal for tomato seed germination and emergence which either fails to take place or becomes increasingly erratic (Bussell and Gray, 1976; DeVos et al., 1981; El Sayed and John, 1973; Jaworski and Valli, 1964; Kotowski, 1926b; Lorenz and Maynard, 1980; Ng and Tigchelaar, 1973; Smith and Millett, 1964; Thompson, 1974).

Soil temperature and water are major factors influencing germination of viable seed (Dubetz et al., 1962); however, these may be compounded by light. Mancinelli et al. (1966;1967), demonstrated phytochrome control of tomato seed germination in the dark. Phytochrome efficacy varied in response to temperature, and at low temperatures (17 - 20^{0}C) sensitivity to P_{FR} influence increased. The probability of seed mortality due to soil saprophytes and pathogens occurs during prolonged periods in cool soils (Harper et al., 1955; Harrington and Kihara, 1960; Pinthus and Rosenblum, 1961; Toole et al., 1951), sometimes resulting in serious reductions in plant populations. These problems arising from direct seeding in cool soils are most important when the field is to be mechanically harvested. Dense populations necessary to obtain a high marketable yield (Bussell and Gray, 1976; DeVos et al., 1981; Oyer and Koehler, 1966), are reduced by the failure of some seeds to germinate and by seedling mortality. Also, cold temperatures delay emergence; thereby, expanding the germination period over several weeks (Cannon et al., 1973; DeVos et al., 1981; Ng and Tigchelaar, 1973). This results in a less uniform stand which matures irregularly. Stand uniformity is necessary to obtain a high concentration of mature fruit for a once-over harvest. Plants developing at variable physiological ages confound the determination of a harvest date when yields will be maximized. Increased probabilities of weed establishment, insect injury, and soil crusting are additional complications associated with a prolonged emergence period (DeVos et al., 1981; Sullivan and Wilcox, 1971).

Cultural techniques and plant breeding provide means of increasing the rate and uniformity of seed germination and seedling emergence in

cold soils. Cultural techniques (Bussell and Gray, 1976) have consisted of seed hardening (Christiansen, 1968; Hegarty, 1970), seed priming (Heydecker et al. 1973; Kotowski, 1926a; Oyer and Koehler, 1966), and pregerminated seed (Sachs, 1977). Variable success has been attained.

Genetic studies have shown sufficient variability in seed germination under cold conditions within L. esculentum and wild Lycopersicon species that may be utilized in breeding programs (Cannon et al. 1973; DeVos et al., 1981; El Sayed and John, 1973; Ng and Tigchelaar, 1973; Patterson and Payne, 1983; Webb, 1973). The number of genes controlling resistance to chilling injury of seeds, or the ability to germinate at sub-optimal temperatures, is not clear. Work by Cannon et al. (1973), implied that low temperature germination of tomato seed was controlled by a recessive gene. Their conclusion conflicts with reports by other researchers who claimed a minimum of 3 - 24 gene pairs determined this characteristic (DeVos, et al., 1981; El Sayed and John, 1973; Ng and Tigchelaar, 1973). According to DeVos et al. (1981) the study conducted by Cannon et al. (1973) was not designed to detect continuous polygenic variation. Furthermore, DeVos et al. (1981) and Ng and Tigchelaar (1973) detected a significant maternal inheritance effect. Abdul-Baki and Stoner (1978) found physiological evidence of a maternal contribution after experimenting with the leachates of seeds that had varying abilities to germinate under cold conditions. Genotypes that performed poorly in the cold contained a germination inhibitor; whereas, those more cold tolerant contained a promoter.

Estimates of nuclear heritability of low temperature seed germination in tomato indicate significant additive gene effects (DeVos

et al., 1981; El Sayed and John, 1973; Ng and Tigchelaar, 1973) Also, dominance and partial dominance were reported by Ng and Tigchelaar, (1973) and DeVos et al., (1981) respectively, to contribute to a proportion of the total nuclear genetic variance. While seed germination and emergence may be genetically improved for tolerance to low temperature conditions, such tolerance is not necessarily correlated to the plant response to chilling temperatures at other developmental stages (Herner and Kamps, 1983; Kemp, 1968; Patterson and Payne, 1983).

Seedlings and Transplants

The widespread use of transplants in the midwest and eastern United States shifts the focus of the effects of chilling temperatures in the spring from the seed to the developing plant. Tomato seedlings may be transplanted to the field well before the danger of frost has passed. Placing caps or row covers over the plants protects them from frost and chilling injury. When future frost probabilities are sufficiently low the protective covers are removed. However, the threat of chilling night temperatures often persists until mid-June. Within this period, tomatoes can shift from the vegetative growth phase to the reproductive stage. Therefore, the effects of chilling temperatures early in the growing season may significantly affect seedling survival, growth, flowering, fruit set, and early fruit development.

Seedlings often respond rapidly to low temperatures (King et al., 1982) and thus provide an *in vivo* system for ascertaining the temperature sensitivity of some physiological processes. The benefit of

response research.

A variety of qualitative and quantitative techniques have been used in attempts to assess chilling injury in seedlings. Techniques range from simple, though sometimes subjective, visual evaluations to quantitative measurements indicative of physiological changes. "Chilling injury evaluated by qualitative visible symptoms is a function of both physiological injury per se, and rate of symptom development in the particular tissue" (Lyons, 1973).

A change of physical structures within the cell may cause the disruption of normal metabolic processes, resulting in physiological injury. Visible changes in ultrastructure of chilled cells of sensitive species have been microscopically detected (Ilker et al., 1979; Moline, 1976; Patterson and Graham, 1979). The more apparent macroscopic symptoms of general concern are loss of turgor, tissue necrosis, external discoloration, reductions in growth and vigor, flowering, and fruit set, abnormal fruit development, surface pitting, (Lyons, 1973) and increased susceptibility to decay organisms (McColloch and Worthington, 1952).

Loss of turgor, an irreversible symptom of chilling injury is caused by water loss and entry of air into the cells (Wright and Simon, 1973). At chilling temperatures turgor loss may be difficult to identify, but becomes more evident once tissue is warmed to non-chilling temperatures (Patterson et al., 1978). Furthermore, subjecting tissue to warmer temperatures following a chilling exposure usually results in rapid development of additional symptoms (Lyons, 1973; Patterson et al., 1978).

Chilling injury of seedlings is determined by low temperature and

interactions with other parameters. The role of water in the development of chilling injury has been examined from several aspects. Studies of the effects of relative humidity at chilling temperatures, and abscisic acid levels, have demonstrated the relationship between water and the expression of chilling injury (Herner and Kamps, 1983; King et al., 1982; Rikin and Richmond, 1976; Rikin et al.,1976; Rikin et al., 1979; Rikin and Richmond, 1979; Rikin et al., 1981; Sasson and Bramlage, 1981; Wright and Simon, 1973). High (100%) humidities or increases in ABA levels inhibit or delay dehydration of cells during a chilling stress. Cell water loss is generally a predecessor to other symptoms of injury such as tissue necrosis. Diurnal responses of tomato seedlings (Herner and Kamps, 1983; King et al., 1982; Patterson et al., 1979) to chilling temperatures illustrates the significance of light as another interacting component of the development of chilling injury.

Subjective evaluation of tissue necrosis is a simple and common method to test interacting environmental and genetic parameters of chilling stress. Genetic applications have been demonstrated by Herner and Kamps (1983) and Patterson et al. (1978) in studies of natural genetic adaptation of selected species in the Solanaceae family. Such a visual test may have limited value, however, due to low sensitivity (Patterson and Payne, 1983), and inherent subjectivity.

Nondestructive and destructive measurements of growth and development are quantitative and perhaps more sensitive assays of the temperature response of plants. Nondestructive measurements, for example, permit repeated observations on the same specimen over time. Collection of fresh and/or dry weight values of specified plant parts

Collection of fresh and/or dry weight values of specified plant parts during the course of an experiment or at its completion are examples of widely used but destructive techniques.

With regard to growth and developmental research the aforementioned technologies, evaluation of tissue damage and the collection of fresh and/or dry weights are inherently limited, even when used in conjunction. Plants of the same chronological age often are not the same physiological age, resulting in a large source of variability that can obscure results (Erickson and Michelini, 1957). Utilization of Erickson's and Michelini's (1957) non-destructive plastochron index (PI), a numerical index of the developmental age of plants, can often minimize this type of variability. In 1880 Askensay proposed the term plastochron to designate the time interval between formation of two successive internode cells, or more broadly defined as the interval between corresponding stages of development of successive leaves (Erickson and Michelini, 1957). The index indirectly relates observations of each experimental unit to time. Therefore, appearance of leaves at successive intervals is one criterion that must be met for the PI to be reliable (Coleman and Greyson, 1976; Lamoreaux et al., 1978). In tomato, flower bud production has been shown to change this time interval, thus limiting application in tomato research to approximately the eleventh leaf stage (Coleman and Greyson, 1976; Stevens et al., 1984; Vallejos et al., 1983).

Growth rate changes can be measured by proper implementation of the aforementioned methods (Coleman and Greyson, 1976; Jaworski and Valli, 1964; Kemp, 1968; Learner and Wittwer, 1953; Martin and Wilcox, 1963; Patterson and Payne, 1983; Rikon and Richmond, 1976; Stevens et

al., 1984; Vallejos et al., 1983; Went, 1944). Detection of these changes or differences can be used to estimate temperature minima, optima, and maxima for growth. The optimum is the temperature range most conducive to rapid growth; an increase or decrease of temperature outside this range reduces the growth rate. In general, the optimal temperature range for tomato is 65 - 75^{0}F (Lorenz and Maynard, 1980). Differences have been reported between tomato varieties and other Lycopersicon species (Kemp, 1968; Learner and Wittwer, 1953; Patterson and Payne, 1983; Vallejos et al., 1983). Species of Lycopersicon which grow naturally at high altitudes are exposed to lower mean temperatures, therefore they would be expected to have evolved a lower temperature optimum. Varieties which undergo a slower rate of change of growth at lower temperatures or have lower temperature optimum should be more tolerant of chilling temperatures than those with a higher optima (Learner and Wittwer, 1953; Patterson et al., 1978; Sutcliffe, 1977).

For the vegetative developmental stage several different approaches may be used to detect tolerance to chilling temperatures. Utilizing PI, Vallejos et al. (1983) demonstrated differential reductions in growth rate, at low temperatures, of selected tomato species. Kemp (1968) detected differences between varieties grown at a sub-optimal temperature (10^{0}C) for two weeks. Went (1944) reported cessation of differentiation of the growing point and stem elongation in a canning variety of tomato subjected to a constant 5^{0}C. Patterson and Payne's (1983) screening technique identifies genotypes which can grow during a light period at 20^{0}C following the 16 hour dark period at 0^{0}C. Temperature optima for growth has been correlated with

Plants more tolerant of low temperatures usually have lower temperature optima (Learner and Wittwer, 1953; Patterson et al., 1978; Sutcliffe, 1977). Went (1957) proposed that the optimal temperatures for stem elongation equaled the optimal temperature for fruit production in tomato, contradicting an earlier report by Learner and Wittwer (1953) which noted varietal interaction.

Flower and Fruit Production

Fruit production is a function of flower formation, successful pollination (fruit set), and fruit development. The effects of low temperatures on these characters has been studied to improve early market tomato production. Went (1957) observed that the tomato inflorescence size and the number of nodes between each inflorescence were related to the night temperature. Warmer night temperatures reduced the size of, and increased the number of leaves between, inflorescences. Experiments initiated after the expansion of the cotyledons of tomato were conducted by Calvert (1957), Lewis (1953), Phatak et al. (1966), and Wittwer and Teubner (1956;1957) to ascertain the temperature sensitive period in flower initiation and development. These researchers obtained results similar to Went (1957). Lewis (1953) suggested three main factors affecting the size of the inflorescence in tomatoes, one of which is environment. Exposure to what is considered a low (14^{0}C) but non-chilling temperature after the expansion of the cotyledons increases the production of flowers in an inflorescence as compared to plants raised at higher temperatures ($25 - 30^{0}$C). A decrease in node number to first inflorescence resulting from a low temperature treatment was also confirmed. Whether this is a result of slower growth is unclear. Clarification is

necessary since the number of nodes below the first inflorescence is considered an index for earliness of flowering in tomato (Phatak, 1964; Phatak and Wittwer, 1965). The temperature sensitive period for changes in node number precedes slightly the temperature sensitive period for changes in floral number. Maintenance of cool temperatures can extend the increased flower effect through the fifth inflorescence in some varieties. Studies by Phatak et al. (1966) and Wittwer and Teubner (1956;1957) included temperatures in the upper range (10^{0}C) of chilling. No mention was made of chilling injury per se except in fruit development (Wittwer and Teubner, 1956). Went (1944) observed that apparently normal flowers were produced at 5^{0}C, but fruit set failed to occur.

Pollen and Fruit Set

Successful pollination is required for non-parthenocarpic fruit set. Poor fruit set on early flowers of tomato can be ascribed to low temperatures adversely affecting the pollination process. Inhibition of pollination by temperature can be attributed to the development of inviable pollen under low temperature conditions, germination failure, or the inability of the pollen tube to successfully grow through stylar tissue to the ovary (Kemp, 1965a). Went (1957) reported that night temperatures below 12.8^{0}C resulted in formation of abnormal and empty pollen grains. Charles and Harris (1972) attributed poor fruit set at low temperatures (10 and 12.8^{0}C) primarily to poor pollen viability and germination. Pollen produced at 10^{0}C was inviable, failing to produce pollen tubes in germination tests. Normal pollen development increased with increasing temperatures. Reductions in

development increased with increasing temperatures. Reductions in percent germination of pollen and rate of pollen tube growth were observed by Smith and Cochran (1935). In vitro germination at 5°C inhibited pollen germination of tomato (Zamir et al., 1981). Some growth regulating substances have been effective in overcoming low temperature restrictions (Mann and Minges, 1949), but plant injury reduces the appeal of such application of these substances to improve early fruit set in field tomatoes. The possibility of genetic improvement would be a preferable alternative.

Differential responses to chilling temperatures of pollen and fruit set of Lycopersicon species and varieties have been observed by Daubeny (1961), Huner and VanHuystee (1982), Kemp (1965a; 1965b), Maisonneuve (1983), Ward (1956), and Zamir et al. (1981; 1982). Maisonneuve (1983) examined pollen developed at 7°C and noted better quality of pollen from accessions of high altitude L. hirsutum. Zamir et al. (1981; 1982) reported a selective advantage of high altitude L. hirsutum pollen for fertilization at low temperatures. Their research concluded that the genes expressed by the haploid pollen grains are responsible for differential fertilization at low temperatures and selection of these gametes could have a corresponding effect on the sporophyte. Studies by Huner and VanHuystee (1982) disagreed, noting there was no vegetative difference in reponse to chilling temperatures of the cultivars tested but was observed for the ability to set fruit. Some varieties of L. esculentum have the ability to set fruit at low night temperatures (Kemp,1965a; 1965b). Kemp (1965b) identified a recessive gene in the variety Earlinorth which permitted fruit set to occur at night temperatures of 40°F. Inheritance was proposed to be

simple with complete dominance. Pollination at low temperatures may result in a reduction in numbers of fertilized seeds within the fruit thereby producing abnormal, "catfaced" fruit (Ward, 1956).

Responses of the Membranes

The physiological responses discussed above are generally considered secondary, or indirect, events of chilling temperatures (Lyons et al., 1979). Comparative physiological studies of chilling-sensitive and -resistant species have provided evidence to suggest cell membranes are the primary temperature sensor (Lyons, 1972; 1973; Lyons and Raison, 1976; Lyons et al. 1979; Raison, 1974). At temperatures critical for chilling injury of intact plants (9-12^{0}C in tomato) "breaks", deviations from expected linearity, occured in Arrhenius plots measuring respiration activity of mitochondria from chilling sensitive tissue (Lyons and Raison, 1976). The "breaks" are a relatively consistant phenomenon of chilling sensitive species and represent an increase in activation energy (Ea) of the membrane bound enzymes (Graham and Patterson, 1982). Corresponding results of electron spin resonance (esr) have been observed; thus, indicating a membrane phase change from a liquid-crystalline form to a less flexible solid gel (Lyons, 1972; Lyons et al., 1979). Chilling resistant species generally maintain a linear temperature dependency to near or below 0^{0}C. The phase change experienced by chilling sensitive species is viewed as the primary mechanism of temperature response followed by physiological dysfunction. Prolonged periods of dysfunction lead to the development of permanent injuries (Lyons et al., 1979).

The various membrane systems within the cell may exhibit differential sensitivity to chilling (Ilker et al., 1979; Thompson,

Jr., 1979). Ilker et al. (1979) studied the sequence of ultrastructural changes in tomato cotyledons during a chilling stress. They concluded that "the ultrastructural chilling symptoms of tomato seedling cotyledons (held at $5^{o}C$ for 2 to 24 hours) manifested themselves primarily as a progression of membrane deteriorations." Damage sustained by the different organelles was dependent upon the period of chilling exposure.

A temperature-induced phase transition from a liquid-crystalline to a coagel form requires a greater order of the lipid molecules composing the membrane (Levitt, 1980). Therefore, a change in the semi-permeable properties characteristic of the membranes could be expected. Levitt (1980) proposed that increased permeability of the plasmalemma measured by solute leakage or ion accumulation in the cell wall and intercellular spaces may result from mechanical and/or metabolic stresses. Solidification of the membranes is often accompanied by contraction and loss of flexibility (Levitt, 1980; Lyons, 1972; 1973). Non-uniform contraction caused by sudden chilling or chilling combined with dehydration subjects the membranes to mechanical stresses likely to produce fractures causing the membrane to become leaky (Levitt, 1980). The enhancement of cellular dehydration on solute leakage and chilling injury was demonstrated by Wright and Simon (1973). Simon (1974) suggested that dehydration of water from the cells is necessary to create a mass flow of electrolytes to the apoplast. The rate of electrolyte leakage from the cell has been shown to increase with increased periods of chilling stress. This may be attributed to the ultimate degeneration (Ilker et al., 1979; Moline, 1976) of the membranes as phosphorylative activity and the energy to

(1973) presented a schematic summarization of the events leading to chilling injury in sensitive plants (Figure 1).

Since the amount of electrolyte leakage is dependent on the duration of the chilling stress (Wright and Simon, 1973) it would be expected that measurement of this parameter would be indicative of the injury incurred. Electrolytes in the cell wall and intercellular spaces will leak from injured tissue submersed in an appropriate liquid medium, commonly water. Subsequent measurement of conductivity of the water provides evidence of the leakiness of the membranes. Increased amounts of leakage have been reported in chilling sensitive tissues as compared to chilling resistant tissues (Nobel, 1974). Differences such as these suggest the possibility of detecting genotypic variation to chilling within species generally considered susceptable (Paull et al. 1979). Van De Dijk et al. (1985) reported differences with respect to electrolyte leakage among 10 genotypes of tomato. Conversly, reports by Miltau et al. (1984) and Stevens et al. (1984) concluded that measurement of electrolyte leakage was not sufficiently reliable as a selection criteria. Conflicting results may be due to differences in treatment methodologies. Consequently, the interaction of treatment parameters and electrolyte leakage should be carefully scrutinized prior to its employment as a selection criteria.

Responses of the Chloroplast

Further evidence of conformational changes in membranes may be acquired by examining changes in the physiological processes directly associated with specific membrane systems. A variety of assays have been utilized to investigate temperature effects on the thylakoid

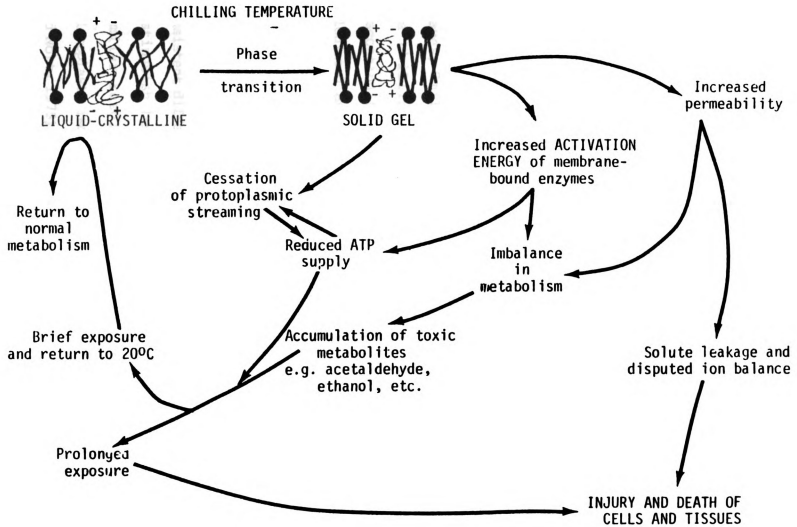

Figure 1. Schematic pathway of the events leading to injury in sensitive plant tissues. Lyons, James M. (1973).

membranes of chloroplasts. The progressive loss of the capacity for oxygen evolution as measured by the membrane-localized Hill reaction activity of chloroplasts is a common feature of chilling sensitive plants (Smillie, 1979). Kaniuga and Michalski (1978) investigated the composition of free fatty acids in relation to Hill reaction activity in isolated chloroplasts of chilling sensitive species. The authors concluded that cold, dark storage of leaves results in damage to the thylakoid structure affecting photosystem II electron transport. After examination of the photoreductive activities of chloroplasts isolated from detached tomato leaves held at $0^{o}C$ for varying periods of time and in vivo measurements of cytochrome-554 photooxidation in chilled leaves, Smillie and Nott (1979) reported that "the site of action of the chilling effect was water donation to photosystem II." Discontinuities observed in Arrhenius plots of osmotic response calculated as reflection coefficients (Nobel, 1974) and photoreduction rates of $NADP^{+}$ (Shneyour et al., 1973) in tomato have been attributed to a phase transition. In contrast, no "breaks" that might indicate membrane lipid phase changes were observed in modified Arrhenius plots of chlorophyll fluorescence (Melcarek and Brown, 1977). However, consistent differences in fluorescence yields enabled the authors to distinguish between chill-sensitive and chill-resistant species. Similar results were observed in photoreductive activity of chloroplasts isolated from chilled, detached leaves of tomato (Smillie and Nott, 1979).

Measurements monitoring chlorophyll fluorescence have been utilized to investigate temperature-induced injuries of green plant tissues (Miltau et al., 1984; Hetherington et al., 1983; Melcarek and

Brown, 1977; Potvin, 1985; Schreiber and Berry, 1977; Smillie, 1979; Stevens et al., 1984; Yakir et al., 1985). Chlorophyll fluorescence yield rises because of the reduction of Q to Q^- (Govindjee and Papageorgiou, 1971). According to Smillie (1979) a factor which inhibits the reducing side of photosystem II, e. g. chilling injury, would be expected to result in a decrease in the rise of variable chlorophyll fluorescence. In a review by Govindjee and Papageorgiou (1971), variable chlorophyll fluorescence is defined as the difference in fluorescence yield between P (a high peak) and O (initial level, origin) of the fluorescence transient. Measured over time, a rapid decline in variable chlorophyll fluorescence has been reported in chill-sensitive species as compared to chill-resistant species (Melcarek and Brown, 1977; Smillie, 1979). The rate of decline of variable chlorophyll fluorescence has been utilized to distinguish differences in sensitivities to chilling temperatures of closely related species and cultivars (Miltau et al., 1984; Potvin, 1985; Smillie, 1979; Stevens et al., 1984). Smillie (1979) suggested that the ability to monitor the progession of damage to the chloroplast membrane by changes in chlorophyll fluorescence provides new approaches to the study of the mechanisms and parameters of chilling injury.

Selection Criteria for Resistance to Chilling Injury

Effective exploitation of genetic diversity by plant breeders requires techniques which can accurately identify desired traits within plant populations. The information obtained from physiological studies benefits the plant breeder in providing new approaches and technologies to develop more accurate and precise screening procedures. The cause(s)

are not yet well understood (Lyons, 1979). Therefore, selection criteria determining the sensitivity/resistance to chilling injury must be carefully assesed. Visual evaluations of damage to plant tissue following exposure to low temperatures have been a common, qualitative method to detect chilling resistance. Subjectivity, low precision, and the necessity to incur deleterious damage are among the disadvantages of the utilization of a visual rating system. The ability to make early associations of a number of physiological events at the cellular level with the occurance of chilling injury may serve to circumvent some of these disadvantages. Several of these primarily quantitative systems have been discussed above.

The degree of association (i.e. correlation) of measurable metabolic events leading to chilling injury and the visible injury that develops after prolonged exposure to chilling temperatures should be determined prior to utilization in a plant breeding program. Furthermore, the researcher should be assured that components of the methodologies do not significantly influence the measured parameter to distort or change the results. For example, injury incurred during a chilling treatment utilizing detached leaves or leaf tissue as the experimental unit could result from, or be confounded with, expected changes in leaf water status or normal senescence. Several studies measuring the change in the rise of the chlorophyll fluorescence induction after specified chilling periods have utilized detached leaves as the experimental unit during the chilling treatment, apparently assuming detached leaves would produce parallel responses to an intact leaf (Hetherington et al., 1983; Miltau et al., 1984; Stevens et al., 1984). However, studies have shown that leaf detachment can

obscure results and cause incorrect conclusions. Potvin (1985) examined the effects of chilling intact vs. detached leaves as measured by chlorophyll fluorescence. Potvin (1985) concluded that detachment of leaves caused a more rapid rate of decline in yield of chlorophyll fluorescence, and detachment also significantly altered the ranking of two of the species in regard to their chilling tolerance. Studies by Kanuiga et al. (1978) on Hill reaction activity as affected by chilling temperatures found that photosystem II was damaged more effectively by darkness and the detachment of leaves from the plant than the cold treatments. The present study on chill-stressed intact tomato plants was conducted to evaluate the correlations between four screening procedures; 1) chlorophyll fluorescence, 2) a visual rating assigned to positioned leaflet samples, 3) electrolyte leakage, and 4) a visual rating assigned to the intact plant. The ability of a selection criterion to rank nine Solanaceous genotypes in regard to chill-tolerance was also examined.

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CORRELATION OF SUBJECTIVE AND QUANTITATIVE TECHNIQUES TO MEASURE CHILLING INJURY OF SELECTED *LYCOPERSICON* SPECIES AND *SOLANUM LYCOPERSICOIDES*.

Correlation of subjective and quantitative techniques to measure chilling injury of selected *Lycopersicon* species and *Solanum lycopersicoides*.[1]

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Abstract. Intact plants of *Solanum lycopersicoides* and eight *Lycopersicon* species ecotypes between the four and eleven leaf developmental stage were subjected to a non-stress condition of 20°C or a chilling stress of 2.5°C for 72 hours. Subsequently chilling injury was assayed sequentially on each plant by a visual rating of damage on specified leaflets (VRL), chlorophyll fluorescence (CF), electrolyte

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leakage (EL), and a visual rating of plants (VRP). Estimates of correlations between genotypic effects for pairs of traits were significant for 1) CF and VRL, 2) CF and VRP, 3) VRL and VRP, and 4) VRP and EL. Correlation estimates of the interaction of temperature by genotypic effects were highly significant between 1) CF and VRL, 2) CF and VRP, and 3) VRL and VRP. A relationship between chilling tolerance and altitude at which wild species ecoptypes were collected was apparent. Chilling resistance found in an intergeneric hybrid of sensitive Lycopersicon esculentum Mill. cv. "Sub-Arctic Maxi" x resistant Solanum lycopersicoides suggested dominant nuclear gene control. Temperature by genotype effects corresponded with leaflet position.

Introduction

Chilling injury results from a physiological dysfunction when sensitive species, primarily those of lowland tropical and sub-tropical origin, are exposed to low, non-freezing temperatures within the 0 - 12^{0}C range (10, 12). The cultivated tomato (Lycopersicon esculentum Mill.) is subject to reductions in vigor, yield, and length of growing season due to sensitivity to chilling temperatures (2, 3, 35, 37). In the norhtern climates of the midwestern United States transplants are predominately used for the establishment of tomato fields. The vegetative tomato plant is the first stage vulnerable to spring chilling temperatures. Through breeding and selection of genetically resistant plants the deleterious effects of chilling temperatures may be significantly reduced.

Selection assays which quantify chilling injury could increase the precision and accuracy in the identification of genotypic responses to

chilling temperatures by elimating the subjectivity associated with rating systems based on visible symptoms. Furthermore, quantitative assays may have the ability to detect chilling injury prior to the development of visible macroscopic symptoms, saving time and preventing the destruction of plant material. The cause(s) of chilling injury are not yet well understood (13), therefore, progress in accurate quantification of injury has been slow.

Physiological investigations of chilling injury have emphasized the deviation of normal physiological processes associated with the functions of the cell membrane resulting from structural and metabolic changes due to temperature (4, 13, 21). Measurements of electrolyte leakage and chlorophyll fluorescence have been examined for their application as quantitative screening criteria in genetic studies of chilling injury. Electrolyte leakage has been measured as either the rate of leakage (11, 36) or as a percent of ions leaked after a specified period of time (15, 16, 24, 26, 31, 34). The influence of temperature on chlorophyll fluorescence has been simply expressed as the change in the rise and slope of variable fluorescence measured over time (14, 15, 20, 27, 29, 31).

The utilization of detached leaves or leaf discs in several studies as the experimental unit during the chilling stress may have distorted or confounded the influence of temperature with events resulting from the detachment, such as, expected changes in water status or normal senescence. In a comparison of the effects of chilling temperatures on intact vs. detached leaves, Potvin (20) reported that the decrease in variable chlorophyll fluorescence was larger when the leaves were detached and, the ranking of two species

with regard to chilling tolerance was significantly altered. Kanuiga et al. (7) reported that photosystem II was damaged more effectively by darkness and the detachment of leaves from the plant than the cold treatment. The results presented by these researchers question the reliability of methodologies which include the use of detached leaves as the experimental unit during a chilling stress.

Prior to their utilization in genetic research, it is desirable to determine the degree of association (i.e. correlation) among quantitative assays and visible injury symptoms that develop after prolonged exposure to chilling temperatures. The present study on chill-stressed intact tomato plants was conducted to evaluate the correlations between four screening procedures; 1) a visual rating assigned to positioned leaflet samples 2) chlorophyll fluorescence, 3) electrolyte leakagem and 4) a visual rating assigned to the intact plant. The ability of the selection criterion to rank nine Solanaceous genotypes in regard to chill-tolerance was also examined.

Materials and Methods

Plant material

The Solanaceous species selected for this study are listed in Table 1. Seeds of #H 2653 and #H 722 were each increased by a single selfing of five plants of each line grown in the greenhouse. Seeds of these lines were germinated on a soil-less planting medium, (65% Michigan peat, 15% perlite, and 20% vermiculite; Michigan Peat Co., Sandusky, MI). Three weeks after sowing, the seedlings were transplanted into the same medium in 10.2 cm clay pots. Each of the remaining genotypes was propagated from a single seed-derived stock

Table 1. Collection sites and sources for selected Solanaceous species.

Genotype	Collection site	Source
Lycopersicon pimpinellifolium (Jusl.) Mill. (P.I. 126430)	Peru	North Central Regional Plant Intro. Station, Iowa State Univ., Ames
Lycopersicon hirsutum f. glabradum Humb. & Bonpl. (LA 1624)	<100 meters, Jipijapa, Manabi, Ecuador	Tomato Genetics Stock Center University of California, Davis
Lycopersicon hirsutum Humb. & Bonpl. (LA 1775)	1000-1500 meters, Rio Casma, 71 km from Panama, Ancash, Peru	Tomato Genetics Stock Center University of California, Davis
Lycopersicon hirsutum Humb. & Bonpl. (LA 1363)	3200 meters, Alta tortaleza, Ancash, Peru	Tomato Genetics Stock Center University of California, Davis
Lycopersicon esculentum Mill. (Heinz #H 2653)	---	H. J. Heinz Company
Lycopersicon esculentum Mill. (Heinz #H 722)	---	H. J. Heinz Company
Lycopersicon esculentum Mill. cv Sub-Artic Maxi (MSU #L 104)	---	H. J. Heinz Company
Solanum lycopersicoides (LA 1990)	CPI 255549, Palca, Taena, Peru	Tomato Genetics Stock Center University of California, Davis
Lycopersicon esculentum Mill. cv Sub-Artic Maxi (MSU #L 104) x Solanum lycopersicoides (LA 1990)	---	Michigan State University East Lansing, MI 48824

plant. Asexual propagation was accomplished by terminal shoot cuttings, insuring genetic uniformity within each species ecotype. Cuttings were rooted in perlite under an intermittent mist system and after three weeks rooted cuttings were transplanted to 10.2 cm clay pots as described above. Potted plants were grown in a glasshouse maintained at 17 ± 2^{0}C minimum night temperature, fluctuating day temperatures and natural photoperiodic conditions present at East Lansing, Michigan from January through March 1985. Standard insect control practices were practiced and the plants were fertilized daily with a solution of 110.9, 92.1, and 61.5 mg/l of N, P, and K respectively through a drip-tube irrigation system.

Six plants of each genotype were selected for controlled chilling temperature treatments based on similarity in developmental age. The multiple meristematic growth habit of P. I. 126430 and LA 1363 produced plants with an average of 9-11 expanded leaves compared to 4-8 leaves typical of the other genotypes. Senescencing leaves and visible flower clusters were removed within 15 hours of the controlled temperature experiment. The youngest 70% (approximately) expanded leaf to be evaluated from each plant was identified and tagged prior to the chilling treatment.

Chilling Temperature Treatment

At the end of a daily natural dark period, the test plants were watered to saturation and subsequently transferred from the greenhouse to two Percival controlled environment chambers (CEC). Each CEC was set to maintain a constant temperature of 20 ± 2^{0}C (control) or 2.5 ± 1.0^{0}C (chilling), providing 10 μE m-2s-1 light intensity (Westinghouse Econ-O-Watt F40cw/rs/ewII) on a 10 hour photoperiod and relative

humidities of 68 - 83% (2.5^{o}C) and 80 - 100% (20^{o}C). Three plants of each genotype were held in each chamber for a 72 hour period and subsequently returned to the greenhouse. Both temperature treatments were replicated four times.

Evaluation Procedures

Five leaflets of the selected youngest leaf were sampled in a terminal to proximal pattern (Figure 1), therefore, leaflets reflected a within leaf position effect. Subsequently, each leaflet was subjected to one qualitative and two quantitative assays to evaluate chilling injury.

1. Visual Rating of Leaflets (VRL)

Immediately following transfer of the plants from the CECs to the greenhouse the leaflets were visually rated on a 1 - 9 scale:

1 - no injury

3 - slight injury, some wilting and dehydration of the leaflet margins - 30% of the leaf affected

5 - moderate injury, 50% of the leaflet wilted and dehydrated

7 - severe injury, 70% of the leaflet wilted and dehydrated

9 - entire leaflet affected, probable death

2. Chlorophyll Fluorescence (CF)

Visually rated leaflets were detached from the leaf petiole, briefly washed once in deionized distilled water and placed on moist filter paper in covered plastic petri dishes. The petri dishes were then placed in the dark for a minimum of 30 minutes at room temperature. Fluorescence was measured using the Branker model SF-10

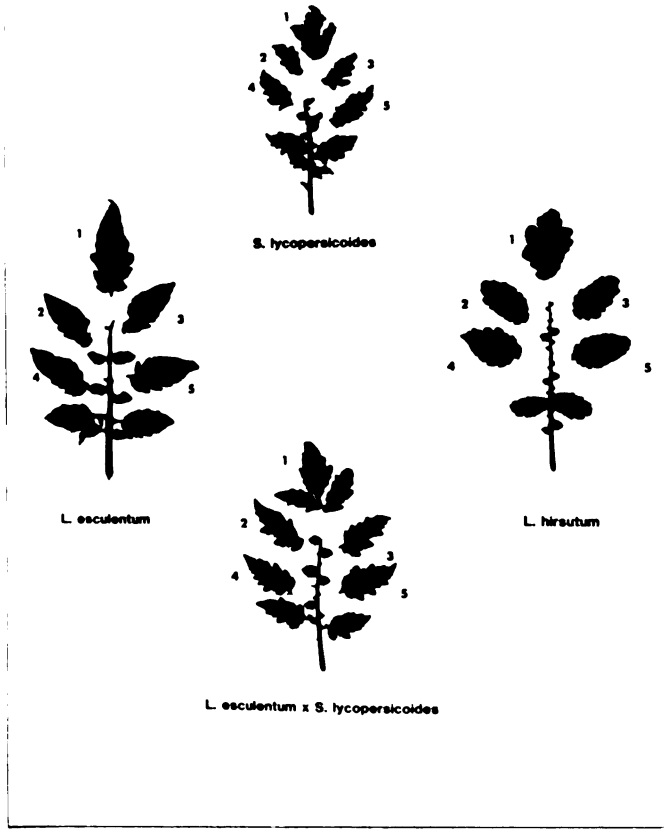


Figure 1. Reprensentative sampling patterns of leaflets of selected Solanaceous species. Sampling pattern of P. I. 126430 was the same as L. esculentum.

plant productivity fluorometer described by Ahrens et al. (1). A 1 mm hole was cut in black cardboard fitted to the sensing probe partially occluding the opening to accommodate narrow and deeply dissected leaves. Acclimated leaflets were surface-dried with Kimwipes and placed with their abaxial sides up on a black piece of cardboard. The above described restriction form was placed on top of the leaflet, taking care to avoid the midrib. Fluorescence signals were displayed on a Nicollet Explorer III oscilliscope and recorded on magnetic disks (Verbatim MD 525-01-18158). Variable fluorescence was calculated by the formula:

$$\frac{f_m - f_o}{f_o}$$

Where:

f_o = the initial level O (origin) in the fluorescence transient of Chl a yield

f_m = the high peak in the fluorescence transient of Chl a fluorescence yield

3. Electrolyte Leakage (EL)

Following the fluorescence measurement, leaflets were prepared to measure electrolyte leakage. Leaflets were immersed in 10 ml deionized distilled water in 18 X 150 mm glass test-tubes which were stoppered with foam plugs. Electrolytes were allowed to leak for 24 hours at room temperature. Samples were placed on a Vortex Geni-mixer for a few seconds and subsequently conductivity was measured at room temperature with the YSI (Yellow Springs Instrument Co., Inc.) Model 32 Conductance Meter. Samples were subsequently autoclaved for 20 minutes, placed

on gyratory shakers and allowed to leak for another 24 hours to obtain total electrolytes leaked. Conductivity is expressed as a percent of the total.

4. Plant Visual Rating (VRP)

Four days after the plants were returned to the greenhouse, they were given a visual rating using the scale described by Herner and Kamps (5).

Statistical Methods

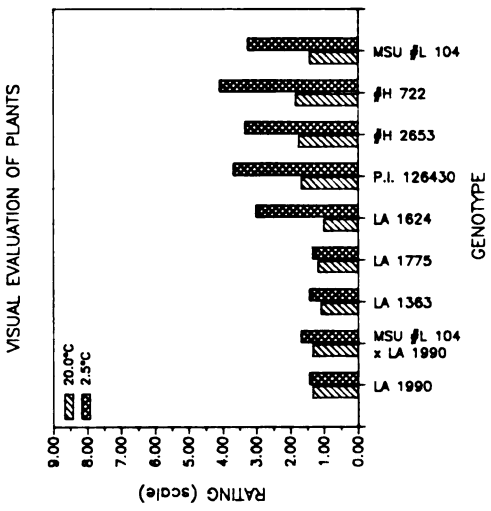
An analysis of variance was calculated for each of the assays. Correlations between the assays were estimated for the treatment effects of 1) genotype, and 2) the temperature by genotype interaction. Coefficients of variation were calculated for each assay to compare precision.

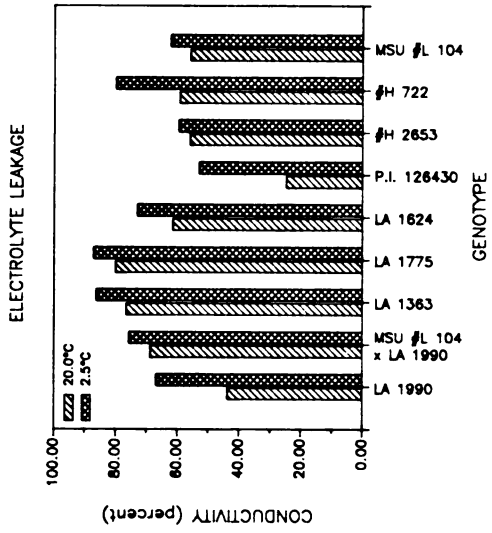
Results

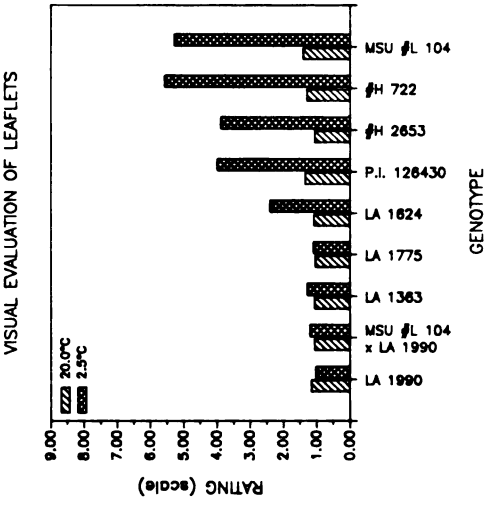
Linear correlation of chilling injury assays - genotypic effects

Plants exposed to 2.5^{0}C compared to those exposed to 20^{0}C resulted in increased values for visual ratings and electrolyte leakage and reduced values of variable chlorophyll fluorescence (Figure 2). Estimated correlations of genotypic effects (Table 2) were significant between the pairs of assays of: 1) chlorophyll fluorescence (CF) and visual rating of leaflets (VRL), 2) CF and visual rating of entire plants (VRP), 3) VRL and VRP, and 4) VRP and electrolyte leakage (EL). EL did not significantly correlate with either CF or VRL.

Figure 2. Genotypic response to two temperatures as measured by each of four assays; VRL, CF, EL, and VRP.


VISUAL EVALUATION OF PLANTS
RATING (scale)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
20.0°C
2.5°C
MSU #L 104
#H 722
#H 2653
P.I. 126430
LA 1624
LA 1775
LA 1363
MSU #L 104 x LA 1990
LA 1990
GENOTYPE


ELECTROLYTE LEAKAGE
CONDUCTIVITY (percent)
100.00
80.00
60.00
40.00
20.00
0.00
20.0°C
2.5°C
MSU #L 104
#H 722
#H 2653
P.I. 126430
LA 1624
LA 1775
LA 1363
MSU #L 104 x LA 1990
LA 1990
GENOTYPE


VISUAL EVALUATION OF LEAFLETS
RATING (scale)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
20.0°C
2.5°C
MSU #L 104
#H 722
#H 2653
P.I. 126430
LA 1624
LA 1775
LA 1363
MSU #L 104 x LA 1990
LA 1990
GENOTYPE

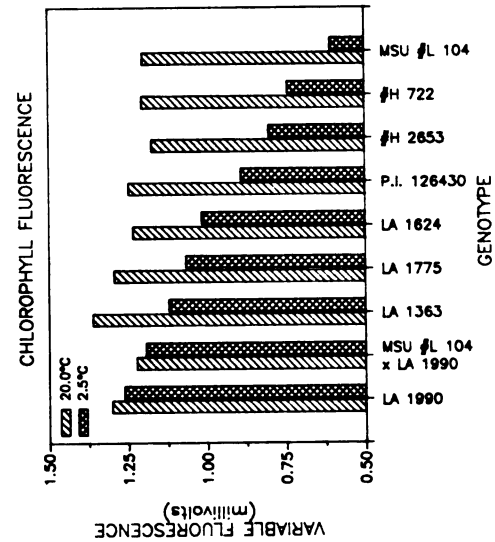

CHLOROPHYLL FLUORESCENCE
VARIABLE FLUORESCENCE (millivolts)
1.50
1.25
1.00
0.75
0.50
20.0°C
2.5°C
MSU #L 104
#H 722
#H 2653
P.I. 126430
LA 1624
LA 1775
LA 1363
MSU #L 104 x LA 1990
LA 1990
GENOTYPE

Table 2. Estimated correlation coefficients (r) of genotype effects between assays evaluating chilling injury: CF - chlorophyll fluorescence; VRL - visual rating of leaflets; VRP - visual rating of plants; EL - electrolyte leakage.

	CF	VRL	VRP	EL
CF	-	-0.926**	-1.000**	NS
VRL		-	0.989**	NS
VRP			-	-0.710*
EL				-

* Significant at the 5% level.
** - Significant at the 1% level.
NS - Nonsignificant.

Linear correlation of chilling injury assays - temperature x genotype effects

Estimates of correlations of the interaction of temperature by genotypic effects were highly significant between 1) CF and VRL, 2) CF and VRP, and 3) VRL and VRP (Table 3). Temperature and genotype interaction effects were not discerned with the EL assay as indicated by a nonsignificant F-test.

Genotypic responses - chlorophyll fluorescence assay

Plants held at the non-chilling temperature of 20 C showed no difference between genotypes with regard to CF activity. Tolerance/sensitivity to the chilling temperature was identified by variable fluorescence reductions following exposure to 2.5^{0}C. Genotypes with detectable differences between the 20 and 2.5^{0}C temperature treatments are chilling-sensitive (Table 4). Significant differences between LA 1990 and MSU L 104 are indicative of chill-resistant and -sensitive genotypes, respectively (Table 4). The response of the intergeneric hybrid MSU L 104 x LA 1990 was resistant, significantly different from MSU L 104, the female parent, but not from LA 1990, the pollen parent.

Effect of leaflet position

Within each leaflet position, genotypic differences were detected between plants treated at 2.5^{0}C, but not at 20^{0}C. Differences associated with the terminal or either of the near proximal positions (Table 5) closely compared with the interaction of temperature by genotype effects at 2.5^{0}C (Table 4). Sensitive genotypes (Table 4) showed significant reductions in CF between corresponding non-stressed

Table 3. Estimated correlation coefficients (r) of the temperature by genotype treatment effects between assays evaluation chilling injury; CF - chlorophyll fluorescence; VRL - visual rating of leaflets; VRP - visual rating of plants.

	CF	VRL	VRP
CF	-	-0.885 **	-1.059 **
VRL		-	1.012 **
VRP			-

** Significant at the 1% level.

Table 4. Effects of temperature and genotype on chlorophyll fluorescence.

Treatment: CEC Temperature (^{o}C)	Genotype means[y]	Variable fluorescence (millivolts)	No. of observations
20^{o} (T_1)	LA 1990	1.30	60
	MSU #L 104 x LA 1990	1.22	60
	LA 1363	1.36	60
	LA 1775	1.29	60
	LA 1624	1.23	60
	P.I. 126430	1.25	60
	Heinz #H 2653	1.17	60
	Heinz #H 722	1.20	60
	MSU #L 104	1.20	60
2.5^{o} (T_2)	LA 1990	1.26 a	60
	MSU #L 104 x LA 1990	1.19 ab	60
	LA 1363	1.12 abc	60
	LA 1775	1.07 abc	60
	LA 1624	1.01 bcd	60
	P.I. 126430	0.89 cde	55
	Heinz #H 2653	0.80 def	60
	Heinz #H 722	0.74 ef	60
	MSU #L 104	0.61 f	60
Standard deviation of the means (s_d) = 0.12			
$T_1 - T_2$			
	LA 1990	NS	
	MSU #L 104 x LA 1990	NS	
	LA 1363	NS	
	LA 1775	NS	
	LA 1624	NS	
	P.I. 126430	*	
	Heinz #H 2653	*	
	Heinz #H 722	**	
	MSU #L 104	**	
Standard deviation of the means (s_d) = 0.14			

[y]Mean separation by LSD at the 5% level
NS - Nonsignificant by LSD at the 5% level
* Significant by LSD at the 5% level
** Significant by LSD at the 1% level

Table 5. Effects of temperature, genotype, and leaflet position on variable fluorescence.

Treatment		Variable fluorescence (millivolts)				
Temperature of CEC (^{o}C)	Genotype	Position 1	Position 2	Position 3	Position 4	Position 5
2.5	LA 1990	1.25 a[y]	1.23 a	1.26 a	1.28 a	1.29 a
	MSU #L 104xLA 1990	1.16 ab	1.19 ab	1.20 ab	1.22 ab	1.21 ab
	LA 1363	1.01 abc	1.07 ab	1.11 abc	1.25 a	1.15 abc
	LA 1775	1.05 abc	1.02 abc	1.04 abc	1.12 abc	1.09 abcd
	LA 1624	0.98 bc	0.97 bcd	1.01 bc	1.06 abc	1.05 abcd
	P.I. 126430	0.81 cd	0.82 cde	0.87 cd	0.98 bc	0.97 bcd
	Heinz #H 2653	0.62 de	0.77 de	0.74 de	0.98 bc	0.90 de
	Heinz #H 722	0.51 e	0.65 e	0.67 de	0.96 c	0.92 cde
	MSU #L 104	0.51 e	0.58 e	0.56 e	0.70 d	0.68 e

Standard deviation of the means (s_d) = 0.12

[y]Mean separation within columns by LSD at the 5% level.

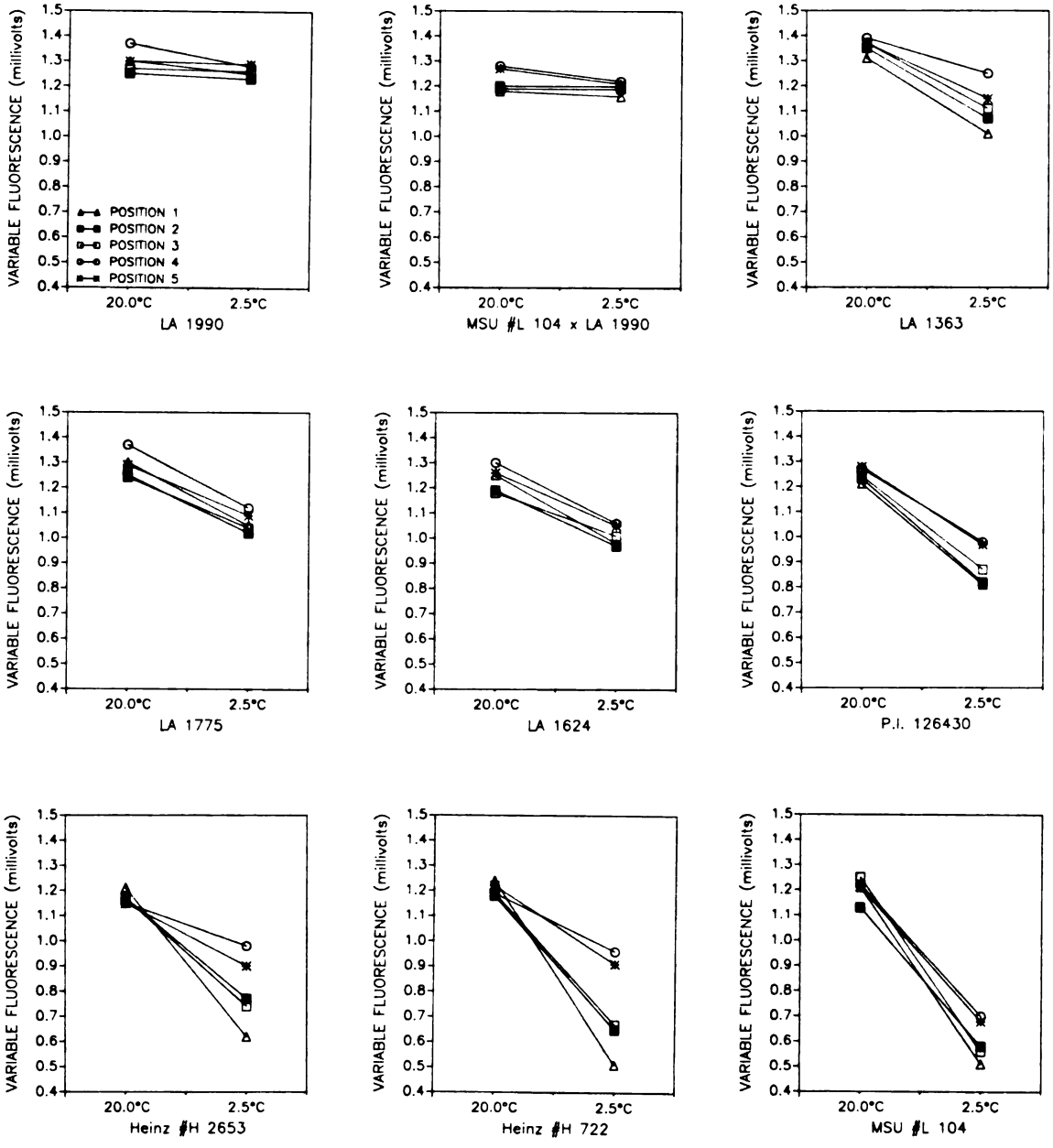
and stressed leaflets (Figure 3).

Discussion

Intact tomato and S. lycopersicoides plants at the vegetative growth stage subjected to a 2.5^{o}C temperature stress for 72 hours developed visible symptoms typical of chilling injury. These symptoms included discoloration of plant tissue, loss of turgor, and necrosis, hereafter referred to as chilling symptoms. Results of the VRL and VRP assays showed that the average amount of chilling symptoms ranged from very slight (<15%) to moderate (55%), depending upon genotype. Since all senescencing and necrotic leaf tissues were removed prior to the chilling exposure, it is reasonable to assume that the injury which occurred afterward reflected actual chilling injury. Nonsignificant r values estimated between the EL assay and either VRL or CF assays for genotype effects indicated that EL was not an accurate method to quantify chilling injury. Furthermore, the nonsignificant F-test for the temperature by genotype interaction suggested that all genotypes responded similarly to the chilling temperature.

Ion leakage has been proposed as a screening criteria for chilling injury, (8, 16, 31, 34) but the studies to date are conflicting on the reliability of this method. Ion leakage has been used to detect the increases in membrane permeability associated with chilling injury and can be measured either as an increase in rate or relative percent of leakage. Patterson et al. (16) measured rates of EL over a 15 day chilling period and reported a relationship between the sensitivity/resistance to chilling temperatures and the natural environments of Passiflora species. Stevens et al. (31) used

Figure 3. Effect of temperature, genotype, and leaflet position on variable fluorescence.


VARIABLE FLUORESCENCE (millivolts)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
POSITION 1
POSITION 2
POSITION 3
POSITION 4
POSITION 5
20.0°C
2.5°C
LA 1990
MSU #L 104 x LA 1990
LA 1363
LA 1775
LA 1624
P.I. 126430
Heinz #H 2653
Heinz #H 722
MSU #L 104

techniques similar to those described by Patterson et al. (16) to measure chilling resistance of Solanaceous species, but concluded the assay was unreliable for identifying chilling tolerance. In the present study EL was represented as a percent of the total electrolytes after leaf tissue was allowed to leak for 24 hours. A comparison between the EL of chilled and non-chilled leaf tissue indicated that this leakage period was too long to determine actual injury. Failure to detect temperature response differences after a 24 hour leakage period and reports by other researchers of significant response differences of plant tissue to chilling temperatures using leakage periods of only 1-6 hours (8, 9, 23, 26, 32, 34, 36) would also imply that the 24 hour period was too long. Visual evaluations of chilling symptoms and CF accurately reflected the response of tomato leaf tissue to the chilling exposure as indicated by significant correlation estimates between the three assays. Although rating visible damage is a simple procedure, subjectiveness in evaluating late manifestations of cellular damage, and low precision may considered inherent limitations. Reliability and accuracy of subjective assays, as opposed to objective assays, largely depends upon the experience and the ability of the researcher to make unbiased observations. Comparisons of coefficients of variation indicated that the subjective assays were the least precise (sensitive) relative to the quantitative assays. CF was the most precise assay in this study and readily detected significant differences between the temperature effects; whereas, this was not possible with either the VRL, VRP or EL assays.

Reductions in variable chlorophyll fluorescence in response to temperature stresses have been shown to be indicative of cell damage

(6, 20, 29). The rates of reduction during the course of a chilling exposure have been related to the degree of chilling sensitivity/tolerance, such that, rapid reduction rates were indicative of chilling sensitive plants. In this study the measurement of chlorophyll fluorescence immediately after completion of the chilling exposure also detected genotypic sensitivities to chilling temperatures. As noted above, the CF assay was significantly correlated with VRL and VRP for genotype effects and effects due to the interaction of temperature by genotype. This indicated that CF is an accurate and reliable means to quantitate the responses of intact plants to a chilling exposure.

Results from the CF assay were used to evaluate genotype response to temperature. Plants subjected to the $20^{0}C$ temperature exhibited no genetic differences with regard to fluorescence activity. These results suggest that chilling-sensitivity and -resistance can be detected by evaluating the responses of plants to low temperature treatments alone. This has apparently been an assumption in earlier studies since non-chilling treatments were not included (15, 20, 31).

Results of the genotypic response to chilling stress showed a clear distinction between the extremes of resistant LA 1990 and sensitive MSU L 104. The intergeneric hybrid produced from the cross MSU L 104 (female parent) x LA 1990 was determined to be chilling-resistant. This suggests that chilling tolerance is primarily controlled by dominant nuclear gene(s), which is in agreement with an earlier report by Robinson and Phillis (25). Differences between the other genotypes were less obvious, but the apparent trend suggested a correlation between the altitude at which the wild genotypes were

collected and the relative ranking of genotype means of the chill-stressed plants. L. hirsutum is naturally distributed over a range of altitudinal elevations from coastal lowlands in Equador to 3,300 m in Peru (33). L. pimpinellifolium is found in coastal Peru (22). Throughout this range the mean temperature decreases approximately $4^{o}C$ for each increase in elevation of 1000 m (28). Genetic adaptation to this natural thermal gradient would be expected, producing ecotypes with increasing tolerance to low temperatures as altitude increased. This study in conjunction with others which correlated temperature responses of such parameters as growth responses (33), pollen germination and fertilization (38), protoplasmic streaming (17), seed germination and chlorophyll development (18), seedling growth and survival (19), electrolyte leakage (16), and photoreductive activity (30) to altitudinal origin, are supportive of this expectation.

Genotypic responses to chilling temperatures were determined by assaying five leaflets, which represented five positions of a leaf, for each plant (see materials and methods). The question arises, does the genotypic response of a particular leaflet to the chilling stress correspond to the low temperature genotypic effects? Our results showed a significant interaction between leaflet position and genotype of plants subjected to the 2.5 but not to the $20^{o}C$ treatment. The differential response of the leaflets of a genotype to the chilling stress were proportional, such that, the overall effect was a change in the scale of measurement, not a change in rank. Genetic differences associated with each leaflet position corresponded with the terminal or either of the near proximal leaflets, which would permit a reduction in the number of leaflets per plant needed to make an accurate

assessment of chilling tolerance. The results of this study show that a single CF measurement per sample after the completion of a chilling stress is an accurate and sensitive assay to quantify chilling injury. Furthermore, the number of leaflets per plant required to assess the genetic response to chilling temperatures may be reduced from five to one, but sampling position of all plants should be consistent.

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