

**PHYSIOLOGICAL BASIS FOR DROUGHT
RESISTANCE IN *Lycopersicon* SPECIES : A STUDY
ON THE VARIOUS DROUGHT AVOIDANCE AND
TOLERANCE MECHANISMS**

MERVAT EDWARD

**DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE**

1989

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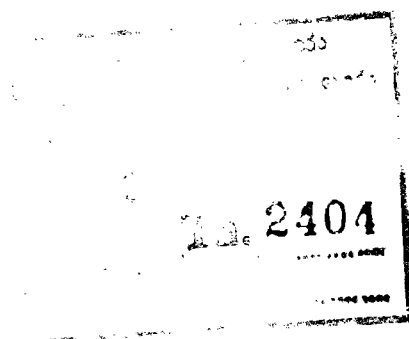


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MERVAT EDWARD

Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
for the award of the Degree of

Doctor of Philosophy

IN

CROP PHYSIOLOGY

BANGALORE

NOVEMBER 1989

*Dedicated to
My Beloved Children
Marian & Mina*

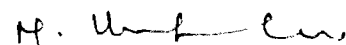
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CERTIFICATE

This is to certify that the thesis entitled
"PHYSIOLOGICAL BASIS FOR DROUGHT RESISTANCE IN
Lycopersicon species : A STUDY ON THE VARIOUS DROUGHT
AVOIDANCE AND TOLERANCE MECHANISMS" submitted in
partial fulfilment of the requirements for the degree
of DOCTOR OF PHILOSOPHY in CROP PHYSIOLOGY to the
University of Agricultural Sciences, Bangalore, India,
is a record of research work carried out by Mrs. MERVAT
EDWARD SORIAL, under my guidance and supervision and
that no part of the thesis has been submitted for the
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Bangalore

November , 1989



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Professor and Head
Major Advisor

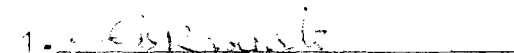
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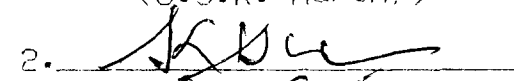
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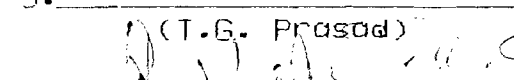
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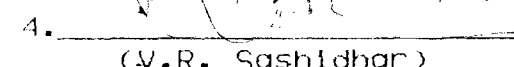
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Mervat

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ABBREVIATIONS

A	-	Assimilation rate
gs	-	Stomatal conductance
CWT	-	Cumulative water transpired
TDM	-	Total dry matter
DAS	-	Days after sowing
E	-	Evaporation
ET	-	Evapotranspiration
FC	-	Field capacity
LAD	-	Leaf area duration
T	-	Transpiration
TQ	-	Transpiration quotient
WUE	-	Water use efficiency
WEE	-	Water extraction efficiency
SLW	-	Specific leaf weight
RWC	-	Relative water content
ψ_w	-	Water potential
ψ_π	-	Solute potential
OA	-	Osmotic adjustment
PEG	-	Polyethylene glycol
SDM	-	Shoot dry matter
rs	-	Stomatal resistance
rm	-	Mesophyll resistance
$\mu \text{ Ei m}^{-2} \text{ s}^{-1}$	-	Micro Einstein square meter second
$\mu \text{ mole m}^{-2} \text{ s}^{-1}$	-	Micromoles, square meter second
$\text{m moles m}^{-2} \text{ s}^{-1}$	-	Millimoles square meter second
$\delta^{13}\text{C}$	-	Data carbon 13
VPD	-	Vapour pressure deficit

INTRODUCTION

I. INTRODUCTION

Droughts are the inevitable and recurring feature of our semi-arid tropics and despite our improved ability to predict their onset, duration and impact, crop scientists are still concerned about it as it remains the single most important factor affecting the yield potentials of crop species. When drought is severe, the yield reduction could be as high as 90 per cent (Swindale and Bidinger, 1981). Although the importance of the problem has been realised, the complexity of the problem has posed a major obstacle and hence there has been no significant breakthrough in developing superior genotypes to stabilise the yield in drought prone areas and under rainfed conditions, where water stress is both unpredictable and intermittent.

The attempts made to develop resistant varieties were unsuccessful because of lack of knowledge, until recently, about plant mechanisms, which favour survival, growth and productivity.

The progress made in the area of drought stress, in recent years has been phenomenal and several aspects of drought stress effect at the cellular, organelle, plant and canopy levels have been understood. The important adaptive strategies,

escape, avoidance and tolerance mechanisms and associated physiological and biochemical parameters have been clearly elucidated (Begg and Turner, 1976, Paleg and Aspinall, 1981; Turner, 1986).

Although the concept of drought resistance is viewed differently by molecular biologists, biochemists, physiologists and agronomists the major concern is to enhance the biomass returns under limited input of water, which is a characteristic feature of rainfed agriculture. Therefore some of the adaptive mechanisms of plants, which do not decrease plant yields to a greater extent, assumes greater importance. They are water harvesting and its utilization efficiency, developmental plasticity and partitioning efficiency.

The importance of root characteristics, their extraction efficiency, water use efficiency (WUE) (Passioura, 1972; Ludlow and Wilson, 1972; Tanner and Sinclair, 1983) leaf characteristics to conserve tissue water by helionastic, stomatal and cuticular characteristics (Jones, 1976; Blake *et al.*, 1984) the higher allocation of carbon to the grain, the morphological traits to minimise heat load (O'Toole and Chang, 1979) as important avoidance mechanisms, and their relevance, have been clearly demonstrated.

However, these avoidance characteristics, which favour maintenance of higher tissue water content under receding moisture stress, only postpone the immediate effect of moisture stress. Therefore, under severe moisture stress conditions, the intrinsic tolerance mechanisms become more relevant. Under rainfed situations, where crop is subjected to cycles of stress, survival at the end of stress and recovery on alleviation is important.

Plants that tolerate moderate stress at low tissue water potential, may do so by virtue of several dehydration tolerance mechanisms like maintenance of membrane integrity (Leopold *et al.*, 1981), osmotic adjustment (Morgan, 1983) and chloroplast integrity (Mohanty and Boyer, 1976).

The empirical screening under field situation is still the major approach to identify drought resistant types. Methods to handle large germplasm lines have been standardised and perfected (ICRISAT, 1985). But, often the important morphological, physiological characters associated with drought resistance are not examined, and analysed.

For a meaningful breeding programme for developing drought resistant genotypes, the major constraint is the lack of information about the genetic sources with different adaptive mechanisms. It is very

relevant in this context to identify diverse genetic sources with different adaptive mechanisms, to assess the stability across the environments and the nature of heritability of such traits.

Although tomato is generally grown under irrigated conditions, its cultivation as a rainfed crop has gained importance particularly in semi-arid regions. Recently, wild species, L. chilense (IBPGR, 1989) has been shown to grow and survive in extremely arid regions of Africa, and Chile. It is therefore imperative to obtain information on the drought resistance mechanisms of such types to enable us to incorporate these traits in a breeding programme for crop improvement.

In view of this, the major approach in this investigation was to identify variability in different drought adaptive mechanisms among *Lycopersicon* species and few *Lycopersicum esculentum* genotypes with diverse genetic background.

The major objectives of the present investigation therefore are;

1. To study the differences in WUE under varying environmental conditions and physiological traits associated with it.

2. To study the variation in two other avoidance mechanisms namely
 - a) Root characteristics
 - b) Cuticular transpiration rates.
3. To assess the relative desiccation tolerance based on empirical approach and examine the relevance of a few dehydration tolerance mechanisms namely osmotic adjustment, protein turnover rates, chloroplast and membrane integrity characteristics.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Amongst the several abiotic stresses affecting the crop growth, moisture stress is the most important one. It affects the growth and development and ultimately the overall productivity of the crop particularly when it is grown under rainfed conditions.

Turner (1986) suggested that the mechanisms of adaptations of water deficits can be divided into three categories.

1. Drought escape: The ability of a plant to complete its life cycle before serious soil and plant water deficits develop (Turner, 1979).

Developmental plasticity or adjustment in phenological development is one such mechanisms to escape severe water deficits. This is achieved by advancement or postponement of flowering. Such an adjustment in flowering has been studied and reported in several crops e.g., in sorghum, Curtis (1968), rice, O'Toole and Chang (1979) wheat, Turner (1966) and sunflower, Takami *et al.* (1981). And plasticity in tillering (Begg and Turner, 1976; Alagarswamy, 1981).

2. Drought resistances at high tissue water potential - Drought avoidance mechanism which maintain high plant water status: The important plant characters associated with this adaptive strategy are;

(a) Maintenance of water uptake

1) Root characters (Passioura, 1981, 1986).

(b) Water utilization efficiency

ii) Characters associated with low transpiration ratio (RT) or transpiration quotient (TQ), Ludlow (1972), Tanner and Sinclair (1983).

(c) Increase in stomatal and cuticular resistances (Jones, 1979; Black *et al.*, 1984).

(d) Reduction in evaporation load (O'Toole and Chang, 1979).

(e) Reduction in evaporation surface (reduction leaf area); Ritchie (1974), Rawson *et al.* (1981).

3. Drought resistance at low tissue water potentials - Drought tolerance mechanisms which help in survival and growth inspite of low tissue water potential: The important plant processes associated with this mechanism are (a) characters at cellular level.

- i) Osmoregulation (Turner and Jones, 1980; Rains et. al., 1980; Borowitzka, 1981).
- ii) Chloroplast integrity (Mohanty and Boyer, 1976).
- iii) Hormonal factors (Milborrow, 1981).
- iv) Membrane integrity (Leopold, et al 1981; Uma Shadnker, 1984).

From a crop productivity perspective it is important to understand which of these mechanisms favour little or no reduction or cause significant decreases in biomass, growth or productivity.

An attempt was made recently to categorize the adaptive mechanisms depending on their relationship with productivity (Udaya Kumar et al., 1988).

Another abiotic stress which often limits crop productivity particularly during the monsoon season is low light stress (Gulmon et al., 1981, 1986). Plants have developed various adaptation to low light stress (Boardman, 1977 and Givnish, 1988). There is no reported work on the low light adaptation of tomato genotypes.

In this chapter, literature pertaining to different aspects of total drought resistance with

special emphasis on tolerance and avoidance mechanisms has been reviewed extensively.

Drought avoidance advantages and disadvantages

The essential feature of plants having avoidance mechanism is the maintenance of a high water status or turgor with decreasing soil moisture (Blum *et al.*, 1977; Jordan *et al.*, 1979).

Avoidance is always considered a more advanced character (Levitt, 1980). Many avoidance parameters for example, increase in water uptake and increase in hydraulic conductance, cause minimum or no decrease in production processes, while a few others which are achieved by decreasing or reducing transpirational (leaf) area or by acceleration of senescence (Turner and Burch, 1982) could cause significant reduction in crop growth rates and productivity.

Water harvesting and utilization efficiency

WUE in general terms, refers to the amount of water used per unit of plant material produced.

Originally Shantz and Piemeisal (1927) termed it water requirement. The term transpiration ratio (RT) or transpiration quotient (TQ) replaced water requirement although both expressions denoted the

amount of water used in transpiration to the dry matter produced during a given growth period.

Monteith (1984) has pointed out, the word "efficiency" (in WUE) is inappropriate in that, a maximum established by theory or observation does not exist for reference. So the best expression of WUE is transpiration quotient (TQ) or transpiration ratio (Stanhill, 1986) which is determined as

$$\text{Transpiration ratio (RT)} = \frac{\text{Amount of water used in transpiration (ml)}}{\text{Amount of dry matter produced (g)}}$$

Fischer and Turner (1978) used the term transpiration efficiency rather than WUE for DM production per unit of transpiration, retaining the term WUE for dry matter production per unit of ET.

$$\text{Transpiration efficiency} = \frac{\text{Dry matter produced (mg) or (g)}}{\text{Water used in transpiration (g) or (kg)}}$$

$$\text{WUE} = \frac{\text{DM produced (mg) or (g)}}{\text{Water lost in ET (g) or (kg)}}$$

and expressed as $\text{g DM kg}^{-1} \text{ H}_2\text{O}$ used in T alone (T efficiency) or ET (WUE).

Further, WUE is defined differently in different contexts.

Agronomical

Agronomists usually define WUE in terms of units of water used per unit of dry matter produced, often using total water lost by both evaporation and transpiration. They compare WUE of crops on the basis of above ground biomass or only the economic yield per unit rainfall received in the growing season (French and Schultz, 1984).

$$WUE = \frac{\text{Grain yield or above ground biomass (kg)}}{\text{Precipitation during growing season (mm)}}$$

Hydrological

WUE has been defined as the ratio of volume of water used in productivity i.e., transpired from the area under study, to the volume of water potentially available for that purpose.

WUE has been defined by Bos and Nugteren (1974) as the increase in the water content of the root zone following irrigation, expressed as a fraction of total quantity of water supplied to the irrigated area.

Physiological

Here, WUE can be defined at the level of single leaf or at the whole plant or crop level.

At the level of the leaf it is the ratio of carbon assimilated to water lost in transpiration and is expressed as $\text{mg CO}_2 \text{ g}^{-1} \text{H}_2\text{O}$ or $\text{m mol CO}_2 \text{ mol}^{-1} \text{H}_2\text{O}$.

Plant carbon and water relations are inevitably linked by the diffusion pathway of CO_2 and water through the stomata. Fischer and Turner (1978) have observed that the differences between the magnitudes of transpiration and CO_2 uptake, which are the two components of instantaneous WUE, are primarily caused by the different atmospheric concentrations of these gases. This would mean that efflux of H_2O vapour would at least 100 fold more than that of carbon dioxide.

At the level of the crop, WUE is the ratio of the total dry matter per unit of water used. However, because of the difficulty in harvesting, WUE is usually calculated based on above ground biomass. Therefore, greater the assimilates, a plant transfers to its roots, the lower will be its WUE (Passioura, 1983). The second complication arises out of the difficulty in delineating evaporation from total evapotranspiration (Turner, 1986). WUE can be looked at from many levels and in literature, there is considerable variation and ambiguity in the units used.

1. At the level of the single leaf

It is the ratio of carbon assimilated to water lost in transpiration at the gas exchange level. In other words it is the balance between the uptake of CO₂ and the loss of H₂O.

At the single leaf level it is expressed as

$$WUE = \text{Millimoles CO}_2 \text{ mol}^{-1} \text{H}_2\text{O}$$

$$\text{Or} = \text{mg CO}_2 \text{ gm}^{-1} \text{H}_2\text{O}.$$

A few important principles are to be considered for the study of WUE at the gas exchange level. Gaastra (1959) studied the diffusion of CO₂ and H₂O through the stomata and the physical laws influencing the rates. The diffusivity of water vapour is 1.56 times that of CO₂ or conversely the diffusivity of CO₂ is 0.64 times that of water vapour. Kramer (1983) suggested that WUE can be defined by the equation

$$WUE = \frac{\Delta C}{\Delta e} \times \frac{D_C (r_a + r_s)}{D_e (r_a + r_s + r_m)}$$

Where, ΔC , Δe are the leaf to air concentration differences for CO₂ and H₂O and D_C and D_e are the diffusivities for CO₂ and H₂O respectively for boundary layer (r_a), stomatal (r_s) and mesophyll resistance (r_m)

This can be expressed as

$$WUE = \frac{0.64 \text{ CO}_2}{H_2O} \times \frac{ra + rs}{ra + rs + rm}$$

Where, Δ is the difference between the internal or external concentrations of CO₂ or H₂O.

2. At the level of the plant or crop

It is the total dry matter per unit of water used in evapotranspiration (ET) under field conditions (Fischer and Turner, 1978). They also reported two types of complications arising out of determinations of WUE based on DM accumulation. One is the water loss through evaporation (field studies) and second biomass of roots is commonly neglected in the determinations of WUE. Although container determinations of transpiration ratio are of interest their practical field application does have some limitations.

WUE in containers or pot studies

Advantages and disadvantages: The environments of pot experiments may not exactly represent that of a field canopy especially with respect to reproducing the canopy microclimate. The balance between the growth of various plant parts may also change due to reduced inter plant competition or reduced soil

volume. However, because of the inherent problems in field genotypic differences in WUE would still be valid in pots because evaporation could be effectively checked to a very great extent.

The first study of transpiration ratio of crop plants was conducted by Lawes (1850). The transpiration of wheat, barley, bean, pea and clover plants was measured by gravimetric method. The containers held 19 kgs of soil whose surface were sealed against evaporation and protected from wetting by rain. These containers were weighed at weekly intervals to arrive at the cumulative water transpired (CWT). Transpiration ratio was found to vary widely. On the basis of both yield and total dry matter basis, the minimum values of transpiration ratio, 148 was for clover and the maximum, 272 was for barley plants when grown at the higher fertilizer level.

Later this technique of container studies for determinations of WUE has also been extended to different C3 and C4 crops by Briggs and Shantz (1914) and Shantz and Piemeisal (1927).

Downes (1969) studied the differences in transpiration rates and WUE between tropical and temperate grasses. They observed that the WUE values for C3 and C4 species were 1.5 mg DM/g H₂O and 3.3 mg DM/g H₂O respectively.

Ludlow and Wilson (1972) studied the effect of several environmental factors, oxygen concentration, light and vapour pressure difference (VPD) on the transpiration ratio (RT) of a C4 grass (Panicum maximum) and a C4 legume (Phaseolus atropurpureus). They observed an increase in the transpiration ratio in both species with decrease in light intensity. However, the RT was always two fold higher in the C3 legume at all light intensities. This study suggests that the high WUE of C4 species was maintained at all light intensities.

Farquhar and Richards (1984) determined the WUE of wheat genotypes on the basis of the dry matter accumulation and water transpired in containers. They observed that the range in WUE among the wheat genotypes was from 4.0 to 6.5 g DM kg⁻¹ H₂O. This is a convenient technique to assess WUE at whole plant level.

Using such techniques, differences in WUE have also been assessed in other crop species and amongst varieties within a species; for example, amongst cowpea varieties; (Nagamani, 1985), soybean varieties, (Arun, 1985), small millets species and varieties of finger millet (Sashidhar, 1987), C3 and C4 species; (Uma, 1987) and (Ravishankar, 1989).

Field studies on WUE by the ET method

There are very few field studies where WUE was determined based on dry matter accumulation. In one such field study Teare *et al.* (1973) compared summer crops of sorghum, a C4 species, and soybean, a C3 species. They found that WUE of sorghum was approximately three times greater than soybean with the maximum value of 7 mg DM g H₂O .

Garrity *et al.* (1982) conducted a field experiment using a modified line source sprinkler system to study the evapotranspiration and yield relationship in grain sorghum. WUE consistently decreased as ET declined below the maximum. It appeared that water stress regardless of timing reduced WUE. The relationship between ET and grain yield and dry matter was linear.

French and Schultz (1984) studied the relationship between yield and water used under mediterranean type environment in wheat. The highest DM and grain yield production were 37 and 12.7 kg/ha respectively for each mm of water used.

Range and genotypic differences in WUE

Fischer and Turner (1978) have reported that at a leaf and air temperature of 25°C and an air relative humidity of 50 per cent the WUE would be not

more than $30 \text{ mg CO}_2 \text{ g}^{-1} \text{ H}_2\text{O}$ for any crop species. Calculations suggest that there is considerable variation in WUE among genotypes and species. Most of the workers found 2 to 3 fold differences in WUE of C3 and C4 crops. Teare *et al.* (1973) reported a three fold greater WUE for sorghum, a C4 crop when compared to soybean, a C3 crop. Similarly, Caldwell *et al.* (1977) reported WUE of $4.3 \text{ mg DM g}^{-1} \text{ H}_2\text{O}$ for a C4 shrub and $2.9 \text{ mg DM g}^{-1} \text{ H}_2\text{O}$ for C3 a shrub community.

Ku and Edward (1978) also found distinct differences in WUE between C3 and C4 species. C4 plants had a higher WUE than C3 plants. Mabrouk *et al.* (1984) showed that WUE of C4 species was higher than those of C3 species. He attributed this to the higher rates of photosynthesis of C4 species rather than a lower transpiration rate. Rawson *et al.* (1977) confirmed the findings of Downes (1970), and concluded that C4 plants have at least a two fold superiority in WUE over C3 plants. The WUE was 364 and 373 $\mu\text{g CO}_2 \text{ mg}^{-1} \text{ H}_2\text{O}$ in sorghum and barnyard grass expressed per millibar vapour pressure differences (VPD) as compared to 127 in the C3 wheat.

In container studies Uma (1987) estimated the transpiration quotient for two C3 species (sunflower and groundnut) and two C4 species (finger millet and amaranthus). TQs for sunflower and groundnut were

found to be 552 and 490 where as it was 292 and 263 for amaranthus and ragi under non-stressed conditions.

Sashidhar (1987) studied WUE differences in six minor millet species and found a TQ range of 201.9 in little millet to 392.4 in kodo millet.

Factors affecting water use efficiency: Influence of environmental factors

The gas exchange determinations of WUE are considerably influenced by environmental factors, the most important factor being atmospheric humidity. WUE is linearly related to the reciprocal of Δe , other things remaining equal WUE decreases as the saturation deficit increases. Rawson and Begg (1977) studied the effect of atmospheric humidity on photosynthesis, transpiration and WUE of leaves of several plant species. WUE decreased in response to VPD change from 5 to 10 millibars in non-stressed plants of barley. In stressed plants, the WUE increased in response to a VPD change from 5 to 15 millibars in whole barley plants.

In a recent study Turner (1984) determined the WUE or transpiration efficiency in five genera; sunflower, cowpea, cotton, almond and pistachio; in response to a change in vapour concentration differences (VCD) from 10 MPa/pa to 25 MPa/pa. They observed that the WUE decreased in all species at

higher VCD. However, differences in WUE at the highest VCD amongst the species was significant, and ranged from 2.3 to 3.3 m mol CO₂/mol H₂O. Sunflower and cotton plants had similar WUE at high VPD (2.3 m mol CO₂/mol H₂O). This was associated with similar degree of decrease in photosynthesis (9 and 4 per cent) and a similar degree of increase in transpiration (196 and 192 per cent) at high VCD, as compared to plants at normal ambient VCD. However, in cotton and pistachio, similar WUE values at high VCD were associated with markedly differing per cent increase in transpiration and decreases in photosynthesis while pistachio plants showed over forty per cent decrease in photosynthesis. They concluded that intrinsic characteristics which maintained higher photosynthesis at high VCD were important.

Schulze et al. (1975) showed that high VPD induced stomatal closure altered the transpiration/photosynthesis (T/P) ratio in apricot (Prunus armeniaca). Low T/P ratios (high WUE) were recorded in the dry season, at a time when the stomata respond strongly to high VPD by increase in resistance (rs). Schulze et al. (1986) demonstrated conclusively that in apricot, stomatal closure was a direct effect of humidity in many cases, and not due to negative feed back as a result of loss of turgor of the leaf.

The major influence of air temperature on WUE operates via its effect on VPD. Khairi and Hall (1976) also observed a decrease in actual WUE in citrus species when the ambient temperature was increased from 22 to 38°C. Schulze *et al.* (1973) made similar observations even when high temperature was associated with water stress and concluded that e or the VPD is closely coupled to air temperature in the field. Rawson (1979) studied the effect of vertical wilting on WUE. When leaves changed from a horizontal to a vertical orientation WUE improved apparently through changes in leaf temperature.

At the single leaf level, gas exchange measurements of WUE appear to indicate that there may be an optimum irradiance for maximum WUE. Downes (1970) investigated the effect of variation in light intensity and leaf temperature on WUE based on gas exchange measurements of photosynthesis and transpiration in wheat and sorghum. In both species WUE decreased as temperature increased at all light intensities. They also observed that WUE in wheat was greater at the moderate light intensity ($0.26 \text{ cal cm}^{-2} \text{ min}^{-1}$) and the highest light intensity but not at the lowest light intensity of $0.06 \text{ cal cm}^{-2} \text{ min}^{-1}$. Thus, there is often an optimum irradiance for maximum water use efficiency (Fischer and Turner, 1978). This

optimum is usually less than the irradiance incident upon a leaf oriented normally to the sun's rays.

Mabrouk *et al.* (1984) studied the influence of light intensities on WUE in cassava using gas exchange techniques at two different leaf to air VPD. At a VPD range of 1.0 to 1.5 KPa the optimum light intensity for maximum WUE was $1100 \mu \text{E l m}^{-2} \text{s}^{-1}$ where as at greater VPD's of 2.0 to 2.5 KPa the optimum light intensity decreased to $600 \mu \text{E l m}^{-2} \text{s}^{-1}$.

Effect of moisture stress on factors affecting the WUE

Amongst the several factors which influence WUE at canopy level, the leaf area, canopy transpiration and assimilation rate are the important plant characters. All these factors are known to be affected by moisture stress. The effect of moisture stress on these three important factors is also reviewed as following:

Garrity *et al.* (1982) reported that canopy evapotranspiration was greatly reduced in the water stressed treatment, but evapotranspiration per unit leaf area was apparently unaffected. Thus the major mechanism for transpiration control under stress is reduction in leaf area rather than increase in stomatal resistance.

Jones (1976) reported that when plants were subjected to a slower field drying, the increase in stomatal resistance was always accompanied by an increase in r_m , which would result in a decrease in WUE under severe stress conditions. They concluded that in such a situation the photosynthetic machinery would also be affected.

Bunce (1981) made extensive measurements of photosynthesis, stomatal and mesophyll conductances of drought tolerant and drought susceptible lines of five genera. They observed that differences in WUE among plants at high leaf water potential were caused by differences in stomatal conductances but differences at low leaf water potentials were caused more by differences in mesophyll conductances amongst the lines. Lines with high mesophyll conductance under severe stress also showed high WUE.

It can be concluded that moisture stress can either increase or decrease WUE depending upon the relative stability of mesophyll factors to moisture stress and accompanying variations in the environmental factors like VPD and temperature. In general the effect of mild stress seems to be more on stomatal factors particularly on r_s , thus enhancing WUE.

WUE and stable carbon isotope composition

To assess the intrinsic WUE by indirect techniques, carbon isotope discrimination is one such method.

Plants with C3, C4 and CAM photosynthetic pathway show characteristically different discrimination against ^{13}C during photosynthesis. C4 plants show lesser carbon isotope discrimination than C3 plants. Troughton *et al.* (1974) found mean $\delta^{13}\text{C}$ value for C4 plants as -13.5 (parts per milli) and for C3 plants as -28.1 (parts per milli). This difference in isotope composition has become one of the standard methods to distinguish C4 plants from C3 plants. O'leary (1981) extensively reviewed the components affecting overall isotope discrimination during photosynthesis.

In recent studies carbon isotope discrimination has been shown to be related to WUE. Farquhar and Richards (1984) reported a three fold range in WUE amongst wheat cultivars under different moisture regimes. WUE increased in all the cultivars in response to a decline in water potential. In the severe stress regime, the range in the WUE amongst cultivars was from 4.0 to 6.5 mmol CO₂ per mol H₂O. They showed a linear relationship between the carbon isotope composition as reflected by $\delta^{13}\text{C}$ values, and

WUE. The increase in WUE under stress was associated with greater decrease in stomatal conductance than assimilation and with positive shifts in $\delta^{13}\text{C}$ value. They observed that with an increase in WUE under stress, $\delta^{13}\text{C}$ values became less negative.

Masle and Farquhar (1988) studied the relationship between WUE and carbon isotope discrimination grown under stress in wheat seedlings. They noticed that WUE of wheat seedlings increased with decreasing soil ψ and that WUE was negatively correlated with carbon isotope discrimination. Decrease in intercellular partial pressure of CO_2 accounted for increase in WUE under stress.

Hubick *et al.* (1986) studied the correlation between WUE and $\delta^{13}\text{C}$ isotope discrimination in diverse peanut genotypes. Variation was found in WUE and also isotope discrimination in *Arachis* species and amongst *A. hypogaea* cultivars. Variation in WUE and discrimination were strongly correlated.

Martin (1988) studied the correlation between WUE and carbon isotope discrimination in *Lycopersicon* species. He found the correlation between $\delta^{13}\text{C}$ and WUE at single leaf level and whole plant level was significant.

Turner (1986) suggested that this technique has offered a new tool to explore the extent of genetic variation for WUE in dry land crops.

It can be concluded from all these studies that carbon isotope analysis may be a useful tool in selection for improved WUE in breeding programme for C4 species particularly under limited water availability.

Significance of WUE

Importance of high WUE genotypes lies in their better performance under stress conditions. But higher WUE is not always associated with high dry matter production. For example, several studies have shown that CAM plants have high WUE, but the dry matter production of CAM plants is not high.

However, in field crops at a given ET differences in biomass are attributed to variation in WUE. At a given water input, species showing high WUE also produced high biomass (Udaya Kumar, 1988).

Arun (1985) also observed that genotypes with high WUE selected from pot culture experiments showed a lesser reduction in economic yield when subjected to stress under field conditions.

The agronomic significance of the transpiration ratio, lies in the opportunity it offers for selecting the correct cropping strategy for the water limited regions (Stanhill, 1986). This can be achieved by choosing the crops and seasons which will minimise evapotranspiration ratio.

Approaches to improve WUE

There are two approaches to improve WUE as (a) by reducing the plant or canopy transpiration (b) by increasing the photosynthetic rate per unit leaf area.

The first approach is based on increasing the stomatal resistance and thereby decreasing transpiration without a concomitant decrease in photosynthesis (Gastrop, 1959; Kramer, 1983). Any increase in stomatal, cuticular, or boundary layer resistances will reduce transpiration more than carbon exchange and thereby increase WUE.

Miskin et al. (1972) identified barley lines with low and high stomatal frequency. They observed that the low stomatal frequency lines had lower transpiration rates but maintained photosynthetic rates similar to that of the high frequency lines. The WUE of low stomatal frequency lines was therefore significantly higher than the high frequency lines.

Another approach to increase WUE is by increasing the ambient CO₂ concentration in which the plants are growing. This approach however has little practical utility under field conditions.

Jones et al. (1985) have shown that the WUE of soybean canopies was significantly higher in a CO₂ enriched atmosphere. In this study, a two fold higher photosynthetic rate of the canopy was observed at high CO₂ level associated with a significant reduction in canopy transpiration. This decrease in canopy water loss was a result of partial stomatal closure.

Kramer (1983) reviewing the approaches to increase WUE at the field levels, reported that increase in WUE without reductions in growth can be achieved more by adjustment of cultural methods and improving the limiting factors of plant development, than by increasing the photosynthetic potential. He suggested that manipulations of either stomatal behaviour or photosynthetic rate per unit leaf area may have limited potential for breeding purposes.

Blake et al (1984) observed lower transpiration rates associated with higher stomatal resistances, smaller stomata, earlier partial opening in the morning and lower stomatal frequency on the adaxial leaf surface, in high water use efficiency clones of poplar.

The rather minor effect of leaf size on RT that holds for most crop plants operates through its effect on the leaf's boundary layer. Leaf structure is of greater significance and RT can be expected to decrease as the ratio of the leaf's internal assimilating surfaces to its external transpiration surface increases (Nobel, 1980). He determined the ratio of mesophyll surface area to the leaf surface area and reported that there was a two-fold difference in this ratio between an extremely xerophytic and mesophytic species. He also observed that this ratio changed in cotton leaves under moisture stress.

Johnson et al. (1983) observed similar differences in isogenic lines differing in wax deposition in leaves of sorghum. WUE was 43 per cent higher in the glaucous lines than in the non-glaucous selections under field conditions.

Leaf movements which orient the leaf parallel to the sun's rays, leaf rolling and flagging, erect leaves and needle like leaves, all common features of dry situations, especially once leaf water potential begins to fall, are considered adaptations to increase WUE (Fischer and Turner, 1978).

Shackel and Hall (1979) observed that diurnal changes in leaf orientation and leaf rolling under

moisture stress can also improve WUE by reducing the effective incident irradiance.

Water harvesting : Influence of root characteristics

Root characters: the second mechanism which could result in lesser decrease in productivity or growth under rainfed conditions is higher water extraction. Better root system, through the development of roots under drought stress is of particular significance to plant, since, extensive and efficient root system is an important drought avoidance mechanism.

Dewitt (1958), Fischer and Turner (1978) and Hanks (1981) reported that, the greater rooting depth and density are constitutive genotypic variation in non-stressed plants. A facultative expression of these traits in response to stress, will result in more uptake of available soil moisture during a rain less period. As a result of this greater capacity of uptake, the crop will be able to maintain relatively high shoot water potential and continue transpiration at near potential rates over a longer period, thus maintaining a high actual to potential evapotranspiration ratio.

Teare et al. (1973) studied the WUE of sorghum and soybean in relation to crop canopy area, stomatal

regulation and root distribution. Higher WUE in sorghum was associated with higher canopy stomatal resistance, higher net assimilation rates and a two fold higher root dry matter per unit soil volume than that found in soybean.

Root characteristics influencing water uptake under stress

Several studies have shown that other root characters apart from a greater root/shoot ratio, can also influence better water harvesting, particularly from the deeper soil profiles where water is still available, although the upper layer would have dried out (Passioura, 1986).

Greater number of thick roots is one of these characters reported in some varieties of upland rice (Chang and Vergara, 1975) and better root osmotic adjustment.

Klepper *et al.* (1973) reported a shift in root density of cotton plants during soil moisture depletion. In a subsequent study Taylor and Klepper (1974) showed that this shift occurred below -1 bar. They concluded that at the start of soil drying, root density was greatest near the soil surface but after drying had progressed, root density was greater in the deeper region of the root profile.

Meguro (1961) emphasised the importance of deep root to shoot ratio. The ability of a variety to absorb water from the deep soil layer is one important characteristic among rice genotypes determining a variety's resistance to drought.

Sharp and Davies (1979) reported an absolute increase in the amount of root formed under mild stress possibly because of a more effective osmotic adjustment in roots than in shoots. It seems likely that this absolute increase can occur when mild water stress inhibits shoot growth more than it inhibits photosynthesis, thus making a surplus of carbohydrates available for root growth.

Martin and Thorstenson (1988) studied three tomatoes, Lycopersicum esculentum Mill. cv. a drought tolerant cultivar and a wild related species. They found that, the season-long water use efficiency was greatest in the wild species and poorest in the domestic plants. Lycopersicum pennelli possessed a great degree of drought tolerance. The drought tolerance was not the result of a deeper penetrating root system but due to other physiological or morphological/anatomical characteristics.

Root/shoot ratios under moisture stress

Several studies have shown the importance of this character as a drought avoidance mechanism. For example Passioura (1981) reported an increase in root/shoot ratios in wheat genotypes, this was confirmed in a later studies by Masle and Passioura (1981). They concluded from this experiment that the increase in root to shoot ratio is primarily due to a greater reduction in shoot relative to root.

However, many instances an absolute increase in root have been reported in case of apricot (Evenari *et al.*, 1977) and in bermudagrass (Bennt and Doss, 1960), and in a few horticultural species (Brouwer 1981).

Many studies have reported an effect of water stress on the root/shoot ratio. For example in Arachis hypogaea genotypes, the mean ratio was 0.23 for the unstressed plants but it was 0.32 for the stressed plants. The stress also increased the root/shoot ratio of both Arachis villosa and A. glabrata (Brouwer, 1981).

Several studies have shown that drought resistant upland rice varieties have a higher deep root to shoot ratio. In a study conducted at IRRI (1982) it was reported that, there was a good

relationship between a greater deep root/shoot ratio and drought resistance of upland rice varieties.

In another study on sorghum genotypes (Alagarswamy and Bidinger, 1975) it was reported that, in three sorghum varieties tested, the shortest variety, Mino Milo Br. 54, had the deepest and most proliferated root system.

Reduction in leaf size/area and other leaf characteristics under stress and their relevance as adaptive mechanism

Leaf expansion was shown to be highly sensitive to water stress (Rawson and Turner, 1982 and Ludlow and Ng, 1976). Leaf expansion have been shown to cease even at water potential of -4 bars (Hsiao, 1974) in maize.

The mechanisms whereby plants avoid the effects of drought stress relates to control of transpirational water loss from all parts of plant. This is accomplished by changes in the surface area of transpiring plant parts. The most obvious morphological change with the onset of drought is a reduction of leaf area, either through a reduction in leaf size or by the shedding or death of leaves.

Turner (1979) reported that the water stress effect in crop species is by adjustment of transpiring

leaf area through accelerated senescence of lower leaves.

Turner et al. (1978) studied the effect of water stress on leaf size of sunflower. They found reduction in leaf size by about two-thirds under moisture stress condition.

Constable and Hearn (1978) found that the expansion was reduced when leaf water potential decreases below -1.0 to -1.2 MPa, whereas leaf senescence and shedding occur only when the minimum leaf water potential falls below -2.0 MPa. They also showed that senescence and shedding reduced the leaf area index and reduced evapotranspiration from an average of 10 mm/day to 3.5 mm/day. Senescence and shedding reduced the leaf area index and reduced evapotranspiration from an average of 10 mm/day to 3.5 mm/day.

Husain and Aspinall (1970) reported that, water stress also affects leaf area through its effect on the initiation of new leaves. For example in barley plants subjected to stress, initiation of new leaves ceased, while existing leaves continued to expand.

Karamanos in 1978 concluded that drought stress was found to control effectively the total active leaf area in field beans as it does in many other plant

species. There is a high positive linear relationship between the final leaf area achieved and the average plant water potential.

Leaf shape, orientation, surface texture and colour also influence the water status of plants. Surface hairiness or colour affect the albedo of the leaf and can reduce radiant heat load and transpiration, leaf rolling has been observed in drought resistant durum wheat cultivars (Hurd, 1976).

Shackel and Hall (1979) observed that diurnal changes in leaf orientation and leaf rolling under moisture stress can also improve drought resistance by reducing the effective incident irradiance, these characteristics were observed to be particularly important under semi arid conditions. Begg and Torssell (1974) reported that, paraheliotropy refers to active orientation of leaves to decrease radiation intercepted when leaf water content decreases. In legumes, leaflets orient horizontally to the incident radiation in the absence of water deficits but parallel to the incident beam in the presence of water deficits.

Ehleringer *et al.* (1976) suggested that, leaf or stem pubescence (hairiness) is often cited as a feature of plants adopted to arid environments. Pubescence can reduce the radiant heat load of leaves by increasing the reflection of the leaf surface.

However, they also showed that pubescence could reduce the absorption of photosynthetically active radiation.

The increased pubescence and waxiness observed under stress in some species and cultivars increased leaf reflectance and reduces the water loss (Ehleringer, 1980).

In wheat Quarrie and Jones (1977) reported increase in pubescence as a result of water stress. Similar responses was observed in sunflower leaves (Turner and Begg, 1981).

Blum (1975), reported that the water deficits increased the amount of epicuticular wax in sorghum.

Stomatal sensitivity as a drought resistance mechanism

The ability of stomata to regulate water loss provides an important mechanism for reducing water loss during drought.

Turner (1974) showed that, under field conditions, stomatal resistance increased markedly at values of leaf water potential ranging from -0.8 MPa in field beans to below -2.7 MPa in cotton.

Similarly Blum (1974) reported that, there is a varietal difference in stomatal response to water stress in sorghum and wheat.

Frank et al. (1973) observed that, as leaf water potential falls below some critical level, stomata begin to close. In wheat, closure occurred at leaf water potentials of -13 to -15 bars in young plants and -18 to -20 bars in headed plants.

Field studies at ICRISAT (Shiva Kumar et al., 1979) comprising sorghum grown under irrigation or on post-rainy season residual soil moisture also indicated that, plants in irrigated plots had higher stomatal conductance at higher leaf water potential than plants grown on limited soil water. Water use efficiency was decreased by continuous exposure to water stress.

Jones (1979) suggested that for crop grown under intermittent moisture stress conditions higher canopy conductance is not desirable for maintenance of high leaf water potentials. Therefore, screening for low maximum conductance could be one way of maintaining higher tissue water potential under stress.

Pearson (1980) observed that tobacco plants reduced canopy conductances during drought by reducing both leaf area index and stomatal conductances.

Prasad et al. (1985) studied the regulation of water loss under moisture stress in sunflower

genotypes. They determined the transpiration rate, diffusive resistances, canopy conductances, and water potential at three canopy positions in non-stressed and stressed plants of five cultivars and hybrids. They observed that cultivars differed in the extent of change in conductance (gs) and transpiration rate at different canopy positions. They showed that in the hybrid BSH-1, conductance was low at all levels in the canopy associated with higher (less negative) water potential. This hybrid had lower transpiration rates at all canopy levels under stress although the number of stomata was high.

Drought tolerance mechanisms

Drought tolerance refers to the ability of plant tissues to withstand water stress. The degree of tolerance varies widely among plant species and among growth stages within genotypes. Tolerance ranges from the ability to maintain photosynthesis and plant growth at low cell water potential to the ability to survive periods of prolonged desiccation.

Extensive studies have been made on the drought tolerance nature of many crop species. Particularly attention has been paid to assess the differences among genotypes within species as part of the effort to increase yield under dry conditions.

Maize is less drought tolerant than sorghum (Beadle *et al.*, 1973). Sorghum was able to photosynthesize at 25 per cent of its maximum rate at low leaf water potential (-11.5 bar), while maize had ceased photosynthesis at this stress level. Differences in the abilities of sorghum genotypes to survive low leaf water potentials have also been demonstrated in several other studies (Sullivan and Eastin, 1974).

Osmotic adjustment an adaptive tolerance mechanism

As water is removed from a cell with the development of water deficits, the solutes inside the plasmalemma are concentrated and the osmotic potential is lowered. The degree of concentration depends on tissue elasticity. For a given change in water potential, the greater elasticity, greater is the concentration of solutes. In some species, water stress does not only concentrate the solutes but also induces solute accumulation a process known as osmotic adjustment (Turner and Jones, 1980)

Significance of OA

Osmotic adjustment is generally regarded as an important adaptation to drought or salinity. Because it helps to maintain turgor and cell volume, it is often thought to promote growth, yield or survival of

plants in dry or saline soils. Osmotic adjustment itself cannot promote growth, the solutes which account for it must be diverted from essential processes such as protein and cell wall synthesis.

The role of osmotic adjustment is to maintain a higher turgor of leaves as water deficits increase, thus leading to survival down to lower water potential and for longer periods during drought. Moreover, the increased tolerance of dehydration allows a large number of leaves to survive the stress and to contribute to the productivity of the plant when stress is relieved.

Definition and changing perspectives

The term osmotic adjustment originated in the salinity literature to describe change in the osmotic pressure of leaves in parallel with that of the soil (e.g., Bernstein, 1961). An increase in the osmotic pressure of leaves or roots mainly due to salts, in response to the increase in the salt concentration in the soil was considered as osmotic adjustment. The term was later used for both drought and salt affected plants.

Osmotic adjustment is now usually defined as an increase in solute potential of cell sap due to the accumulation of solutes which helps to maintain turgor at low water potential.

Such accumulation of solutes however does not delineate the extent to which the solutes accumulate by virtue of the passive concentration due to decrease in cell water content upon dehydration, and that due to the active synthesis of solutes during stress.

Few workers such as Wilson *et al.* (1979) and Morgan (1983) have attempted to separate these two components and have suggested the calculation of an additional parameter namely osmotic adjustment.

Osmoregulation in plants subjected to stress has been noticed in several studies.

Itoho (1986) confirmed that osmoregulation occurred in young intact mungbean seedlings exposed to high osmotic stress. Despite a decrease in the leaf water potential due to slow water depletion, mid day leaf turgor in non-irrigated sorghum was maintained at levels comparable to that in irrigated, due to a concomitant decrease in the solute potential (Feres *et al.*, 1978).

They also reported similar osmotic regulation in maize though the turgor was significantly lower compared to the irrigated plants.

The maintenance of turgor itself depends upon the extent to which the leaf water potential is reduced. The extent of osmotic adjustment(OA) is not

uniform for the various tissues in the plant. Oosterhuis and Wulfschleger (1987) for instance found that the leaves of cotton osmoregulate to a lower extent than their roots. The significance of osmoregulation was clearly demonstrated in two distinct hybrids sorghum (Wright et al, 1983).

A comparison was made with a sorghum hybrid (E-57) and another hybrid (TX-671) with similar maturity, E-57 was able to maintain stomatal opening to a lower leaf water potential, than TX 671 because of its greater capacity to osmoregulate. In E-57 osmotic potential declined due to active solute accumulation, thus maintaining turgor as leaf water potential declined to -2.0 MPa, where as in TX 671 it could only maintain this adjustment to -1.4 MPa. The differences in osmotic adjustment were associated with maintenance of leaf area, water extraction and root growth by E-57. This indicated that OA enabled photosynthesis to continue and a significant amount of the resultant assimilate may have been diverted to root growth (Wright et al, 1983).

Fereres et al. (1978) indicated that sorghum in contrast to maize can maintain full turgor even at grain filling because of a superior ability to adjust

osmotically as a physiological response to increasing drought stress.

Culter and Rains (1978) indicated that cotton plants maintained turgor to lower values of water potential when the plants were conditioned by several cycle of water stress.

Tal and Shannon (1983) observed that the wild relatives of the cultivated tomato L. cheesmanii, L. peruvianum and L. pennellii, are distinguished from the cultivated species by a lower absolute elongation rate of the stem under control conditions and a small relative decrease of this rate under high salinity. The wild species, especially Lycopersicon pennellii, showed high accumulation of Na and a greater decrease in K^+ content under salinity as compared with the cultivated plants. In all three species, Na probably substitutes for K^+ for some of the physiological functions. Similar results were obtained in a recent container study with six diverse Lycopersicon species by Rajasekaran and Aspinall (1988), who showed that Lycopersicon esculentum was very susceptible, while Lycopersicon pimpinillifolium was moderately resistant to salinity.

Morgan et al. (1986) examined the association between osmoregulation and grain yield of wheat. The yields of wheat genotypes which were high in

osmoregulation were 11-17 per cent higher in bread wheats and 7 per cent higher in durum wheats than those which were low in osmoregulation.

Flower and Ludlow (1986) reported that, pigeonpea leaves have the capacity for substantial osmotic adjustment, most of which is due to the accumulation of solutes.

Solutes contributing to osmotic adjustment

Since the documentation of the nature of osmotic solute accumulation in diverse organisms over the last two decades, it has been realised that at least theoretically any low molecular weight nontoxic compound capable of forming water micelles can serve as osmotic solute (Wyn Jones and Gorham, 1983).

Of the number of osmotic solutes reported, some of the most commonly occurring are potassium, sucrose, amino acids, sugar alcohols, quaternary ammonium compounds, etc. (Barnett and Naylor, 1966; Singh *et al.*, 1973a; Hellebust, 1976; Jager and Meyer, 1977).

Examination of various solutes have shown that among the inorganic ions, potassium is most preferred because it is the least inhibitory monovalent cation; it has less tendency to alter the protein conformation

(Paleg and Aspinall, 1981). Polyols and other ring containing sugars are also favoured as the accumulation does not vastly perturb the water structure. Most of the amino acids that accumulate such as glycine, alanine and proline behave as zwitterion at physiological pH and hence do not interfere with the macromolecules functioning.

Yancey (1982) observed that the dependence of plant tissues on accumulation of inorganic ions during water stress appears to be a suboptimal adaptation when compared with the organic osmotic solutes. This is because though many biochemical functions require specific inorganic ions, increasing the concentration often leads to the disruption of functions. For example the salts of Na^+ and K^+ affect both the catalytic rate (Borowitzka and Brown, 1974) and apparent Michaelis constant (k_m) (Bowlus and Samero, 1979) of the enzymes.

Polyols such as glycerol and mannitol occur in many algae and in certain salt tolerant plants (Cutler *et al.*, 1980; Yancey *et al.*, 1982). In each of these instances, polyols aid in the cell water retention while remaining compatible with the macromolecular functioning. An illustrious example of the role of glycerol is given by Borowitzka and Brown (1974) who showed the accumulation of glycerol in the unicellular

alga Dunaleila viridis exposed to the medium containing 4.25 M NaCl. It was suggested that glycerol maintains, the enzyme activity under conditions of high extracellular salt concentration and low water activity.

Among the amino acids, the imino acid proline is one of the prominent solutes reported to accumulate under moisture stress (Rajgopal et al., 1977; Richards, 1978; Bansal and Nagarajan, 1986; Venekamp et al., 1987).

A major portion of the accumulated proline is found in leaves and leaf sheath with very low levels in the root (Singh et al., 1973c). However, Barnard and Daks (1970) reported high concentrations in root tips of maize as well. A unique feature of proline is that on rehydration of the stressed tissue, its accumulation ceases followed by a sharp decline in its level (Stewart, 1972; Singh et al., 1973b,c; Blum and Eberlein, 1976 and Parameswara, 1978).

Since, the early reports of proline accumulation under water deficit stress, many workers have tried to relate its accumulation with the performance of the plants under stress. For instance, Barnett and Naylor (1966) found that the drought resistant Bermuda grass accumulated nearly four fold more proline than the susceptible species. They

suggested that the accumulated proline served as a storage compound for the carbon and nitrogen requirements of the plant once the water stress alleviated.

Maintenance of membrane integrity as a drought tolerance mechanism

Several metabolic, physical and biochemical changes occur in the biological systems undergoing desiccation. These changes can be ultrastructural level, or a change in membrane integrity, protein synthesising capacity and production of free radicals.

There is currently much interest in the structure and role of plant cell membranes in relation to drought and cold resistance (Lyons et al., 1980).

One of the primary injuries caused by moisture stress is loss in cell compartmentation due to the disruption of membrane. Bell et al. (1971) were among the first to suggest that desiccation may cause change in membrane permeability. The leakage of electrolytes as a measure of membrane permeability has been found to be different for desiccation tolerant and susceptible bryophytes (Gupta, 1976, 1977).

Viera De Silva (1976) investigated change in membrane integrity under moisture stress in drought tolerant species Gossypium anomalus and in drought

susceptible species G. hirsutum. The latter showed excessive swelling of thylakoids and disappearance of chloroplastic ribosomes.

Dhindsa and Bewley (1977) also found that membrane damage consequent to desiccation stress was less in drought tolerance than in susceptible species of moss. The leakage of solutes was small and reversible in the drought tolerant types while it was large and irreversible in drought susceptible species.

Marathe (1989) studied the maintenance of membrane integrity in excised leaves of soybean and finger millet leaves under severe stress. Soybean showed significantly greater degree of membrane damage than finger millet as revealed by the leakage of solutes absorbing at 273 nm.

A number of reasons have been proposed for loss of membrane integrity and consequent leakage of solutes on imposition of moisture stress. Physical changes at the membrane sites involved in the maintenance of Lipoprotein association has been suggested by Luzzati and Hsiao (1962) and Finian (1969). Alteration in hydrophobic hydrophilic interactions within the membrane below 20 per cent hydration lead to changes in the structure of membrane.

The displacement of the divalent calcium ion from their anionic binding sites on the membrane phospholipids could also result in the loss of membrane integrity (Bewley, 1979; Uma Shanker, 1984).

The relationship between desiccation tolerance and protein synthesis

A feature of drought resistance may be the ability to continue protein synthesis at moderate or even fairly severe water deficits.

The effect of water stress on protein is often studied by following the incorporation of the amino acids into protein. By using the leucine incorporation, during an osmotic stress, (Koretskaya Zhokevich, 1966) and salinity (Ben-zion *et al.*, 1967; Dhindsa and Cleand, 1975) protein synthesis was assessed.

A significant difference in drought tolerance has been demonstrated in mosses (Bryophytes) Bewley (1973) suggested that, the protein synthesis capacity was intact on desiccation. In extensive studies of desiccation tolerance in poikilohydric or resurrection plants; he showed that when the moss, Tortula ruralis was rehydrated after dessication it rapidly resumed the capacity for protein synthesis.

Hsiao (1970) studying the effect of mild to moderate water stress on protein synthesis in corn seedling, showed that it reduced the ability of the plant to synthesize proteins in coleoptile segments.

Dhindsa and Bewley (1976) studied the responses of the drought tolerance in moss, Tortula ruralis. They found a decrease in fresh weight, an inhibition of amino acid incorporation into protein and a decline in polysome population, with increasing degree of water stress.

Bewley et al. (1983) observed a reduction in protein synthesis when maize seedlings were subjected to water stress. Water stress induces quantitative changes in the pattern of protein synthesis.

In another study, Bewley and Larsen (1980) examined the effect of water stress in pea roots and maize mesocotyl segments. They observed that when the magnitude of water stress was lethal, there was no recovery of protein.

Similarly in Aveng sativa Coleoptiles, Dhindsa and Cleland (1975) reported that, osmotic stress causes a differential inhibition of protein synthesis, showing that the synthesis of some protein is affected to a greater extent than others. They mentioned also, if water stress is not too severe, recovery of protein

synthesis can occur on rehydration but excess water loss causes irreversible damage.

From the information available on the effect of stress on protein synthesis, it can be concluded that mild or moderate stress decreases the protein synthesizing ability both during stress and also on alleviation. In view of the importance of this metabolic phenomenon, several attempts have been made to look in to the role of ribosomes and the effect of stress on them (Bewley and Larsen, 1982).

Decrease in both quantity of polyribosomes and their activity has been reported in pea roots and maize mesocotyls subjected to PEG stress (Bewley and Larsen, 1980) in Tortula ruralis subjected to different levels of stress (Bewley, 1979). However, the extent of decrease in polyribosomal content or activity is relatively less in non-growing regions of the mesocotyls compared to growing tissue (Bewley and Larsen, 1982).

The polyribosomal activity has been implicated as a measure of protein turnover in tissues subjected to varying abiotic stresses.

Though it is unequivocally proven that overall protein turnover and its synthesis is reduced under several abiotic stresses like temperature, salinity

and moisture stress, a particular class of proteins are known to be synthesized in tissues subjected to stress. These proteins are broadly termed as stress shock proteins (Key *et al.*, 1982).

Synthesis of heat shock proteins (HSP's) have been reported from different types of organisms both prokaryotes and eukaryotes, both in animal and also plant system (Key *et al.*, 1982), Plesofsky-vig and Bramble, 1985). Though the overall nature of HSP's has been understood to a large extent, the type of proteins synthesized seems to be species specific and also may vary from tissue to tissue with in a plant. Since they are synthesized in response to an abiotic stress, more interest has been generated in recent years to study the relevance of these proteins to thermo and stress tolerance (Lin *et al.*, 1984).

The role and the mode of action of these HSPs is yet another area where active research is being carried out.

Recovery growth on alleviation of stress - Recovery growth as a measure for intrinsic tolerance

Leaf expansion is more sensitive to water deficits than most other plant processes. The extent to which water deficits reduce growth and yield by reducing leaf area depend upon the relationship between expansion rate and leaf water potential both

during the stress period and during recovery following irrigation.

This ability to produce leaf area upon relief of stress may be particularly important to yield.

The recovery growth on alleviation of stress can be measured by (a) seeing the increase in leaf area, leaf area duration or leaf area index immediately or a week or two after alleviation (b) recovery in stomatal opening and photosynthetic capacity, (c) recovery in expansion rates and (d) recovery in biomass production or in flowering.

Parameswara and Krishna Sastry (1982) reported that when there was no moisture stress CSH-6 sorghum hybrid had the highest elongation rate followed by CSH-5. Moisture stress caused cessation of leaf elongation within 48 hrs in some and 72 hrs in others. On alleviation of stress there was death (firing) of leaves to varying degrees. The percentage leaf firing was the lowest in hybrid CSH-6. In view of the importance of the net functional leaf area available after alleviation of stress for regenerative growth, less damage CSH-5 during stress and on alleviation was a desirable character. Since, genotypic differences exist for this parameter it could be used as an index for drought tolerance.

The contribution to the recovery of leaf area changed between the expansion and the duration components depending upon leaf age. Old leaves completing their "normal" expansion period of approximately 15 days had very little capacity for increased growth rates when water was applied. On the contrary, young leaves had normal durations and recovered by growing faster.

Ludlow and Ng (1976) reported that following rewatering in either the light or the dark, there was marked stimulation of elongation rate of leaves which had previously experienced water stress compared with control leaves of Panicum maximum Variety trichoglume.

The potential for recovery was smallest in the oldest leaves and greatest in those leaves that emerged after rewatering. The late emerging leaves of Seneca were significantly larger in stressed plants than the leaves of a similar time of emergence in plants that received no stress throughout their development (IRRI, 1982).

However, field studies have shown that full compensation of leaf area index can occur in sunflower. Leaves that emerge after relief of stress replace the loss of leaf caused by early stress. This ability to recover from stress is an important drought resistance mechanism (IRRI, 1982).

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The main objective of the present investigation was to assess the variations amongst a few *Lycopersicon* species and *Lycopersicon esculentum* genotypes in several drought avoidance and drought tolerant characters. Three aspects were studied under avoidance mechanisms viz., (a) WUE (b) root characters, (c) non-stomatal rate of water loss. A wide range of tolerance characters like seedling and leaf survival at comparable water potentials or RWC, osmotic adjustment, protein synthesizing capacity and chloroplast and membrane integrity characteristics were studied.

To examine the variation in these drought avoidance and tolerance mechanisms, several pot culture and laboratory experiments were conducted. All the pot culture experiments were conducted at the Botanical Garden, Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India. The place is situated at latitude of 12° 58' north, longitude of 77° 35' east and on altitude of 930 m from mean sea level. The average annual rainfall of the area is about 890.9 mm.

**Description of Lycopersicon species and Lycopersicum
esculentum genotypes used**

Variations in the several physiological characteristics were examined in the following Lycopersicon species and Lycopersicum esculentum genotypes. The numbers given against each species are their accession numbers.

1. 990 - L. peruvianum
2. 1301 - L. Pimpinillifolium
3. 1086 - L. auforetut
4. 987 - L. esculentum var. Cerasiforme
5. 1048 - L. esculentum
6. 564 - L. esculentum
7. Pusa Ruby - L. esculentum

Tomato belongs to the family solanaceae and genus Lycopersicon. The genus includes 12 species, all native to South America.

According to Mullar (1940) and Luckwill (1943), the genus is further subdivided into two subgenera:

1. Eulycopersicon - Annual edible species characterized by red fruits with carotenoid pigmentation and annual. Cultivated tomato L. esculentum and L. pimpinillifolium species are included in this group.

2. *Eriopersicon* - Green fruited species with anthocyanin pigmentation and it includes the species *pissisi* (Luckwill, 1943), *peruvianum* (Mullar, 1940), *hirsutum* (Mullar, 1940), *glandulosum* (Mullar, 1940).

For the present investigation species from both groups were included. 990, 1048, 987 and 1086 were collected from National Bureau of Plant Genetic Resources, 1301 from Dr.C.M. Rick, University of California Davis, and 564 Local collection. Pusa Ruby is a recommended variety widely grown both under irrigated and rainfed conditions. 990, 987 and 1301 are wild types and known to have certain degree of drought and salinity (Aspinall, 1981) resistance.

3.1 Studies on WUE

There are two approaches to determine WUE at the whole plant level based on dry matter accumulation and cumulative water transpired (CWT).

(1) **Intrinsic WUE:** In this technique, the amount of water added to maintain different moisture regimes is based on actual water use, therefore, although the pots are brought back to either field capacity or a lower water regime each day, water added for each variety or species differs as the CWT varies due to intrinsic differences in transpiration. This approach

is widely followed (Turner, 1986; Hubick et al., 1988).

(b) Known Input WUE: This approach is closer to the actual field situation, where genotypes or species receive the same amount of precipitation irrespective of their actual water use. Their WUE will be governed chiefly by their conversion efficiency, or the efficiency with which they produce dry matter with the given water input. This known input could either be "non limiting or limiting". The latter is commonly referred to as "known limited water input situation".

3.1.1 Experiment-I: Differences in intrinsic WUE in genotypes of *Lycopersicon* (Summer season)

A pot culture experiment was conducted during the summer season, 1988. This experiment was conducted using empty battery containers made of carbonised rubber measuring 28 x 14.4 x 12.5 cm³ (L x B x H). These containers could be effectively used for gravimetric determination of water use efficiency.

Design: CRD pot culture experiment

Replications: Seven pots were maintained for each treatment.

Preparation of pots

Each battery container was filled with 8 kg of red loamy soil containing sufficient FYM. Ten pots

2.37

were used for each Lycopersicon genotype. Fertilizers (N, P, K) was applied at the rate of 45:40:24 kg NPK per acre and the quantity required to each pot was determined based on the weight of the soil. Twice the recommended dosage was applied to each pot at the time of sowing to compensate for initial leaching losses.

Raising the plant material

The seedlings were thinned twice at 15 and 20 DAS and two plants per pot were retained. The pots were watered daily and maintained at field capacity upto 30 DAS. The amount of water required to bring the soil to field capacity was determined. For red loamy soil, which was used in this experiment, the moisture content at field capacity is 16 to 18 per cent. After bringing the pots to the field capacity, the drainage holes of the pots were closed by plugging with clay and cement. The exposed part of the soil in the pots was completely covered with plastic pieces (500 g) to prevent evaporation from the soil surface. The weight of the individual pots with plants and pot pieces was recorded with the help of salter bench plot form type of balance of 20 kg capacity with a resolution of 50 g. The pots were weighed daily to record the amount of water lost essentially through transpiration since "E" was checked to considerable extent.

After each weighing, required amount of water was added to bring back the soil moisture content to field capacity. Using this procedure the cumulative water added (CWA) to each pot was recorded. The CWA represents essentially the water lost in transpiration (CWT). However, to correct for E losses another set of containers without plants but only the soil surface covered was maintained and weighed every day along with the other set. The CWA in this case is nearly similar to the water lost due to E in the pots having plants with the soil surface covered, this amount was deducted from CWT. The meteorological data for the experimental growth period is given in Appendix-1.

The following observations were recorded in this experiment:

1. Initial and final leaf area at the end of the experiment.
2. Leaf area duration (functional leaf area).
3. Initial dry matter and the final dry matter at the end of the experiment.
4. Total dry matter accumulation during the experimental period (23 days) (Δ TDM).
5. Cumulative water transpired (CWT).
6. Transpiration quotient (TQ).
7. rate of water loss (CWT/LAD)

8. LAD/DM ratios
9. LAD/shoot DM ratios
10. Water use efficiency (WUE)
11. Root dry matter
12. Root/shoot ratios

1. Leaf area

The initial leaf area on the 30th day and the final leaf area on the 53rd day were measured using LICOR leaf area meter (Model LI-3000) and expressed as cm² per pot. For determining the initial leaf area, three pots (each with three plants) were used. These nine plants were uprooted and the leaf area was determined. On the 53rd day, the final leaf area of all plants in the remaining seven pots was determined.

2. Functional leaf area during the experimental period (leaf area duration LAD)

The LAD is a reflection of the functional leaf area available for assimilation during the active growth period. LAD was calculated using the standard formula given by Hunt (1983) for container studies.

$$LAD = \frac{L1 + L2}{2} \times t2 - t1$$

Where; L1 - Leaf area dm² at time t1

L2 - Leaf area dm² at time t2

t_2-t_1 - Duration in days between initial and final samples (23 days)

LAD - was expressed as $\text{dm}^2 \text{ day}^{-1}$.

3. Initial and final DM

The initial dry matter on 30th day and the final dry matter on 53rd day was recorded from same plants used for leaf area estimation. The plant material was oven dried at 80°C for 48 hours. The weights were recorded and expressed in g dry weight per plant. The total dry matter accumulated from 30th to 53rd day was computed as the difference in final and initial dry matter.

4. Cumulative water transpired (CWT)

The pots were weighed daily and the amount of water to be added to bring back the soil moisture content to field capacity was determined. This is the reflection of the amount of water used by plant for transpiration and also includes evaporation losses. The sum of water added to the pots during the experimental growth period is the cumulative water added. The cumulative water transpired by the plants was determined by deducting the evaporation losses determined in the similar way from the pots without plants. Cumulative water transpired was expressed as ml per pot.

5. Transpiration quotient (TQ)

The transpiration quotient was estimated as per the method of Ludlow (1972) as the amount of water required to produce unit dry matter. Transpiration quotient (TQ) or transpiration ratio RT (Stanhill, 1986) is given as;

$$\frac{\text{Cumulative water transpired;} \\ \text{(ml/plant) during experimental period}}{\text{Total dry matter produced during the} \\ \text{same period (g/plant)}}$$

The TQ was expressed in ml H₂O g⁻¹ dry weight.

6. Water use efficiency (WUE)

Water use efficiency is defined as the amount of dry matter produced per unit amount of water transpired.

WUE was estimated as the ratio of the dry matter accumulated during the duration of the experiment (23 days) to that of the cumulative water transpired during the same period and expressed as g DM kg⁻¹ H₂O.

In all experiments WUE was expressed in two ways (a) on the basis of total dry matter which included the root dry weight,)b) on the basis of shoot dry matter alone (shoot DM).

7. Rate of water loss

The cumulative water transpired (CWT) divided by the functional leaf area (LAD) gives a reflection of the rate of water loss on a whole plant basis in other words it is a reflection of the mean transpiration rate per plant per day. This was calculated by the formula;

$$\text{Mean transpiration rate (rate of water loss)} = \frac{\text{Cumulative water transpired (CWT)}}{\text{Functional leaf area (LAD)}}$$

and expressed as $\text{ml H}_2\text{O dm}^{-2} \text{ day}^{-1}$.

8. LAD/DM

This ratio is an indication of the assimilation capacity per unit leaf area. In other words, it is an indirect reflection of net assimilation rate. This was computed using the dry matter accumulated during the experimental period and the functional leaf area of that period. Lower the ratio, greater is the assimilation capacity.

$$\text{LAD/DM} = \frac{\text{LAD}}{\text{TDM}}$$

9. Root biomass and root/shoot ratios

At the end of the experiment, the roots were carefully extracted and the root biomass and root to shoot ratios were computed.

3.1.2 Experiment-II: The differences in WUE in Lycopersicon species during monsoon conditions

In an other pot culture, the variation in WUE was determined during the monsoon season, in all the Lycopersicon genotypes.

Season - Monsoon

Design - CRD pot culture experiment

Treatments - 7 Lycopersicon genotypes.

The method adopted and the observations recorded were same as described for Experiment-I.

This experiment was conducted with an objective to study the influence of environmental factors on WUE. To avoid interference from rain the plants were grown under rain shelter. Therefore the radiation received by the plants was relatively less (range between 415-476 $\mu \text{Ein m}^{-2} \text{day}^{-1}$, PAR) compared to the summer experiment. The duration of this experiment was 24 days. The meteorological data for the period of the experiment are given in Appendix-2.

3.1.3 Experiment-III: Effect of light intensity on WUE in selected Lycopersicon species

A pot culture experiment was conducted using battery containers during summer season to study the differences in WUE among the selected species in relation to light intensity. The results of the I

(Summer) and II (monsoon) experiments, indicated the WUE is affected by light intensity. Further it was genotype specific. In some Lycopersicon genotypes like 1301 and 1086 there was an increase in WUE and in some others like 990 a decrease was noticed. Therefore the effect of light on WUE was again assessed in an another pot experiment by growing the plants at two light regimes. The Lycopersicon genotypes selected for this study and the treatments imposed are;

Design - CRD pot culture experiment

Main treatments - 3 Lycopersicon genotypes

1. 1301 - L. pimpinillifolium
2. 990 - L. peruvianum
3. Pusa Ruby - L. esculentum.

Light regimes - Two levels

1. Full sunlight
2. 40 per cent sunlight.

Preparation of pots, sowing and watering and observations recorded were same as described in Experiment-I.

In addition to the parameters associated with WUE in this experiment gas exchange parameters like assimilation rate(A), stomatal conductances(g_s) were recorded.

Gas exchange parameters using portable photosynthesis system - ADC

Photosynthetic rate and stomatal conductances were monitored using a portable carbon dioxide analyser model ADC - LCA-2. This instrument measures the CER in an open system. This ADC system is designed to measure instantaneously CO₂ and water vapour exchange. The major components of the system are (a) air supply unit (ASU), the main function of which is to supply moisture free air at a specific flow rate which is adjustable from 200 to 600 ml per minute, (b) Parkinson's leaf chamber, this unit houses the sensors necessary to measure PAR, RH, temperature. The volume of the leaf chamber where leaf is clamped is 16 cm³, (c) The IRGA - LCA-2 measures the changes in CO₂ concentration in differential mode, (d) the data logger, the main function is to log the data and do the primary computations. The primary and computed data are exposed as follows.

1. PAR - $\mu \text{ mol m}^{-2} \text{ sec}^{-1}$
2. Leaf temperature $^{\circ}\text{C}$
3. Conductances $\text{m mole m}^{-2} \text{ sec}^{-1}$
4. Transpiration $\text{moles m}^{-2} \text{ sec}^{-1}$
5. Photosynthetic rate $\mu \text{ mole CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$

In the plants grown in both light regimes, the gas exchange characters were determined after 10 days after initiation of the experiment. The gas exchange parameters were recorded at growth light intensities in all the three *Lycopersicon* genotypes during morning hours around 11.00 AM. The light intensities in the open field grown plants ranged from 1600 to 1800 $\mu\text{mole m}^{-2}\text{sec}^{-1}$, where as in the plants grown under low light regime under rain shelter, the light intensity was about 40 per cent of the open field conditions.

Fully expanded 4th leaf from top was clamped in the leaf chamber. RH in the system was manipulated to maintain the ambient RH while taking the observations in all the treatments. For each observation 5 to 6 data were logged and the mean was computed.

3.1.4 Experiment-IV: Effect of moisture stress on WUE and related parameters

The intrinsic differences in water use efficiency in general were assessed by maintaining the plants at a particular moisture regime. However, under the field conditions (rainfed situations) the input of water by precipitation will remain same for all the species. So, it is more appropriate to assess differences in WUE among the species at a given input of water. All the 7 *Lycopersicon* genotypes were

supplied with a similar input of water and differences in water use efficiency were estimated under two moisture regimes.

Main treatments

Lycopersicon genotypes:

1. 990 - L. peruvianum
2. 1301 - L. pimpinillifolium
3. 1086 - L. aufretut
4. 987 - L. esculentum Var. cerasiforme
5. 1048 - L. esculentum
6. 564 - L. esculentum
7. Pusa Ruby - L. esculentum.

Sub-treatments

Moisture regimes:

1. Adequate input of water
2. Limited input of water

In adequate input moisture regime the pots were maintained at near field capacity. In limited input moisture regime the plants received 60 per cent of the water applied to control adequate water input plants.

Replications

Seven pots were maintained for each treatment.

The procedure adopted for preparation of the pots, raising of the plant material and fertilizer

application was similar as in the first experiment, except that the size of the containers was increased substantially to facilitate better root growth. These containers (49 x 29 x 21 cm³ - L x W x H) were filled with 16 kg of red loamy soil containing sufficient FYM. Two plants per pot were maintained in each treatment. Evaporation was minimised by mulching the soil surface with plastic pieces. However, since this experiment did not involve actual weighting of pots no effort was made to delineate the possible evaporation losses from soil surface from that of actual transpiration as was done in the first experiment. The experimental growth period was 22 days for all species except in 990 *L. peruvianum* where it was 29 days. In the initial stages the water required for 990 *L. peruvianum* was less and hence to give the same total input of water the duration of crop growth period was extended by 7 days in 990.

Moisture regimes

The daily water loss through transpiration was determined in each genotype by measuring soil moisture per cent before and after watering and the amount of water required to maintain the plants at around near field capacity was approximated. Care was taken to see that there was no water logging or water stress.

The mean amount of water required to maintain field capacity was given to all Lycopersicon genotypes under moisture regime 1 and 60 per cent of that was given for the second moisture regime. The plants were irrigated twice in a day to the respective moisture regimes.

The observations recorded in this experiment were same as in the previous experiments. But while computing other parameters, the cumulative water added was used instead of CWT. No correction was given for evaporation losses. Even the rate of water loss was computed based on CWA instead of CWT.

In addition to these parameters, observations were also recorded in this experiment on root water extraction efficiency.

3.2.1 Assessment of differences in root characters and their relationship to water extraction efficiency index

Water uptake associated with root characteristics is one of the most important drought avoidance strategies adapted by the plant to maintain a higher water status.

In addition to the root volume and other root characters other characters such as hydraulic conductance and root osmotic adjustment also influence water extraction efficiency from the soil. These

characters become even more important under receding soil moisture conditions.

To determine the variation in water extraction efficiency among the *Lycopersicon* genotypes, the soil moisture content measured at near field capacity at the beginning of the experiment and at the end of the experiment 22 days after cessation of watering. The water retained at the end is the reflection of water extraction efficiency. If this is high lesser is the efficiency and vice versa. A similar assessment was also made in plants grown in the limited input moisture regime.

The water extraction efficiency index was determined based on this data for a known input of water.

$$\text{Water extraction efficiency index} = 10 - \left[\frac{\text{Amount of water remaining at the end of experiment} \times 100}{\text{Cumulative water added}} \right]$$

3.2.2 Variations in the non-stomatal rate of water loss

As one of the moisture conservation mechanisms, at leaf level, the rate of water loss from cuticle was determined in *Lycopersicon* genotypes. The measurements were made in the leaves of adequately watered plants.

The principle in this method was that, in the excised leaves preincubated in the dark, when subjected to live wilting the decrease in fresh weight (rate of water loss) is mainly attributable to cuticular loss of water. However, during the initial periods loss of water through stomata may contribute to certain degree.

For measuring the rate of water loss, the fully expanded fourth leaf from the top was taken and randomly four leaflets were taken from each of the seven *Lycopersicon* genotyp. Initial fresh weight of the leaflets was noted and was kept in dark. The leaves were then weighted in a precision balance at 30 minutes intervals until loss of 50-70 per cent of initial fresh weight. Four replications were maintained for each genotype. In three such experiments the following observations were taken.

Observations

1. Per cent reduction in fresh weight
2. Leaf area
3. Dry weight
4. Specific leaf weight.
5. Rate of water loss.

3.3 Studies on desiccation tolerance

The following experiments were conducted to study the desiccation tolerance and other drought tolerance mechanisms.

1. Assessment of desiccation tolerance based on empirical screening methods.
2. Variation in proline accumulation.
3. Studies on osmotic adjustment.
4. Effect of moisture stress on metabolic activities.
5. Membrane stability characteristics.

3.3.1 Assessment of desiccation tolerance based on empirical screening techniques

The objective of this experiment is to evaluate the intrinsic desiccation tolerance based on seedling mortality, leaf desiccation and recovery growth rates.

Three different types of experiments were conducted.

3.3.1.1 Evaluation of the desiccation tolerance at a comparable tissue water status based on leaf survival

The objective of these studies was to evaluate *Lycopersicon* species for drought tolerance at low but

comparable tissue water potential at the seedling stage. To achieve this objective, different pot culture studies were conducted.

Experiment-I

In pot grown plants moisture stress of different durations and intensities was imposed at seedling stage. The extent of differences in leaf survival at different but comparable leaf water status was assessed. In addition to leaf survival, leaf proline content at different tissue RWC was also determined.

The species *Lycopersicon* genotypes studied in this experiment are;

1. 990 - *L. peruvianum*
2. 1301 - *L. pimpinillifolium*
3. 1086 - *L. aufretut*
4. 987 - *L. esculentum* var. *Cerasiforme*
5. 1048 - *L. esculentum*
6. 564 - *L. esculentum*
7. Pusa Ruby - *L. esculentum*

Stress regimes

Moisture stress of different durations and intensities was imposed at seedlings stage in pot grown plants. The extent of differences in leaf

survival at different but comparable tissue water status was assessed.

In addition to leaf survival the leaf proline content at different tissue RWC levels was also determined.

Preparation of pots

Big empty battery container was filled with 16 kg of red loamy soil containing sufficient FYM, each species was sown in six pots. The amount of fertilizers (NPK) applied was at the rate of 45:40:24 kg NPK per acre, calculated based on the weight of the soil. Twice the recommended dosage was applied to each pot at the time of sowing.

Sowing

Seeds were sown in four rows and after germination two thinnings were done, one on 16th and the other on 30th DAS. Two plants were maintained per each row. The pots were watered daily and maintained at field capacity upto 30 DAS.

Imposition of stress

To maintain at field capacity in big battery containers with 8 seedlings, the approximate water required was 1500 ml per pot. To impose gradual and mild stress, from 30th day onwards the pots were

irrigated with 1000 ml per day instead of 1500 ml. From the second day after imposition of stress, visual wilting was observed. The following observations were recorded on 4th and 5th day after imposition of stress. The observations were recorded on both days with an objective to achieve comparable RWC values which is essential to assess the relative survival ability.

Observations recorded

1. RWC
2. Leaf survival percentage

Relative water content (RWC)

The relative water content was estimated based on method of Barrs and Weatherly (1968). Leaflet of leaves of plants subjected to moisture stress were weighed to indicate the fresh weight. Immediately after weighing, the leaf material was transferred to petridishes containing water. After 12 hours, the leaf material was surface blotted and weighed once again to indicate the turgid weight. The leaflets were then oven dried at 80 °C and dry weight recovered. The relative water content was calculated as follows.

$$RWC = \frac{\text{Initial weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Survival percentage

The intrinsic desiccation tolerance was assessed based on the per cent survival of the leaves. The stress imposed was not severe enough to cause any seedling mortality. The per cent leaf survival was determined by measuring the total leaf number, desiccated and green at the end of the stress period and the total number of green and dessicated leaves 24 hours after stress alleviation. The number of green leaves that recovered were counted and expressed as percentage.

$$\text{Per cent survival} = \frac{\text{Green leaves}}{\text{Total leaves}} \times 100$$

Experiment-II

The species and genotypes studied, and the technique adapted to raise the material were same as in Experiment-I. However, in this experiment, the stress imposed was severe and fairly rapid. From 30 DAS, the stress was imposed and the pots were irrigated with 500 ml of water instead of 1500 ml, which was needed for maintaining the field capacity. From 2nd day onwards the plants showed visual wilting symptoms, but the observations were recorded on 4th and 5th day. The following observations were made.

1. RWC
2. Leaf survival percentage.

The method adopted for determining these two parameters was the same as described in the previous experiment.

Experiment-III

The approach in this experiment and also the objective similar to those of the two previous experiments. However, while raising the plant material in each pot, in addition to one of the species of *Lycopersicon*, Pusa Ruby was also sown in each pot to get a comparative assesment of survival at a given tissue water status. As in the previous experiments, plants were grown at field capacity for 30 DAS. On the first day of imposition of stress, the pots were irrigated with 1500 ml water and on the second day 1000 ml. Subsequently water was with-held completely and the tissue water status was monitored by determining the tissue water potential psychrometrically. The observtaions on the following aspects were recorded and on 8th day after with-holding water when in many of the species tissue water potential decreased to -2.0 to -2.5 MPa. The observations recorded were;

1. Leaf water potential
2. RWC

3. Solute potential
4. Turgor potential
5. Per cent survival of the leaves.

Leaf water potential

The leaf water potential was estimated using a HP-115 water potential data system, in the dew point mode. The thermocouple psychrometer directly measures the vapour pressure of water from intact plant tissue (total water potential) including the contribution of turgor potential. The second or third leaf from randomly selected plants was excised and the leaf punches used to estimate the leaf water potential. To determine total water potential, 1.0 cm diameter leaf discs were placed in sealed psychrometer chamber which contained a thermocouple junction. After a stabilization period (70 to 90 minutes). The leaf discs were then used for determining the solute potential using vapour pressure osmometer Wescor-5100C.

Solute potential

The solute potential was determined using a vapour pressure osmometer (WESCOR-1500C). Leaf sections were dry frozen after wrapping in aluminium foil in liquid nitrogen for 5-10 seconds and allowed to thaw for 30 minutes. Later, the thawed tissues was

centrifuged quickly for 2 minutes at 4000 rpm to liberate the intracellular contents (sap). The solute potential of the collected sap was determined using a calibrated vapour pressure osmometer.

Experiment-IV

The protocols of this experiments were the same as Experiment-III except that the data on water status and leaf survival was recorded on 12th day after imposition of stress when the leaf water status decreased further to -2.0 to -3.0 MPa. Here also the per cent survival of leaves was determined in addition to leaf water potential.

3.3.1.2 Evaluation of desiccation tolerance based on seedling survival and leaf desiccation under gradual but severe stress conditions

In this experiment the emphasis was to evaluate the relative survival when severe stress imposed was gradually under field situations. A small plot experiment was conducted during rainfree period, summer. The extent of desiccation tolerance was determined based on seedlings survival at the end of the stress cycle. In addition to this during the stress period at different days after imposition of stress, the RWC and the number of green leaves survived was also recorded. In addition to that a few other physiological characteristics were also recorded.

preparation of the land, sowing and imposition of stress

Two plots each with an area of 7.5 m^2 were used for this experiment. Each species was sown in two rows in each plot which served as replications. In this study 20 days old seedlings were transplanted. In each row 6 uniform plants were maintained with spacing of 30 cm between the plants and 30 cm between the rows.

Stress was imposed 15 days after planting, by stopping irrigation. The duration of the stress cycle was for 2 to 3 days, and for 21 days after imposition of stress, the following observations were recorded, three days after stress alleviation the recovery rating was also recorded.

observations

1. Per cent mortality of the seedlings
2. Per cent leaf survival
3. RWC
4. Proline accumulation
5. Per cent recovery.

Per cent recovery calculated based on the number of green leaves that recovered after stress alleviation expressed as percentage.

$$\text{Recovery percentage} = \frac{\text{Total green leaves after recovery}}{\text{Green leaves at the end of stress}} \times 100$$

3.3.1.3 Assessment of desiccation tolerance based on seedling survival under rapid desiccation

From the results from the previous small plot field experiment, four genotypes which distinctly differed in survival percentage were selected and in another pot culture experiment under rapid stress condition, seedling survival was determined. Species studied were 987, 1301, 990 and Pusa Ruby.

In addition to the seedling survival, the osmotic adjustment, the per cent leaf survival and recovery percentage was also determined.

The method adopted for raising the plant material was same as described in previous pot culture experiments. Stress was imposed by with-holding water in 20 days old seedlings and when the leaf tissue water status decreased to 35 to 40 per cent, the following observations were recorded.

1. RWC
2. Water potential
3. Solute potential
4. Leaf survival percentage
5. Seedling survival percentage
6. Recovery percentage.

3.3.2 Variation in proline content of Lycopersicon species subjected to different levels of moisture stress

To examine this aspect, no specific experiment was conducted to raise plant material, the leaf proline content was determined from two experiments. The data on RWC and proline content were estimated in pot culture experiment (3.3.1.1 Experiment-I) and in small plot experiment where stress imposed was severe but gradual. The proline content was determined at different tissue RWC level but an attempt was made to compare Lycopersicon genotypes at a similar RWC levels.

Leaf proline content

Whenever sampling was made to determine RWC, in similar type of leaves proline content was estimated by adopting the method of Bates et al. (1973).

Proline estimation

Reagents: The reagents used were 3 per cent sulphosalysilic acid, toluene, and acid ninhydrin. Acid ninhydrin was prepared by dissolving 2.5 g ninhydrin in 100 ml of glacial acetic acid and orthophosphoric acid (6M) mixed in the ratio of 3:2.

Procedure

A known weight of leaf samples was homogenised in a known volume of 3 per cent aqueous sulphosalysilic acid and the homogenate filtered. The filtrate was made upto the original volume in all cases. To 2.0 ml aliquot of the filtrate was added 2.0 ml of acid ninhydrin and 2.0 ml of glacial acetic acid. These were shaken well and kept for one hour at 100 °C in a hot water bath. The reaction was terminated by placing the test tubes in ice bath. The content of each were then transferred to a separating funnel and 4 ml of toluene was added. The separating funnel was shaken vigorously and allowed for few minutes for the two layers to separate out. The lower layer was discarded and the upper toluene layer having the colour due to proline ninhydrin chromophore was collected in test tube. The colour intensity was read at 520 nm in a colorimeter (Sepectronic-21).

The proline content in the leaf material was calculated as follows.

$$\text{Proline weight} = \frac{36.231 \times \text{optical density}}{\text{volume of extract}} \times \frac{\text{Volume of aliquot}}{\text{Dry weight of the leaf}}$$

Where, 36.231 is the factor derived from standard curve of pure proline (Bates *et al.* (1973).

3.3.3 Variation in osmotic adjustment in genotypes differing in desiccation tolerance

Based on the overall ranking of species in survival at low tissue water potential, both in pot and field conditions on the leaf and whole plant level, two species distinctly differing in their survival capacity under moisture stress were selected.

In another container experiment, RWC, leaf water potential, osmotic adjustment on different days after withholding water was determined in Pusa Ruby and 987.

Species studied were

1. Pusa Ruby - L. esculentum (susceptable)
2. 987 - L. esculentum var. cevasiforme (resistant).

Observations

The following observations were recorded on day 1, 4, 9, 14 and 16 after imposing the moisture stress.

1. Water relations - RWC, water potential, osmotic potential
2. Starch
3. Solutes accumulated in the leaves
 - a) Sucrose

b) Hexoses and pentoses

c) Total amino acids.

4. Root characters - Root dry weight and root to shoot ratio.

5. Turgid weight, dry weight ratios.

Preparation of pots

Big battery containers filled with 16 kg soil were used for this experiment. The method adapted for preparation of pots sowing and after care was the same as described for other pot culture experiments.

Imposition Stress

The pots were watered daily and maintained at field capacity upto 30 DAS. After that the amount of water was decreased gradually and subsequently withheld completely.

Water potential and solute potential were measured in the 3rd leaf from the top. The total sugars, starch, sucrose and amino acid content were also measured in similar leaves.

Osmotic adjustment

When plant cells are subjected to water stress they loss water and result in the increase in the concentration of solutes in the cell. This contrasts

to the active accumulation of solutes through the process of osmoregulation which helps in the maintenance of the turgor by reducing the osmotic potential. It is necessary therefore to delineate the extent of active osmotic solute accumulation (osmotic adjustment) from that due to passive concentration build up of solutes.

There are two methods of estimating osmotic adjustment, namely that of Wilson et al. (1979) and Morgan (1980).

(a) In Wilson's method, a cell is assumed to behave as a perfect osmometer

For an ideal osmometer

$$\psi \text{ II} = \frac{\psi \text{ IIo } V_o}{V} \dots\dots\dots 1$$

Where V_o is the osmotic volume and 'o' signifies a reference state like full turgor

Equation (1) could be approximated to,

$$\psi \text{ II} = \frac{\psi \text{ IIo } RWC_o}{RWC} \dots\dots\dots 2$$

Where, RWC_o represents V_o and RWC is the volume at reduced water status. To correct for the osmotic potential contributed due to the passive concentration build-up because of a decreasing cell

volume or RWC, Wilson et al. (1979) expressed the osmotic potential at a given water content, to that at 100 per cent RWC using the following equation derived from equation 2.

$$\psi_{IIo} = \frac{\psi_{II} (RWC - B)}{(100 - B)}$$

OR

$$\psi_{IIo} = \frac{\psi_{II}(RWC)}{100}$$

Where 100 refers to a tissue in the reference state of complete turgidity (100 per cent RWC). Though the apoplast water(B) also theoretically would contribute to the concentration of solutes of a tissue when it dehydrates it is excluded since it is negligible and does not increase with stress (Wilson et al., 1979). The differences between the ψ_{IIo} of tissues that are stressed and those that are fully irrigated indicates the extent of osmotic adjustment.

(b) Morgan's method: The method of Morgan (1980) involves comparing the observed accumulation of solutes with that expected from an ideal osmometer in which concentration of solute is changed only by the gain or loss of water from the cell.

Transforming equation(2) into its linear form we get;

$$\log \psi_{II} = \log (\psi_{II} / RWC_0) - \log RWC$$

Where the intercept is $\log (\psi_{II} / RWC_0)$ and the slope is -1. Thus by evaluating the slope of the observed response of $\log \psi_{II}$ to $\log RWC$ it is possible to determine the extent to which the accumulation is due to active processes.

Extraction and estimation of starch, soluble sugars and amino acids

In the same leaf sample both starch and other soluble compounds were extracted and estimated. Initially the reducing sugars, pigments and other soluble fractions were extracted in methanol, chloroform water (MCW, 12:5:3 by volume). About 200 to 300 mg of leaf material was taken and these soluble compounds were extracted by adding 5 ml of MCW for a period of 10 minutes at room temperature in a water bath. The samples were then centrifuged and supernatant was separated. This procedure was repeated 3 to 4 times and the supernatant was pooled for estimation of soluble compounds. The residue was dried at 50 °C to remove the chloroform.

To the residue 0.2 ml of 50 per cent ethanol and 4 ml of water was added and starch was gelatinized

by keeping in a vigorously boiling water bath for 15 minutes.

To the MCW extract water was added (at the rate of 3 ml per 5 ml MCW extract) and the chloroform pigment phase was separated and discarded. The alcohol phase was evaporated and residue was dissolved in 2 ml of 50 per cent isopropanol.

Estimation of starch using enzymatic method

The gelatinized starch was hydrolysed to glucose by enzymatic method according to Haising and Dickson (1979).

Reagent

1. **DNS reagent:** 1 g of DNS (dinitrosalicylic acid) and 30 g of sodium potassium tartrate were added to 16 ml of 10 per cent NaOH and 50 ml of water. After dissolving the salts, the volume was made upto 100 ml (Clarke, 1964).

2. Enzyme preparation

Mixture of amylase and amyloglucosidase (1:2) was diluted one 1:200 times by using acetate buffer pH 4.2, and used for hydrolysing the starch. This enzyme preparation contained approximately 0.5 and 15 units per ml of amylase and amyloglucosidase respectively.

3. **Acetate buffer:** To 1 M sodium acetate buffer, 1 M acetic acid was added until the pH of solution reaches 4.2.

Procedure

After extracting the soluble compounds in MCW, the residue was gelatinized. To the gelatinized starch solution, 1 ml of the enzyme preparation was added and incubated at 40 °C for 30 minutes in a water bath. Then 2 ml of DNS reagent was added and kept in the boiling water bath for 10 minutes. After cooling the test tube, 10 ml of water was added and optical density was taken at 540 nm. The standard curve was prepared by using soluble starch. The starch content of sample was expressed as µg starch/g dry weight of plant sample.

Estimation of sucrose

The sucrose content was determined in MCW fraction enzymatically by using invertase as described by Jones *et al.* (1977). The reducing sugars formed are determined by using DNS reagent. About 0.2 to 0.3 ml of the extract (MCW extract taken in isopropanol) was made up to 1 ml and 1 ml of invertase enzyme (12.5 units) was added and kept for incubation at 45 °C for 30 minutes. then the reducing

sugars in the incubation medium was determined by adding 2 ml of DNS reagent.

The sucrose content in the samples were determined by using standard curve.

Reagents:

1. DNS reagent was prepared as per the procedure described by Clark (1964).

2. **Enzyme:** Commercial preparation of invertase was used in this experiment. 0.5 g of invertase was dissolved in 10 ml of citrate buffer pH 4.6 and centrifuged for 10 minutes at 3000 g. The supernatant was diluted one in three. Approximately 1 ml of this enzyme preparation contains 12.5 units per ml.

Estimation of hexoses and pentoses by phenol sulphuric acid method

The simple sugars, oligosacchrides and their derivatives with a free or potentially free reducing group give an organic yellow colour when treated with phenol and concentrated sulphuric acid. The reaction is sensitive and colour is stable.

Procedure

The alcohol phase of MCW fraction was used for determining pentoses and hexoses. A known aliquot of

alcohol extraction (0.2 to 0.4 ml) was made up to 1 ml with water. To this 0.5 ml of phenol reagent and 5 ml of sulphuric acid was added, mixed well and allowed to stand for 15 minutes and then placed in water bath at 25 to 30 °C for 30 minutes. The optical density was read at 490 nm for hexoses and 480 nm for pentoses.

A standard curve for sugars was prepared by using glucose for hexoses and xylose for pentoses.

Reagent

1. Phenol reagent: 5 g of redistilled phenol was dissolved in 95 ml of distilled water and stored in brown bottle.

The estimation of amino acids

The total amino acids determined in MCW extract by using neutral ninhydrin reagent method as described by Rawson et al. (1957).

Reagent

Preparation of citrate buffer: Citrate buffer (pH 5) prepared by dissolving 1 g NaOH in 250 ml distilled water to which 2.6 g of citric acid was added. The volume was made upto 62.5 ml with distilled water.

Neutral ninhydrin reagent

Reagent-A: 40 g stannous chloride dissolved in 25 ml of citrate buffer.

Reagent-B: 1 g ninhydrin dissolved in 25 ml of methoxy ethanol.

Reagent 'A' and 'B' were mixed in equal proportion to get ninhydrin reagent.

Procedure

Alcohol phase of MCW was used to estimate total amino acids. To 0.1 ml of aliquot, 1.0 ml of ninhydrin reagent was added and mixed vigorously. The tubes were placed in a water bath at 100 °C for 10 minutes. After cooling the test tubes to room temperature 5.0 ml of isopropanol, diluted 1:1 with distilled water was added. After thoroughly mixing the solution it was left for 10 minutes to develop the colour. The absorbance was read at 570 nm in a spectrophotometer (Spectronic-21). The total amino acid content in the samples was calculated by using the leucine standard curve (Rasen, 1957).

Computation of solute concentrations

The solutes concentration (Hexoses, pentoses, sucrose and total amino acids) was initially calculated as mg per 45 leaf discs and then converted

Based on water content into either millimoles or μ moles of solute, as suggested by Lee and Tsai (1985) and Brooks *et al.* (1982). The water content of the 45 leaf discs was calculated as μ litres of H₂O, based on determination of fresh weight and dry weight of the leaf discs, on each day of the stress and nonstress treatments (Appendix-9).

Turgid weight/dry weight ratio

TW/DW ratio was measured in selected species, Pusa Ruby and 987 during the experimental period. The observations had been taken on 1, 4, 9, 14 and 16 days after imposing to stress. The ratio was calculated based on the turgid and dry weight of samples.

3.3.4.1 Potential photosynthetic rates in plants subjected to stress

This method is used to assess the differences in photosynthetic rate when the substrate carbon dioxide concentration is at saturation level at the site of carboxylation. In principle it reflects the potential carbon fixation ability of the chloroplast.

This method often employed to assess chloroplast integrity in leaves subjected to environmental stress. The chloroplast functional ability cannot be measured in plants subjected to stress by using gas exchange instruments. This is mainly due to the effect of environmental factors on

the other diffusive characters of leaves like g_s and the liquid phase resistances. In view of this reduction in CER observed need not be due to loss of chloroplast integrity. therefore, the functional ability are determined by assessing the potential photosynthetic rate at saturated carbon dioxide level. In mesophytes, the most of the crop species approximately 50 per cent of ambient carbon dioxide concentration has been shown to overcome any stomatal limitation for carbon dioxide entry.

A new sensitive method

A method developed by Department of Crop Physiology, Univerisity of Agricultural Sciences, Bangalore, India, was used to study potential photosynthetic rate in leaves subjected to different levels of moisture stress. In this method excised leaflets were exposed to 5 per cent carbon dioxide concentration containing a known per cent of $^{14}\text{CO}_2$ in assimilation leaf chamber under natural high light condition. Because of the high carbon dioxide levels maintained in the chamber the C_i will be at saturated level for carboxylation. After exposing leaves for known time the rate of assimilation can be determined by assessing the radioactivity in the leaves.

Description of assimilation chamber and release of CO₂

The assimilation chamber was constructed with transparent perspex sheets, and contains two distinct compartments. The bigger compartment contained a perspex tray divided into twenty sub-compartments where the leaflets were placed while exposing them to elevated CO₂ levels. The leaflets are kept in each compartment on a nylon mesh in such a manner that there will be free diffusion of carbon dioxide from both the leaf surfaces.

In the other compartment provision was made to release ¹⁴CO₂ and the required concentration of ¹⁴CO₂. Inside the chamber two battery operated fans were fitted to facilitate uniform distribution of CO₂ after it is released from a bicarbonate source.

The total volume of the chamber was 2.4 litres, and the net volume 2 litres. To get a 5 per cent CO₂ concentration, 375.4 mg of NaHCO₃ was completely acidified using 10 ml of 10 N perchloric acid. The radioactivity of ¹⁴CO₂ maintained was 25 µci per litre of air.

The leaflets were numbered and arranged in the assimilation chamber. Under dark conditions CO₂ (with known ¹⁴CO₂ concentration) was released and the leaves were exposed to saturated light intensities of 1500 -

1600 μ Ei m⁻² sec⁻¹ for a period of 15 minutes. The RH in the chamber was maintained at near 100 per cent, so that the excised tissues maintain their original tissue water status. After exposing the leaves for a known time, the radioactivity was determined using a Geiger Muller Counter (GCS-16) and the radioactivity was expressed as cpm/100 mg dry weight and cpm/cm² LA.

By using this method, two experiments were conducted.

Experiment-I: Variation in potential photosynthetic rates in Lycopersicon species

Differences in potential photosynthetic rate as a measure of mesophyll factors were determined in all the seven Lycopersicon genotypes. The leaf-lets from well watered pot grown plants were exposed to saturated carbon dioxide concentrations and the extent of incorporation of labelled ¹⁴C was determined as a measure of potential photosynthetic rate. Three leaf-lets from each genotype were present in the assimilation chamber at the same time. Since, all the genotypes were exposed to similar condition in terms of RH, light intensity and carbon dioxide concentration. Three such experiments were conducted.

Experiment-II: Effect of moisture stress on potential photosynthetic rate

The effect of moisture stress on photosynthetic rate was determined in four *Lycopersicon* species/genotypes namely (a) Pusa Ruby (b) 987 *L. esculentum* var. *cerasiforme* (c) 990 *L. peruvianum* (d) 1301 *L. pimpinillifolium*.

The leaflets from the fully expanded third leaf were brought to the laboratory from well watered plants. These leaflets were subjected to stress under laboratory conditions.

At 100, 80, 70 and 50 and 40 per cent tissue moisture content the potential photosynthetic rate was assessed. At each time, the leaf material with different tissue water status of a single genotype was exposed to saturated carbon dioxide concentrations in the assimilation chamber. Therefore the differences in assimilation rate was mainly due to differences in tissue water status because the RH, temperature, light intensity and carbon dioxide concentration was same. The absolute values for different *Lycopersicon* genotypes are not comparable because, at a time only one genotype was studied.

The effect of stress on the potential photosynthetic rate was expressed as the per cent decrease over the turgid leaf.

$$\text{Per cent reduction in potential photosynthetic rate at a given tissue water status} = \frac{\text{cpm g dry weight in stressed tissue}}{\text{cpm g dry weight in turgid tissue}} \times 100$$

3.3.4.2 Effect of moisture stress on protein synthesis

Protein synthesis was studied by determining ^{14}C leucine incorporation into protein (Dhindsa and Bewley, 1976 and Bewley et al., 1983).

The extent of ^{14}C incorporation was studied in plant tissues during the rehydration period after subjecting them to stress using an osmoticum PEG (6000 MW). By using this technique the protein synthesis ability was studied in four *Lycopersicon* species namely (a) Pusa Ruby (b) 987 *L. esculentum* var. *cerasiforme* (c) 990 *L. pruvianum* (d) 1301 *L. pimpinillifolium*.

About 500 mg of leaf punches were subjected to stress by floating them in PEG (-1.5 and -3.0 MPa) for a period of 18 hours. The leaf discs were washed thoroughly and rehydrated in water for a period of 1 hour, and then transferred to an incubation medium (Tris-HCl buffer, pH 7.0 containing 0.3 mM leucine (leucine + ^{14}C leucine). Required amount of ^{14}C leucine was added to get the activity of $1.5 \mu\text{Ci ml}^{-1}$ of incubation medium.

At the end of the 3 hour incubation period the leaf discs were thoroughly washed and the supernatant was extracted in buffer. The proteins were precipitated in the supernatant by TCA. The radioactivity in all the three fractions (the residue, the precipitated protein, and the remaining supernatant) was determined to arrive at the total uptake. The precipitable protein content was also determined by using the FCR method (Lowry *et al.*, 1953).

From this primary data, the following aspects were computed.

- (a) Total uptake of labelled ^{14}C leucine (cpm). It is the total activity recorded in residue, precipitated protein, and supernatant.
- (b) Total incorporation of label into protein. It is total activity in precipitated protein.
- (c) Incorporation of label per mg protein ;

$$\frac{\text{Total incorporation}}{\text{Protein content in mg}}$$

- (d) Incorporation as per cent of uptake ;

$$\frac{\text{Total incorporation(b)}}{\text{Total uptake (a)}} \times 100$$

- (e) Incorporation per mg protein as per cent of uptake

$$\frac{\text{Incorporation of label/mg protein(c)}}{\text{Total uptake(a)}} \times 100$$

3.3.5 Studies on membrane integrity

The loss of membrane integrity in leaf tissue subjected to moisture stress was studied in four Lycopersicon genotypes, 987, 990, 1301 and Pusa Ruby. The leaf punches from the turgid leaves were subjected to live wilting under laboratory conditions and the decrease in fresh weight was maintained by periodical weighting and at 75 and 50 per cent loss of fresh weight, the membrane integrity was studied.

To indicate the extent of membrane damage in tissues subjected to moisture stress measurements on the leakage of solutes, absorption at ultraviolet wavelength 273 nm was determined following the method of Leopold et al. (1981).

Procedure

A known number of leaf discs or sections from leaves of plants subjected to moisture stress were incubated in beakers containing 10 ml of distilled water. They were allowed to leak their cell contents into the bathing medium for three hours. A three hour

period was taken as optimum as much of the solutes absorbing in the ultraviolet ranges leaked out by then.

After three hours of incubation the absorbance of bathing medium at 273 nm was estimated using an UV-Double Beam Spectrophotometer (Shimadzu-UV 2005). This was referred to as the initial absorbance due to the leakage of solutes from the tissue, following this, the contents of the beaker were incubated in a hot water bath 100 °C for 15 minutes. After suitable dilutions, the absorbance of the bathing medium was once again taken to indicate the final absorbance. The final absorbance is due to the total solutes contained in the tissues. The percentage leakage of solutes was calculated as;

$$\begin{array}{l} \text{Per cent leakage} \\ \text{of substances} \\ \text{absorbing at} \\ \text{273 nm} \end{array} = \frac{\begin{array}{l} \text{Initial absorbance of the} \\ \text{bathing medium} \end{array}}{\begin{array}{l} \text{Final absorbance at the} \\ \text{bathing medium} \end{array}} \times 100$$

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The main objective of the present investigation was to study the differences in several adaptive mechanisms for drought resistance in a few genotypes of *Lycopersicon*. The genotypes studied in the present investigation were:

1. 990 - *Lycopersicum peruvianum*
2. 1301 - *L. pimpinillifolium*
3. 1086 - *L. aufretut*
4. 987 - *L. esculentum* var. *Caroseforme*
5. 1048 - *L. esculentum*
6. 564 - *L. esculentum*
7. Pusa Ruby - *L. esculentum*

Species 990, 987 and 1301 are wild types and have been reported to possess certain drought resistant characteristics. The *Lycopersicum esculentum* genotype 564 is a local collection and 1048, 1086 were selections from germplasm material and on an empirical basis, showed a certain degree of resistance (personal communication). The local cultivated variety, Pusa Ruby has a very wide adaptability and is being cultivated both as irrigated and rainfed crop. This local variety, Pusa Ruby is included in all the experiments as a check variety to compare the other genotypes studied with regards to their adaptive mechanisms. Although 987, 564 and Pusa Ruby belong to

Lycopersicum esculentum, significant variation in the morphological characteristics, exists between these genotypes. In the foregoing chapters these species, L. peruvianum, L. pimpinellifolium, L. aufretut and different genotypes of L. esculentum are all referred to as Lycopersicon genotypes.

In this study differences in a few important adaptive mechanisms were examined amongst the Lycopersicon species and a few genotypes of Lycopersicum esculentum.

1. WUE as an avoidance mechanism
2. Root characters and water extraction efficiency.
3. Non-stomatal rate of water loss.
4. The intrinsic tolerance mechanisms based on extent of survival under stress water relations and other biochemical parameters.

4.1 Water use efficiency

In rainfed areas where stress is not severe enough to pose a problem for survival, drought avoidance characteristics may have a greater relevance. Under such conditions, the productivity predominantly depends on water extraction and its utilization efficiency. Therefore the plant avoidance mechanisms like root characteristics and physiological

and biochemical characteristics associated with WUE are important.

Keeping this in view, several experiments were conducted to assess the differences in WUE amongst these selected species of *Lycopersicum* and genotypes of *L. esculentum* with distinct growth habits. Although, the WUE is genotypic specific, the environmental factors also may alter the WUE. An attempt was made in this investigation to assess the variation in WUE among these genotypes under varying environmental conditions.

WUE in general terms is defined as the amount of dry matter produced per unit amount water transpired and often it also expressed as transpiration quotient (TQ) or transpiration ratio or transpiration efficiency. At the canopy level under field conditions, WUE is determined by the ratio between evapotranspiration (ET) and also dry matter produced. The major drawback in these field studies is to delineate the 'E' and 'T' from 'ET' problems associated with extraction of root system which otherwise contributes to the total dry matter. Therefore a better analysis of WUE at the whole plant level can be obtained through container studies despite the fact that the limitation is inadequate soil volume affecting in turn, the full expression of genotype.

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In this present investigation experiments on WUE were carried out in battery containers made of carbonized rubber. By adopting a gravimetric technique the daily water loss through ET was computed. In this technique, the actual amount of water lost in transpiration is determined by weighing the pots with plants.

4.1.1 Experiment-I: Differences in intrinsic WUE in genotypes of *Lycopersicon* (Summer season)

To assess the differences in WUE the DM and CWT were determined in pot grown plants from 30 to 53 days after planting.

Leaf area and leaf area duration (LAD)

There was significant difference in initial LA between the species, suggesting that initial growth rates were different in these genotypes. Genotypes 1086 and 987 recorded higher initial leaf area compared to others. However, the differences in the final leaf area between the genotypes was not very marked except in genotype 990 (Table 1).

The LAD, is a reflection of the functional leaf area available for transpiration and assimilation during the experimental period, Except in 990 the differences in LAD amongst the other genotypes was not significant.

Table 1. Leaf area, functional leaf area (LAD), total dry matter accumulation and cumulative water transpired (CWT) per pot during experimental period in Lycopersicon species under nonstress conditions (Summer, 1988).

Genotypes	Initial LA (cm ²)	Final LA (cm ²)	LAD dm ² day ⁻¹	Initial DM (g)	Final DM (g)	Δ DM (g)	Δ DM shoot (g)	CWT(ml)
Pusa Ruby	69a	4329a	505.8a	0.285c	53.62c	53.34c	48.04c	22840c
1048	271b	4428a	540.4a	1.305b	64.47ab	63.16ab	49.94bc	23079c
990	167c	2125b	363.6b	0.662c	24.00d	23.29d	12.95d	6290d
564	144cd	4795a	568.0a	0.489c	70.71a	70.22a	59.74a	24356c
1086	444a	4408a	558.0a	1.989a	65.81ab	63.82ab	55.23ab	25396c
1301	160c	4438a	528.8a	0.678c	60.87bc	60.19bc	50.22bc	29842b
987	369a	5053a	623.6a	1.348b	72.00a	70.70a	61.29a	33200a
CD at 5%	87.83	1636.85	121.71	0.597	9.18	9.290	6.170	307159

Dry matter accumulation

The initial and final DM showed significant variation amongst the genotypes. At final sampling, dry matter accumulated in root and shoot was assessed separately. Based on the initial and final DM differences, the shoot DM and TDM were calculated.

In this experiment species 987, 1086, 564 and 1048 recorded higher shoot and total DM compared to other genotypes. The shoot DM and the TDM recorded in 990 was significantly less than other genotypes.

Root characters

One of the plant characters associated with water harvesting is root volume and its activity. Since, water harvesting is an important avoidance mechanism, an attempt was also made to study the differences in root volume and biomass among the species. The objective is to identify species with greater root proliferation and higher root to shoot ratios. Keeping this in view, an attempt was made to carefully extract the root system and record root biomass while taking the final biomass observations. The data on the root biomass, shoot to root ratio and the per cent root biomass to TDM are given in Table 2.

The root biomass showed marked variation amongst the species. Highest root biomass was recorded

Table 2. Root dry weight and root/shoot ratios among Lycopersicon sps.
(Summer, 1988).

Genotypes	Pusa Ruby	1048	990	564	1086	1301	987	CD at 5%
Root dry weight(g)	5.30f	13.22a	8.34e	10.48b	8.59de	9.97bc	9.41cd	0.98
Root/shoot ratio	0.11	0.26	0.55	0.17	0.15	0.19	0.15	-

in 1048 and least in Pusa Ruby. In genotypes 564, 1086, 1301 and 987, the root to shoot ratios did not differ markedly and ranged from 0.154 to 0.199. However, highest root/shoot ratio was recorded in 990 followed by 1048. In Pusa Ruby not only was the root biomass less but also the root to shoot ratio was lowest.

Cumulative water transpired (CWT)

Though the differences in LAD was not significant amongst the species except for 990, the CWT were significantly higher in 987 compared to other species. Even in species 1301 also the CWT was high. There was no significant difference in CWT between the genotypes Pusa Ruby, 1048, 564 and 1086. In genotype 990 which recorded the lowest LAD, the CWT was also lowest. The data on CWT suggest that there were significant differences in mean transpiration rate amongst the species studied.

Water use efficiency (WUE), rate of water loss and LAD/DM ratios

In this experiment plants were maintained at near field capacity by irrigating the pots twice a day and giving the required input of water after determining the cumulative water transpired gravimetrically. Based on the CWT and DM, the intrinsic WUE differences were computed for all the

genotypes. In all the experiments WUE was computed based on shoot DM and TDM. Since, the root DM contributes to the TDM it is appropriate to compute WUE based on TDM. Hence the WUE arrived at based on TDM alone was considered while discussing the differences in WUE.

WUE values showed significant variation amongst the species. Highest WUE was recorded in 990 (3.7 g DM/kg H₂O) water - Table 3). However, the range in WUE in the remaining six species was 2.02 to 2.88 g DM/kg water. Amongst these species 564 and 1048 showed a significantly higher WUE than Pusa Ruby, 987 and 1301, The lowest WUE was recorded in 1301. Although the TDM recorded was highest in 987, the amount of DM produced per unit water transpired was not high in this genotype. The data obtained on intrinsic WUE under non-limiting water available conditions suggests that species differ in this character and the absolute growth rates are not associated with the absolute differences in WUE.

Rate of water loss

One of the important physiological characters associated with WUE is the transpiration rate. In all the experiments conducted on WUE by adopting gravimetric technique, accurate data on the total water transpired was collected. Therefore it was

Table 3. Intrinsic transpiration quotient (TQ) and water use efficiency (WUE) based on actual water used, rate of water loss and LAD/ DM ratio, in Lycopersicon species under non-stressed conditions (Summer, 1988).

Geno- types	TQ ₋₁ (ml g ⁻¹)	WUE ₋₁ (g DM kg ⁻¹ H ₂ O)	Rate of water loss (ml H ₂ O dm ⁻¹ d ⁻¹)	LAD/DM ratio	LAD/shoot DM ratio
Pusa- Ruby	428ab	2.34cd	45.16bc	9.48a	10.53a
1048	365bc	2.74bc	42.71c	8.56a	10.82b
990	270d	3.70b	17.29d	11.32a	17.63a
564	347c	2.88c	42.88c	8.09a	9.51b
1086	398bc	2.51a	45.51bc	8.74a	10.10b
1301	496a	2.02c	56.43a	8.79a	10.53b
987	470ab	2.13c	53.24ab	8.82a	10.17b

CD at 5%	73.04	0.417	8.59	4.13	5.95

possible to compute rate of water loss from the primary values on CWT and LAD. The CWT/LAD (rate of water loss) is an accurate reflection of the mean transpiration per unit leaf area over the experimental growth period. The differences if any due to diurnal variations and due to differences between the leaves are taken care of when the rate of water loss was computed from CWT and mean functional leaf area.

In this important physiological parameter also genotypes differed significantly. The rate of water loss was very less in 990 and is significantly different from the other species. Even amongst the other six species, a wide range in CWT, from 42.71 ml $\text{dm}^{-2} \text{day}^{-1}$ to 56.43 ml $\text{dm}^{-2} \text{day}^{-1}$ was recorded. The rate of water loss was significantly high in 1301 compared to the other species. In this experiment especially amongst the six species, the rate of water loss was the major factor for the observed differences in CWT.

LAD/DM ratio

This ratio indicates the functional leaf area required for the production of unit biomass. The lower the ratio, the higher the assimilation rate and vice versa. This parameter is a reflection of mean assimilation rate of all the leaves for the experimental growth period. The mean net assimilation rate assumes greater significance when the differences

in functional leaf area for the crop growth period between the genotypes are marginal.

It is interesting to note that in this experiment, the difference in LAD/DM ratios were not significant amongst the species. Similar trend was also observed when mean assimilation rate was computed based on the LAD and shoot biomass. It is therefore evident that the observed differences in WUE were mainly due to the variations in the rate of water loss.

Relationship between physiological parameters and WUE

In this experiment, the relationship between the physiological characters like LAD/DM, rate of water loss with WUE was studied. One interesting observation was that genotypes with mean high assimilation rate as reflected by LAD/DM values also had high rate of water loss. A significant negative relation was seen between LAD/DM and rate of water loss ($r = -0.749$ - Table 4).

The LAD and rate of water loss showed a highly negative significant relationship with WUE. However, the LAD/DM did not show a significant relationship with WUE. Therefore it can be inferred that the differences in intrinsic WUE were due to variations in rate of water loss. Genotypes with low rate of water loss 990 and 564 showed significantly higher WUE compared to other genotypes.

Table 4. Correlation values showing relationship between WUE with different physiological parameters (Summer, 1988).

Physio-logical parameters	LAD	DM	DM shoot (g)	LAD/DM shoot	Rate of water loss
WUE	-0.804*	-0.726NS	-0.774	0.812*	-0.975**

Physiological parameters	LAD/DM	DM	Physiological parameters	WUE based on SDM	
CWT/LAD	-0.749*		WUE based on TDM	0.822*	

From the results obtained from this experiment it can be concluded that:

1. Significant variation in intrinsic WUE exist amongst Lycopersicon species when grown under irrigated conditions.
2. Even amongst the six species (Pusa Ruby, 1048, 564, 1086, 1301 and 987) which showed a similar growth rate in this experiment, intrinsic WUE showed significant variations.
3. Amongst the six species (apart from 990) the genotypes with high WUE were 564, 1048 and 1086.
4. TDM, a reflection of absolute growth rate did not show any difference amongst the species. The observed differences in WUE were mainly due to differences in rate of water loss. Genotypes with low rate of water loss showed high WUE.
5. In this experiment in general, the rate of water loss was high in all the genotypes. It can be mainly attributed to the environmental factors prevailing during experimental growth period. The experiment was conducted during summer season during which mean maximum temperature, light intensity and VPD were relatively high (Appendix I).

4.1.2 Experiment-II: The differences in WUE in Lycopersicon species during monsoon conditions

One of the characteristic features of monsoon season is low light intensity, relatively high RH, low minimum mean temperature. Amongst these variables, the reduction in light intensity compared to rainfree summer season is more marked and often considered as a constraint for higher crop growth rates. Since, the light has direct effect both on stomatal and mesophyll characters of the leaves, low light may affect the WUE significantly. However, species with differential adaptations to low light may also differ in their WUE characteristics. Since, the major cropping season is monsoon, an attempt was made in the present investigation to study the difference in WUE during monsoon low light conditions and compare with rainfree summer conditions.

The experiment was conducted in a rain shelter to avoid the interference of rain during the experimental growth period. Inside the rain shelter the light intensity was less, approximately by 30 to 40 per cent. But the variations in ambient RH and temperature are marginal.

The plants were raised in battery containers and the intrinsic WUE at whole plant level under adequate water input was studied by adopting

gravimetric technique as described in the previous experiment. For computation of WUE, the dry matter, CWT were recorded from 30 to 53 days after planting (24 days duration).

Leaf area and LAD

The initial leaf area recorded on 30th day after sowing showed significant difference among the genotypes emphasising that even in monsoon season also initial growth rates varied amongst genotypes (Table 5).

The final leaf area also showed significant variation. Genotypes 1301, 1086 and 546 had significantly higher leaf area compared to other genotypes. The LAD was also significantly high in these species. Low LAD values were recorded in Pusa Ruby and 990.

DM accumulation

The TDM showed a trend very similar to the final DM. Amongst the genotypes 1048 showed higher total biomass and also the shoot biomass. Pusa Ruby, 990 and 1086 recorded relatively less biomass during the experimental growth period.

Table 5. Leaf area, functional leaf area (LAD), total dry matter accumulation and cumulative water transpiration (CWT) (per pot) during the experimental period in Lycopersicon species under non-stressed condition during the monsoon season (1988) under light limiting conditions.

Genotypes	Initial LA (cm ²)	Final LA (cm ²)	LAD dm ² day ⁻¹	Initial DM (g)	Final DM (g)	Δ DM (g)	Δ DM shoot (g)	CWT(ml)
Pusa Ruby	368ab	2849bc	386bc	2.17a	31.77abc	29.60b	24.75bc	11782bc
1048	212c	3379abc	430ab	1.15bc	36.76a	35.61a	30.16a	13138a
990	73d	2602c	312c	0.38d	27.32c	26.94b	22.31bc	10706c
564	449a	4293a	569ab	2.09a	34.07ab	31.98ab	26.62ab	12165ab
1086	318b	4173ab	539ab	1.26bc	29.40bc	28.14b	22.01c	8115d
1301	415a	4960ab	645a	1.46b	33.84ab	32.38ab	26.85ab	7412d
987	201c	4084ab	514ab	0.86c	32.20abc	31.34ab	25.55dc	12658ab
CD at 5%	84.97	1199.34	183.3	0.437	6.05	5.93	4.58	1283.64

Root characteristics

At the end of the experiment roots were carefully extracted to determine root biomass (Table 6). The range in root biomass was from 4.09 g plant to 6.64 g plant. Highest root biomass was recorded in 987 and least was in 990. Root to shoot ratios also showed a considerable variation amongst the species. In genotypes 987 which showed highest root biomass also showed highest root to shoot ratio (0.260). The root to shoot ratios were relatively less in 564 (0.150) and 990 (0.180). Although the total root biomass recorded in monsoon low light conditions was less, the partitioning of biomass to root was not markedly affected under low light conditions. However, genotypic differences exist in this regard.

Cumulative water transpired

The duration of the experiment was almost same as that of summer experiment, the CWT was less. It ranged from 7412 in 1301 to 13,138 ml 1048. The mean CWT value recorded in summer experiment was 23,572 ml. However, in this experiment it was only 10,854, thus recording 54 per cent reduction in CWT in this experiment. Though LAD was more in some genotypes like 1301 and 1086, the CWT was significantly less compared to other genotypes. For example the LAD

Table 6. Root dry weight and root/shoot ratios among Lycopersicon sps. during monsoon, 1988.

Genotypes	Pusa Ruby	1048	990	564	1086	1301	987	CD at 5%
Root dry weight(g)	4.85cd	6.45ab	4.09d	4.16d	5.13bcd	5.79abc	6.64a	1.41
Root/shoot ratio	0.196	0.22	0.18	0.15	0.22	0.21	0.26	-

recorded in 1086 and 564 were same. However, the CWT values were 8,115 and 12,165 ml, respectively.

The lack of relationship between functional leaf area and CWT indicates that under low light conditions, there was significant difference in rate of water loss. This suggests that the environmental factors differentially affected the leaf expansion rates and stomatal behaviour of species. Both 1301 and 1086 had better adaptation to low light conditions with high leaf expansion rate and low transpiration rates.

WUE, rate of water loss and LAD/DM ratios

The WUE was significantly higher in 1301 and 1086 compared to other genotypes (Table 7). In the remaining 5 genotypes, the differences in WUE were not significant. The range in WUE in these 5 genotypes was from 2.48 to 2.71 g DM/kg H₂O where as the WUE values recorded in 1086 and 1301 were 3.47 and 4.37 respectively. This clearly indicates that under low light conditions particularly in rainy season genotypes 1086 and 1031 showed better adaptation and higher WUE.

Amongst all the growth characteristics and physiological parameters associated with WUE, maximum differences were observed in rate of water loss. The

Table 7. Intrinsic transpiration quotient (TQ) and water use efficiency (WUE) based on actual water used and LAD/ DM ratio in Lycopersicon species under control condition during monsoon season under light limiting conditions.

Geno- types	TQ ₋₁ (ml g ⁻¹)	WUE ₋₁ (g DM kg ⁻¹ H ₂ O)	Rate of water loss ₂ (ml H ₂ O dm ⁻² d ⁻¹)	LAD/DM ratio	LAD/shoot DM ratio
Pusa Ruby	398a	2.50c	30.52ab	13.04bc	15.60b
1048	369ab	2.71c	30.55ab	12.08bc	17.57ab
990	397bc	2.52c	33.35a	11.92c	14.39b
564	380a	2.63c	21.38b	17.79ab	21.37ab
1086	288cd	3.47b	15.06c	19.15a	24.49a
1301	229d	4.37a	11.49c	19.92a	24.02a
987	404a	2.48c	24.63b	16.40abc	20.12ab
CD at 5%	68.99	0.494	7.34	5.82	7.29

range was from 11.49 in genotype 1301 to 33.35 ml dm⁻² day⁻¹ in genotype 990. Even in the species 1086 the rate of water loss was significantly less compared to other Lycopersicon genotypes. Therefore the major contributing factor for low CWT in 1301 and 1086 was low rate of water loss.

The differences in LAD/DM ratios were not as marked as the observed differences in rate of water loss. Mean LAD/DM ratios were high in 1301 and 1086 indicating that mean net assimilation rate in these two genotypes was less. Genotypes 990, 1048 and Pusa Ruby recorded low LAD/DM ratios.

Though LAD/DM values were high in both the genotypes 1301 and 1086 the WUE was significantly high mainly because of considerably low mean transpiration rates. For example, the LAD/DM ratios in these two species was 64.9 and 58.5 per plant higher than 1048. However, the rate of water loss in these two genotypes was 62.4 and 50.7 per cent less compared to 1048. Hence, in these two genotypes WUE was significantly high than genotype 1048.

Relationship between physiological parameters and WUE

When the plants were grown under low light conditions, there was a highly negative significant relationship between LAD/DM ratios and rate of water loss indicating that genotypes with higher mean

transpiration rate also had higher assimilation efficiency (Table 8).

In this experiment also the WUE was computed both based on CWT and TDM and CWT and shoot biomass. There was a highly positive significant relationship between the WUE arrived from TDM ($r = 0.993$) and WUE arrived from shoot biomass.

Neither LAD nor the TDM was related to the WUE. However, the rate of water loss showed highly significant negative relationship with WUE.

A positive significant relation between LAD/DM and WUE ($r = 0.755$) suggests that genotypes with low net assimilation rate showed higher WUE. This signifies that under low light conditions, the reduction in transpiration rate is a better adaptive mechanism to achieve high WUE.

From the results obtained in this experiment, it can be concluded that;

1. The intrinsic WUE even under low light monsoon conditions showed significant variations. However, only two genotypes 1086 and 1031, were better adapted to low light conditions and hence recorded higher WUE values compared to other genotypes. In the remaining 5 genotypes, the differences in WUE were not significant.

Table 8. Correlation values showing relationship between WUE with different physiological parameters (monsoon season 1988).

Physio- logical parameters	LAD	DM	DM shoot (g)	LAD/DM	LAD/DM shoot	Rate of water loss
WUE	0.673NS	0.102	0.026	0.755*	0.739NS	+0.852*

Physiological parameters	LAD/	DM	Physiological WUE based parameters on SDM			
CWT/LAD	-0.973**		WUE based on TDM	+0.993**		

2. Amongst the growth characteristics and physiological parameters, genotypes differed significantly in the mean rate of water loss.

The range in the DM was from 26.9 to 35.61 g. in LAD/DM from 11.92 to 19.92 whereas in rate of water loss it was 11.49 to 33.35 $\text{ml dm}^{-2} \text{ day}^{-1}$.

3. A high negative significant relationship between rate of water loss and WUE further emphasizes that the differences in WUE was mainly due to observed differences in mean transpiration rates.

Comparative assessment of WUE and growth characteristics of *Lycopersicon* species grown in summer and monsoon

The results obtained in the first and second experiment indicate that the environmental factors prevailing during experimental growth period affect the physiological and growth parameters associated with WUE and hence WUE values. The mean rate of water loss, LAD/DM and WUE values (mean of all genotypes) in the summer experiment were $43.4 \text{ ml dm}^{-2} \text{ day}^{-1}$, 9.1 and $2.62 \text{ g/kg H}_2\text{O}$ respectively. The corresponding values in monsoon were 23.85, 15.76 and 2.95 (Fig.1). Although net assimilation rates decreased in monsoon low light conditions (as reflected by the higher

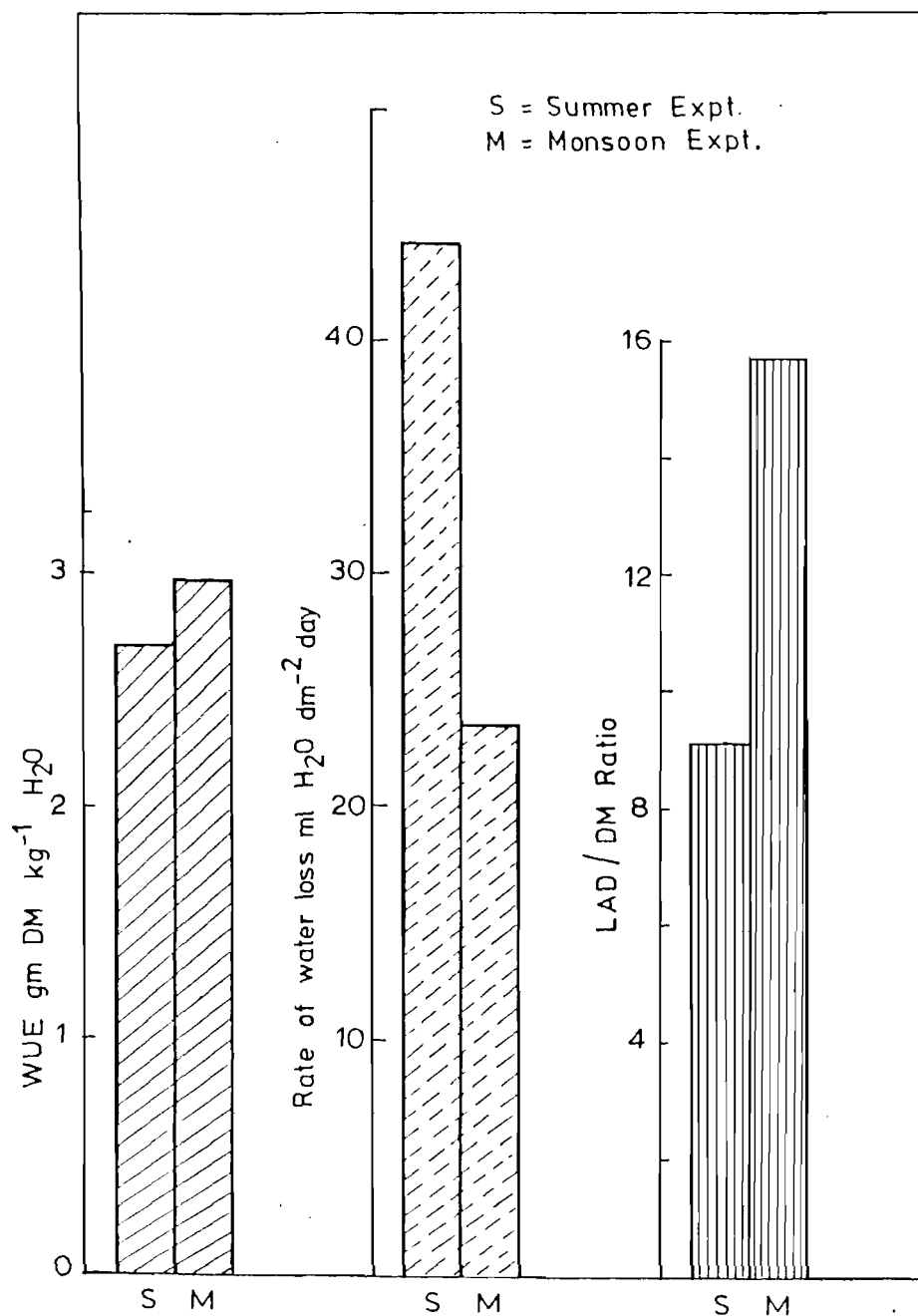


Fig.1: WUE, rate of water loss and LAD/DM ratio in Lycopersicon species in summer and monsoon seasons.

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LAD/DM ratios), because of reduction in rate of water loss, the WUE was 12.56 per cent higher.

The WUE and associated characters were compared for monsoon and summer experiment and the comparative data are given in Figures 2a, 2b, 2c and 2d. In all genotypes, the rate of water loss, mean net assimilation rate and DM were more in summer compared to monsoon experiment. In four genotypes, Pusa Ruby, 1048, 987 and 564 the differences in WUE between summer and monsoon seasons were very marginal. However, in genotypes 1301 and 1086, a significant increase in WUE was observed in the monsoon experiment. Only in genotype 990, a decrease in WUE was recorded under low light.

Therefore it can be inferred that the variations in environmental factors differentially affected the conductances and also the assimilation rate. Both the genotypes 1301 and 1086 showed better adaptation to low light where as adaptation of 990 was poor.

The major environmental factor which differed drastically between the two seasons was light intensity. These genotypes namely Pusa Ruby, 1301 and 990 showed differential response with regards to WUE. When grown under summer high light and monsoon low light conditions. Comparative data on WUE and physiological characteristics of these three genotypes are presented

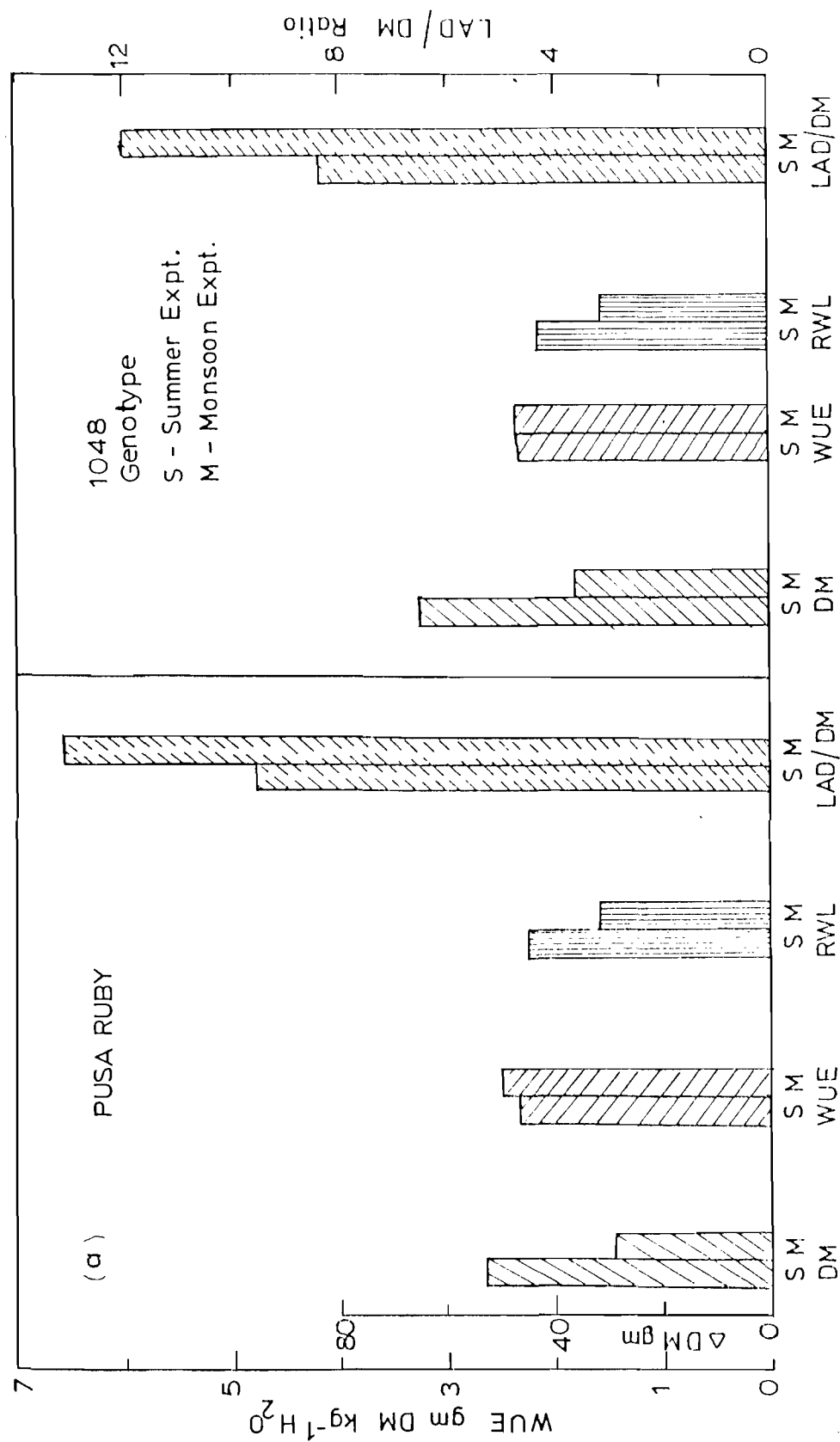
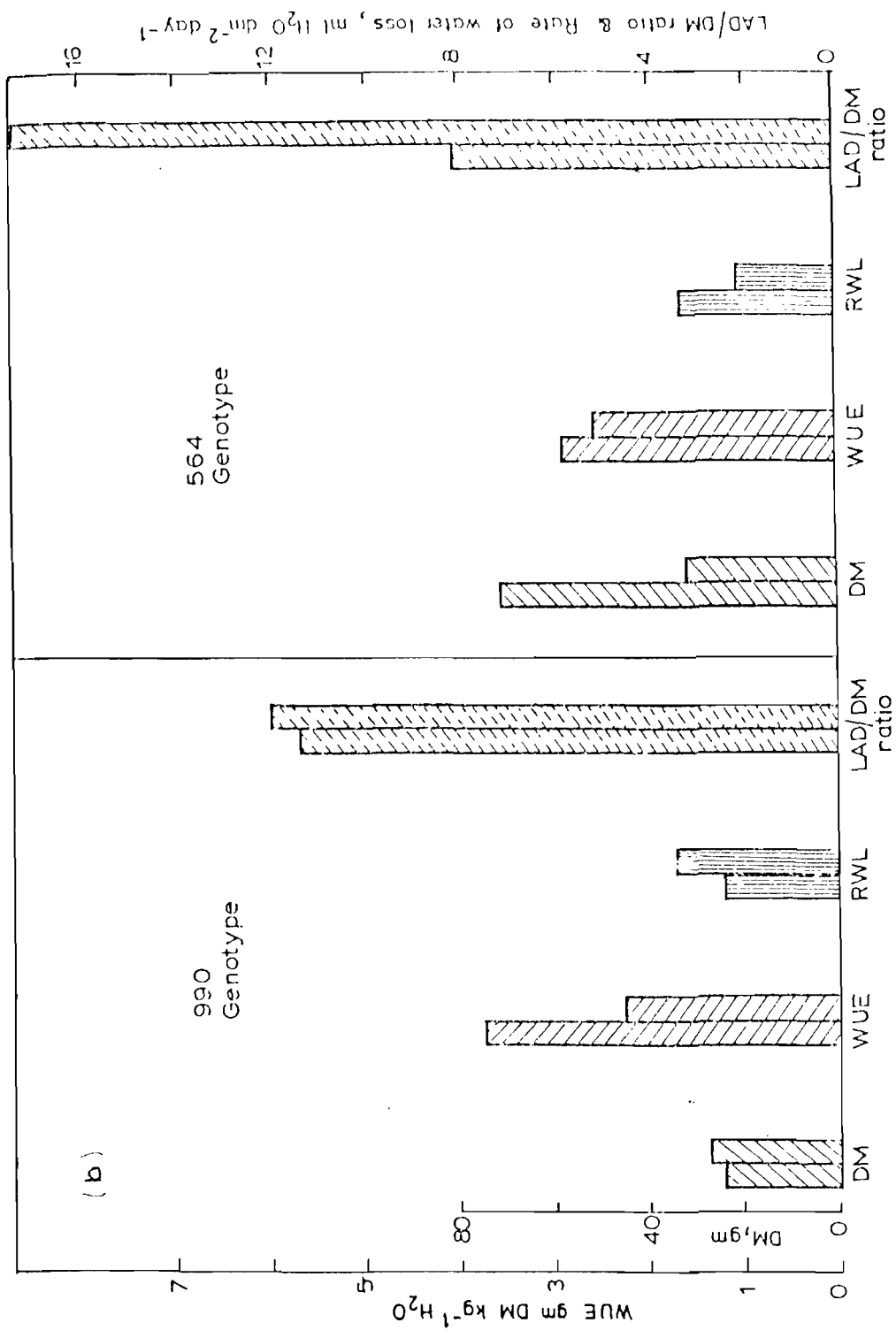
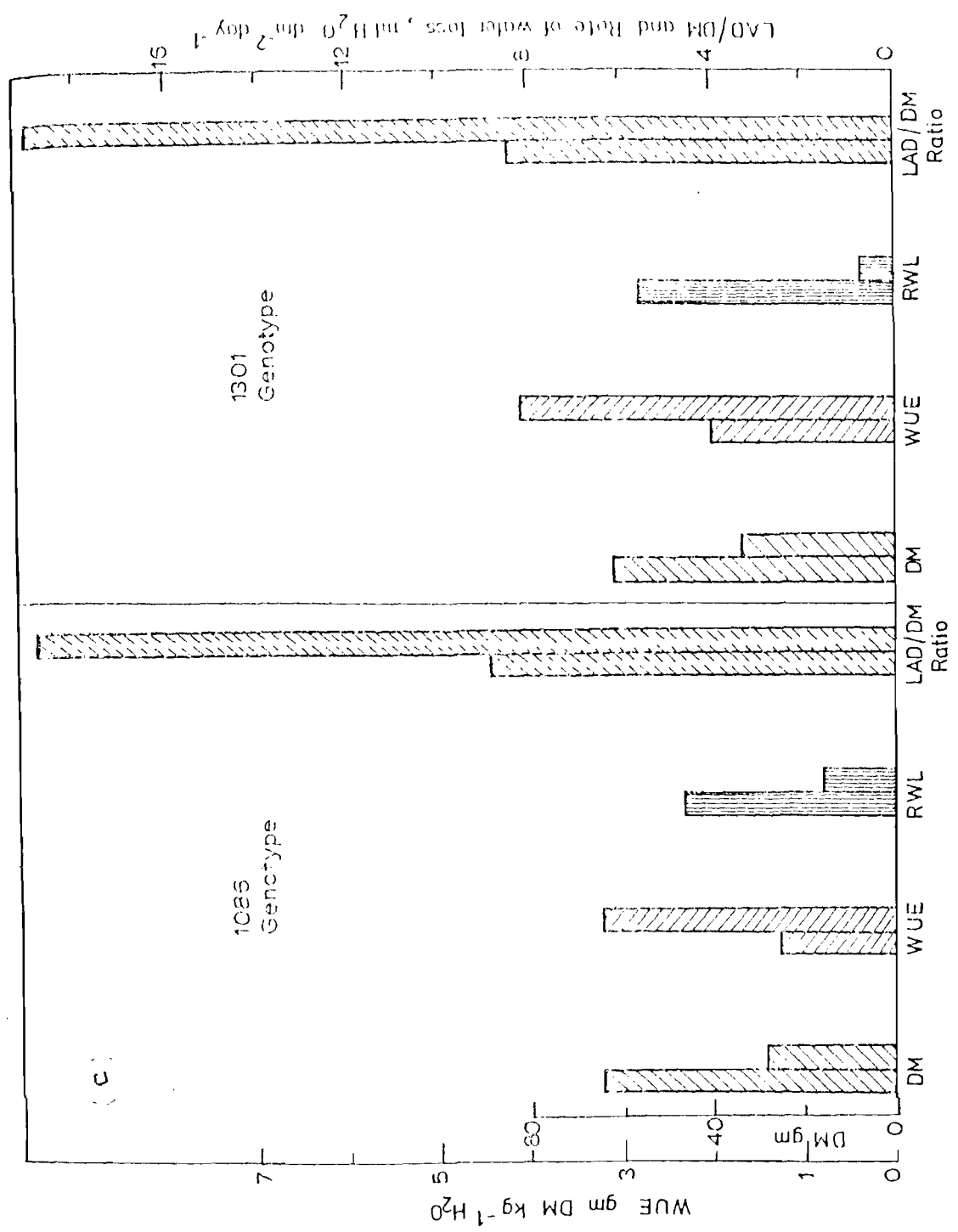
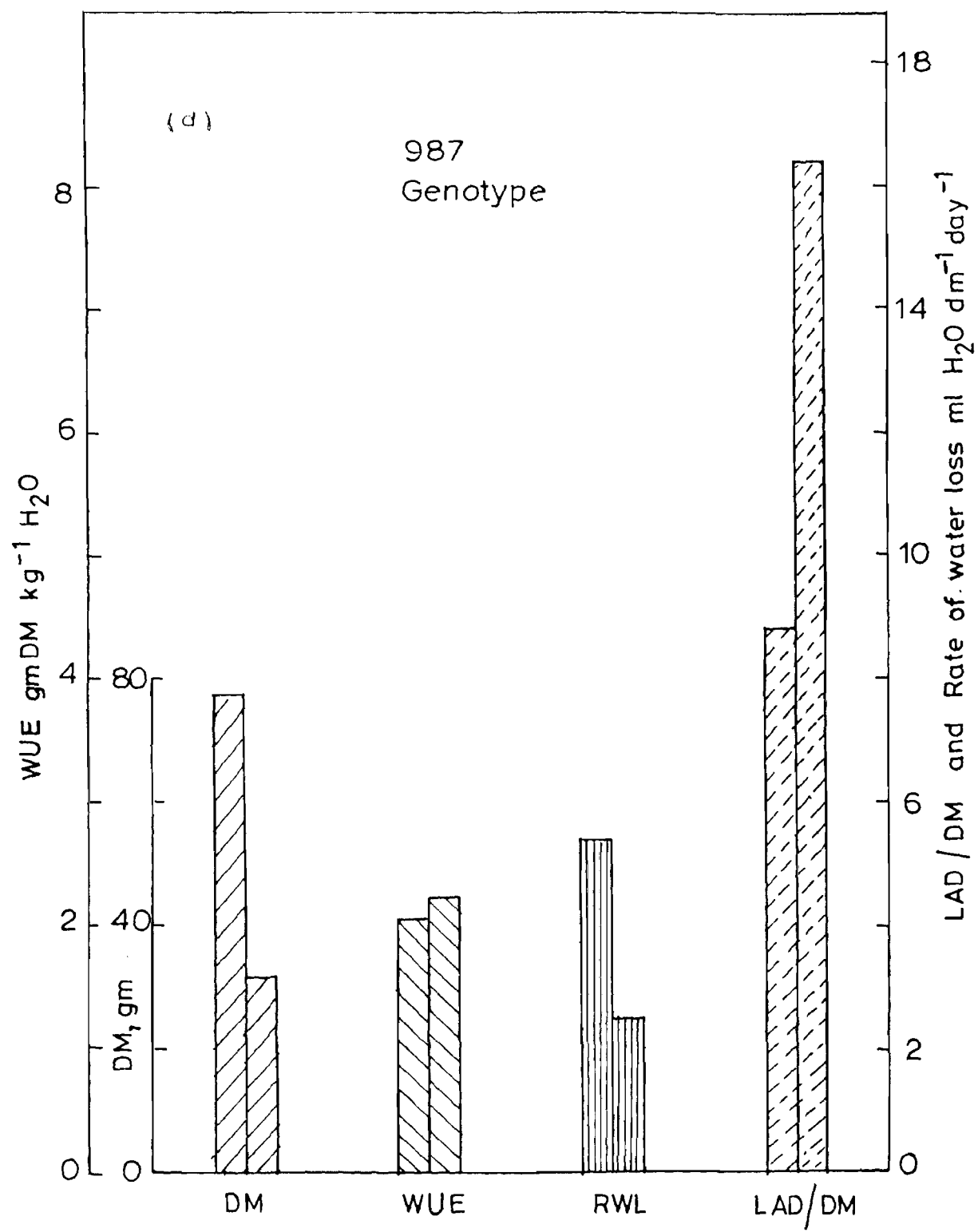


Fig.2a,b,c,d: Dry matter accumulation (DM), WUE, rate of water loss (RWL) and LAD/DM ratios under two light regimes during summer and monsoon seasons in seven Lycopersicon species.







in Table 9. In genotype 1301 a significant increase WUE in monsoon was seen compared to the summer high light conditions where as in 990 a significant decrease was noticed in WUE from 3.7 in summer to 2.5 in monsoon.

An analysis of growth characteristics clearly indicate that in genotype 1301 the per cent decrease in rate of water loss in monsoon compared to summer was 79.6 where as the per cent decrease in net assimilation rate was only 55.9 per cent. Hence, WUE was high in monsoon by 53.8 per cent. However, in 990 though there was significant decrease in net assimilation rate in monsoon, rate of water loss was relatively high in monsoon and hence resulted in a decrease in WUE. Therefore in species 1301 under low light the carbon gain per unit water transpired was higher but it was significantly less in 990.

Interestingly the carbon gain per unit water transpired (WUE) was nearly similar in both the seasons in Pusa Ruby.

To confirm the observed variations in WUE of these species as affected by the environmental factors mainly light intensity, another experiment was conducted with these three distinct genotypes.

Table 9. WUE and physiological characteristics of a few Lycopersicon species showing different adaptation in summer and monsoon.

	Full light (Summer)			Low light (monsoon)		
	Pusa Ruby	990	1301	Pusa Ruby	990	1301
TQ	428	270	495.8	398	397.4	228.88
WUE	2.34	3.70	2.02	2.50	2.52	4.37
Rate of water loss	45.16	23.86	56.43	30.52	33.35	11.49
LAD	505.8	363.6	528.8	386	321	645
LAD/DM	9.48	11.32	8.79	13.04	11.92	19.92

4.1.3 Experiment-III: Effect of light intensity on WUE in selected Lycopersicon species

To study the light adaptation of these three distinct species, one set of plants were grown under reduced light intensity. The reduction in light intensity was 40 per cent. The mean light intensity under low light regime was 199 cal/cm²/day where as it was 500 cal/cm²/day in high light regime. Though the light intensity under low light conditions was comparable with monsoon conditions, the RH was low but the temperature was relatively high compared to monsoon season.

Initially the plants raised in battery containers were grown under high light regime untill 20th day. One of the set was shifted to low light intensity (rain shelter) and the light treatments were continued for a period of 22 days. During this period the plants were maintained at near field capacity by adopting gravimetric method and CWT was determined.

In both the Pusa Ruby and 990 there was no significant difference in LAD between the low and high light regimes. But in genotypes 1301, the LAD increased substantially under low light recording an increase of 192 per cent compared to the high light regime (Table 10).

Table 10. Leaf area, functional leaf area (LAD), total dry matter accumulation and cumulative water transpired (CWT) (per pot) during experimental period in Lycopersicon species grown at two light regimes.

Genotypes		Initial LA (cm ²)	Final LA (cm ²)	LAD dm ² day ⁻¹	Initial DM (g)	Final DM (g)	ΔDM (g)	ΔDM shoot (g)	CWT(ml)
Pusa Ruby	LL	295	3298	467	3.2	24.86	21.66	17.47	4907
	FL	295	3468	489	3.2	34.20	31.0	24.14	9167
990	LL	154	2719	373	0.69	17.88	17.19	13.0	4500
	FL	154	2372	328	0.69	25.83	25.14	20.2	5600
1301	LL	428	5396	757	2.39	30.78	28.38	21.89	6587
	FL	428	1560	259	2.39	23.44	21.04	14.40	8267
CD at 5%	G	49.97	536.18	84.913	0.60	2.90	2.87	3.08	1614.11
	L	77.54	513.31	69.33	0.49	2.36	2.34	2.51	1313.92
	6xL	134.31	889.08	120.09	0.85	4.10	4.05	4.35	2282.70

FL - High light
LL - Low light

Both in Pusa Ruby and 990, the DM was significantly high in plants grown in full sunlight. The per cent increase in DM in full light was 43.1 and 46.2 in Pusa Ruby and 990 respectively whereas in genotypes 1301 which showed a greater adaptation to low light DM was more in low light treatment. Shoot DM also showed a similar trend in all the genotypes.

Root characters: In plants grown under high light regime, root biomass was more compared to the plants grown under low light regime. However, only marginal increase in root to shoot ratios was observed in plants grown under high light (Table 11).

Cumulative water transpired

The CWT decreased significantly under low light regime. However, the genotypic response was different. The decrease in CWT was 46.5, 19.6 and 20.3 per cent in Pusa Ruby, 990 and 1301 respectively (Table 12). In both Pusa Ruby and 990, the LAD did not show any significant difference between light regimes. However, CWT showed a substantial decrease indicating that under low light regime in these three genotypes also rate of water loss showed a decline. The interesting observation was in 1301 even with 192 per cent increase in LAD under low light, the CWT decreased by 20.3 per cent.

Table 11. Root dry weight and root/shoot ratios among three Lycopersicon species grown at two light regimes.

	Low light			High light		
	Pusa Ruby	990	1301	Pusa Ruby	990	1301
Root dry weight(g)	4.19	4.19	6.49	6.86	4.94	5.64
Root/shoot ratios	0.24	0.32	0.29	0.28	0.24	0.36

Table 12. Transpiration quotient (TQ), water use efficiency (WUE), based on actual water used, rate of water loss and LAD/DM ratios in Lycopersicon species at different light regimes.

Genotypes		TQ (ml g^{-1})	WUE ($\text{g DM kg}^{-1} \text{H}_2\text{O}$)	Rate of water loss ($\text{ml H}_2\text{O dm}^{-2} \text{d}^{-1}$)	LAD/DM ratios	LAD/DM shoot ratios
Pusa Ruby	LL	227	4.41	10.46	21.56	26.73
	FL	296	3.38	18.75	15.77	20.26
990	LL	262	3.82	9.13	21.71	28.69
	FL	223	4.48	12.98	13.15	16.24
1301	LL	232	4.31	8.70	26.61	34.58
	FL	393	2.55	31.92	12.31	16.82
CD at 5%	G	55.12	0.762	5.63	3.80	6.39
	L	45.01	0.626	4.60	3.10	5.15
	GxL	77.95	1.085	7.96	5.38	8.93

FL - High light

LL - Low light

WUE, rate of water loss and LAD/DM ratios

The data on rate of water loss indicates that in all genotypes there was a significant decrease in plants grown under low light regime. Even in this character the response of genotypes different. In 1301 which has better adaptation to low light, the rate of water loss was less.

Net assimilation rate decreased in low light in all the genotypes. However, the per cent decrease in net assimilation rate was less compared to the observed reduction in rate of water loss in Pusa Ruby and 1301. Hence, in these two genotypes an increase in WUE was recorded under low light. In genotype 990 the per cent reduction in net assimilation rate was more than the per cent reduction in mean transpiration rate and hence the WUE decreased in this genotypes under low light.

From the data of this experiment it can be concluded that genotype 1301 which showed greater adaptation to low light, there was a significant increase in leaf expansion rate and TDM under low light. More interesting aspect is that a marked decrease in rate of water loss resulting in a higher carbon gain per unit amount of water transpired. Such a trend was also observed when a comparison was made

between monsoon and summer experiments and also in plants grown under low and high light regimes.

In 990 which is adapted better for high light conditions, the WUE was more in summer compared to monsoon conditions. Even in the second experiment the WUE was high in high light regime. In 990, the sensitivity of stomata is less under low light and hence the rate of water loss did not decrease significantly. In genotype Pusa Ruby there was a marginal but non-significant increase in WUE under low light conditions. The increase in WUE though marginal was mainly due to a higher decrease in rate of water loss compared to the net assimilation rate under low light.

Gas exchange characteristics

The gas exchange parameters contributing for the intrinsic WUE at single leaf level, 'A' and 'gs' were studied in these three genotypes grown at high and low light conditions by using a Portable Photosynthesis System, ADC, LCA-2. The gas exchange characters were determined at growth light regimes, in fully expanded third leaf from the top. The light intensity under low light regime was $560 \mu \text{Ein m}^{-2} \text{sec}^{-1}$ where as it was $1400 \mu \text{Ein m}^{-2} \text{sec}^{-1}$ under high light regime. However, 'A' was determined at the same RH and VPD in leaf chamber. From the primary data on 'A'

Plate 1: Pusa Ruby L. esculentum, 990, L. peruvianum and 1301 L. pimpinillifolium, plants grown at two light regimes - High light (FL) and low light (LL).



and g_s , the A/g_s was computed and the data are given in Table 13.

At saturated light intensity, 'A' was highest in 990, followed by Pusa Ruby. 'A' was only $8.2 \mu \text{mol m}^{-2} \text{sec}^{-1}$ in 1301 compared to 15.6 in 990 indicating that at saturated light intensity P_{max} was relatively less in genotype 1301. However, the differences in g_s between these genotypes was marginal ranging from $145.9 \text{ m mol m}^{-2} \text{ s}^{-1}$ in 1301 to 189.5 in 990.

The A/g_s intrinsic WUE at single leaf level was high both in 990 and Pusa Ruby at saturated light intensities.

Under low light 'A' and ' g_s ' reduced in all the species. Highest 'A' at low light intensities was recorded in 1301 followed by 990. Though in 1301, the P_{max} at saturated light intensities was less, the 'A' was high under low light indicating that in this genotypes, the light saturation for 'A' is low. Although ' g_s ' decreased in all the genotypes, the per cent decrease in g_s was less compared to the observed decrease in 'A'. Hence, in all the genotypes, there was a decrease in A/g_s . However, amongst the genotypes, the highest A/g_s was recorded in 1301 and the least was 990. From the results of this experiment it can be concluded that;

Table 13. Gas exchange characteristics in three selected species of tomato differing in whole plant response in WUE to low light: Assimilation rate ($\mu\text{ mol m}^{-2}\text{ s}^{-1}$), stomatal conductance ($\mu\text{ mol m}^{-2}\text{ s}^{-1}$) and C_i (intercellular CO_2 concentration).

Genotypes	High light				Low light			
	A	gs	C_i	A/gs	A	gs	C_i	A/gs
Pusa Ruby	13.7	169.2	220	0.08	5.5	119.9	238	0.04
1301	8.2	145.9	230	0.05	7.9	156.0	248	0.05
990	15.6	189.5	187	0.08	6.9	173	254	0.04

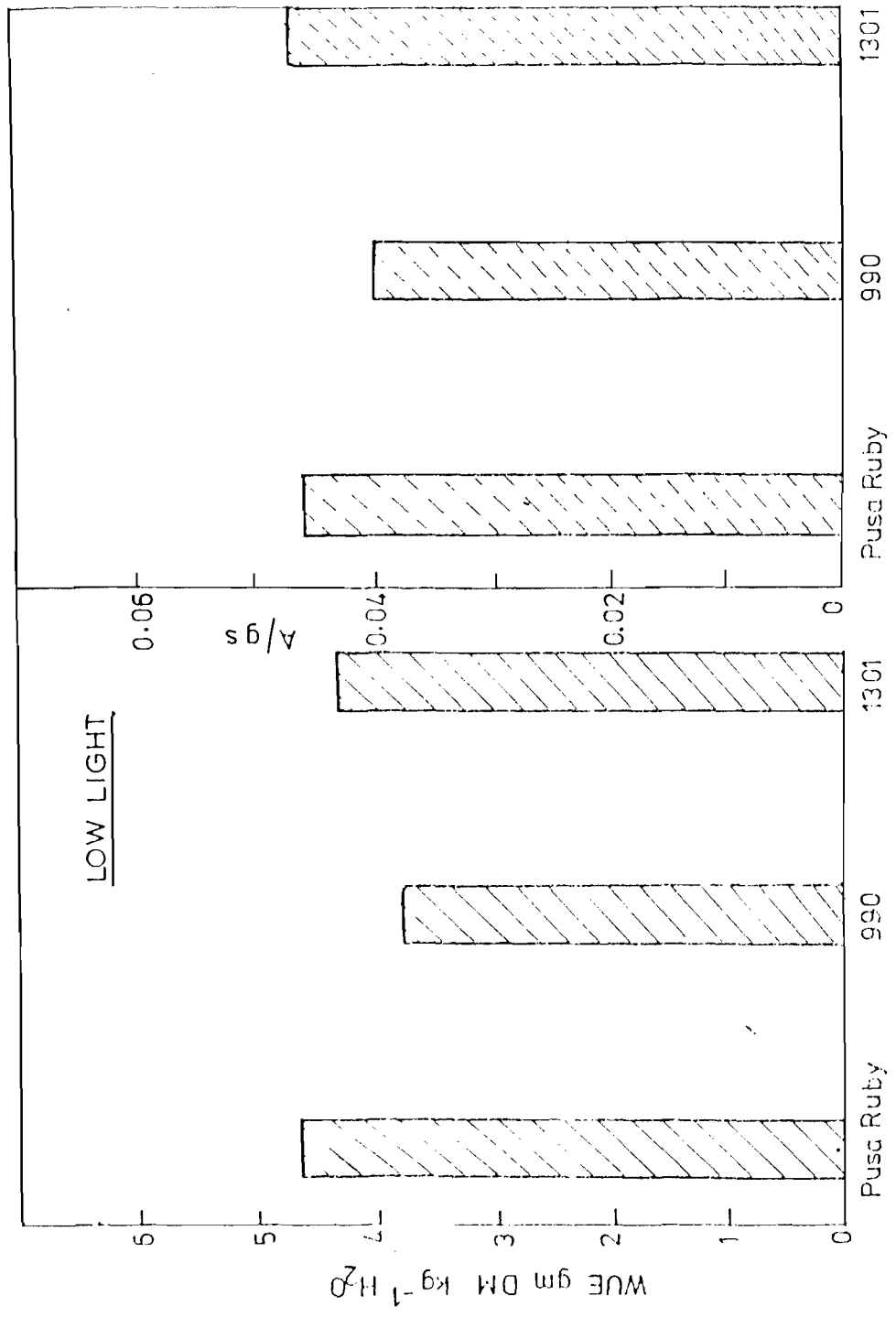
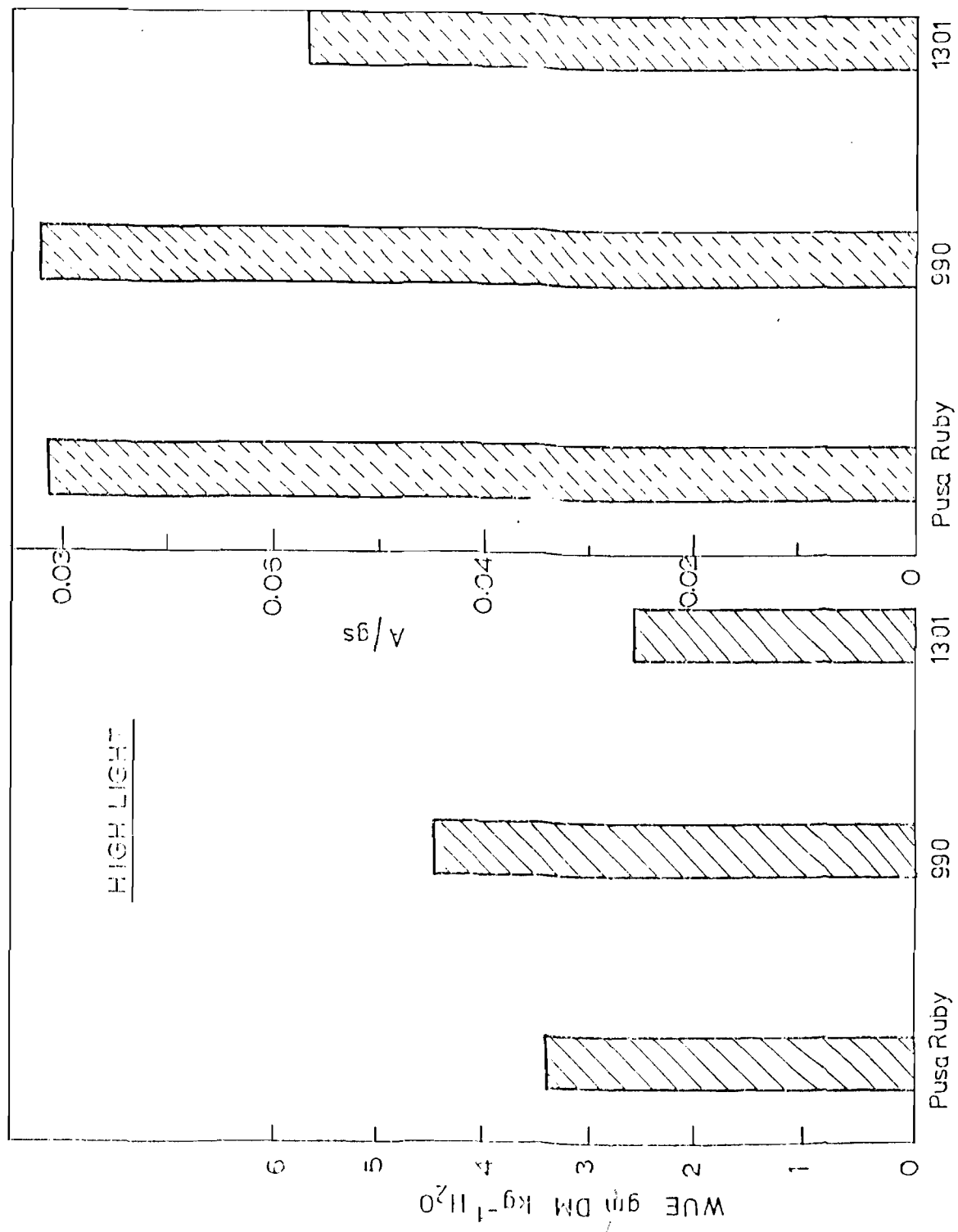


Fig.3a: WUE and A/gs amongst four Lycopersicon species under low light condition.



F19..3b: WUE and A/gs, amongst four Lycopersicon species under high light condition.

1. At saturated light intensities, the P_{max} and A/gs was high in 990 and lowest in 1301.
2. Under low light conditions, though 'A' decreased in all the genotypes, the per cent reduction compared to P_{max} was less in 1301.
3. Since, the reduction in gs was less compared to 'A' the A/gs decreased in all the genotypes under low light.
4. A/gs under low light was high in 1301 compared to other two genotypes.
5. The gas exchange traits were determined in fully exposed third leaf and it may not be a true reflection of the WUE at whole plant level. In 1301 the WUE at whole plant level was significantly high under low light when assessed by gravimetric method where as the A/gs , values were relatively less compared A/gs values at saturated light intensities.
6. Genotypes which showed higher A/gs at a particular light regime also showed higher WUE at whole plant level determined by gravimetric method (Fig.3a and 3b).

4.1.4 Experiment-IV: Effect of moisture stress on WUE and related parameters

Under rainfed conditions, when the factors associated with evaporation and water extraction are not different amongst the species or between the varieties within a species, the biomass differences are predominantly due to differences in WUE. Therefore higher WUE is an important adaptive mechanism under moisture stress conditions.

In this experiment the effect of moisture stress on WUE and other physiological characteristics were determined in all *Lycopersicon* genotypes. In the previous experiments, the amount of water lost by transpiration was determined by gravimetric technique and added to maintain the plants at field capacity. Since the total transpiration varied amongst the genotypes, the cumulative water added was different for different genotypes. However, in this experiment all the genotypes were supplied with the same input of water, adequate or inadequate and the differences in WUE were estimated. In this experiment plants were grown under two moisture regimes termed as adequate and inadequate moisture regimes. In the former the plants were adequately irrigated to maintain near field capacity during the greater part of the day. The plants that are maintained under inadequate moisture regimes received 40 per cent less water than adequately

irrigated plants. The pots which received adequate input of water are termed as control plants and the ones which received 60 per cent of the control input are termed as stressed plants. The experiment was initiated only after 45 days with an objective to achieve adequate leaf area in all the genotypes. The DM and CWT were determined from 45 to 71 days after sowing.

At the beginning of the experiment in all the genotypes except in genotype 990, the leaf area was approximately 2000 cm^2 plant⁻¹. In 990 it was only 1194 cm^2 plant⁻¹. Uniform initial leaf area between the genotypes/varieties is an advantage in known input experiments. Until the initiation of the experiment both sets of pots received adequate water to maintain the plants at near field capacity.

Leaf area and LAD

The final LA and LAD showed significant differences amongst the genotypes. Highest LAD was recorded in 987 followed by 1048. Under stress in all genotypes, a significant reduction was observed. However, the per cent reduction varied and it was maximum in genotypes 1301 followed by 987. The per cent reduction was very less in 990 (11.51%) and 564 (18.19%) (Table 14).

Table 14. Leaf area, functional leaf area (LAD), total dry matter accumulation and cumulative water transpired (CWT) (per pot) during experimental period in Lycopersicon species non-limiting (field capacity) and limited water input situation.

Genotypes		Initial LA (cm ²)	Final LA (cm ²)	LAD dm day	Initial DM (g)	Final DM (g)	DM (g)	DM shoot (g)
Pusa Ruby	C	1931	3565	604	10.3	63.59	53.29	45.79
	S	1931	2395	476	10.3	48.41	38.19	30.97
1048	C	2778	4838	837	12.2	73.58	61.38	44.69
	S	2778	3215	659	12.2	43.30	31.10	22.78
990	C	1194	2802	597	5.16	32.48	27.32	21.59
	S	1194	2219	495	5.16	31.50	26.34	18.79
564	C	2495	4050	720	7.62	62.59	54.98	46.80
	S	2495	2860	589	7.62	42.09	34.47	27.09
1086	C	2290	3772	667	11.2	60.06	48.86	40.48
	S	2290	2392	515	11.2	43.80	32.60	26.96
1301	C	1715	4924	730	6.28	68.20	61.92	53.16
	S	1715	2373	450	6.28	50.68	44.86	35.06
987	C	1720	6707	927	10.06	65.69	55.63	43.63
	S	1720	4029	632	10.06	44.11	34.05	20.95
CD at 5%	G	270.51	491.58	57.34	1.27	4.67	4.494	3.56
	S	144.59	262.76	30.64	0.67	2.44	2.40	1.90
	6xS	382.55	695.20	81.08	1.79	6.59	6.35	5.04

C - Control .
S - Stress .

Dry matter

The final DM and the TDM showed a similar trend in all the genotypes. DM recorded during crop growth period however was not related to LAD. Genotypes 987 and 564 recorded similar TDM though LAD was 927 and 720 respectively. The DM decreased significantly under inadequate input in all the genotypes. However, the per cent reduction varied in different genotypes. Less reduction in DM under stress was seen in 990. Even in 1301 and Pusa Ruby per cent reduction was relatively less than the other genotypes. Genotype 1048 recorded the highest per cent reduction in TDM. In this genotype the per cent reduction in LAD was only 21.27 under stress where as the reduction in biomass was as high as 49.33. A similar trend was seen in genotype 564. In genotype 1301 which showed a highest per cent reduction in LAD (38.36) recorded a reduction in DM of only 27.55 per cent. These results suggest that in some of the genotypes in addition to a reduction LAD a significant reduction in assimilation rate was also observed under stress. The shoot DM also showed a similar trend as TDM.

Root characters

Moisture stress affects the carbon allocation to the roots and often alters root to shoot ratios.

Greater allocation of carbon under stress to the root is an useful adaptive mechanism for water harvesting. Keeping this in view, root biomass was recorded while taking the final observations. The mean root DM (mean of all species) was 9.4 g plant under control whereas it was 8.4 g plant under stress recording a reduction of 10.7 per cent (Table 15; Fig.4). However, the per cent reduction in TDM was 33.5 under stress. Therefore a significant increase in root to shoot ratio was seen under stress. Though the root to shoot ratios increased under stress in all genotypes, in some the per cent increase was very high as in genotypes 987, Pusa Ruby, 990 and 1301. This suggests that genotypes differ in relative allocation of carbon to root system when they are subjected to stress condition. Genotypes 987 and 990 showed greater allocation of biomass to roots when subjected to stress.

Water use efficiency

Genotypes differences exist in WUE even when input of water was same. In control plants highest WUE was recorded in 1301 followed by 1048 and least was observed in 990 (Table 16).

In many genotypes a significant increase in WUE was observed under stress. The increase in WUE was marked in 990 (60.50%), 1301 (20.52%) and Pusa Ruby

Table 15. Root dry weight and root/shoot ratios among Lycopersicon species under a non-limiting (field capacity) and limited water input situation.

Genotypes	Pusa Ruby	1048	990	564	1086	1301	987	CO ₂ 5%
Control:								
Root dry weight(g)	6.58d	16.69a	5.43e	10.18b	8.38c	8.76c	9.80b	0.63
Root/shoot ratios	0.14	0.37	0.24	0.22	0.20	0.16	0.22	-
Stress:								
Root dry weight(g)	8.00bc	8.32bc	7.55c	7.38c	5.64d	8.76b	13.10a	1.39
Root/shoot ratios	0.25	0.36	0.40	0.27	0.20	0.25	0.62	-

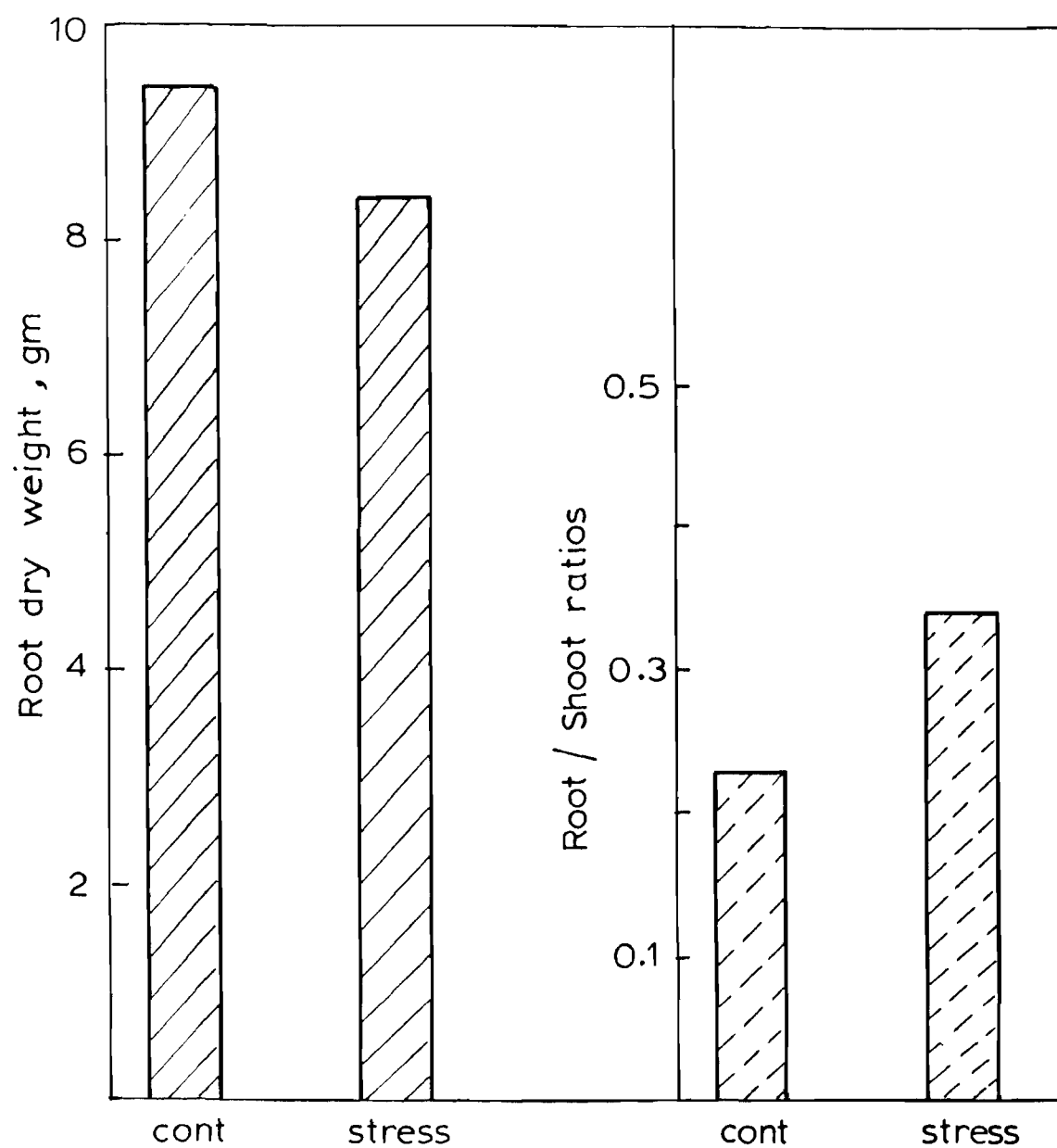


Fig.4: Root dry weight(g) and root/shoot ratios under two moisture regimes amongst seven *Lycopersicon* species.

16. Transpiration quotient (TQ), water use efficiency (WUE), rate of water loss and LAD/DM ratio in Lycopersicon species at a limiting and non-limiting known water input.

	TQ (ml g ⁻¹)	WUE ₁ (g DM kg ⁻¹ H ₂ O)	Rate of water loss (ml H ₂ O dm ⁻² d ⁻¹)	LAD/DM ratio	LAD/shoot DM ratio
C	302	3.31	26.65	11.33	12.93
S	253	3.94	20.49	12.46	15.36
C	262	3.81	19.24	13.64	18.73
S	311	3.22	14.66	21.19	28.93
C	421	2.38	19.86	21.19	26.45
S	262	3.82	13.94	18.73	26.34
C	293	3.41	22.36	13.10	15.38
S	280	3.57	16.40	17.10	21.74
C	329	3.03	24.14	13.65	16.48
S	296	3.87	18.76	15.80	19.10
C	260	3.85	22.05	11.79	13.73
S	215	4.64	21.47	10.03	12.84
C	289	3.46	17.37	16.66	21.25
S	248	3.50	15.28	18.56	30.17
G	21.78	0.28	1.53	2.10	2.44
S	11.64	0.15	0.81	1.12	1.30
SxS	30.80	0.40	2.15	2.98	3.45

C - Control.

S - Stress.

(19.0%) (Fig.4). However, in some genotypes like 987 (1.16) and 564 (4.69) WUE did not increase under stress and in one of the genotypes, 1048 there was a reduction by 15.49 per cent.

Rate of water loss

Since, the input of water was same for all the genotypes, rate of water loss at a given moisture regime will be inversely proportional to LAD. The genotypes which showed high LAD like 987, 1048 and 1301, the rate of water loss was less compared to other genotypes. The rate of water loss reduced in all the genotypes under stress. However, even in this parameter also genotypes differed significantly and highest per cent reduction was recorded in 564 (26.65) followed by Pusa Ruby (23.88) and 1048 (23.8). However, in 1301, the per cent reduction was only 2.63 (Fig.5).

LAD/DM ratios

Under adequate input regime, genotypes 990 and 987 recorded higher LAD/DM ratios and both 1301 and Pusa Ruby recorded low LAD/DM ratios. Except in 990 and 1301, in all other genotypes LAD/DM increased under stress indicating that mean net assimilation rate decreased. A marked decrease in net assimilation rate

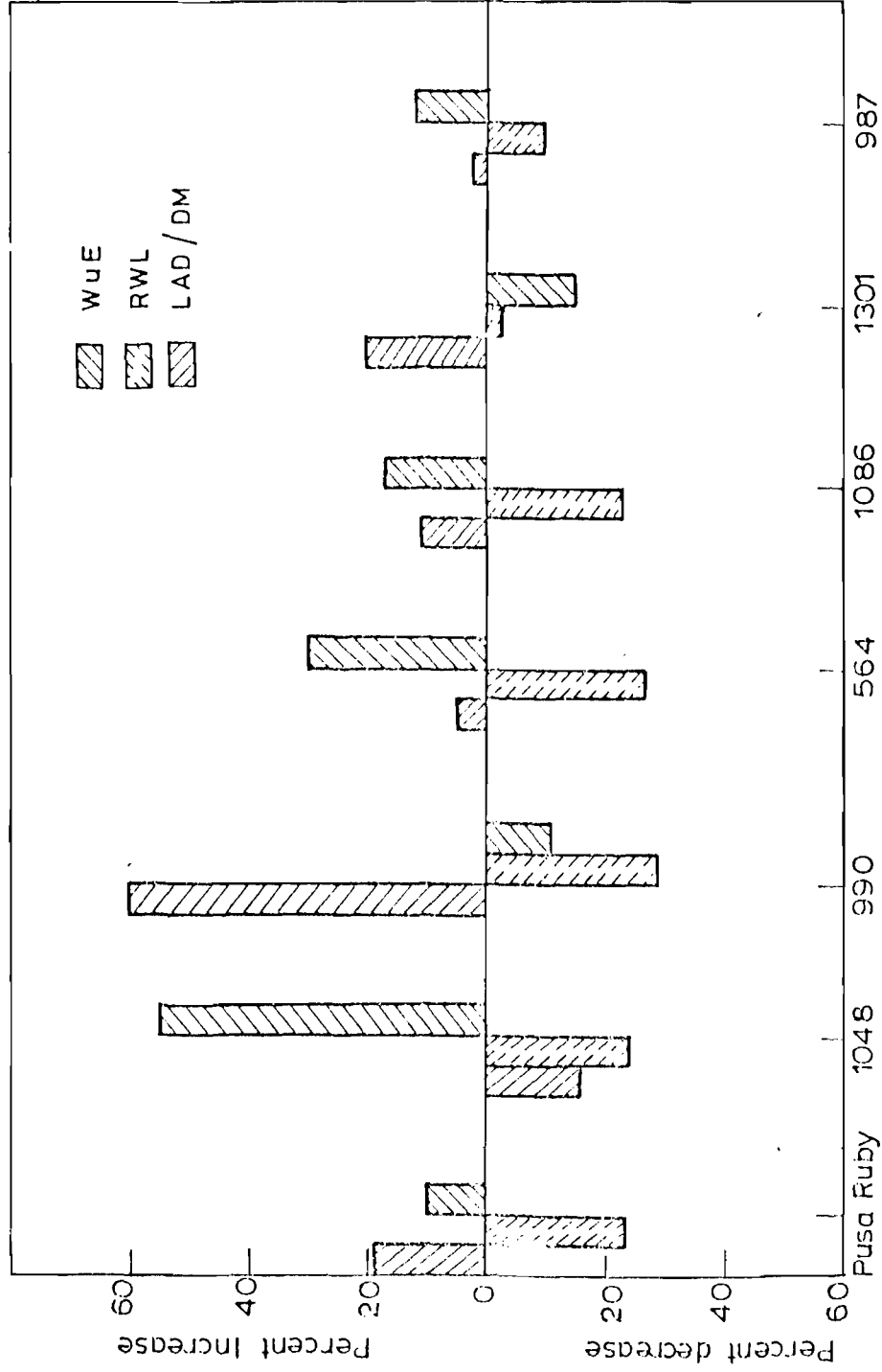


Fig.5: Per cent increase or decrease in WUE and associated physiological parameters at two moisture regimes.

as observed in genotype 1048 resulted in a decrease in WUE under stress (Fig.5).

Physiological traits associated with WUE

An attempt was made to study the relationship between WUE and physiological parameters in stressed conditions with those under non-stressed conditions. Only LAD showed a positive significant relationship indicating that genotypes with intrinsically high LAD also showed a high LAD under stress. However, other growth parameters and WUE did not show any relationship. This suggests that some genotypes which showed low intrinsic WUE (non-stress) showed higher carbon gain per unit conductances under stress ($r=0.762^*$).

Under control non-stressed conditions DM showed a very high positive significant relationship with WUE. However, this relationship was non-significant under stress conditions. Neither LAD/DM nor rate of water loss showed any relationship with WUE in both the moisture regimes. This indicates that at a given input of water either adequate or inadequate, the relative ratios between carbon gain and rate of water loss is more important than any one of these characters. The results obtained from this experiment it can be concluded that;

Table 17. Correlation values showing relationship between WUE with different physiological parameters at two known moisture regimes.

Control:

Physio- logical parameters	LAD dm ² days ⁻¹	DM (g)	Shoot DM	LAD/DM	LAD/shoot DM	Rate of water loss

WUE	0.587NS	0.980**	0.729NS	-0.480NS	-0.643NS	0.063

Physiological parameters	LAD/DM	Physiological parameters	WUE based on SDM
CWT/LAD	-0.608NS	WUE based on TDM	0.831*

Stress:

Physio- logical parameters	LAD dm ² days ⁻¹	DM (g)	Shoot DM	LAD/DM	LAD/shoot DM	Rate of water loss

WUE	-0.434NS	0.723NS	0.623NS	-0.704NS	-0.530NS	0.459

Physiological parameters	LAD/DM	Physiological parameters	WUE based on SDM
CWT/LAD	-0.909**	WUE based on TDM	0.865**

1. Genotypic variation in WUE both under stressed and non-stressed conditions can be determined by providing similar input for all the genotypes.
2. There was no relationship between WUE values recorded under stress and non-stressed conditions.

In some species like 1301, 990 1086 under stress, a significant increase in WUE was recorded where as in genotypes like 564, Pusa Ruby the increase in WUE was marginal. In one of genotype 1048, even a reduction in WUE was observed under stress (Fig.5).

3. Genotypes differed in the extent of reduction of water loss and net assimilation rate under stress.
4. In genotypes 1301 and 990 which showed higher increase in WUE, one of the characteristic feature was the stability in assimilation rate under stress. Infact mean assimilation rate increased under stress in these two genotypes.
5. In general root to shoot ratios increased under stress. But in some the per cent increase was more in genotypes like 990 and 987 signifying their better adaptation.

4.1.5 Potential photosynthetic rate in Lycopersicon species

Carbon gain (assimilation rate) is one of the physiological characters associated with WUE. At a single leaf level A/g_s reflects WUE. A higher assimilation rate as a result of high mesophyll conductances is desirable rather than due to high g_s , thereby resulting in high A/g_s ratio. Keeping this in view as a measure of mesophyll conductances potential photosynthetic rate of these genotypes was determined.

Potential photosynthetic rate is a reflection of chloroplast ability to fix carbon when C_i is at saturated level. C_a is maintained high enough so that there is no stomatal or liquid phase resistance for CO_2 diffusion. This is often referred to as P_{max} at saturated CO_2 concentration. For such studies in mesophytes, the photosynthetic rates are measured at 5 per cent ambient CO_2 concentration.

Turgid leaves were excised and exposed to 5 per cent CO_2 concentration ($^{12}CO_2 + ^{14}CO_2$) in a assimilation chamber. During exposure period, RH of the chamber was maintained high enough to maintain the turgidity of the leaves. The amount of $^{14}CO_2$ fixed was determined and expressed as cpm/g and cpm/unit leaf area. The data of three such experiments are given in

Table 18. Results showed that significant differences exist amongst the genotypes in two experiments. In both the experiments, 564 showed high potential photosynthetic rate. In second experiment potential photosynthetic rate in 564 was significantly high than all other genotypes, except in 1301. In all experiments, Pusa Ruby showed least potential photosynthetic rate. In addition to Pusa Ruby potential photosynthetic rates were also less in 987 and 1086.

The mean potential photosynthetic rate (mean of all these experiments) of different *Lycopersicum* species expressed both as cpm/g and cpm/unit leaf area given in Fig.6. When $^{14}\text{CO}_2$ fixation rate expressed in cpm/g dry weight, highest potential photosynthetic rate was observed in 1301 followed by 990, 564. However, when expressed in cpm/unit leaf area, highest activity was seen in 564 followed by 1301 and 990. In general potential photosynthetic rate was high in these three *Lycopersicon* species. Based on the results the *Lycopersicon* species and *Lycopersicum esculentum* genotypes can be categorised as :

1. High potential photosynthetic types	-	564, 1301, 990
2. Moderate potential	"	- 1086, 1048, 987
3. Low potential	"	- Pusa Ruby

Table 18. Potential photosynthetic rate in excised leaf tissue of Lycopersicon species under control condition based on cpm/cm² LA and cpm/100 mg dry weight.

Genotypes	I Experiment		II Experiment		III Experiment	
	cpm/cm ² LA	cpm/100 mg dry wt.	cpm/cm ² LA	cpm/100 mg dry wt.	cpm/cm ² LA	cpm/100 mg dry wt.
Pusa Ruby	209.1	4410.3	161.0d	1868.0d	92.67d	1399.0b
1048	377.4	10039.1	283.3cd	3671.7cd	189.33ab	2613.6bc
990	432.4	11552.9	460.7bc	4600.0bc	278.33a	3695.3ab
564	442.4	7082.2	771.3a	7354.3	300.33a	4063.3a
1086	436.7	10804.6	311.0cd	3317.0cd	254.0a	3523.6cd
1301	349.9	10930.9	657.0ab	6108.7ab	265.3	3404.6cd
987	293.9	6891.8	266.3cd	3718.7cd	249.3ab	2605.6bc
CD at 5%	NS	NS	214.3	2318.5	118.5	1149.5

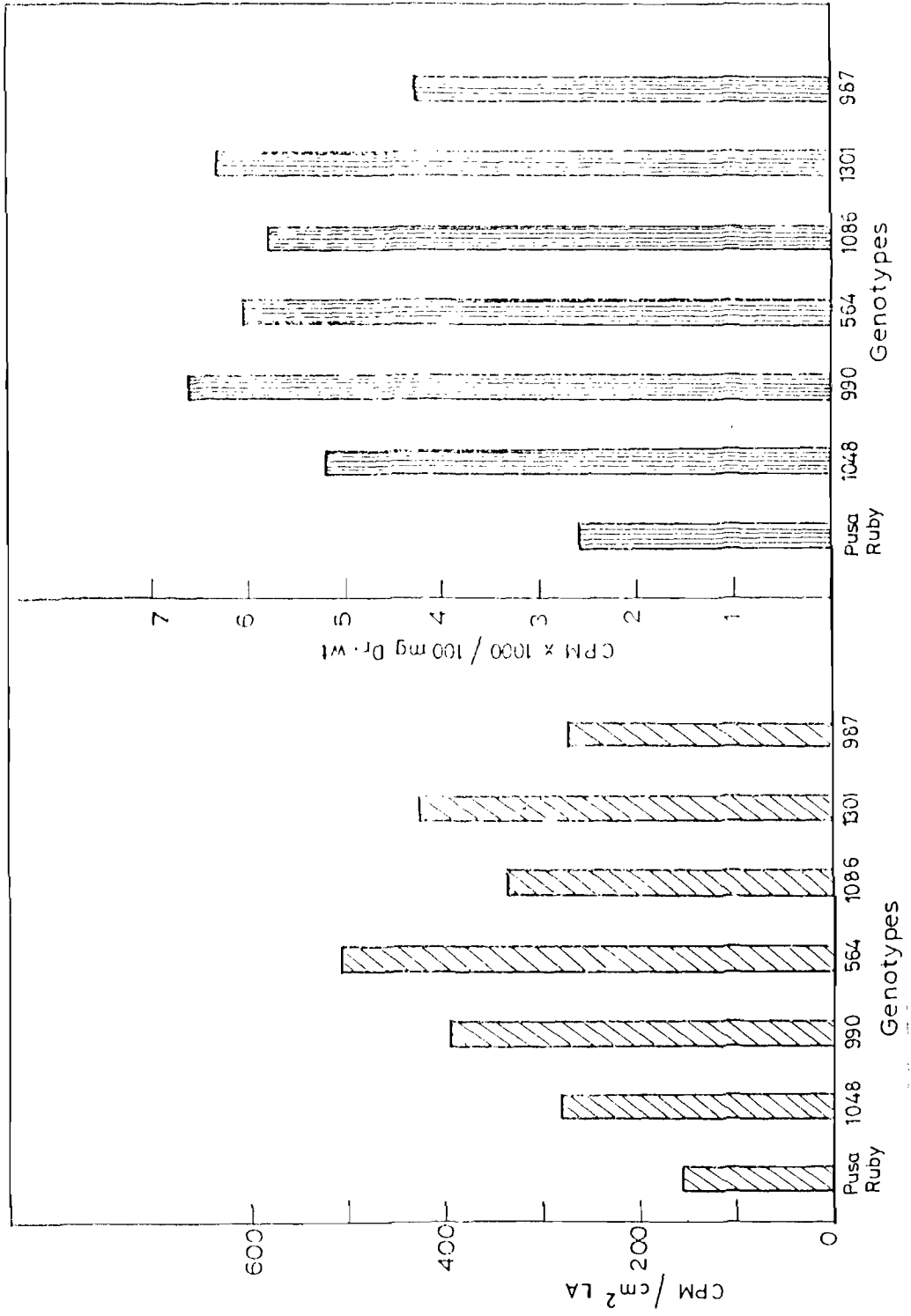


Fig.6: carbon dioxide fixation amongst seven *Lycopersicon* species under control conditions.

4.2 Water extraction efficiency and non-stomatal rate of water loss

Water extraction is an important adaptive mechanism often to maintain higher tissue water potential and thus avoid any substantial reduction in physiological processes. Not only enhancement of ET but also maintenance of relatively higher tissue water potential often depends on root characteristics, volume, spread and uptake. Yet another avoidance mechanism to maintain tissue water status is moisture conservation at leaf level either by stomatal regulation or minimizing cuticular transpiration. The later has been shown to be of substantial percentage of total T in stressed leaves.

4.2.1 Differences in water extraction efficiency in *Lycopersicon* species

The differences in root volume, root DM and shoot root ratios amongst the *Lycopersicon* species and *Lycopersicon esculentum* genotypes were recorded in WUE experiments (Table 19). In addition to the differences in root biomass, in one of the experiments as a measure of root activity, water extraction efficiency index (WEE) was arrived for all the genotypes grown under control and stress situation. The results are given in Table 19.

Table 19. The relationship between soil moisture content, water retained in the pots at the end of the experiment, shoot/root ratio, root dry matter, final LA and water extraction efficiency index.

Genotypes		Root dry weight(g)	Root/Shoot ratio	Final LA (cm ²)	Water retained and SM at the end of Expt.	Water extraction efficiency index	Root/LA ratio (mg/cm ²)
Pusa	C	6.58	0.141	3565	900	4.41	1.85
Ruby	S	8.00	0.258	2395	550	4.31	3.30
1048	C	16.69	0.373	4838	850	4.72	3.40
	S	8.32	0.365	3215	530	4.51	3.60
990	C	5.73	0.248	2802	750	3.48	2.00
	S	7.55	0.402	2219	410	4.06	3.40
564	C	10.18	0.227	4050	580	6.40	2.50
	S	7.38	0.272	2860	370	6.17	2.60
1086	C	8.38	0.207	3772	700	5.65	2.20
	S	5.70	0.209	2392	420	5.65	2.40
1301	C	8.76	6.165	4924	650	5.96	1.80
	S	3.76	0.250	2373	390	5.96	3.70
987	C	9.80	0.225	6707	560	6.52	1.50
	S	13.10	0.6-5	4029	328	6.60	3.30
CO at 5%		1.42	1.06	491.58	-	-	-
		0.76	0.57	262.76	-	-	-
		2.01	1.50	695.20	-	-	-

C - Control.

S - Stress.

Water extraction index was high in Lycopersicum esculentum genotype 987 both under stress and non-stress conditions and it was relatively low in Lycopersicon species 990. A marginal increase in WEE index was observed under stress in Lycopersicon species 990 and Lycopersicum esculentum genotype 987. From these results Lycopersicum esculentum genotypes 987, and 564 were more efficient in water extraction.

The water extraction efficiency index arrived based on cumulative water input and water retained in the soil could be influenced by four important characteristics.

1. Total functional LA per pot.
2. The LA per unit dry weight of the root
3. The root biomass.
4. Intrinsic root extraction efficiencies.

The LA which was significantly different amongst the genotypes was not related to WEE index. For instance, Lycopersicum esculentum genotypes 987 and 564 though has similar WEE index, the LA was different. Same is true in other genotypes also.

Since, significant variation in LA exists, root weight to LA ratio (mg dry weight/cm²/LA) was computed both in control and stress. In all genotypes there was an increase in root to leaf LA ratio under

stress. Though marked differences were observed in root to LA ratios both in control and stress amongst the genotypes, no relationship could be noticed with this parameter and WEE index of roots.

The WEE seems to more related to the observed differences in root dry weight. In general both under control and stress genotypes with high root biomass also showed high WEE index. Such a trend was not seen only in Lycopersicum esculentum genotype 1048 under control and 564 under stress. However, it can be concluded that WEE index is related to root biomass and possibly due to differences in differential root extraction efficiencies.

4.2.2 Species/genotypic variation in cuticular transpiration

In excised leaves after incubating in dark for one hour in turgid conditions, the rate of water loss was determined by live wilting the leaves under laboratory conditions. The reduction of fresh weight was considered mainly due to loss of water due to cuticular transpiration. The data of three such experiments are given in Fig.7a,b,c and Appendix-4,5,6.

Experiment-I

In the first experiment (Fig.7a) the rate of water loss was monitored for 13 hours until the per cent reduction in tissue water status was 28 to 50 per

cent. The per cent water loss was maximum in *Lycopersicon* species 990 followed by 1301 and *Lycopersicum esculentum* genotype Pusa Ruby.

Even from third hour after initiation of experiment, the rate of water loss was high in 990 compared to any other genotype. At all sampling times and also at the end of the experiment, the rate of water loss was low in 564, 1048 and 987.

Experiment-II

In this experiment the rate of water loss was monitored for 16 hours. The per cent water loss ranged from 45 to 65 (Fig.7b). In this experiment also rate of water loss was significantly high in 990, Pusa Ruby 987 and 1301. However, the differences in rate of water loss was observed only 5 hours after initiation of the experiment. *Lycopersicum esculentum* genotypes 564, 1048 and 1086 showed low rate of water loss.

Experiment-III

In another similar study also the rate of water loss was significantly high in 990 even from second hour after initiation of the experiment. Pusa Ruby and 1301 also showed higher rate of water loss compared to other genotypes. Amongst the latter group, *Lycopersicum esculentum* genotype 564 showed lowest rate

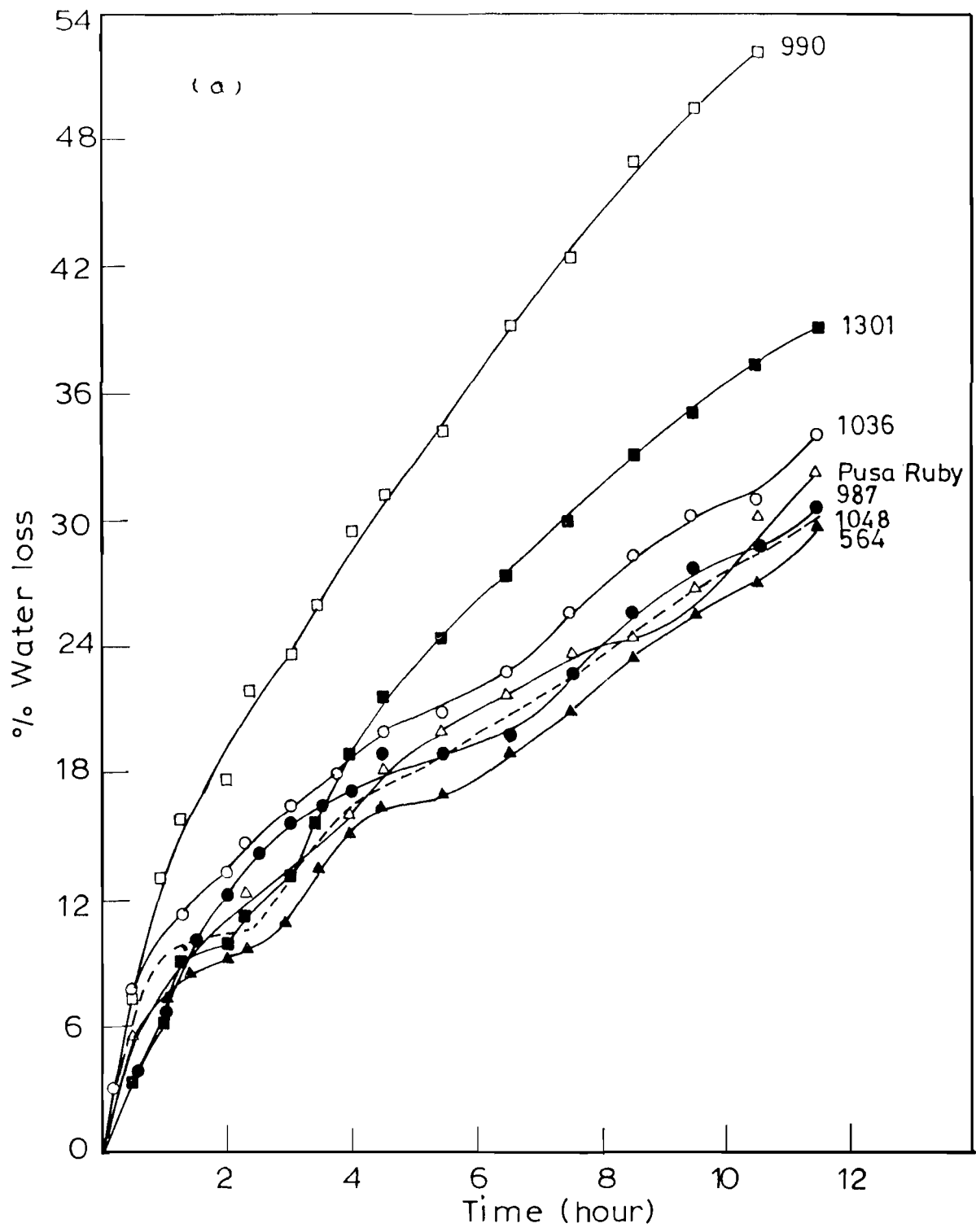
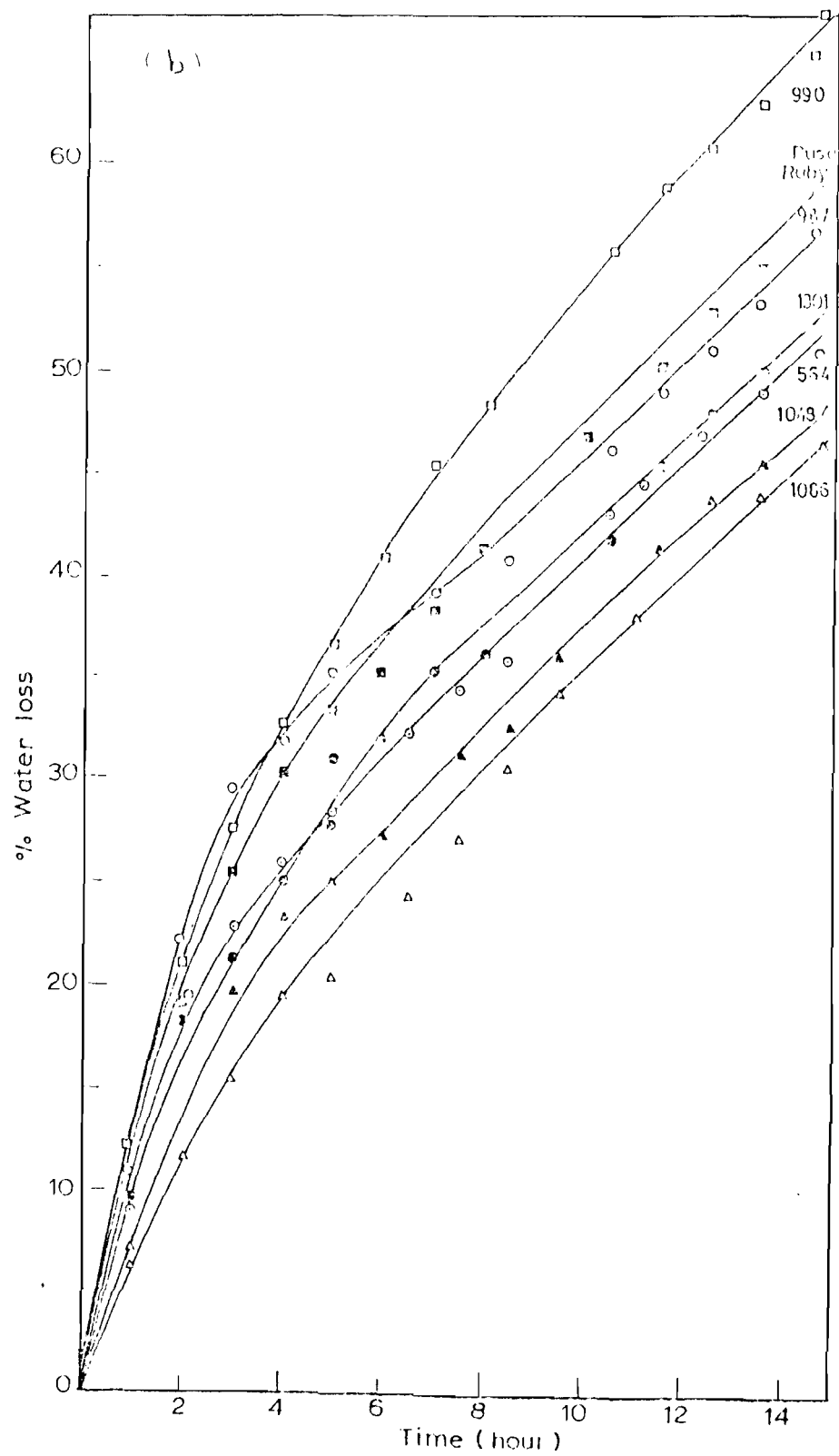
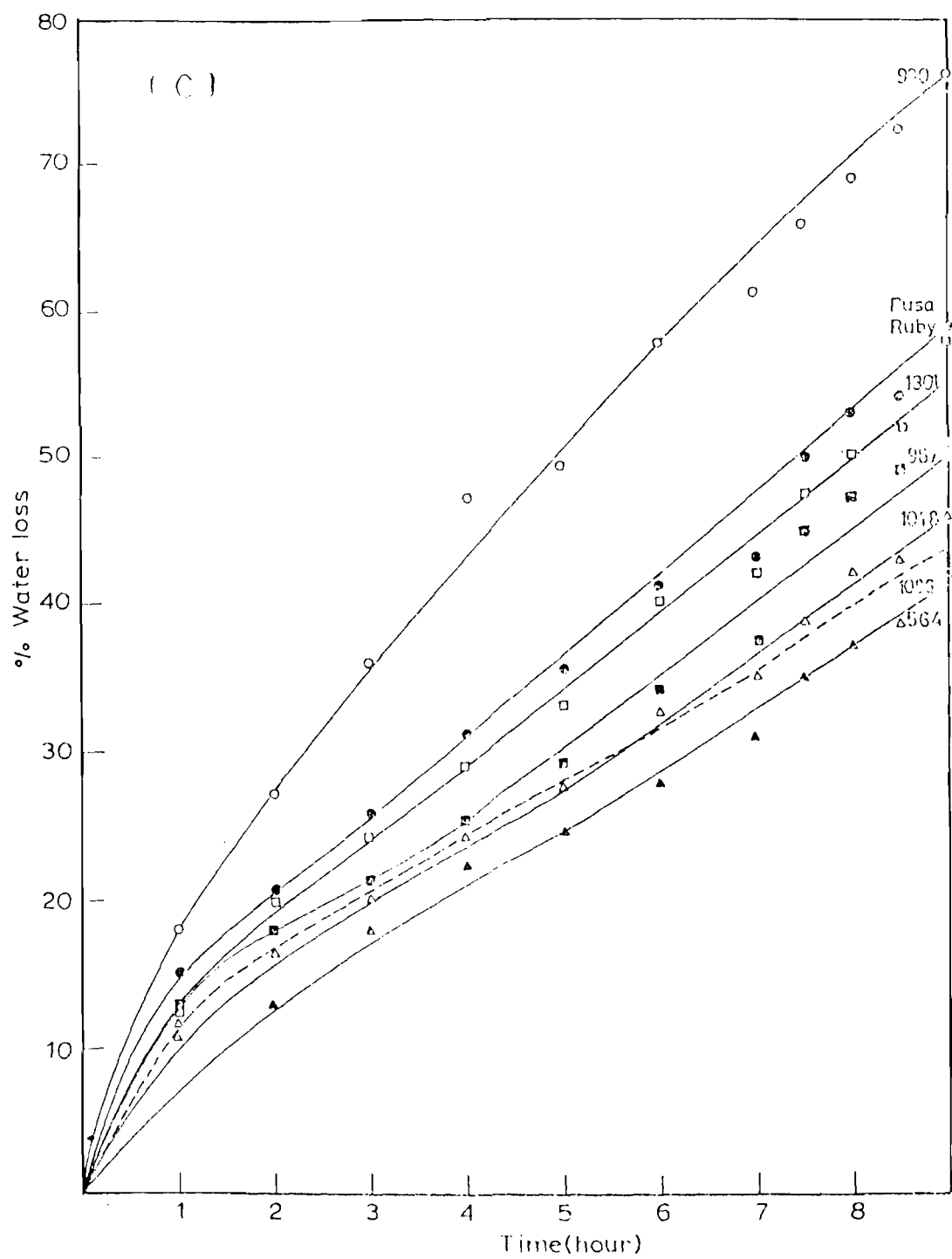


Fig.7a,b,c: Differences in rate of water loss amongst seven Lycopersicon species (a) Experiment-I, (b) Experiment-II and (c) Experiment-III.





of water loss at all sampling times. From results obtained in these experiment, the genotypes can be grouped as follows.

990 - High rate of water loss

Pusa Ruby, 1301 and 987 - Moderate rate of water loss,

1048, 1086 and 564 - Low rate of water loss.

In two of the three experiments, the specific leaf weight was determined with an objective to assess the relationship between leaf thickness and rate of water loss (Table 20). There were marked differences in SLW which ranged from 1.59 to 4.42 (Experiment-1). Genotype like 990 with low SLW showed higher rate of water loss. But such a trend was not seen in the second experiment.

4.3 Variation in desiccation tolerance and associated parameters among the Lycopersicon species

Drought avoidance characteristics facilitates maintenance of tissue water status and growth under moderate stress situations. When stress levels intensity, the survival and regrowth of the plants depends on the intrinsic desiccation tolerance of plants. Physiological and biochemical characteristics associated with desiccation tolerance become more relevant when duration of stress cycle and its

Table 20. Specific leaf weight amongst Lycopersicon genotypes under stress conditions in vitro.

Genotypes	SLW I Expt.	SLW II Expt.
Pusa Ruby	1.59	3.40
1048	2.70	2.90
990	2.65	2.50
564	2.49	2.60
1086	3.40	3.00
1301	4.42	3.90
987	2.91	3.20

intensity is more. At the time of seedling establishment under rainfed conditions and when moisture stress is too severe, intrinsic drought tolerance becomes relevant for survival and regrowth on stress alleviation.

Certain genotypes tolerate moisture stress despite low tissue water potential. This is accomplished often either by mechanisms which maintains turgor pressure of cell (osmoregulation/osmotic adjustment) or by minimising desiccation injury to the cell organelles. The factors associated with osmotic adjustment like solute accumulation and cell elastic characteristics and their relevance has been well elucidated.

There are several mechanisms that facilitate the metabolic activity of cells under low tissue water potential and those which minimize the desiccation injury. Maintenance of membrane integrity, protein synthesis, protoplast characteristics are a few examples.

In view of the importance of drought tolerance mechanisms in addition to drought avoidance mechanisms, the differential desiccation tolerance of these *Lycopersicon* species was studied. The following aspects related to tolerance were examined.

1. Dessiccation tolerance of genotypes was studied based on;
 - a) The degree of desiccation of the leaves in plants subjected to stress.
 - b) Seedling survival.
 - c) Seedling survival, leaf desiccation and growth rates under gradual but severe stress under field conditions.
 2. Variation in proline content of Lycopersicon species subjected to different levels of moisture stress.
 3. The relative osmotic adjustment in genotypes differing in survival ability.
 4. The tissue metabolic activity under stress in genotypes differing in survival based on carbon assimilation and protein synthesis.
 5. The membrane stability characteristics in tissues subjected to desiccation.
- 4.3.1 Relative desiccation tolerance amongst the Lycopersicon species

Emperical assessment: Several experiments were conducted to arrive at the relative intrinsic desiccation tolerance of these Lycopersicon species and Lycopersicum esculentum genotypes based on

empirical evaluation. The characteristics considered for evaluation are;

1. The extent of survival of the leaves at the end of drying cycle.
2. The per cent survival of seedlings at the end of drying cycle.
3. The recovery growth rates as reflected by the rate of production of new leaves on stress alleviation.

Differential desiccation tolerance

An assessment based on leaf survival under rapid stress conditions.

Even when plants were subjected to rapid soil moisture depletion conditions in containers (pots), the tissue water status in different genotypes may differ because of the intrinsic avoidance mechanisms. For instance, differential root characteristics and water retention characteristics of the tissues may result in the differences in tissue water potential or RWC in different species even when they are subjected to similar stress conditions. Therefore the main approach in these experiments was to assess the survival characteristics at a given tissue water status based on measurements of either RWC or water potential. The

data of several such experiments are given in Table 21).

4.3.1.1 Experiment-I

In this experiment pot grown plants maintained at near field capacity until 30 days were subjected to stress by with-holding water. The tissue water status was maintained by determining RWC periodically after imposition of stress. The duration of stress cycle was about 20 days to decrease the tissue water status to 45 per cent of RWC. Number of leaves prior to imposition of the stress and the total number of green and dessicated leaves at the end of stress cycle were recorded independently for each tagged plant. Based on the total leaves and the green leaves, the per cent survival at a given RWC was computed. Higher the ratio of green to desiccated leaves, higher the per cent survival and vice versa.

At RWC of 40 to 45 per cent, the per cent of desiccated leaves, was low in 1048 followed by 564 and highest was recorded in 1086 (Table 21).

The data on the extent of leaf desiccation indicates that even under moderate stress conditions, species differences exist. The per cent survival of the green leaves is not associated however, with the total number of leaves produced by the plant. For

Table 21. Leaf survival at low water status (RWC range 40-45 per cent) as a reflection of desiccation tolerance: Total leaves, green leaves and percentage of desiccated leaves.

Genotypes	Total** leaves	Green* leaves	Per cent** desiccated leaves
Pusa Ruby	15	10	33.33
1048	16	12	25.00
990	13	9	30.77
564	23	17	26.09
1086	26	15	42.31
1301	27	19	29.61
987	24	15	37.50

*Data taken at the end of stress period

**Data taken 24 hours after alleviation of stress

***The extent of leaf survival was assessed amongst the genotypes of a comparable RWC. Therefore the number of plants for observation in each genotype varied anywhere between 6 to 9. Each value is a mean of at least 6 to 9 replicates.

instance with a similar number of leaves (26-27) the per cent of desiccated leaves in genotypes 1301 and 1086 was 29.61 and 42.31 respectively.

4.3.1.1 Experiment-II

In this experiment the stress was imposed for a longer period until leaf RWC reached 30 to 40 per cent. The relative survival of green leaves and the per cent desiccated leaves was computed and the data are given in Table 22.

When the plants were subjected to severe stress, there were more marked differences in terms of desiccation tolerance. Percentage of desiccated leaves was less in genotypes 987 and 1086 and it was relatively high in genotypes 1301, 990, 1048 and Pusa Ruby. Even in this experiment, the per cent desiccated leaves was not related to the total number of leaves produced by the plant.

4.3.3.1 Experiment-III

In an other study after imposing stress in pot grown plants from 30 days after planting periodically the leaf tissue water potentials were determined psychrometrically and when tissue water potential decreased to -2.2 to -2.5 MPa, the differential desiccation tolerance was determined by taking observations on number of leaves dessicated in

Table 22. Leaf survival at low water status (RWC range 30-40 per cent) as reflection at dessication tolerance. Total leaves green leaves and percentage of desiccated leaves.

Genotypes	Total leaves	Green leaves	Per cent desiccated leaves
Pusa Ruby	25	7	72.00
1048	33	9	72.73
990	15	3	80.00
564	21	8	61.90
1086	40	18	55.00
1301	30	5	83.33
987	32	15	53.12

*** Refere Table 21

different genotypes. The extent of desiccation was less in genotype 1048 and relatively high in 1301, where as in other three genotypes 990, 987 and Pusa Ruby, the percentage of dessicated leaves ranged from 72 to 54 (Table 23). In this experiment, due to lack of establishment, genotype 564 was not included.

At the end of the stress cycle, water relation characteristics, were also determined for all the genotypes prior to determining relative mortality of leaves. The data on these characteristics are given in Table 24. Even when the ψ reduced to more than -2.0 MPa, in all the genotypes positive turgor was maintained. However, a marked variation in pressure potential was observed amongst the genotypes. A significant decrease in osmotic potentials was seen in 987, 990 and also 1301. The pressure potentials were relatively low in 1048 and Pusa Ruby. It can be inferred from the data that there was osmotic adjustment and maintenance of greater positive turgor in 987 and 990.

4.3.3.1 Experiment-IV

In another set of pot grown plants subjected to relatively severe stress, the extent of desiccation tolerance was assessed when the leaf water status decreased to -2.9 MPa. Even in this experiment, the per cent dessicated leaves was less in 1048 and 1086

Table 23. Leaf survival at low water status (water potential range as -2.2 to -2.5 MPa) as a reflection of desiccation tolerance: Total leaves, green leaves and percentage of desiccated leaves.

Genotypes	Total leaves	Green leaves	Per cent desiccated leaves
Pusa Ruby	25	7	72.00
1048	35	16	54.29
990	14	5	64.29
1086	42	22	47.62
1301	35	12	65.72
987	35	15	57.20

Data for 564 variety not available
 ***Refer Table 21.

Table 24. Differences in osmotic potential (ψ_{π}) and turgor potential in six species when subjected to nearly similar intensities at moderate water stress.

Genotypes	RWC(%)	ψ_w MPa	ψ_{π} MPa	ψ_p MPa
Pusa Ruby	31.9	2.17	2.49	0.32
1048	33.4	2.11	2.30	0.19
990	45.4	2.38	3.24	0.86
1086	32.2	2.49	3.00	0.51
1301	24.8	3.08	0.50	0.42
987	30.5	2.39	3.33	0.94

Each value is a mean of 6 to 9 replications.

and maximum was observed in Pusa Ruby (Table 25). The tolerance was equally high in 987.

Leaf desiccation - Relative assessment amongst the genotypes

The data on per cent survival of the green leaves at a given tissue water status and relative ranking of the genotypes from different experiments are given in Table 26.

Genotypes 1048, 1086 and 564 can be grouped as types which showed less percentage of the dessicated leaves followed by 987. Both Pusa Ruby and 990 showed relatively same level of survival of leaves when plants were subjected to different stress levels. Least per cent survival of green leaves was recorded in 1301.

Based on the leaf survival, at a given tissue water status three distinct groups can be categorised.

1. High survival - 1048, 1086 and 564
2. Moderate survival - 987
3. Low survival - 990, Pusa Ruby and 1301.

4.3.1.2 Differential desiccation tolerance of Lycopersicon species subjected to gradual but severe stress under field conditions in small plots

During the rainfree summer, a small plot experiment was conducted to assess the relative

Table 25. Leaf survival at low water status (water potential < 2.9 MPa) as reflection at desiccation tolerance: Total leaves, green leaves and percentage of desiccated leaves.

Genotypes	Total leaves	Green leaves	Per cent desiccated leaves
Pusa Ruby	24	11	54.15
1048	23	15	34.78
990	14	6	57.14
1086	42	24	42.86
1301	32	11	65.62
987	32	15	53.12

Data for 564 variety not available.

*** Refer Table 21.

Table 26. Overall ranking of the Lycopersicon species for dessication tolerance based on leaf survival experiments (Experiment 1, 2 and 3).

Genotypes	Survival per cent at RWC of 40-45%	Survival based on		Survival based on RWC at 30-45%	Mean survi- valvol(%)	Ranking based on mean survival
		-2.2 to -2.5 MPa	> -2.9 MPa			
Pusa Ruby	66.67	45.85	28.00	28.00	42.13	V
1048	75.00	65.22	45.71	27.27	53.3-	II
990	69.23	42.86	35.71	20.00	41.95	VI
564	73.91	-	-	38.10	55.35	I
1086	57.69	57.14	52.38	45.00	53.05	III
1301	70.39	34.38	34.28	16.67	38.93	VII
987	62.50	46.88	42.80	46.88	49.77	IV

- Data not available -

tolerance of genotypes based on the extent of leaf mortality at the end of stress cycle and the per cent recovery on stress alleviation. Until 15 days after transplanting, the plants were grown under well watered conditions by irrigating the plots once in three days to field capacity. Duration of stress cycle was 21 days (Table 11).

From 7th day after imposition of stress, at regular intervals the per cent dessicated and green leaves were recorded until the end of stress cycle. In all six observations were made on tissue water status and per cent survival during the stress cycle. At the end of the stress cycle, the plots were irrigated to field capacity. Four days after stress alleviation, the per cent recovery was assessed based on the number of leaves produced per plant.

The tissue water status per cent survival were determined after a specific day during the stress cycle. On the 7th day after stress imposition a significant variation was observed in RWC between the genotypes predominantly due to relative water uptake properties and the water retention properties of the genotypes. Highest RWC was recorded in genotype 990 followed by 1086 and 564. In Pusa Ruby and 1301, the RWC was as low as 45.93 per cent. However, there was no leaf mortality in these genotypes.

Table 27. Leaf survival under field conditions in small plots on imposition of different intensities of water stress.

Genotypes	T1 (7 days) RWC(%)	T2(9 days)		T3(12 days)		T4(14 days)		T5(19 days)		T6(21 days)		Mean survival per cent	Recovery per cent based on mean survival (%)	Ranking
		Survival (%)	RWC(%)	Survival (%)	RWC(%)	Survival (%)	RWC(%)	Survival (%)	RWC(%)	Survival (%)	RWC(%)			
Pusa Ruby	48.90c	89	43.04	75c	42.29	30.0	41.47	4c	34.79b	60d	0b	28	0d	VII
1048	48.63c	91	44.45	96ab	43.16	68ab	42.09	50cd	35.72b	47ab	32.42a	68	72ab	IV
990	62.60a	96	45.73	81ab	42.66	66ab	40.84	41d	38.91ab	33bc	38a	61	38c	V
584	53.44abc	78	46.84	78b	44.69	61b	40.63	54bcd	40.01ab	21c	29.24a	55	41c	VI
1086	59.48ab	93	47.22	91.67a	46.29	76	44.21	73a	43.55a	60a	37.66a	79	68b	I
1301	45.93c	84	44.60	78b	43.51	72ab	41.95	64ab	40.97ab	51a	37.52a	69	83ab	III
987	49.29b	88	48.32	86ab	43.30	67ab	38.54	62abc	35.07b	51a	34.82a	71	89a	II
CD at 5%	10.41	NS	NS	13.01	NS	13.77	NS	19.14	5.05	15.31	1.43	-	19.13	-

The RWC showed a further decrease when the observations were made on 9th day (T2). However, on this day the genotypic variation in RWC was not significant. Though the per cent survival of the green leaves ranged from 78 to 97, the differences were not-significant.

On 12th day (T3) there was a further decrease in RWC and it ranged from 42.66 to 47.21 per cent. Though, the RWC was not significant, a significant variation was observed in per cent dessicated leaves. In Pusa Ruby the per cent dessicated leaves was as high as 43.33 where as it is only 8.33 in 1086 and 19.33 in 990.

RWC showed a further decline when observations were made on 14th (T4) day after imposition of stress. However, genotypic differences were not significant. Marked differences in survival percentage of green leaves was observed. Highest percentage of dessicated leaves was observed in Pusa Ruby. In other genotypes, the per cent dessicated leaves did not show any significant variation.

By 19th (T5) day there was significant differences in RWC and survival percentage among the genotypes. Genotypes 1086, 1301, 564 and 990 maintained relatively higher RWC compared to Pusa Ruby and 987. Though the RWC was same in 987, 1048 and Pusa Ruby, the per cent

survival was 61.67, 50.0 and 4.4 respectively, 1086 in which RWC was maximum showed still higher percentage of survival of green leaves. Though several genotypes maintain approximately similar RWC the relative survival percentage was different indicating that significant variation could be noticed in the percentage of leaf dessication at a given RWC.

The data on 21st (T6) day was recorded only in other six genotypes except in Pusa Ruby since there were no green leaves. In the remaining six genotypes, there was no significant difference in RWC. However, the per cent survival of green leaves was more in 1086 followed by 987. Only 21 per cent of leaves survived in 564.

On stress alleviation, the per cent recovery was assessed based on the total green leaves at the end of recovery period. The total number of leaves includes both the ones survived and the new leaves produced. Species differed in recovery percentage. Highest was observed in 987 followed by 1086 and 1048. Since, all the leaves died before the alleviation of stress, the recovery percentage scored for Pusa Ruby was zero. Amongst other genotypes 564 and 990 showed low recovery percentage.

Seedling survival at the end of the stress cycle

At the end of the stress cycle before rewatering the per cent mortality of the seedlings was also determined. For this purpose initially in each row 5 to 6 plants were tagged and the number of plants survived at the end of drying cycle was recorded. The per cent survival was very high in 3 genotypes, 1086, 987 and 1301 (Table 28). Both in 564 and 990, the per cent survival was less. By the end of drying cycle, none of the plants survived in Pusa Ruby. Based on the per cent survival of seedlings, the relative ranking of the genotypes is given in Table 28.

Based on the mean survival percentage and the recovery percentage, the relative ranking of the genotypes is as follows:

1. Tolerant - 987, 1086 and 1301
2. Moderately tolerant - 564 and 990
3. Susceptible - Pusa Ruby.

4.3.1.3 Assessment of desiccation tolerance of *Lycopersicon* species based on seedling survival

Based on the result obtained from the small plot experiment, four distinct genotypes differing in their tolerance were identified and another experiment was conducted for empirical assessment of seedling survival under rapid stress conditions in containers.

Table 28. Differences in whole plant (seedling) in survival in small plots under field conditions in *Lycopersicon* species.

Genotypes	Total seedlings	Green seedlings	Per cent survival	Ranking
Pusa Ruby	24	0	0	VII
1048	22	14	64	IV
990	20	6	30	V
564	22	6	27	VI
1086	20	16	80	I
1301	20	14	70	III
987	24	18	75	II

Each value is a mean of 2 replications and each replication had 10 to 12 tagged plants.

The genotypes selected for this experiment were 987 and 1301 from tolerant group, 990 from moderately tolerant group and Pusa Ruby as a susceptible check.

As in the previous pot culture experiment, stress was imposed by with-holding water. After 20 days after imposition of moisture stress, observations on water relations, survival percentage based on the total and number of dessicated leaves and seedling survival based on the seedling mortality were recorded (Table 29). On 4th day after alleviation of moisture stress, the recovery percentage based on initial (prior to imposition of stress) and the final (after recovery growth period) values on total leaf number per plant was computed.

Significant difference in RWC was observed, genotypes 990 and 1301 recorded higher RWC values compared to other genotypes studied. Water potential also showed the similar trend. Both 1301 and 990 had higher water potential (less negative water potential values). In addition to water potential and RWC, an attempt was made to determine solute potential with an objective to assess the turgor potential differences at the end of stress cycle. The solute potential was significantly less (more negative) in 987 and also in Pusa Ruby.

Table 29. Differences in whole plant (seedling) survivals at low tissue water potential and the recovery ranking on alleviation of stress.

Genotypes	RWC(%)	ψ_w MPa	ψ_{tr} MPa	Per cent recovery	Leaf sur- vival in seedling	Survival per cent per pot
Pusa Ruby	34.25c	3.30b	3.98b	146.00b	40.5c	40.0c
990	43.25a	2.57c	3.03d	235.45a	50.75a	50.0b
1301	39.75ab	3.10b	3.64c	180.00b	42.50c	57.0a
987	39.00b	3.5a	4.05a	221.68a	75.00b	64.5a
CD at 5%	3.67	0.26	0.042	35.36	5.55	7.69

In this experiment stress was imposed during the early stages of plant growth specifically to look into the seedling survival under stress. Number of seedlings survived at the end of stress cycle was computed based on initial and final seedling number per pot. Prior to stress imposition number of seedlings per pot were 6. The seedling survival was significantly high in 987 and least in Pusa Ruby.

Per cent dessicated leaves, a reflection of leaf survival was significantly less in 987 than other genotypes. Both in 1301 and Pusa Ruby, the percentage of dessicated leaves was as high as 58-68 per cent.

Though the leaf survival percentage was not very high in 990, the recovery per cent was on par with 987 and it was significantly more than Pusa Ruby and 1301 (Table 29).

Results of seedling survival experiment also confirmed that 987 showed relatively high tolerance and the Pusa Ruby least tolerance based on all the characteristics. However, amongst these two genotypes 1301 and 990, the leaf survival and recovery percentage was significantly more in 990, indicating that these two genotypes are moderately tolerant at seedling stage when soil moisture depletion rate was very fast.

Desiccation tolerance - Relative ranking of the genotypes

Three different approaches were attempted to assess the relative desiccation tolerance of these genotypes.

1. The extent of leaf mortality at given water status (Table 26). The mean ranking of all the four such experiments are presented in Table 30.
2. Extent of leaf mortality as a measure of susceptibility in plants subjected to severe but gradual stress under field conditions (Tables 27, 28, 30).
3. Per cent seedling survival and leaf mortality in young seedlings subjected to rapid wilting (Table 29, 30).

For assessing the relative drought tolerance amongst the genotypes, the data obtained on per cent survival (leaves and seedlings) and the per cent recovery of the field experiment was given greater importance. Based on these results, further analysis of the seedling survival was made and the data obtained on the leaf survival of four genotypes was assessed. Amongst these four genotypes, based on survival studies, the following three categories can be made.

Table 30. Overall ranking of Lycopersicon species based on leaf and whole plant (seedlings) from different container and small plots experiments.

Genotypes	Leaf survival Mean table 1,2 & 3	Leaf survival in seed- ling table 23	Seedling survival per pot Table 28	Leaves survival Table 27	Seedling survival Table 27
Pusa Ruby	V	IV	IV	VII	VII
1048	II	-	-	IV	IV
990	VI	III	II	V	V
654	I	-	-	VI	VI
1086	III	-	-	I	I
1301	VII	II	III	III	III
987	IV	I	I	II	II

1. Tolerant - 987
2. Moderately tolerant - 990 and 1301
3. Susceptible - Pusa Ruby.

Though the genotype 1086 showed better survival characteristics in small plot experiment and leaf survival at a given water status this genotype was not used further studies because of its intrinsic susceptibility to virus disease.

4.3.2 Variation in proline content of *Lycopersicon* species subjected to different levels of moisture stress

Experiment-I

Plants experiencing different abiotic stresses have been shown to accumulate proline. Though the role of proline as an osmoticum has not been proven, the possible role of proline to impart desiccation tolerance has been elucidated. The tissue proline content increases with decreasing tissue water status. But differences in proline content at a given tissue water status alone may have some relevance in reflecting the proline synthesising ability of the genotype. Keeping this in view, the relative proline accumulation in a few *Lycopersicon* species and genotypes of *Lycopersicon esculentum* was studied in plants subjected to moisture stress under field conditions in small plots.

The duration of stress cycle was 21 days. From 7th day after imposition of stress, at regular intervals, in addition to tissue water status and leaf survival, proline content also was determined (Appendix-7). In general with decrease in RWC the proline content showed an increase (Fig.8a). However, at a given RWC, there were significant differences in the extent of proline accumulation amongst various *Lycopersicon* genotypes (Fig.8b). For example at RWC of 50-55, highest proline content recorded was in 987 followed by 1048, and lowest was in 564. Similarly, significant variation was also seen in the extent of proline accumulation amongst genotypes when they are compared at a leaf RWC of 40-45 or at 35-40. The proline content was significantly high in 1048 and least was in 564 and 990. Even in Pusa Ruby and 1301 also the amount of proline accumulated was relatively less.

Results of this experiment clearly suggests that at a given RWC there were significant genotypic differences in proline accumulation.

Experiment-II

Proline accumulation amongst all these *Lycopersicon* genotypes was studied in an other experiment by subjecting the plants to rapid wilting in pots. During drying cycle proline content was

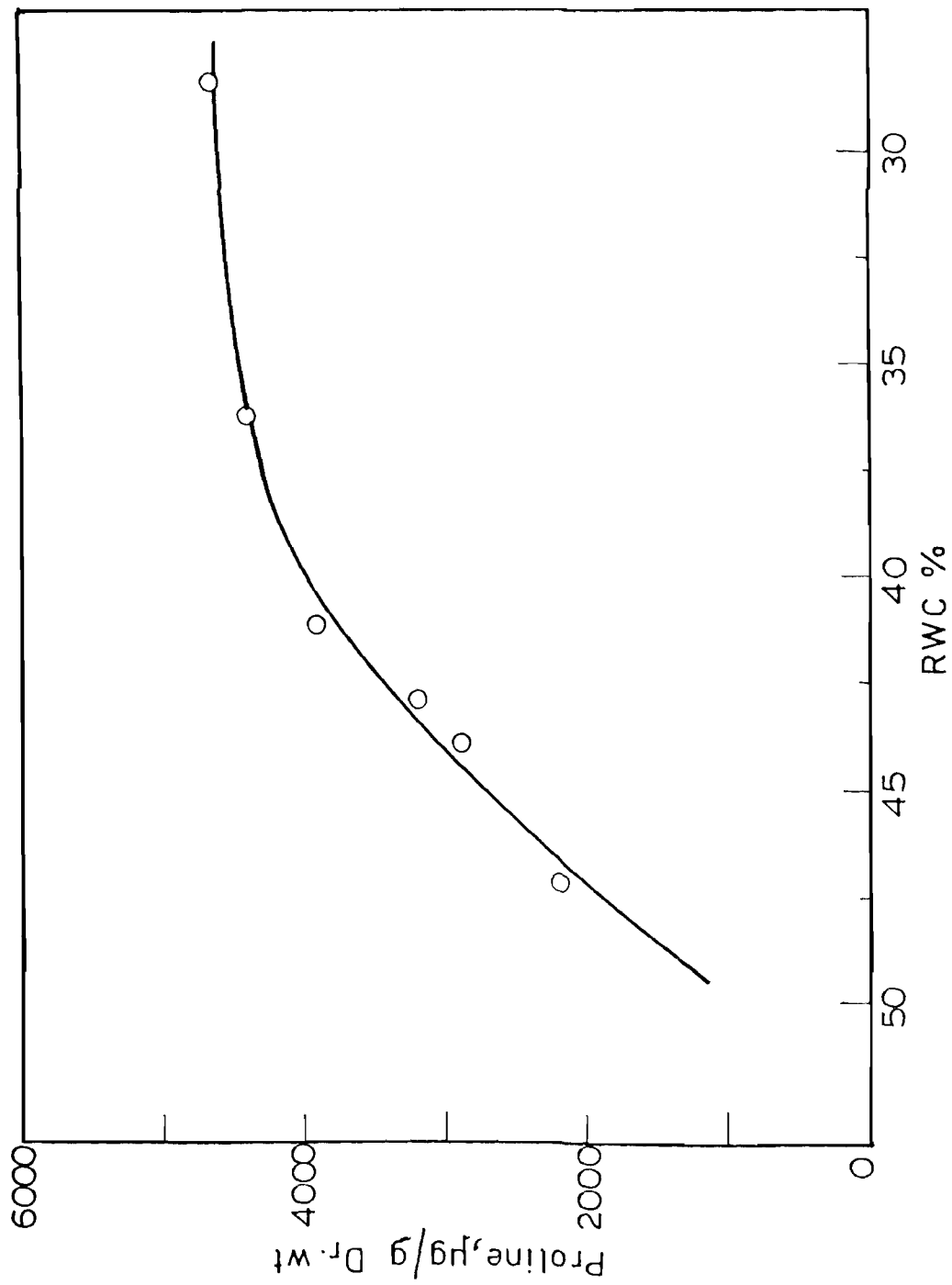


Fig.8a: Proline accumulation in *Lycopersicon* species under moisture stress condition.

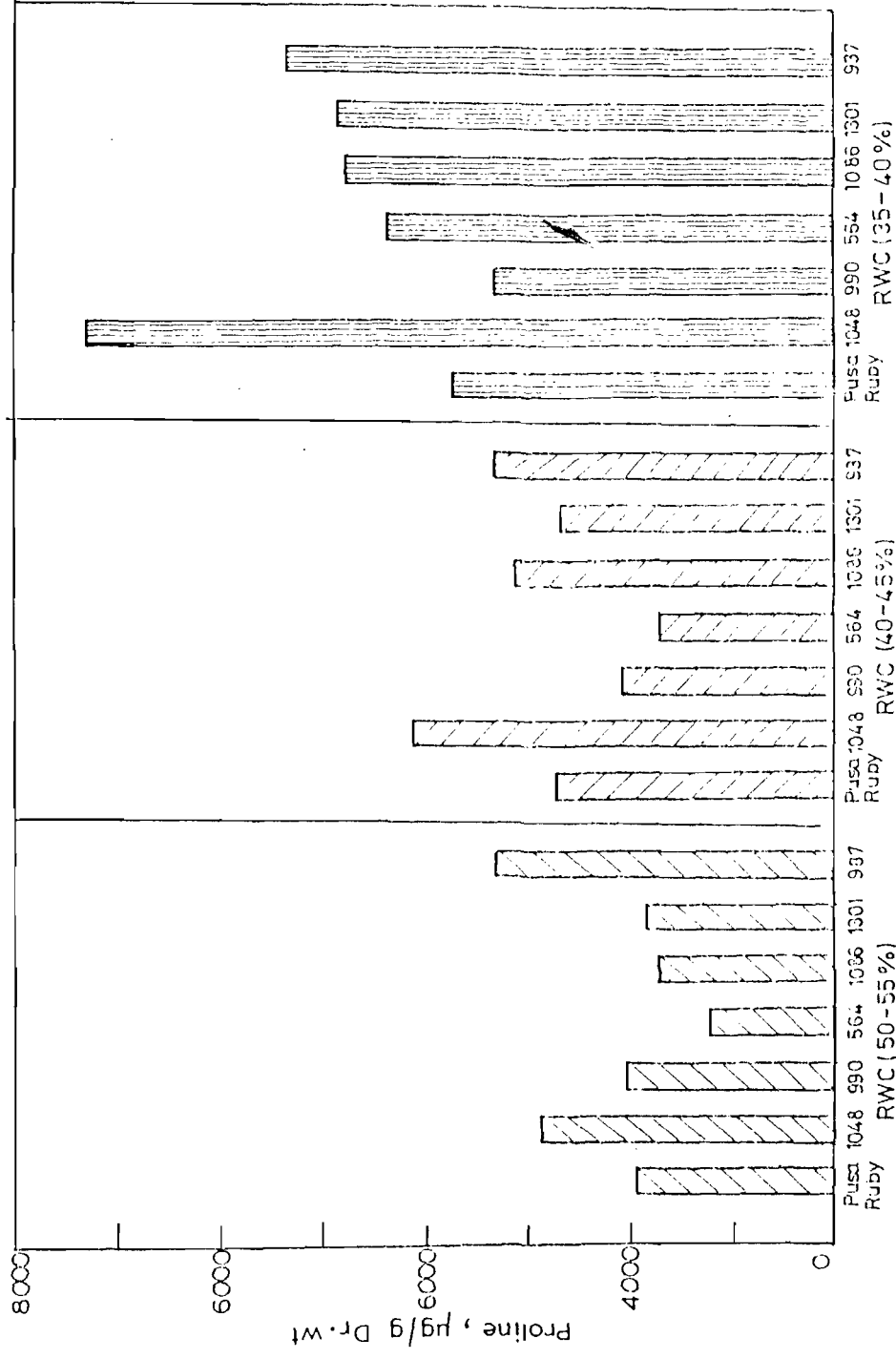


Fig.8b: Proline accumulation in seven Lycopersicon species under moisture stress condition.

determined. The proline values for a comparable RWC of 40-45 amongst Lycopersicon genotypes were given in Fig.8c. Highest proline accumulation was in 1048, 987 and least was in 990 compared to other genotypes.

The following relative ranking of these genotypes can be made with regards to proline accumulation based on the results obtained from the two experiments. (1) 1048, (2) 987, (3) 1086, (4) Pusa Ruby, (5) 1301, (6) 564 and (7) 990.

It can be concluded that 1048, 987 and 1086 as high proline accumulator, Pusa Ruby 1301 as moderate proline accumulators and 564 and 990 as low proline accumulators.

4.3.3 Variation in osmotic adjustment in genotypes differing in desiccation tolerance

Maintenance of the positive turgor by osmotic adjustment could be a mechanism for desiccation tolerance amongst the genotypes. To prove the relative importance of osmotic adjustment in desiccation tolerance (seedling or leaf survival), a pot culture experiment was conducted with two genotypes 987 and Pusa Ruby. These genotypes selected differed in the desiccation tolerance and also relative accumulation of osmotic solutes under stress. Genotypes 987 was categorised as tolerant based on the ranking in survival experiment (Table 26-30 Table 30 overall

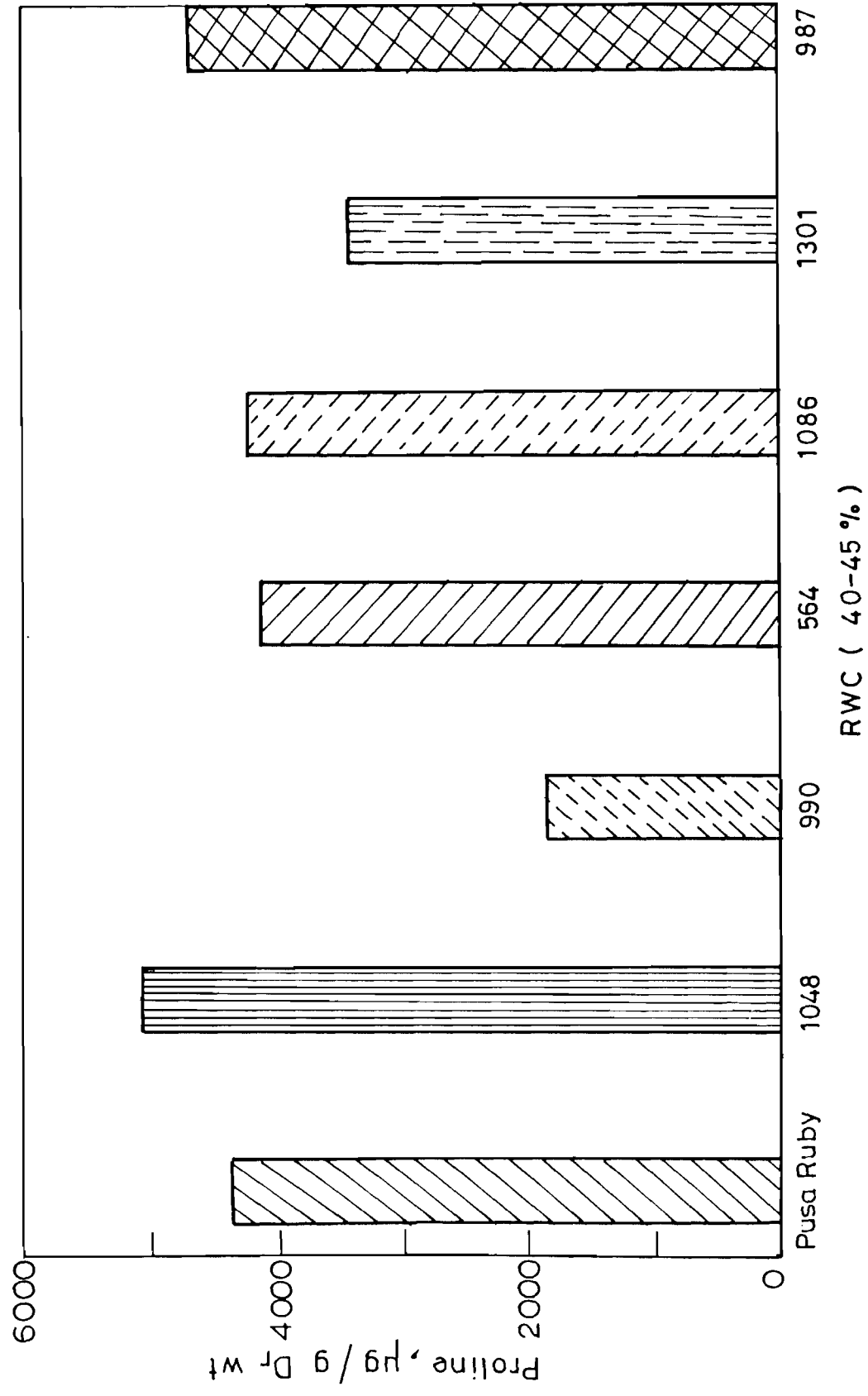


Fig.8c: Proline accumulation at 40-45 per cent RWC in seven Lycopersicon species.

ranking). It is interesting to note that this genotypes also showed a relatively lower solute potential (Table 25 and 29) associated with high turgor potential in seedlings survival experiment compared to Pusa Ruby.

To confirm this, another pot culture experiment was conducted where at different days after stress imposition, water relation characteristics and osmotic adjustment were determined for these two genotypes. Also the data on shoot and root biomass at the end of the stress cycle and the per cent recovery was recorded. As the soil moisture was depleted, there was a decrease in RWC and water potential in both the genotypes. At the end of the stress cycle the water potential and RWC were lower in Pusa Ruby than in 987. For instance at the end of drying cycle, the tissue water potential in Pusa Ruby was -2.6 MPa where as the corresponding value in 987 was -1.96 MPa and the respective RWC values were 33.6 and 49.7. Irrespective of relatively high water status in genotype 987, the osmotic adjustment was more. From the 4th day after the stress imposition, there was higher osmotic adjustment in 987. The osmotic adjustment values for Pusa Ruby and 987 on day 9 was 0.25 and 0.36 MPa respectively. Even under severe stress on day 14 also 987 showed higher osmotic adjustment than Pusa Ruby.

In Pusa Ruby the RWC reached to 30 per cent on day 14 itself. Hence, no further data on water relations were recorded in this genotype. But in 987 the water relation data on day 16, the last day of stress cycle was also recorded. The data clearly indicates that there was significant increase in OA values in 987 (OA 0.43 MPa); (Table 31,32).

Based on the primary data on solute potential and RWC, an attempt was made to determine the intrinsic osmoregulation behaviour from that of passive concentration build up. For this purpose the relationship between log solute potential and log RWC was examined for these two genotypes, Pusa Ruby and 987 (Fig.9a and 9b). Fitting the linear regression between the log solute potential and log RWC, also indicate that there is active accumulation of solutes in the two genotypes as the calculated slopes for the linear regression was -1 that would be expected if the cells were behaving as ideal osmometer. However, the extent of OA between the two genotypes differed substantially.

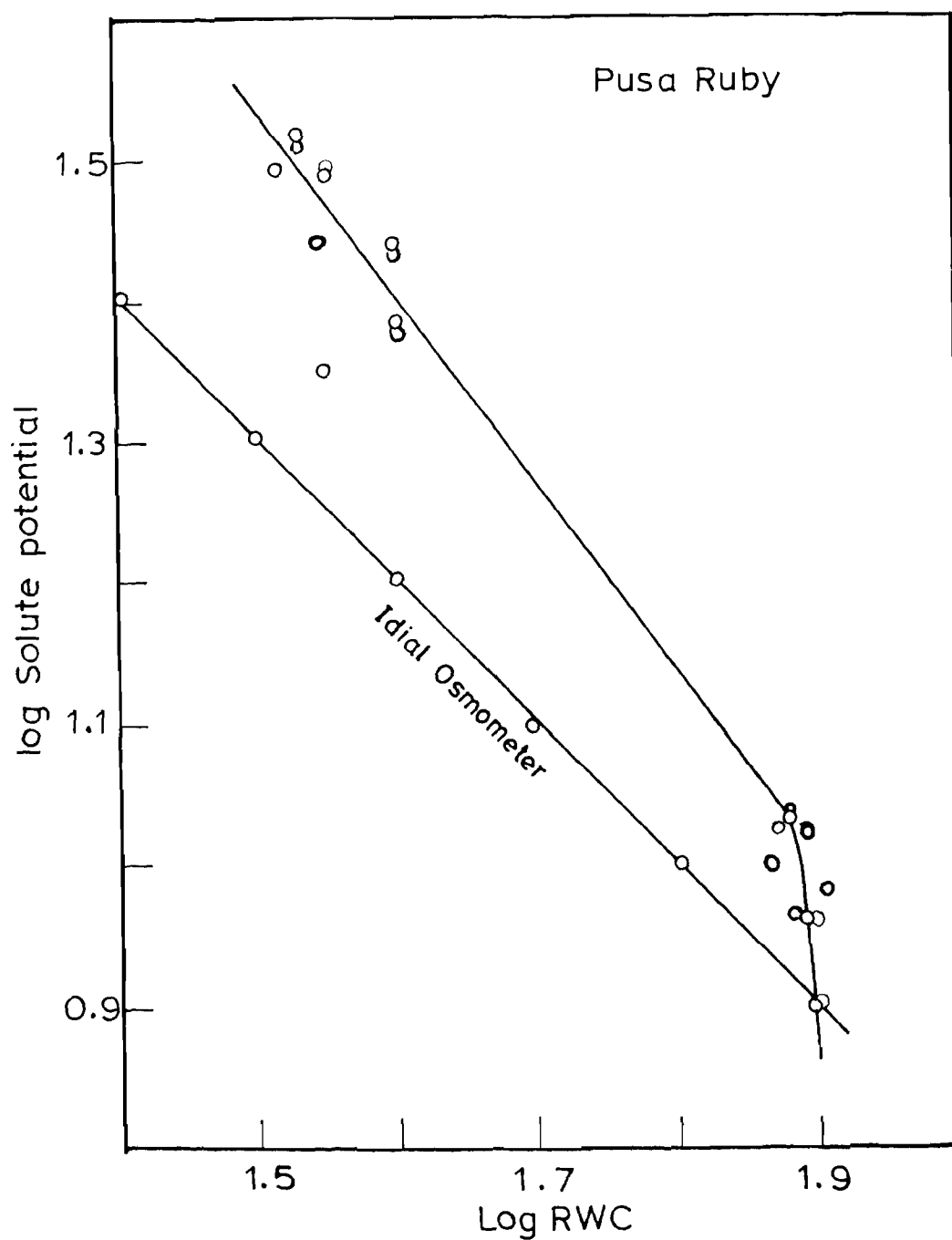
A plot of log RWC and log solute potential for the two varieties showed that there were two linear phases. For e.g., in 987 in the first linear phase there was little change in RWC as solute potential declined (became more negative) (upto log 1.75). The initial response in 987 did not follow ideal osmometer behaviour and it can be attributed mainly to osmotic

Table 31. Differences in osmotic adjustment in two *Lycopersicon* species distinctly differing in desiccation tolerance and intrinsic solute potential.

Genotypes	Days to stress	MPa	MPa	RWC(%)	OA MPa
Pusa Ruby	d0	0.92	0.99	82.5	-
	d4	1.46	1.36	47.8	0.05
	d9	1.76	2.69	38.8	0.25
	d14	2.6	3.14	33.6	0.31
987	d0	0.93	1.14	75.2	-
	d4	1.33	1.29	69.3	0.12
	d9	1.36	1.79	67.8	0.36
	d14	1.96	2.55	47.9	0.43
CD at 5%	G	0.589	0.583	2.045	0.03
	S	0.830	0.824	2.892	0.04
	GxS	1.179	1.166	4.089	0.05

Table 32. Osmotic adjustment under stress in two Lycopersicon species differing distinctly in drought tolerance and intrinsic differences magnitudes of stress.

Genotypes	Days 16 (Severe stress)				Per cent recovery	Root characteristics at the end of experiment (16 days)			
	Ψ_{L}	Ψ_{R}	RWC(%)	OA		Root	Root/shoot	Root dry	Shoot dry
	MPa	MPa		MPa		length (cm)	ratio(mg/g)	weight(mg)	weight(g)
Pusa Ruby	-	-	-	-	47.7	15.8	176.5	126	0.97
987	2.13	3.37	41.9	0.61	59.4	27.7	301.7	310	1.10



19.9a: Relation between log solute potential and log RWC in 987 genotypes under stress. The linear line indicates relation expected if cells were behaving as osmometer.

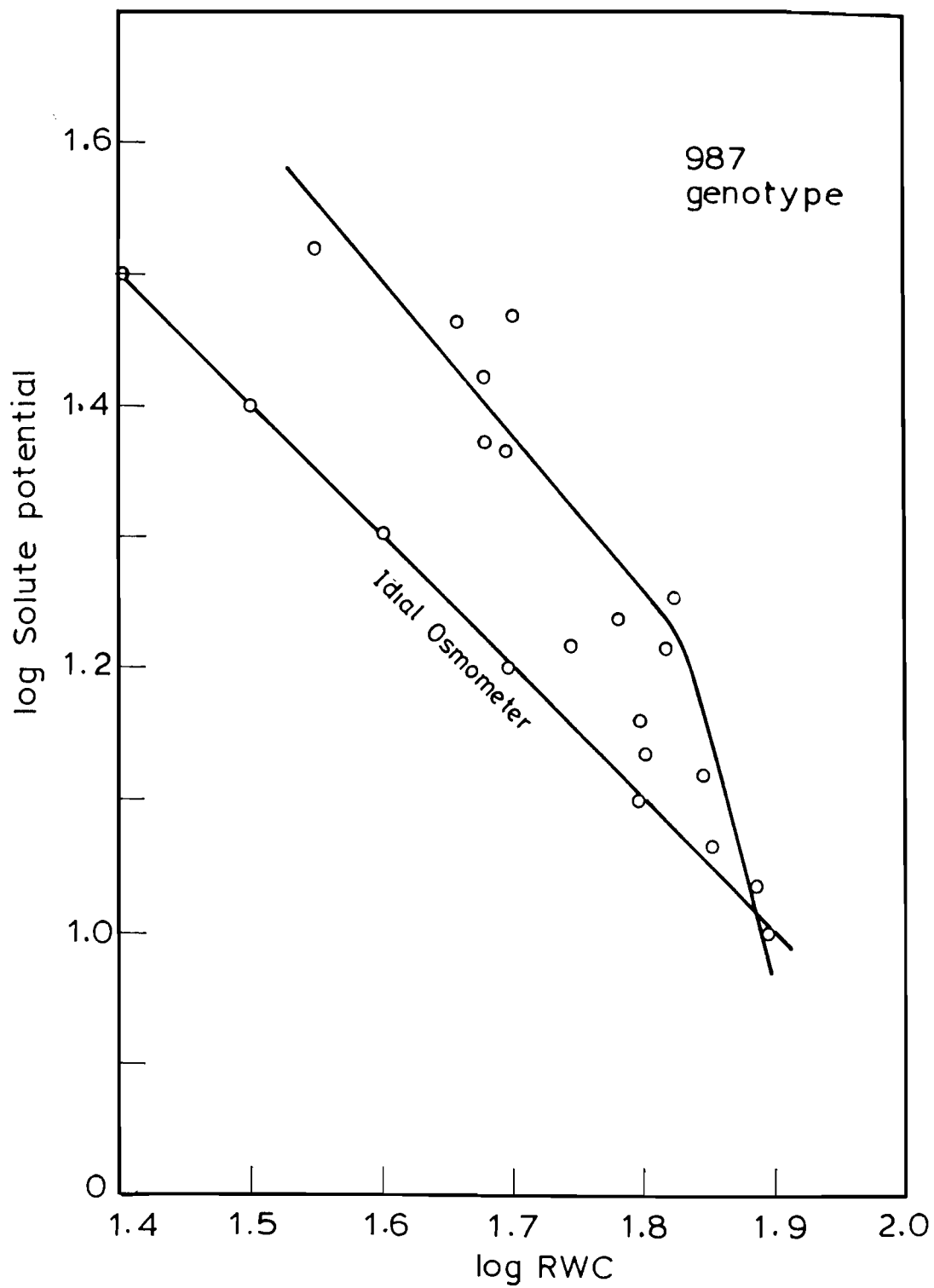


Fig.9b: Relation between log solute potential and log RWC in Pusa Ruby genotype under stress. The linear line indicates relation expected if cells were behaving as osmometer.

adjustment. After this in 987 there was largely a linear phase (from log RWC 1.75 to log 1.45). However, this linear phase followed the line for ideal osmometer behaviour and can be thus attributed to passive solute concentration due to water loss. In the case of Pusa Ruby the first linear phase of true OA was very short (only upto log RWC 1.85 from log RWC 1.9), indicating that although osmotic adjustment occurred it was evident only for a very short period after water was withheld. Thereafter (from log RWC 1.85 to 1.45), the line was linear and closely followed the ideal osmometer line suggesting that further change in UII was only due to passive concentration due to water loss.

Turgor was thus maintained for a longer period (as indicated by a longer first linear phase) in 987 as compared to local.

Biochemical constituents of osmotic adjustment

The analysis of the different solutes which contributed to osmotic adjustment and the degree of their contribution is of considerable importance in studies on drought tolerance due to osmotic adjustment.

The solutes can be either sugars, amino acids, potassium, polyols etc. In addition, the changes in starch content during stress is also important as a

higher level of starch could on hydrolysis lead to greater increase in the levels of sugars. Such an analysis was made in the two varieties during the start of stress day (d0) and upto the end of stress (day 14).

The data are presented in Figures 9c,d,e for the two varieties. Total amino acids, total soluble sugars (Hexoses, pentoses), sucrose were the solutes that assessed with the development of stress. The amount of amino acids increased from 110 mM to 425 mM in 987 at the end of stress, while Pusa Ruby showed only a marginal accumulation from 95 in (d0) to 155 mM (d14), (Fig.9c).

A similar trend was observed when the amount of hexoses and pentoses were analysed as a reflection of total soluble sugars. In general the pentose sugars were present in higher concentration than hexoses in 987, while in Pusa Ruby, the concentrations were nearly similar. On d14 the concentrations of these solutes were nearly two fold higher in 987 than in Pusa Ruby. These differences were seen, although to a lesser marked extent even under non-stressed condition (Fig.9d).

The sucrose content was separately determined by a sensitive enzymic hydrolysis method. Generally, sucrose levels were low in both genotypes under non-stressed conditions. A significant increase was

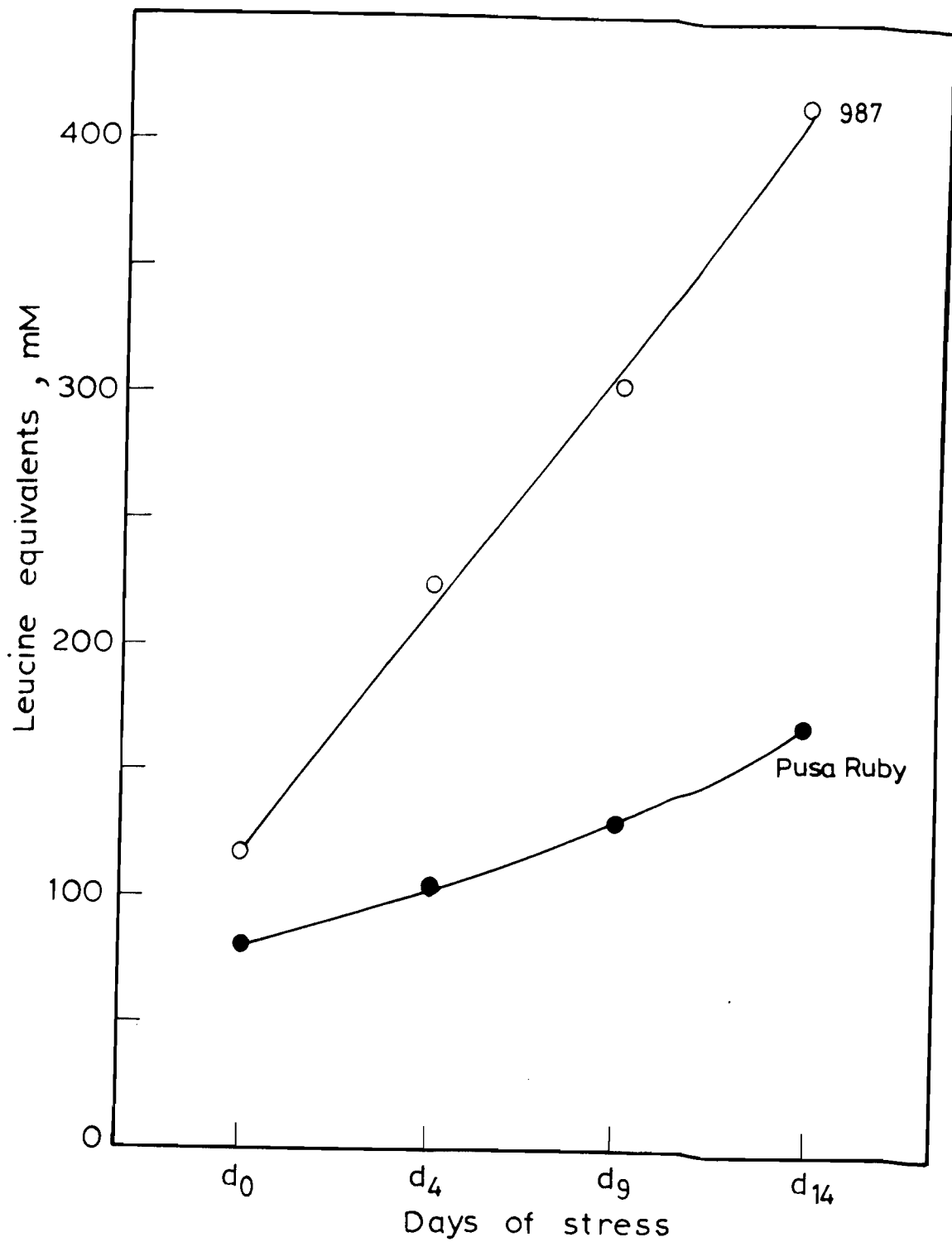


Fig.9c: Total amino acids as leucine accumulation in Pusa Ruby and 987 genotypes under moisture stress condition (each point is mean of eight samples).

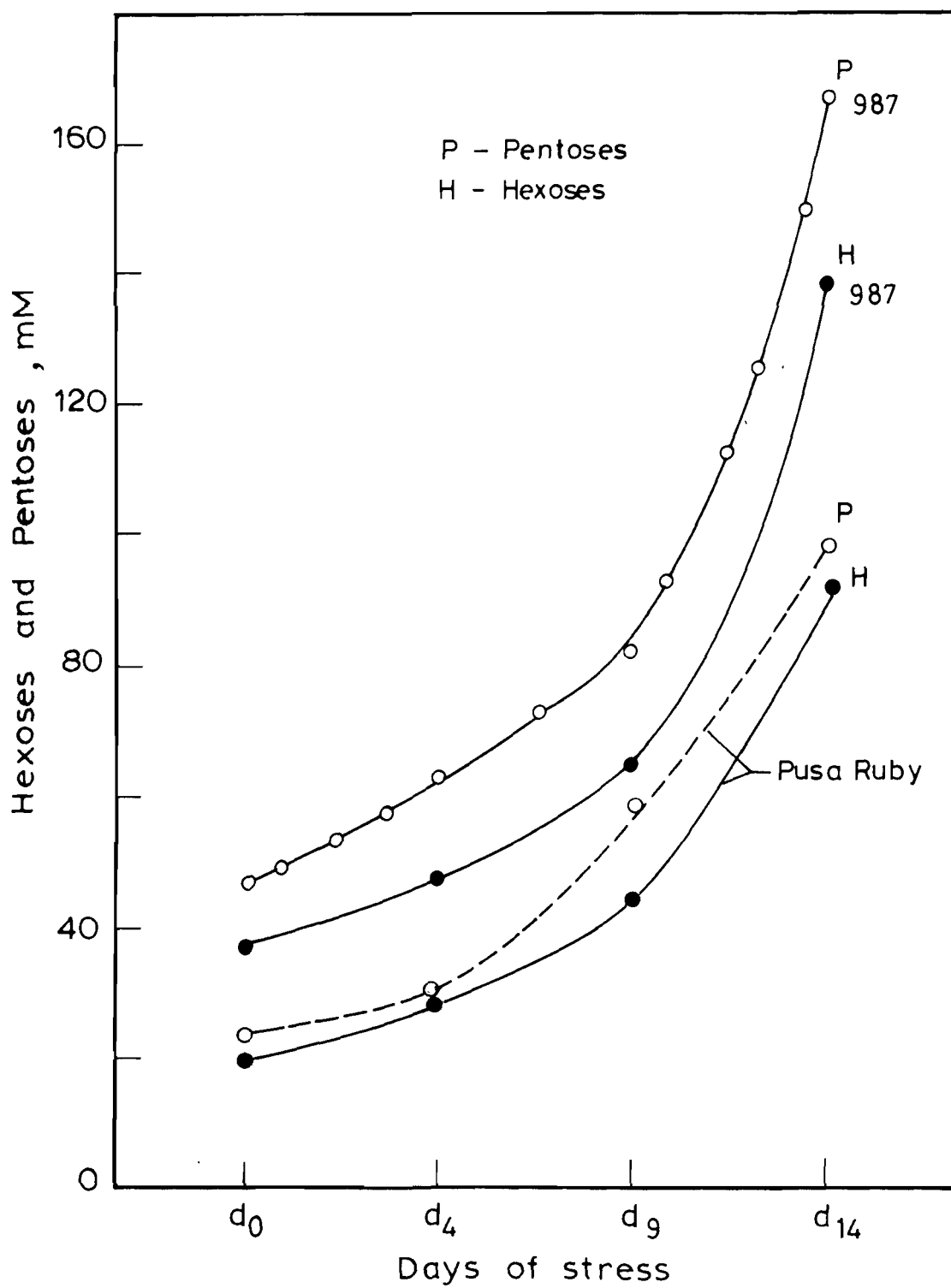


Fig.9d: Total soluble sugars (pentoses and hexoses) accumulation during stress period in Pusa Ruby and 987 genotypes (each point is mean of eight samples).

observed in both varieties under stress resulting in a two fold increase on d14 in both varieties as compared to non-stressed plants. However, in 987 sucrose was still maintained at higher levels in all days of stress (Fig.9e).

As seen from Figures 9c, d, and e, variety 987 accumulated significantly higher amounts of all these solutes than Pusa Ruby at all levels of stress. This suggested that variety 987 showed higher osmotic adjustment by higher solute synthesis accumulation on each day of stress. At the end of stress period (d14) the accumulation of solutes was much more in 987 compared to Pusa Ruby. However, it is also interesting to note that 987 had a significantly higher initial starch content as compared to Pusa Ruby (Fig.9f).

Turgid weight/dry weight ratio

Although osmotic adjustment by accumulation of solutes results in turgor maintenance at low water potential, turgor maintenance can also occur or arise due to other reasons. For example tissues with high elasticity exhibit large changes in volume per unit change in turgor. This allows the RWC to decrease to a relatively low value before turgor is completely lost, and the cell solutes can thus be concentrated to a greater degree, although there is very little change in solute potential at a any given RWC.

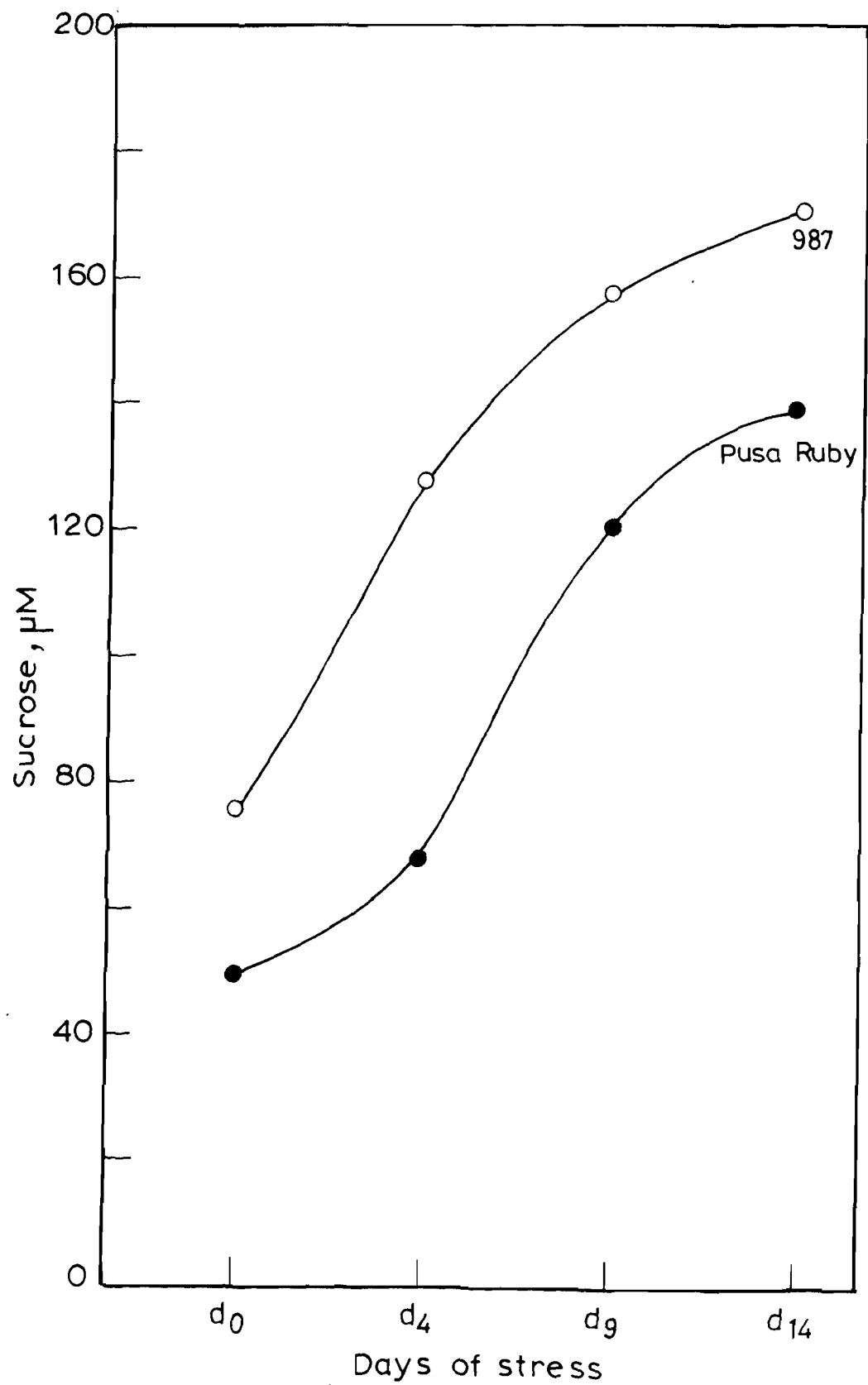


Fig.9e: Sucrose accumulation in Pusa Ruby and 987 under moisture stress conditions (each point is mean of eight samples).

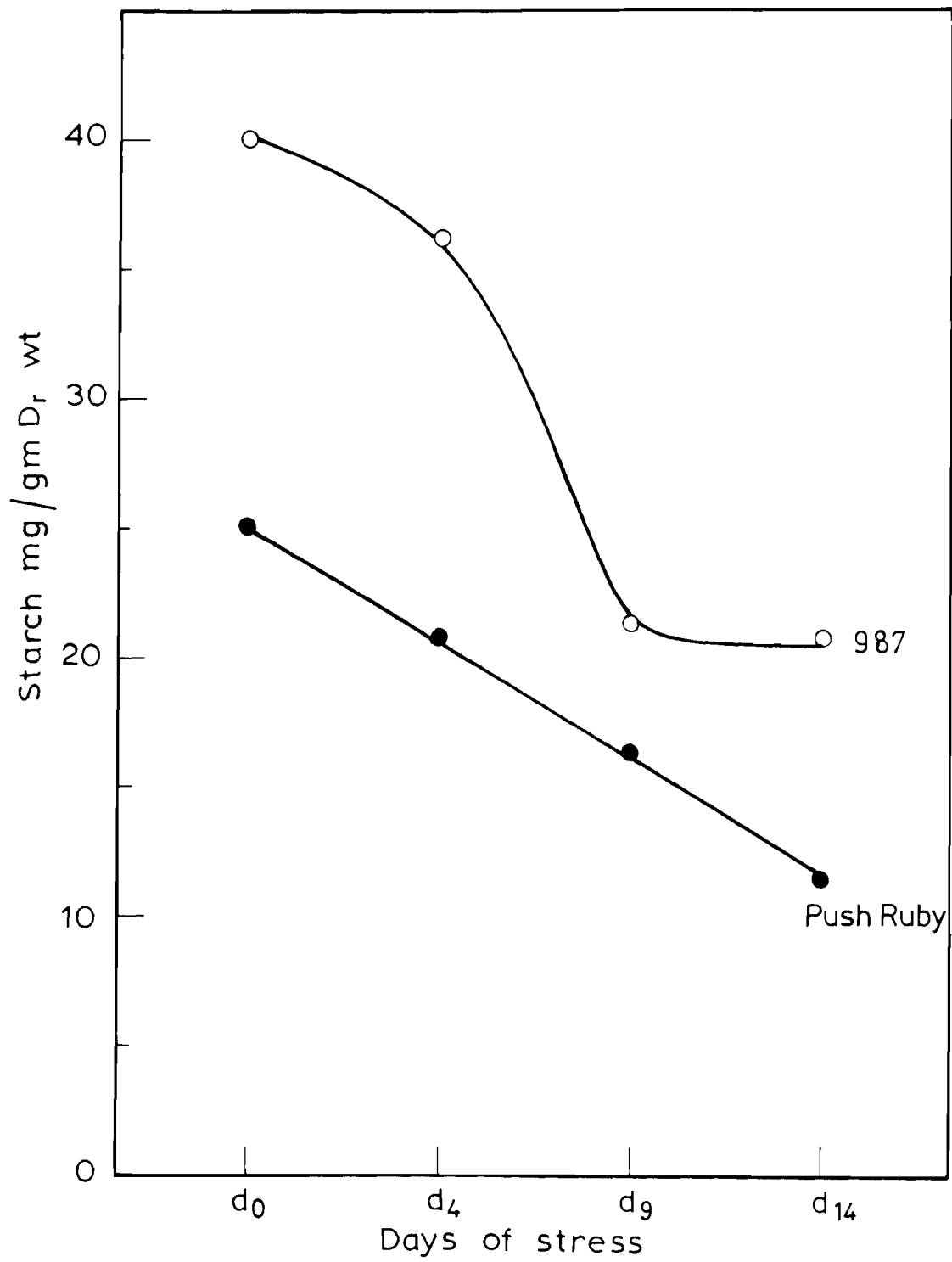


Fig.9f: Starch accumulation in Pusa Ruby and 987 genotypes under moisture stress condition (each point is mean of eight samples)

Plate 2: Pusa Ruby L. esculentum and 987 L. esculentum variety ceraseforme plants subjected to moisture stress (at d14)

A decrease in this ratio, under stress conditions, from a change (decrease in cell elasticity) resulting in a greater increase in water content or due to an accumulation of osmotically active or osmotically inactive material in the cytoplasm. Several studies have shown a relationship between the change in this ratio and the degree of cell turgor (e.g., 1959, 1960, 1961, 1962, 1963, 1964, 1965, 1966, 1967, 1968, 1969, 1970, 1971, 1972, 1973, 1974, 1975, 1976, 1977, 1978, 1979, 1980, 1981, 1982, 1983, 1984, 1985, 1986, 1987, 1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025).



The ratio indicates that changes in cell elasticity during an increase in turgor contribute significantly to the change in ratio. Other factors contributing to the change in ratio (or osmotic potential) which could cause a larger change in dry weight.

A decrease in this ratio under stress can arise, from a change (decrease in cell elasticity) resulting in a greater decrease in water content or due to an accumulation of osmotically active or osmotically inactive material in the tissue. Several studies have shown a relationship between the changes in this ratio and the degree of OA (Wilson et al., 1980; Turner, 1987).

The data on this ratio at different days of stress, corresponding to increasing magnitude of stress, are presented in Table 33.

The data suggested that there was an increase in the ratio in both genotypes with increasing intensities of stress. For e.g., in Pusa Ruby the ratio increased from 8.5 to 11.5 on day 14 while it increased from 9.0 to 13.4 on day 14 in 987. In 987 data on day 16 showed a further increase from 13.4 to 14.9.

The data suggested that changes in cell elasticity leading to changes in turgid weight contributed significantly to the change in ratio, rather than active accumulation of osmotically active (or non-osmotic) solutes which could cause a major change in dry weight.

Table 33. Turgid weight/dry weight ratio on different days of stress in two distinctly differing Lycopersicon species.

Genotypes	D0	D4	D9	D14	D16
Pusa Ruby	8.5	9.9	10.0	11.5	-
987	9.0	10.4	12.3	13.4	14.9

4.3.4 Assessment of desiccation tolerance based on some physiological and biochemical parameters

Decrease in tissue water status affect several cell metabolic activities like carbon assimilation, protein synthesis, nitrogen metabolism etc. Stability of any of these characteristics is a reflection of intrinsic tolerance due to dehydration. An attempt was made to study the effect of moisture stress on carbon assimilation, protein synthesis in these four selected genotypes which showed differences in degree of survival in empirical screening.

4.3.4.1 Potential photosynthetic rates in plant subjected to stress

Carbon assimilation has been shown to be decreased under stress and it was attributed due to decrease in both stomatal as well as mesophyll factors. The environmental factors like VPD and the internal factors like C_i and ABA affect the stomatal factor and hence the carbon assimilation rate. Though it has been shown that stomata offers less limitation to carbon exchange under moisture stress conditions, CER can not be a reflection of intrinsic tolerance of photosynthetic organel to the desiccation. Therefore in recent years, to study the effect of moisture stress on chloroplast functioning, the carbon fixation ability of leaves is determined at saturated CO_2 concentration. The leaf discs are often exposed to

high ambient CO₂ levels of 5 to 15 per cent and either carbon gain or oxygen evolution is determined as a function of chloroplast activity. A method to determine potential photosynthetic rates in excised leaf tissue using ¹⁴CO₂ was adopted after suitable modification. In this method excised leaf punches were exposed to 5 per cent CO₂ concentrations (¹²CO₂ + ¹⁴CO₂) in assimilation leaf chamber and exposed to saturated light intensities under normal conditions. The RH was maintained high enough not to alter significantly the tissue water status during the experimental period.

The turgid leaves from pot grown pots were brought to laboratory and subjected to live wilting for different periods of time. The rate of water loss was periodically monitored and at 20, 30, 50 and 60 per cent reduction in fresh weight, the carbon fixation was determined at saturated CO₂ and light intensities as described earlier. All the leaves with different tissue water status were present at the same time in the assimilation chamber and the observed reduction in assimilation rate was mainly due to differences in tissue water status since other environmental factors like light, CO₂ concentration and RH were same for all the treatments.

The per cent reduction in carbon assimilation was computed based on carbon fixed by the control turgid leaves. The data on per cent reduction in these four genotypes are given in Table 34 and Fig.10a. Similar trend in $^{14}\text{CO}_2$ fixation was seen when it was expressed either per gram dry weight or per cm^2 leaf area. Even a 20 per cent decrease in tissue turgid weight, significantly decreased carbon assimilation. With increase in stress intensity as reflected in per cent decrease in turgid weight, the per cent decrease in mean carbon assimilation was more. For instance when turgid weight was decreased to 30 per cent, the carbon gain decreased to 71 per cent and when turgid weight decreased to 60 per cent it was as high as 93 per cent. However, at all stress levels, there was significant, genotypic difference in per cent reduction of carbon assimilation. The per cent reduction in carbon fixation was less in Pusa Ruby followed by 987. In both 1301 and 990, it was significantly high. For instance when tissue water status was reduced to 30 per cent carbon fixation was reduced to 53.3 per cent in Pusa Ruby and 71.5 in 987. However, in 1301 and 990 the corresponding values 80.0, 79.3 respectively. Under severe stress conditions, when the tissue water status was decreased to 60 per cent, there was no marked difference between the genotypes in terms of per cent reduction in carbon fixation. Upto 50 per cent

Table 34. Per cent reduction in potential photosynthetic rate in excised leaf tissue of Lycopersicon species subjected to different levels of dessication based on cpm 100 mg.

Genotypes	Stress levels				Mean
	80	70	50	40	
987	57.1 (49.08)	71.5 (57.73)	89.3 (70.91)	91.6 (73.15)	77.38 (62.72)
1301	63.8 (53.0)	80.0 (63.44)	90.6 (72.15)	93.0 (74.66)	81.85 (65.82)
990	75.0 (60.0)	79.3 (62.94)	92.3 (73.89)	94.3 (76.19)	85.23 (68.26)
Pusa Ruby	41.22 (39.93)	53.30 (46.89)	74.70 (59.8)	93.40 (75.11)	69.35 (57.56)
Mean	62.9 (52.63)	71.03 (57.75)	86.73 (69.19)	93.08 (74.78)	78.44 (63.59)
CD at 5%	G	5.1**			
	S	5.7**			
	G×S	11.4NS			

Values in paranthesis indicate angular transformation.

CARBON DIOXIDE FIXATION UNDER STRESS

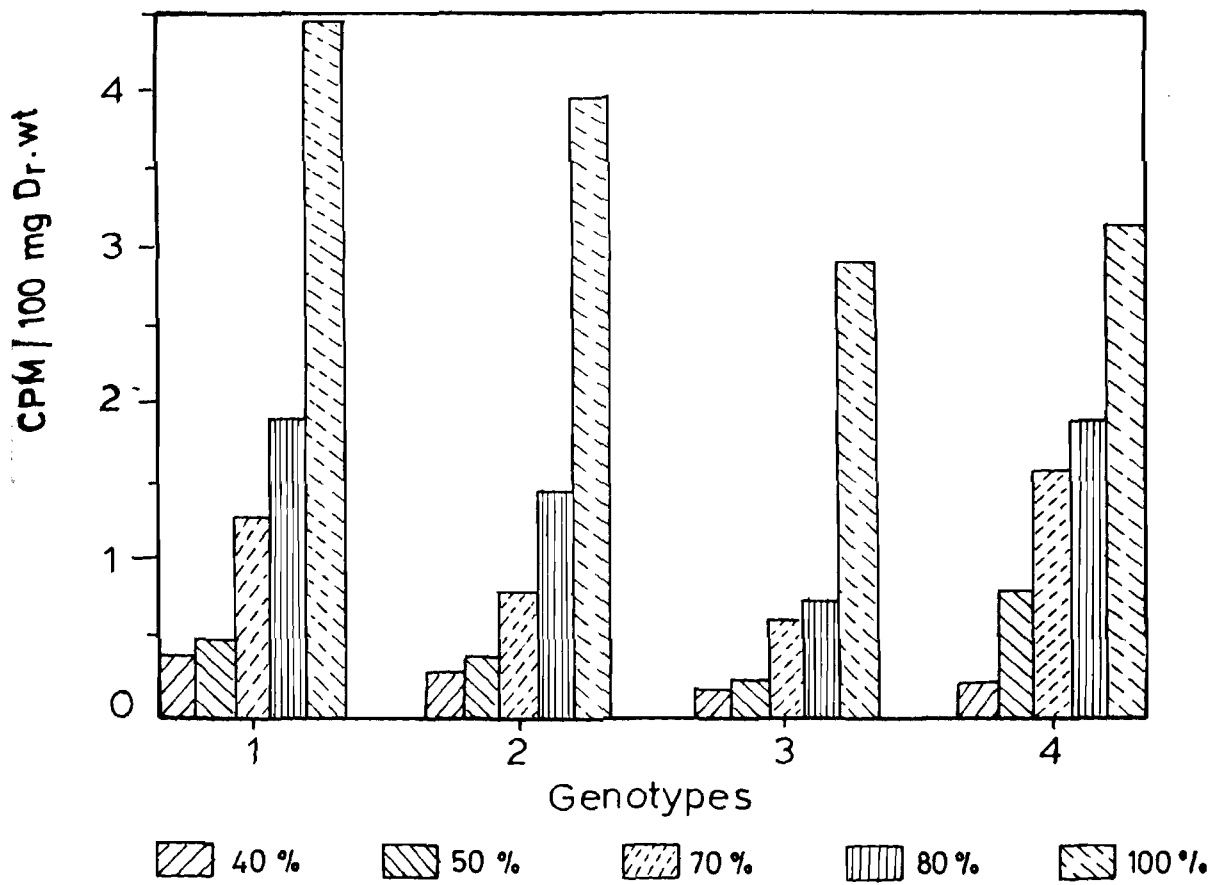


Fig.10a: Carbon dioxide fixation under stress condition amongst four Lycopersicon species (1) 987, (2) 1301, (3) 990 and (4) Pusa Ruby, expressed as cpm/100 mg dry weight.

loss in turgid weight, Pusa Ruby showed significantly lesser reduction compared to other genotypes. But at moderate stress conditions the performance of 987 was on par with Pusa Ruby (Table 35 and Fig.10b).

4.3.4.2 Effect of moisture stress on protein synthesis

The capacity for protein synthesis is an important cellular metabolism which is known to be affected in tissues subjected to dehydration. Even a mild water stress has been shown to affect protein synthesis and its turnover. However, in many plants, if stress is not severe enough, protein synthesis can resume on recovery of plants to full rehydration. Keeping the importance of this metabolic process, protein synthesis under stress has been studied as a measure of desiccation tolerance in different plant species. There are two approaches to study the effect of stress on protein synthesis machinery, to assess the incorporation of labelled compounds in to proteins when tissues are subjected to dehydration and the other approach is to study the rate of incorporation of label during the rehydration of the dessicated tissues.

An experiment was conducted to study the extent of ^{14}C leucine incorporation in four genotypes of *Lycopersicon* which showed differences in intrinsic tolerance.

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Table 35. Per cent reduction in potential photosynthetic rate in excised leaf tissue of Lycopersicon species subjected to different levels of dessication based on cpm cm² LA.

Genotypes	Stress levels				Mean
	80	70	50	40	
987	59.0 (50.18)	71.2 (57.50)	89.8 (71.37)	91.5 (73.05)	82.87 (66.18)
1301	59.9 (50.71)	79.1 (62.8)	89.3 (70.91)	90.0 (71.56)	79.57 (63.99)
990	81.1 (64.23)	82.8 (65.5)	92.2 (73.78)	94.1 (75.94)	87.55 (69.86)
Pusa Ruby	36.9 (37.41)	45.0 (42.13)	68.5 (55.86)	92.8 (74.44)	65.78 (55.34)
Mean	67.17 (55.30)	71.55 (58.34)	84.95 (67.98)	92.1 (73.75)	78.94 (63.84)

CD at 5%	G	4.91**			
	S	5.5**			
	G×S	10.97*			

Values in paranthesis indicate angular transformation.

CARBON DIOXIDE FIXATION UNDER STRESS

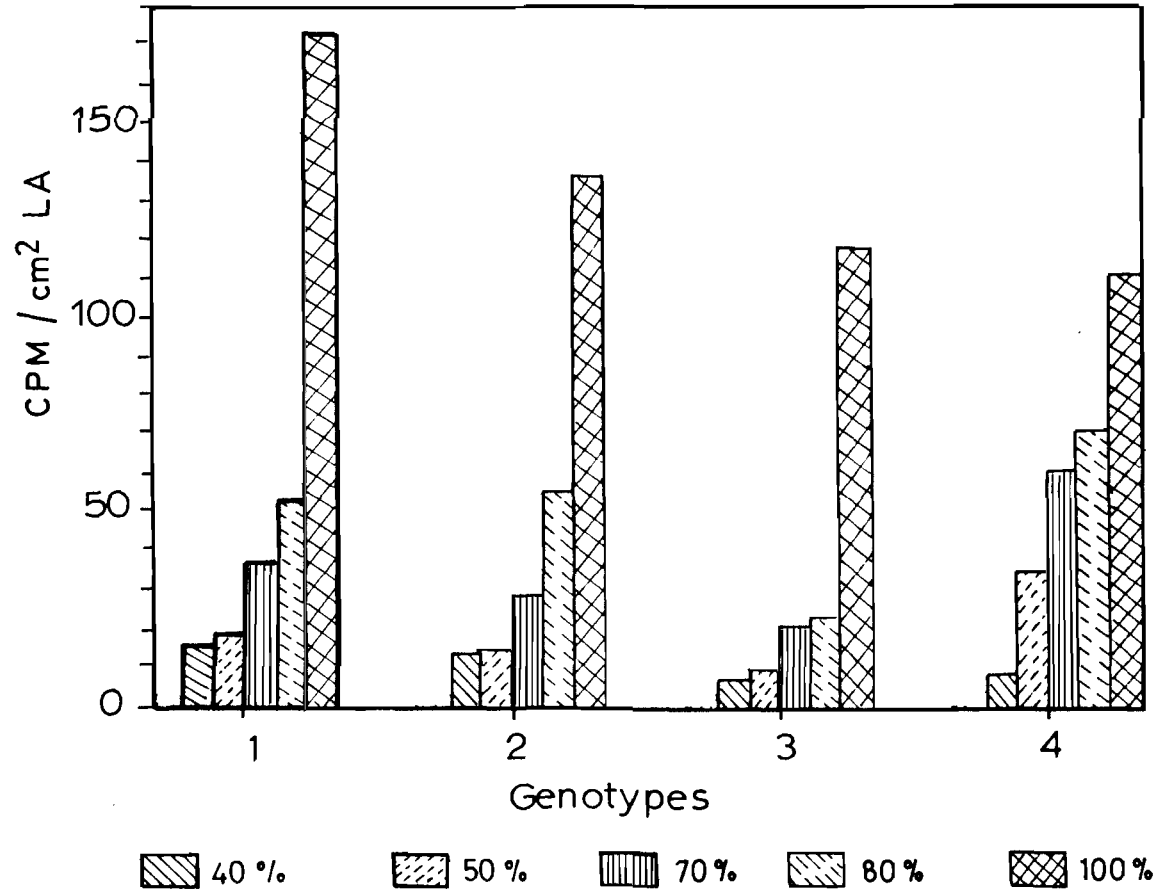


Fig.10b: Carbon dioxide fixation under stress condition amongst four Lycopersicon species (1) 987, (2) 1301, (3) 990 and (4) Pusa Ruby, expressed as cpm/cm² LA.

In excised leaf tissues, stress was imposed by using an osmoticum (PEG) for 18 hours and during the first two hours of stress alleviation period (rehydration period) the extent of incorporation of labelled leucine was determined. In addition to the control treatment, there were two stress levels (-1.5 and -3.0 MPa). The effect of moisture stress on uptake of labelled amino acid, its incorporation and the tissue protein levels were determined and the data are presented in Table 36.

With increase in stress levels, there was a significant decrease in uptake of labelled amino acid. The per cent reduction in uptake was not same in all the genotypes. It was affected to a lesser extent (33.8%) in 987 compared to other genotypes under moderate stress conditions. However, in tissues subjected to higher levels of osmotica (-03.0 MPa), the per cent reduction in uptake ranged from 69.3 to 79.3 indicating a significant decrease in all genotypes. Therefore decrease in the incorporation of label in to protein in all genotypes during stress could be due to decrease in the uptake.

In all the genotypes the precipitable protein content decreased. However, in this regard also significant genotypic variation was observed. Interestingly in genotype 987, the protein content

Table 36. Protein synthesis on alleviation in four Lycopersicon genotypes subjected to osmotic stress.

Treatments		Total uptake cpm *	Incorporation in to protein cpm *	Protein content (µg g fr.wt.)	Incorporation into protein cpm µg protein	Incorporation of per cent of uptake	Incorporation/ unit protein protein as per- cent of uptake
Pusa Ruby	Control	4421	4461	10.0	68.9	7.8	1.6
	-1.5 MPa	2605(41.1)*	244.0(29.5)	8.9(11.0)	54.8(20.5)	9.4(20.5)	2.1(31.3)
	-3.0 MPa	1183.0(73.2)	133.0(61.6)	4.5(55.0)	40.9(40.6)	11.4(46.2)	3.6(125.0)
1301	Control	5618	518	1269	82.2	9.2	1.5
	-1.5 MPa	2372(53.7)	275.0(46.9)	10.0(20.6)	55.0(33.1)	11.6(26.1)	2.2(46.7)
	-3.0 MPa	1293(74.7)	133(74.3)	5.7(54.8)	46.6(43.3)	13.3(44.6)	3.3(120.0)
987	Control	5187	3270.0	12.5	52.4	6.3	1.0
	-1.5 MPa	3.403(33.8)	268.0(59.0)	10.6(15.2)	50.6(3.4)	7.9(25.4)	1.5(50.0)
	-3.0 MPa	1272(79.3)	181.0(72.3)	8.9(28.8)	38.4(27.5)	17.7(85.7)	3.3(23.0)
990	Control	3762	417	13.5	61.7	10.4	1.6
	-1.5 MPa	1805(52.0)	187(54.9)	11.9(11.9)	31.5(52.5)	10.8(3.8)	1.9(18.8)
	-3.0 MPa	1135(69.8)	123.0(70.5)	8.4(137.8)	29.3(52.5)	11.1(6.7)	2.6(62.5)
CD at 5%		6	34.46	0.68	6.50	1.64	0.50
		5	270.68	29.84	0.59	5.63	1.42
		1	541.36	59.68	1.17	11.26	2.84

*Values for 500 µg fresh weight
Values in paranthesis indicate per cent reduction over control.

decreased from 12.48 in control to 10.6 in moderate stress and to 7.6 in severe stress. However, the corresponding values for Pusa Ruby was 10.03, 8.9 and 4.5 respectively. Even in 990 also the per cent reduction in protein content was less under severe stress. Compared to Pusa Ruby and 1301, relatively the protein content under severe stress was high in 987 and 990.

Incorporation of the labelled ^{14}C lucine per mg protein also decreased under stress in all the genotypes. However, the per cent decrease in incorporation of label per mg protein was less in 987 compared to other genotypes especially in tissues subjected to moderate stress.

Since, the stress affected the uptake of labelled amino acid considerably the incorporation of label into the protein as per cent of uptake was computed. One interesting observation was that in control (under non-stress condition) per cent incorporation was more in genotypes 990 and 1301 compared to genotype 987 and Pusa Ruby. In general, in all the genotypes, there was an increase in incorporation under stress when the per cent incorporation was computed based on the total uptake of the label. Even in this parameter also genotypes differed. Per cent increase in the incorporation of the protein was more in 987 followed by Pusa Ruby.

However, in 990 which showed less reduction of protein content under severe stress, a marginal reduction was recorded even when the per cent incorporation was computed based on the uptake.

Similarly the incorporation per unit protein based on the uptake was also computed. Even here a significant increase in incorporation was observed in all the genotypes under stress. However, the per cent increase was much greater in 987 and relatively less 990 and 1301. The observed increase in incorporation of label into the protein as per cent of uptake (either total or per mg protein) under stress conditions can not be explained clearly. However, the possibility is that incorporation was not studied at a similar uptake of the label. The comparison of the stress effect on amino acid incorporation will be more meaningful if the differences in total uptake are marginal.

Amongst the several parameters studied to assess the stress effect in the protein synthesis the extent of incorporation of labelled amino acids per unit protein may give a better picture of the stress effect on protein synthesis. Based on the data on incorporation in to unit protein, the per cent reduction in 987 was relatively less in moderate stress and to certain degree even in severe stress. Even in Pusa Ruby, the per cent reduction was less compared to

990 and 1301. Based on the results on protein synthesis, both 987 and Pusa Ruby maintained relatively higher protein synthesising ability under stress. Amongst these two genotypes, the per cent reduction in protein content was less in 987 further emphasizing the desiccation tolerance of this genotype even based on protein turnover characters.

4.4.5 Studies on membrane integrity

As an important parameter of desiccation tolerance, the maintenance of membrane integrity was studied. The loss of membrane integrity as indicated by the leakage of cellular solutes absorbing at 273 nm was estimated. In all the experiments leaf punches from the turgid leaves were subjected to live wilting and at 50 and 75 per cent loss of water, the extent of membrane damage was assessed. Results of three such experiments are presented.

Experiment-I

When leaf punches were subjected to dehydration there were no marked differences in time taken for 50 per cent or 75 per cent loss of moisture. At 50 per cent loss of moisture, the per cent leakage was very less in Lycopersicum peruvianum 990 and the observed differences in other three Lycopersicon genotypes were not significant (Fig.11a). In all the genotypes except Lycopersicum peruvianum 990. there was

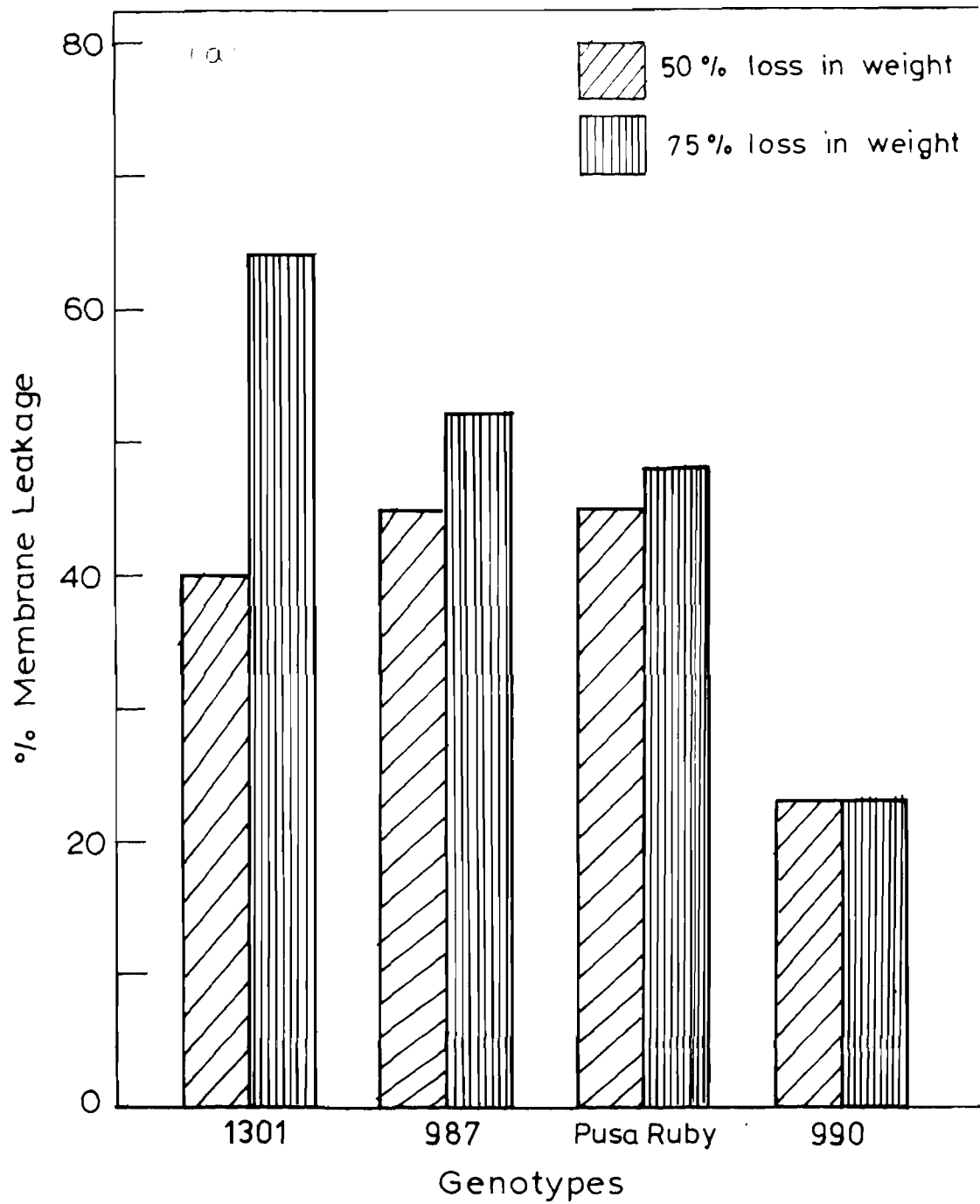
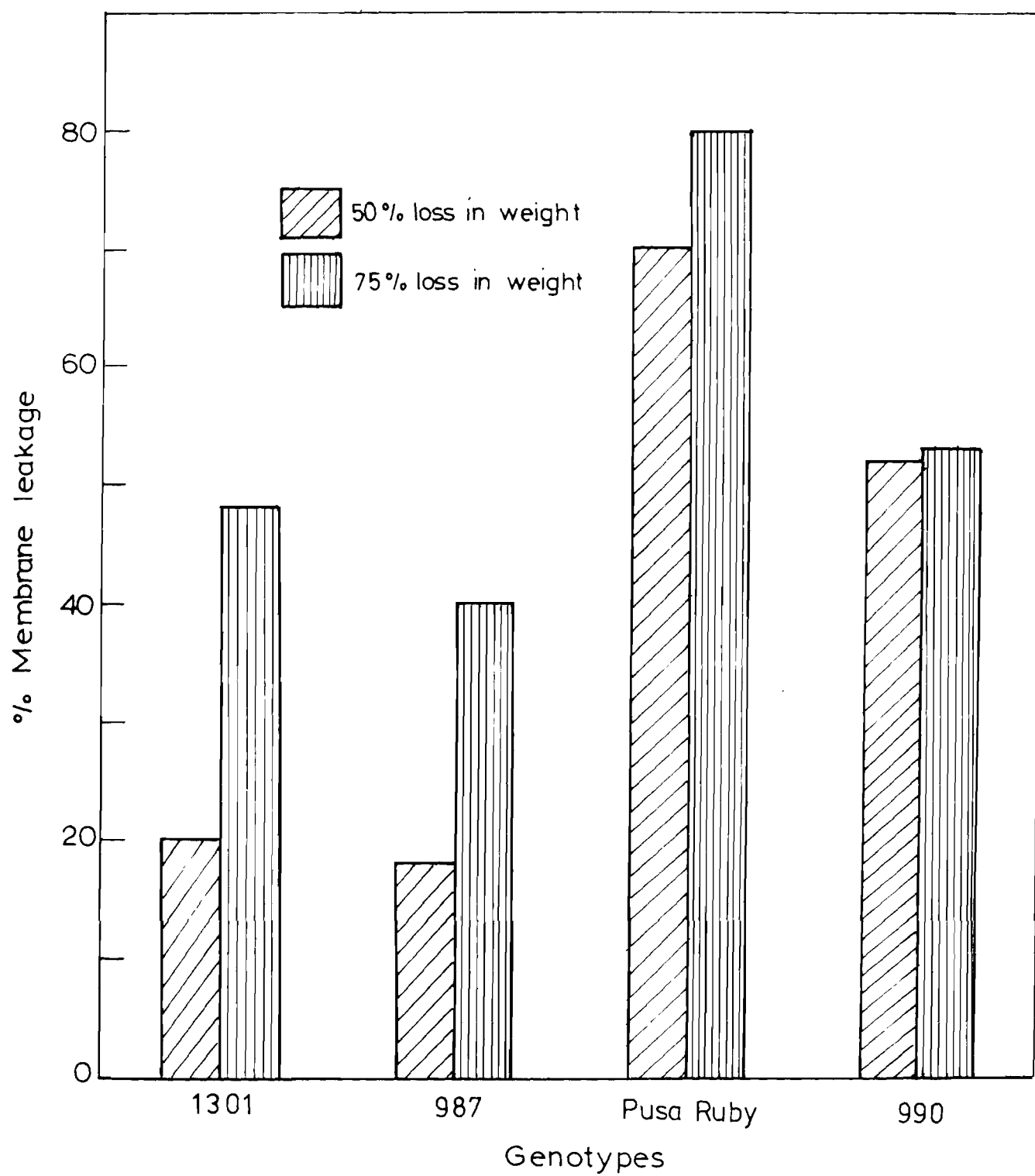
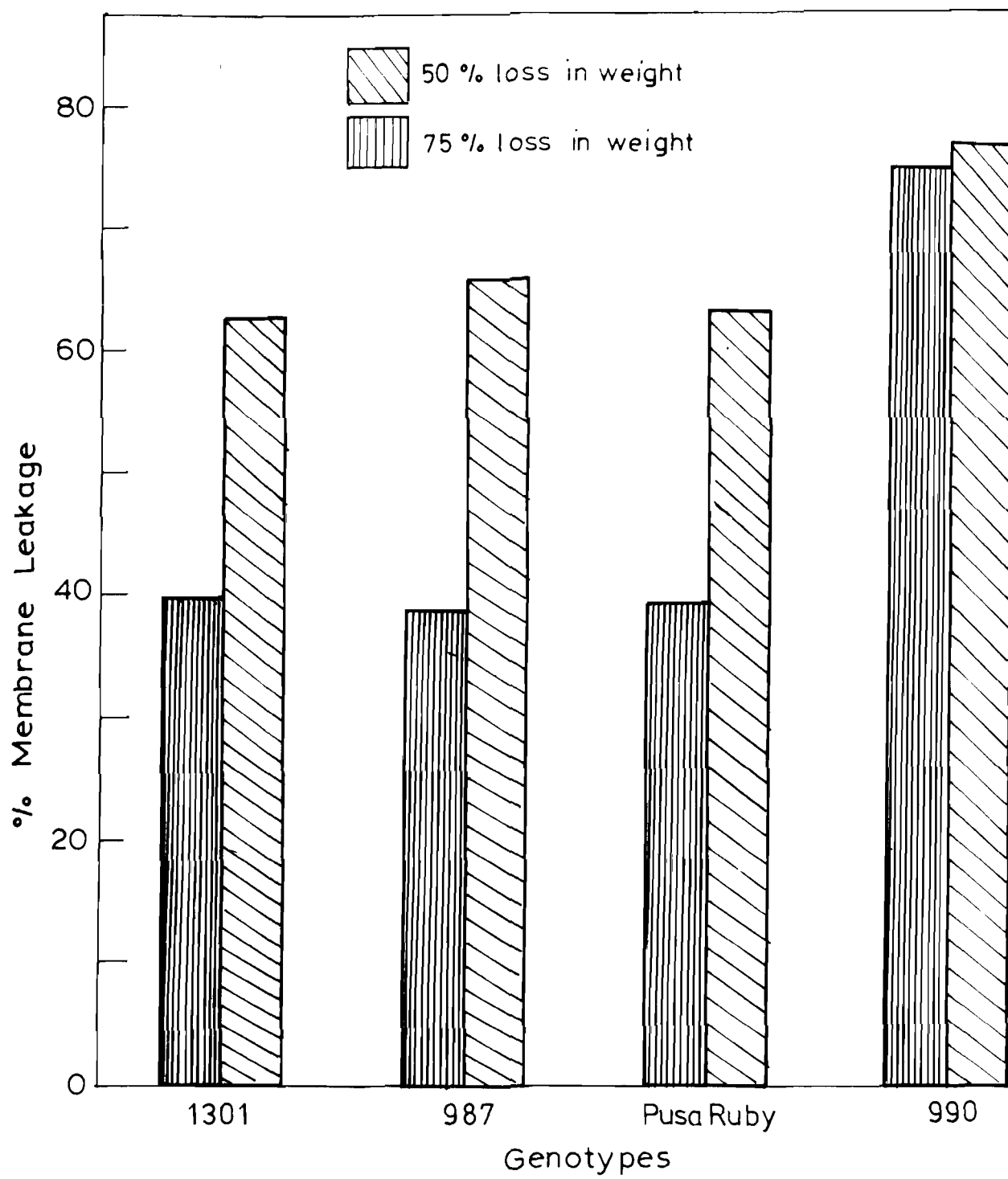


Fig.11a,b,c: Relation between 50 and 70 per cent loss in weight and per cent membrane leakage amongst four *Lycopersicon* species in vitro (a) Experiment-I, (b) Experiment-II and (c) Experiment-III.





an increase in per cent leakage of the solutes at 75 per cent water loss. At 75 per cent water loss, the per cent leakage was significantly high in 1301 which recorded approximate 64 per cent where as it was only 48 per cent in Pusa Ruby and 52 per cent in 987 and in 990.

Experiment-II

In this experiment also all the genotypes lost 50 per cent of their fresh weight in one hour and 75 per cent three hours. When the stress was more rapid than in the previous experiment, the per cent leakage at 50 per cent loss of fresh weight was high in 990 (Fig.11b). In this genotype the per cent leakage was more than 50 per cent compared to 987 (18%) and 130 (20%). However, in 990 there was no further significant increase in per cent leakage when tissue fresh weight was further decreased to 75 per cent, but a substantial increase in per cent leakage was seen in 987 and 1301. Though the per cent leakage increased at 75 per cent of water loss, the extent of leakage was relatively less in 987 compared to other genotypes. Per cent leakage at 75 per cent water loss was high in Pusa Ruby in this experiment.

Experiment-III

In another similar study, the per cent leakage at 50 per cent water loss was significantly high in 990. But the differences in per cent leakage was not significant in other three genotypes at 50 per cent loss of fresh weight like in the previous experiments. There was no further increase in the leakage of solutes in genotype 990 when per cent loss of water increased from 50 to 70 per cent. But in other three genotypes, the per cent leakage increased under severe stress. But the differences between these genotypes were not significant. Even at 75 per cent loss of water highest leakage was seen in 990.

The results on membrane integrity suggests that there is no consistent trend amongst the genotypes studied. But based on second and third experiment where the time taken for depletion of 50 or 75 per cent of tissue fresh weight was similar, the loss of membrane integrity was more in genotype 990 compared to other genotypes. But there were no noticeable differences in this parameter amongst the other three *Lycopersicon* genotypes studied. In all the genotypes the per cent leakage increased with increasing per cent water loss. But in all the three experiment there was not any substantial increase in per cent leakage in 990 when tissues were subjected to severe dehydration.

In all the three experiments the stress imposed was too rapid (approximately 60 to 90 minutes for 50 per cent loss of water). This can attributed as one of the reasons for the lack of consistent trend in these experiments (Appendx-8).

DISCUSSION

V. DISCUSSION

Drought stress causes more yield losses than the combined effect of all other abiotic stresses. But the progress made in developing crop varieties with stable productivity under drought prone environments is not very encouraging. The reason being the complexity of drought stress situation where magnitude, duration and time of occurrence of stress varies from season to season and often compounded by variations in temperature, VPD and soil characteristics.

However, the progress made in recent years in drought stress research has been phenomenal and several adaptive strategies have been clearly understood and their relevance to productivity has been highlighted in several recent reviews (Turner, 1986).

Plants when subjected to moisture stress adapt predominantly three major strategies namely drought escape, drought avoidance and drought tolerance. More recently attempts have also been made to categorise the different avoidance and tolerance mechanisms based on their effect on productivity (Turner, 1986 and Udaya Kumar *et al.*, 1988). In view of the fact that achieving higher biomass yields under moisture limited conditions is the major objective more emphasis has

been laid on plant avoidance characteristics like water extraction, (root characters) its utilization efficiency and higher partitioning to reproductive organs. But the importance of certain intrinsic drought tolerance mechanisms can not be ignored. Desiccation tolerance mechanisms have their own relevance during seedling stage when desiccation is rapid and severe and also under conditions of prolonged stress. Survival under stress and regrowth on alleviation are equally important.

One of the major objectives is to identify the resistant genetic sources for different adaptive mechanisms from amongst the wild species and germplasm lines. Therefore the major approach in the present investigation was to study the different adaptive mechanisms of diverse *Lycopersicon* species and also a few selected germplasm lines of *Lycopersicum esculentum*. The important avoidance and tolerance mechanisms studied during the course of investigation are;

1. Differences in intrinsic WUE under varying environmental situations.
2. Root characteristics as an important character to maintain higher tissue water potential.
3. Differences in cuticular transpiration.

4. Differences in desiccation tolerance based on empirical screening and a few intrinsic tolerance mechanisms.

Water use efficiency (WUE)

Although the total ET is related to biomass, at a given level of ET, difference in WUE alone is the major factor contributing for differences in productivity among the genotypes or species (Garrity *et al.*, 1982 and Johnson *et al.*, 1983). Therefore attempts to identify intrinsic differences in WUE and to identify morphological and physiological traits associated with WUE gained considerable importance in recent years.

Method to determine WUE at whole plant level

At the whole plant and the canopy level in field studies, it is difficult to determine the WUE because of the problems associated with accurate determination of ET and delimitation of E from T. The root biomass which otherwise contributes to total biomass is also not considered under field conditions.

Therefore the intrinsic WUE at the whole plant level was determined in this investigation by adopting a gravimetric technique. At a given growth period by determining the CWT and DM, differences in WUE can be determined by adopting this method. Since, the E is

checked or appropriate correction is given, from the primary values of leaf area (LAD), CWT and the DM, it is possible to determine physiological factors associated with WUE like mean transpiration water loss (rate of water loss) and the mean net assimilation rate.

Similar method to determine intrinsic WUE has been reported earlier (Fischer and Turner, 1978; Pasioura, 1983). In several species (Sashidhar, 1987; Uma, 1987; Ravishankar, 1988) and in genotypes of cowpea (Nagamani, 1985), soybean (Arun, 1985), finger millet (Sashidhar, 1987).

In addition to this method, a new method was adopted to determine WUE in containers at a given input of water. The results obtained clearly indicated that even when a known input of water was periodically supplied and CWT arrived at, the differences in WUE can be accurately determined. Such an approach has two advantages. One is daily weighing can be avoided since the input of water is same for all the crops and another advantage is that rainfed situation can be simulated where input of water for all crops always remains the same.

By using both techniques significant genotypic variation was noticed in the WUE across different environments (Table 3, 7, 9, 12, 16; Fig.1, 2, 3, 4).

Differences in WUE among Lycopersicon species

WUE though an intrinsic character, which is species specific, has been shown to be affected by the environmental factors basically light intensity, VPD and moisture stress. Only stable physiological or biochemical traits across the environments are alone amenable for genetic manipulation in crop improvement programme. Therefore it is more relevant to study differences in WUE under varying environmental conditions.

Effect of light - An assessment based on summer and monsoon experiments

To study the differences in WUE, and the effect of radiation input as an important environmental variable, two experiments were conducted, one during the summer and the other during monsoon under non-stress conditions.

The difference in the light intensities, RH and temperature between the two seasons are given in Appendix-1 and 2. Though significant variation was observed in all environmental factors between two seasons, the major difference was seen in light intensity received during crop growth period. When the plants were grown under high light conditions during summer, highest WUE was recorded in genotype 990 which was distinctly more than other species. However,

among the other 6 species 564 and 1048 showed high WUE.

Since, the differences in LAD were not significant amongst the six species except 990, the CWI dependend only on rate of water loss differences, which was relatively less in 564 and 1048. The mean rate of water loss in these two high WUE types was 42.8 where as the mean of other three low WUE types, 1301 and 987 was 54.8. The respective mean values of LAD/DM for these two groups is 8.3 and 8.8. Since the differences in LAD/DM were marginal, a negative significant relationship exists between WUE and rate of water loss (-0.975).

When the plants were grown in monsoon season under a rain shelter, differences in rate of water loss were very marked. The range in TDM, LAD and LAD/DM was from 26.9 to 35.61 g pot⁻¹, 312 to 645 and 11.9 to 33.0 respectively. But the range in rate of water loss was from 11.3 to 33.9 ml. dm⁻² day⁻¹. This suggests that under low light also, the difference in rate of water loss seems to be the major contributing factor for the observed differences in WUE amongst the species. There was a negative significant relationship between rate of water loss and the WUE ($r = -0.975^{**}$). Both high WUE types 1301 and 1086 had low rate of water loss compared to 1048 and 564.

The results obtained from WUE and associated physiological characteristics in these two experiments clearly indicated that environmental factors markedly affect intrinsic WUE.

In plants grown in low light intensity, in general all the growth characteristics (LAD, DM) and physiological characteristics (mean assimilation rates and rate of water loss) showed a decline. However, the carbon gain per unit conductances increased in low light resulting in an increase in WUE. If we take the mean WUE of all the genotypes there was a marginal increase in monsoon (2.95) compared to summer (2.6). But the response of these Lycopersicon genotypes was different. In 990 there was a marked reduction whereas in 564 and 1048 there was a marginal decline. In Pusa Ruby a marginal increase was noticed. But in 1301 and 1086 there was a marked increase in WUE.

In 564, 1048 and in Pusa Ruby in general the carbon gain per unit water transpired remained same in both the seasons. In 1301 and 1086 a significant increase in leaf expansion rates and a decrease in rate of water loss was noticed when grown in low light.

Effect of light on WUE in distinct Lycopersicon genotypes differing in their response - Comparison at two light regime

To study the differential adaptation of these three distinct genotypes, another pot culture experiment was conducted during summer at two different light regimes. The other environmental factors like T, RH and VPD remained same for both the light regimes. Hence, the differences if any on growth and other parameters can be attributed to the light effect.

In this experiment also WUE efficiency increased under low light in both Pusa Ruby and 1301 where as in 990 in fact, a decrease was observed. In 1301, which was adapted to low light, the per cent increase in WUE was much higher than Pusa Ruby and it can be mainly attributed to greater decrease in rate of water loss than mean assimilation rate.

Though it has been well established that light is one of the important environmental factors which affects both the T and photosynthesis, the effect of light on T is often positive showing a curvilinear or linear relationship with increase in irradiance mainly because of an increase in temperature and a decrease in r_s (Fischer and Turner, 1978), while the net photosynthesis (especially in C3 plants) may show a downward trend with increasing irradiance. therefore

an optimum irradiance exist for maximum WUE which would be species specific. Since, a curvilinear relation exists between irradiance and photosynthesis, in C3 species with low light saturation, the WUE would likely to increase under low light conditions (Ludlow and Wilson, 1972). Even in a C4 crop like sorghum WUE was shown to be highest in medium light intensities (Downes, 1970). A study conducted in finger millet also showed higher WUE in crops grown in monsoon compared to crops grown in summer (Sashidhar, 1987).

Since, light affects both g_s and g_m and in the light of recent evidences, g_s is under the feed back control of g_m (James Jacob, 1989), A/g_s may not substantially change due to variation in the light. But A/g_s may decrease in high light mainly due to the effect of VPD on T . At a given g_s , the difference in T are mainly due to difference in VPD (Turner, 1986). Such a phenomenon can be explained in plants grown in monsoon and summer since VPD was more in summer. However, in the light regime experiments conducted during summer, the only variable was light since the VPD was same. But even under these conditions, there was a significant increase in WUE from 2.55 to 4.31 in a light adapted species like 1301. This indicates that in some species, there is ²greater adaptation to the low light in terms of stomatal sensitivity and stability in g_m .

The gas exchange data under these two light regimes, also indicated that under low light both A and gs decreased. However, the decrease in A was to a greater extent than gs resulting in a decrease in A/gs under low light (Table 13 and Fig.3). But in species 1301, A/gs ratio was significantly higher under low light regime compared to other two species.

Effect of moisture stress - A comparative assessment in Lycopersicon genotypes under adequate and inadequate water regimes

Yet another variable which affects WUE is moisture stress. Since, the WUE is having greater relevance under water limited conditions, the effect of stress on WUE in different species of Lycopersicon and the reasons for variations in WUE under stress were studied in another pot culture study where plants were grown under two moisture regimes, one adequate and another inadequate input of water.

In plants grown under inadequate water input a significant reduction in LAD (mean of all 7 species 25%) and biomass (30%) was noticed in all the genotypes. The reduction in biomass under stress was less in 990, 1301 and 1086. The genotypes which showed higher WUE under adequate input did not show similar trend under inadequate (stress conditions) input water regimes. In some species like 1301, 990, 1036 and Pusa Ruby, a significant increase in WUE was

recorded, where as in species 564 and 987, the increase in WUE was marginal. In one of the species, 1048, there was a marginal decline in WUE.

A significant increase in WUE at whole plant level in container studies has been reported amongst the species (Sashidhar, 1987; Uma, 1987; Ravishankar, 1989) and amongst genotypes of cowpea (Nagamani, 1985) and finger millet (Sashidhar, 1987). It has been suggested that high intrinsic WUE types may also show a ~~similar~~ response under moisture stress conditions (Ravishankar, 1988). When pooled analysis was made across the species, a significant positive relation between non-stressed and stressed plants was observed. Even such a relationship was seen amongst the genotypes of a few species (Anonymous, 1989). but in this study no such relation was seen. In few species like 1301 and 1086 with low intrinsic WUE values, WUE not only significantly increased under stress but also absolute values were high.

Since the control and stressed plants were grown under similar environmental conditions, the differential response to genotypes, with regards to increase or decrease in WUE, under stress has to be assessed based on the two physiological parameters, assimilation rate and stomatal conductances (rate of water loss). An increase in A/gs ratio under stress

compared to control plants increase WUE and vice versa. Therefore Osmond (1980) emphasised the importance of A/g_s ratios under moisture stress conditions when partial stomatal closure occur. A change in A/g_s ratio reflect the relative extent to which the stomatal and non-stomatal factors are affected during moisture stress. A decrease in A/g_s ratio indicates that the mesophyll characters are affected to a greater extent than stomatal factors and vice versa.

To achieve higher WUE, ΔA (change in the assimilation due to stress) should be less compared to g_s (change in g_s) under stress (Udaya Kumar *et al.*, 1988). So species or genotypes which intrinsically show a higher WUE under moderate or severe stress situations will show a lesser $\Delta A / \Delta g_s$ values. It has been shown in recent studies (James Jacob, 1989) that decrease in A under stress is more due to greater limitations in g_m than g_s . In other words the observed decrease in A is mainly due to the direct effect of stress on chloroplast characteristics and g_s may not impart any significant limitations on A . However, the decrease in g_s under stress is largely attributed to the feed back control of g_m on g_s and possibly to a certain extent the direct effect of tissue water status on g_s (Guehl and Aussenae, 1987). It has been shown that in plant subjected to stress, higher stomatal loop gains results in a higher A/g_s . Therefore to achieve

higher A/g_s under stress, two important characteristics would be greater stability of g_m or chloroplast characteristics or greater sensitivity of g_s in plants experiencing water deficit.

Even in this study species in which WUE increased under stress showed a greater stability in assimilation rate. In fact in 1301 and 990, the mean assimilation rate increased under stress as evidenced by decrease in LAD/DM ratios (Table 16). Though the rate of water loss, a reflection of stomatal conductance, decreased in all the species including in 1048, lack of stability in assimilation rate (increase in LAD/DM ratios from 13.64 to 21.19 under stress) resulted in a lower WUE in this species.

WUE - Effect of environmental factors overall assessment of relative performance of Lycopersicon genotypes

The main objective of the WUE experiments was to identify genetic sources with intrinsically high WUE and to study their stability over two important environmental stresses, the light and moisture. In the first experiment (summer), the plants were grown under saturated light intensities and adequate irrigation and hence considered as a non-stress situation. In the second experiment (monsoon), the light intensity was low and hence can be considered as light stress experiment. The comparison between the

two experiments gives information on the effect of low light on WUE and response of genotypes to this environmental variable. Similarly the adequate input treatment of the moisture stress experiment, can be compared with inadequate input treatment of the same experiment to assess the effect of moisture stress amongst the *Lycopersicon* genotypes.

From the seven distinct *Lycopersicon* species and *Lycopersicum esculentum* genotypes with diverse genetic background, two distinct groups can be identified which differ in their intrinsic WUE and their response to environmental stresses. 990 with poor initial growth rates was not considered for this composition.

In the first group (1048, 564 and Pusa Ruby), the intrinsic WUE under non-stress conditions was always high but their adaptation to low light and moisture stress was not marked and hence no substantial increase in WUE under these situations, in these genotypes (Table 37; Fig.12).

In the second group, (1301, 1086 and 987) in general, the intrinsic WUE values under non-stress conditions were low, but their adaptation to low light and moisture stress was marked and a substantial increase in WUE in genotypes belonging to this group was observed (Table 37; Fig.12).

Table 37. DM and LAD in distinct group of Lycopersicon genotypes differing in their stress adaptation. .

	Expt.I (Summer)	Expt.II (Monsoon)	EXpt.IV ----- Control Stress	
Mean of 3 genotypes from each group				
LAD:				
Mean of high WUE type (3 genotypes)	538	462	720	575
Mean of low WUE types (3 genotypes)	570	566	775	532
DM:				
Mean of high WUE types (3 genotypes)	62	32	56	34
Mean of low WUE types (3 genotypes)	64	30	52	37
Mean of 2 distinct genotypes from each group				
LAD:				
Mean of high WUE types (2 geotypes)	554	499	779	624
Mean of low WUE type (2 genotypes)	543	592	699	483
DM:				
Mean of high WUE types (2 genotypes)	66	33	58	32
Mean of low WUE types (2 genotypes)	61	30	56	39
High WUE group 1048*; 564*; Pusa Ruby				
Low WUE group 1301*; 1086*; 987				
*Distinct genotypes in each group.				

To assess the possible reasons for such adaptation, an attempt was made to analyse the extent of increase in WUE and associated characteristics, rate of water loss and DM/LAD under low light and moisture stress, between these two groups of genotypes. For this analysis the mean values of three genotypes belonging to the distinct groups were used (Fig.12).

Light stress

The adaptation to low light stress was very marked in the second group of genotypes which showed 46 per cent increase in WUE when grown under low light where as, in the first group mean values show a marginal decrease (-1.5 per cent) in WUE. In the first group, the extent of decrease in mean assimilation rate and rate of water loss was same and hence the carbon gain per unit amount of water loss remained same, and hence similar WUE values under low and high light conditions (Fig.12). However, in the second group the decrease in mean net assimilation rate and rate of water loss was more than the first group. However, the decrease in rate of water loss was more than mean assimilation rate.

Though the assimilation rate decreased to a greater extent in low light adapted group, the absolute growth rates were not substantially decreased under low light. The TDM, reflection of growth rate

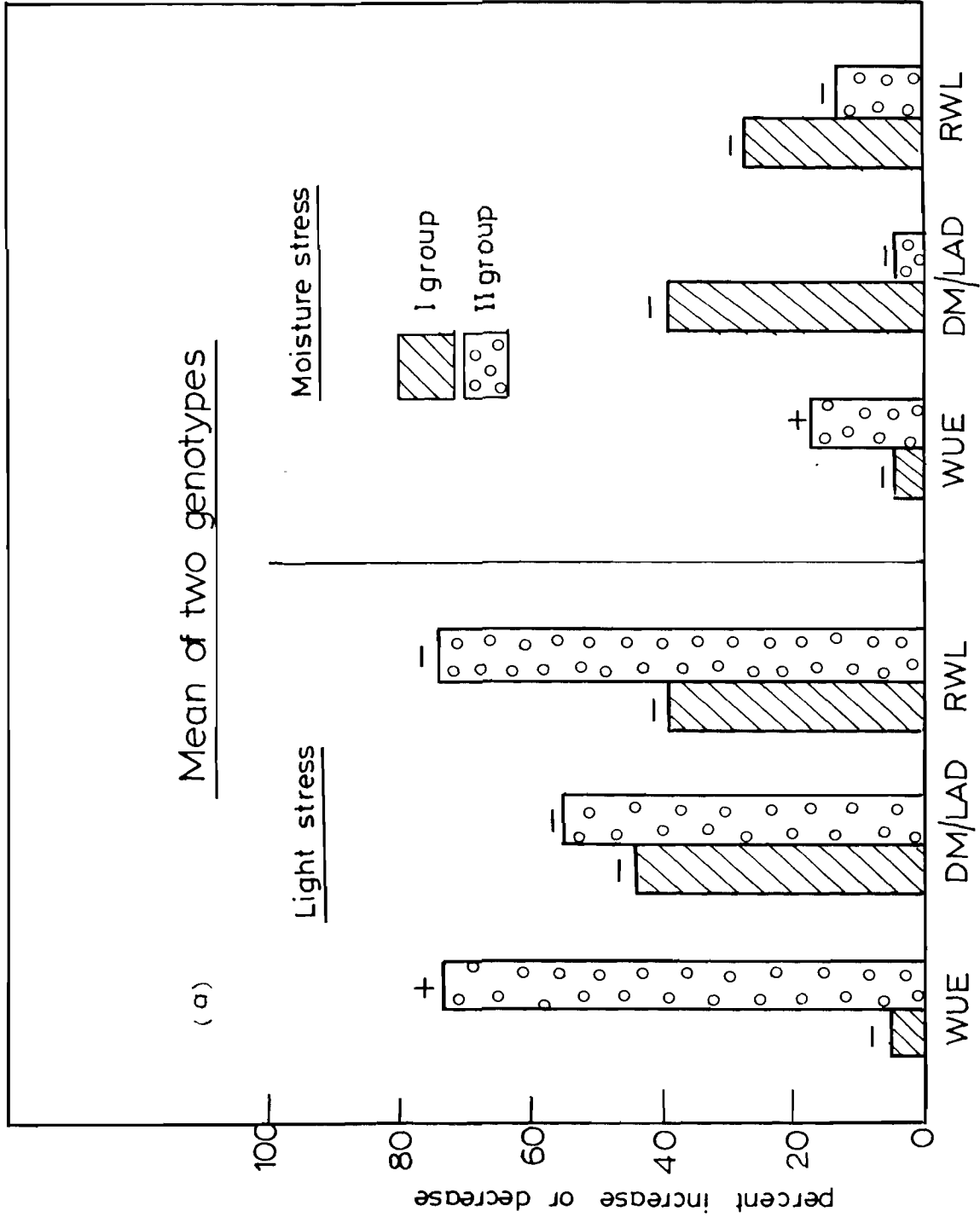
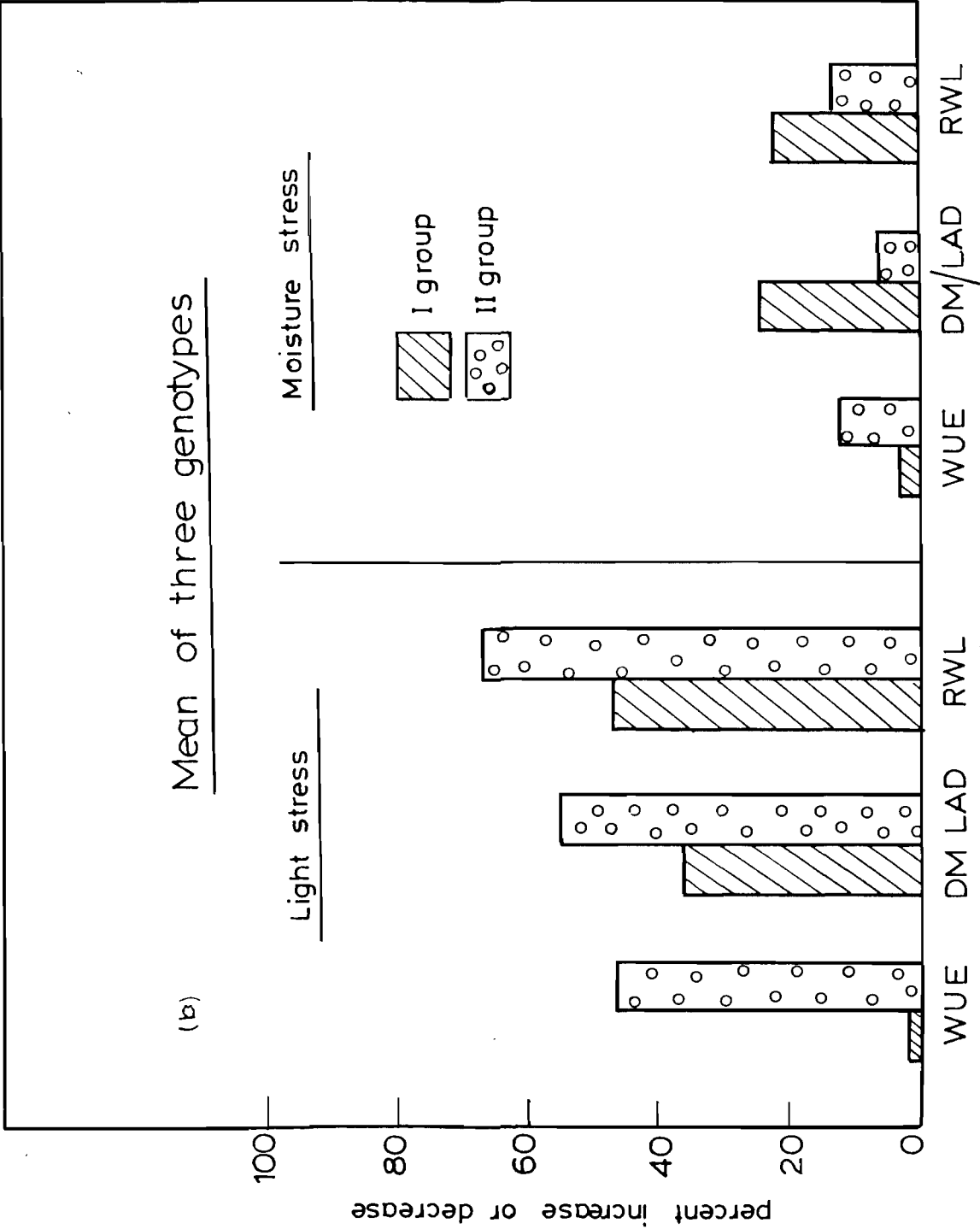


Fig.12: Change in WUE and associated physiological traits in two groups of Lycopersicon genotypes differing in their stress adaptation (Group 1: 564, 1048 and Pusa Ruby; Group 2: 1301, 1086 and 987).



during experimental growth period did not show any substantial decrease compared to first group of genotypes with high intrinsic WUE. This was mainly, because in the second group, the leaf expansion rate was high and resulted in a greater LAD values which contributed for higher DM (Table 10). For instance the LAD in high light for the I and II groups were 538, 570 respectively where as the corresponding values for low light were 462 and 566.

Moisture stress

The interesting aspect is that even under moisture stress, the group of genotypes which showed greater adaptation to low light also showed greater adaptation to moisture stress. But the adaptation was more marked to low light stress than to moisture stress.

In both the groups, I and II, there was an increase in WUE, but it was only 3 per cent in the I group where as it was 11 per cent in the II group. the per cent decrease in mean assimilation rate and rate of water loss was similar in the I group and hence a marginal increase in WUE. But in the II group, the per cent decrease in mean assimilation rate was much less compared to per cent decrease in rate of water loss resulting in a considerable increase in WUE.

Yet another interesting fact is that, the increase in WUE in the second group of genotypes is due to a greater sensitivity of stomata, maintaining low rate of water loss in low light. But under moisture stress, the advantage of these species was predominantly due to maintenance of assimilation rate possibly due to stability of mesophyll characteristics. Infact the reduction in mean assimilation rate (DM/LAD) in this group was very marginal and increased in one of the species, 1301.

The genotypes were grouped in to I and II based on overall performance. But in each group there are two distinct genotypes which showed significant adaptation. In the I group 1048 and 564 with intrinsically high WUE showed least adaptation to light and moisture stress and hence did not record any increase in WUE under these stresses. But in the II group, 1086 and 1301 showed better adaptation in both abiotic stresses and resulted in higher WUE values grown under both the conditions. The WUE and physiological characteristics of there distinct genotypes from these groups are given in Fig.12. Though the absolute values were different, the trend remained exactly the same when the mean data of all the three genotypes or two distinct genotypes was analysed for stress adaptation.

Based on the results of these experiments the Lycopersicon genotypes can be categorised as follows;

1048, 564 - High intrinsic WUE, no adaptation to light and water stress.

1301, 1086 - Low intrinsic WUE, greater adaptation to low light and moisture stress.

Pusa Ruby - Moderate intrinsic WUE and moderate adaptation to low light and moisture stress.

Root characters - Differences in root biomass and water extraction efficiency

Another aspect which was examined in this investigation was root characteristics and their water extraction efficiency.

In an initial evaluation, genotypes of rice categorised as drought resistant, were shown to have higher deep root to shoot ratios (IRRI, 1982). The importance of root volume, density and extraction efficiency as drought resistant mechanism to maintain high tissue water potential has been documented in several studies (Alagaraswamy and Bidingar, 1975; Passioura, 1981). However, the progress made was limited in root studies because of intrinsic problems associated with root extraction and quantification under field conditions.

Further, environmental factors and soil compaction also affect the root growth in soil, which again differ amongst genotypes. Attempts are made to evaluate root characteristics in root boxes, collapsable bricks structures and in containers. In these structure though root extraction is possible, soil volume is a constraint for expression of full genetic potential. However, a comparative assessment between genotypes of similar growth pattern is possible (Blum and Arkin, 1984).

In view of this, in all the WUE experiments, an attempt was made to quantify root characteristics in these Lycopersicon species. Since, this character is having relevance under stress situations, a comparative assessment of the root characters was made in the stress experiment. The root biomass though showed a decrease in many genotypes grown under stress, the root/shoot ratios showed an increase in all the genotypes. The root/shoot ratio increased from 0.23 in control to 0.34 under stress (Fig.4). In three genotypes, Pusa Ruby and 987 an increase in root biomass was recorded in plants subjected to stress and it was also associated with a significant increase in root to shoot ratios.

In addition to the root volume and proliferation, the importance of root hydraulic

conductance and osmotic adjustment in water extraction and translocation has been highlighted by Passioura (1986). Under low soil water potential, further extraction of water from soil is of significant relevance to postpone shoot desiccation. The variation in the root water extraction efficiency was examined among these *Lycopersicon* species. Though no specific data was collected in root osmotic characteristics, it can be inferred, that the higher water extraction efficiency observed in *Lycopersicon* genotypes like 987 and to certain degree 564 was predominantly due to the differences in root dry matter. However, the importance of other root characteristics can not be ruled out.

From the results obtained on root characteristics, it can be concluded that 990 and 987 showed higher root to shoot ratios under stress and also absolute root biomass. In species 987 and 564, the water extraction efficiency was higher than the others.

Differences in rate of water loss - A measure of cuticular transpiration

Apart from water extraction efficiency in plants subjected moisture stress conditions, avoidance of leaf desiccation can also be achieved by minimising the cuticular water loss. Even when stomata are closed, cuticular water losses can be substantial

(Oppenheimer, 1960; Nobel, 1974) reaching more than 15 per cent of total transpiration rate measured in the light as shown in sorghum (Jordon et al., 1984). Cuticular losses for a genotypes or species are not constant. But they are dependent mainly on VPD (Jordon et al., 1984).

One of the often used technique to determine cuticular rate of water loss was by determining the fresh weight of leaf tissue exposed to live wilting (Jordon et al., 1984). From the several experiments it was possible to identify the variation in this character. 990 showed high rate of cuticular water loss. Pusa Ruby, 1301 and 987 showed moderate rate of water and the remaining three 1048, 1086 and 564 showed very low rate of water loss.

Unlike many adaptive mechanisms, the heritability of this trait has been shown to be very high (Clarke and Townley Smith, 1986) and hence could be a useful drought avoidance character. However, it has been shown that, the cuticular wax composition, though species/genotypic specific known to be affected by environmental conditions, VPD and tissue water status (Blum, 1975).

Desiccation tolerance - Empirical assessment and biochemical characteristics associated with it

The third major objective of the investigation was to characterise the species with desiccation tolerance based on not only empirical assessment but also physiological and biochemical characteristics associated with desiccation tolerance.

Since, the tolerance to desiccation is the sum effect of several physiological and biochemical characteristics, empirical screening still has been the major approach to characterise genotypic differences in the extent of desiccation tolerance. As a measure of desiccation tolerance, either seedling mortality or extent of leaf desiccation are studied. A field evaluation for desiccation tolerance is often difficult (except at seedling stage), because of other avoidance characteristics like, water extraction and its conservation by leaves alter tissue water status. Comparison of intrinsic desiccation tolerance amongst the genotypes/species is relevant only at a given tissue water status. In a series of pot culture experiments, the difference in desiccation tolerance at a comparable RWC was attempted. However, in all these experiments, the stress imposed was rapid and some times too severe. The major character assessed was extent of leaf survival.

Based on these studies the relative ranking of genotypes with regards to survival is as follows.

1. Species with high survival - 1048, 1086 and 564.
2. Species with moderate survival - 987.
3. Species with low survival - 990, Pusa Ruby and 1301.

The other approach adopted for assessing the desiccation tolerance empirically was by subjecting the young seedlings to moisture stress under field conditions, where stress imposed was gradual but of considerable magnitude. The water uptake and other avoidance mechanisms may not pose a problem in this study, because the stress was imposed in 30 day old seedlings immediately after establishment. As a measure of tolerance, leaf desiccation, seedling survival and also recovery growth on stress alleviation were considered. Though it was a field assessment, at later sampling time, the differences in RWC were not marked and hence the extent of leaf survival is comparable amongst the species (Table 27).

The relative ranking of the genotypes based on survival ability is as follows.

1. Tolerant - 987, 1086 and 1301
2. Moderately tolerant - 564 and 990
3. Susceptible - Pusa Ruby.

The relative ranking of genotypes with regard to survival under rapid wilting conditions in pot culture experiments was not same as ranking of genotypes noticed under slow wilting conditions in small plot field experiment.

However, the data obtained on per cent survival and the per cent recovery of the field experiment was given greater importance, and four distinct genotypes were selected for further analysis. Species 1086 though showed high survival in both studies, it was not included because of its susceptibility to virus. Based on the data of another experiment, the relative desiccation tolerance was assessed in these four genotypes. The data of this experiment also confirms the previous results. Though the survival percentage was low in 990, in general recovery rate was high in this species. The relative ranking for desiccation tolerance of these four species are;

1. Tolerant - 987
2. Modertely tolerant - 990 and 1301
3. Susceptible - Pusa Ruby

The plants that show desiccation tolerance, despite a low tissue water potential, may do so either by certain intrinsic dehydration tolerance mechanisms or by maintaining positive turgor. Some of these

mechanisms may explain the differences in survival when subjected to severe stress.

A few mechanisms are examined namely osmotic adjustment, accumulation of an osmoprotectant like proline, carbon assimilation, protein synthesis and membrane integrity.

Proline

Among several solutes that accumulate in tissues subjected to abiotic stresses, proline accumulation is rapid and hence considered as an osmoticum having relevance to stress tolerance. The importance of this solute in stress tolerance has been shown in some studies (Singh *et al.*, 1973a; Stewart and Lee, 1979) in lower organisms and also in cell cultures (Handa *et al.*, 1986). Though the role of proline as an osmoticum has been disputed by several workers (Paleg and Aspinal, 1981), the importance of proline as a compatible solute (Schobart, 1978 and *et al.*, 1982) and as an osmoprotectant and also as a cytoplasmic osmoticum (Wataid *et al.*, 1983 and Pahllich *et al.*, 1983) was favoured by a few workers.

The amount and rate of accumulation of proline has been shown to be directly related to tissue water status and rate of dehydration (Paleg and Aspinal, 1981).

1981). Therefore even it has been argued that the proline content in tissue is a reflection of tissue susceptibility because in genotypes where rapid desiccation occurred, the proline accumulated was also high (Hanson et al., 1977).

Therefore it is essential to compare the extent of proline accumulated at a comparable tissue water status. The relative proline accumulation amongst the *Lycopersicon* genotypes was compared at a comparable RWC. At any RWC the amount of proline accumulated was significantly high in 1048, 987 and very low levels were accumulated in 564 and 990.

The main approach here is to relate the proline accumulated at a comparable RWC with desiccation tolerance arrived at based on empirical assessment. No relationship could be noticed between the extent of proline accumulation and leaf survival under rapid stress situations. Both 1048 and 564 with differential proline accumulation ability showed similar desiccation survival.

Even in the small plot field experiment also, seedling survival and leaf survival did not show any relationship to the proline accumulating capacity of the genotypes. However, both the low proline accumulators 564 and 990 showed less leaf survival under slow wilting conditions compared to 987, 1086 and

1301. The recovery percentage of different genotypes in the field experiment showed some relation with intrinsic proline accumulation ability.

The interesting aspect noticed in this study was that proline accumulation is genotype specific and not a mere reflection of tissue water status. Lycopersicon genotype 1048 showed high proline synthesising ability while 990 and 564 can be categorised as low proline accumulators. These contrasting genotypes can be used further in understanding the role of proline and its relevance in desiccation tolerance.

Osmotic adjustment

With crop plants, the maintenance of function and ultimately of survival depends on the maintenance of a relatively high water content or turgor. Maintenance of positive turgor by osmotic adjustment could be a mechanism for desiccation tolerance amongst the genotypes (Morgan, 1983; Rain, 1980; Morgan, 1984). Osmotic adjustment is an adaptive process which can alleviate some of the deleterious effects of water deficits (Flower and Ludlow, 1986) particularly under a slow or gradual stress under field situations (Acevedo *et al.*, 1979; Morgan, 1986). There is evidence that osmotic adjustment delays the onset of death in excised rice leaves until lower water potential are reached

(Hsiao et al., 1984). Similar results were obtained in pigeonpea (Flower and Ludlow, 1986). Under field situations diurnal growth trends have also been related to diurnal variations in OA (Acevedo et al., 1979). In pot grown seedlings of Eucalyptus species, OA has been shown, when subjected to one or two cycles of drought (Myers and Neales, 1986).

Therefore the differences in OA were studied in two distinct Lycopersicon genotypes differing in desiccation tolerance based on empirical assessment.

In a container study, the extent of OA was determined in two genotypes, Pusa Ruby (susceptible) and 987 (a tolerant genotype). The active OA as described by previous techniques (Wilson and Ludlow, 1983; Morgan, 1983) was studied to delineate the effects of passive concentration of solutes due to loss of water from true osmotic adjustment. Although it is a container study, the stress imposed was not rapid, therefore considerable OA was observed in both the genotypes. On any given sampling date the extent of OA was significantly higher in 987 (Table 31, 32). Particularly during the initial stages of stress (moderate stress), turgor was maintained for a longer period (Fig.9b, 9a) by OA in 987. Even in Pusa Ruby which consistently showed less drought tolerance, also showed a certain degree of OA, although for a shorter duration.

Several studies have shown that although a number of solutes are involved in OA, the contribution of soluble sugars, and amino acids are substantial (Morgan, 1984; Johnson *et al.*, 1984). With decrease in tissue water status, increase in the severity of a significant accumulation in amino acids, sucrose, hexose and pentose sugars was observed. In 987, on all days after imposition of stress, the amount of pentoses, hexoses, was two fold higher than in Pusa Ruby. With regards to the amino acids, in 987 there was a linear increase in content whereas the rate of accumulation was much less in Pusa Ruby.

Turgid weight/dry weight ratio

The importance of turgid weight/dry weight ratio has been shown in cellular water relations under stress (Turner *et al.*, 1987). A decrease in turgid weight/DM ratio has been attributed to greater accumulation of osmotically or non-osmotically active solutes (Turner *et al.*, 1987; Myers and Neales, 1986). In some studies, however, an increase has also been reported under stress which was attributed to changes in both cell elasticity and apoplastic water content (Conroy *et al.*, 1988).

In 987 though there is significantly higher amounts of osmotic and non-osmotic solutes, a higher TW/DM ratio was observed in this genotype, on all days

of stress. This can be attributed to a greater degree of change in cell elasticity or apoplastic water content as compared to Pusa Ruby.

Variation in potential carbon fixation rates under stress

A now well known phenomenon which illustrates the consequence of moisture stress is, inhibition of photosynthetic machinery due to exposure of plant to combination of moisture stress and high light intensities. Photoinhibitory damage need not be considered as primary water stress effect, since it is not observed at relatively low light intensities (Powles and Critchley, 1980).

A concomittent decrease in 'A' and 'gs' under stress shown in several studies (Boyer, 1971; Krideman and Downson, 1984), often emphasised the stomatal limitations for carbon assimilation under stress. Even in recent review Kaiser (1987) concluded that moisture stress decreases 'A' but mesophyll factors were not affected until tissue water potential decreases to a greater extent. In excised leaf slices, even when RWC decreased to 30 per cent, there was no substantial decrease in chloroplast functioning as measured by oxygen evolution. However, it has been clearly demonstrated that the decrease in 'A' is direct effect of moisture stress on mesophyll factors.

In stressed plants exposed to high light intensities, a decrease in 'A' even at high internal carbon dioxide concentration (C_i) has been shown, suggesting that mesophyll factors were affected (Bojrkman and Powles, 1984; Johnson *et al.*, 1987; Ludlow and Bjorkman, 1984).

A decrease in 'gs' in plants subjected to stress is mainly due to feed back inhibition of 'gm' on 'gs' (Guehl and Aussenee, 1987; James Jacob, 1989) suggesting that the 'gm' poses a greater limitations to 'A' under stress but not 'gs'.

An attempt was made in the present investigation to study the effect of dehydration on chloroplast carbon dioxide fixation ability. To avoid the resistances offered by stomata and liquid phase factors for carbon dioxide diffusion, $^{14}\text{CO}_2$ incorporation at its saturated concentrations was studied.

Carbon fixation ability under stress was assessed by computing the per cent reduction based on the carbon fixed by control turgid leaves. The results of this experiment clearly indicates that, even a 20 to 30 per cent reduction in tissue water status, significantly decrease the ability of chloroplast for carbon fixation at saturated light intensities. When the tissue water status was decreased to 50 per cent,

there was 74-92 per cent reduction in carbon fixation ability (Table 34). Since, the carbon dioxide diffusion and availability is not a limitation, the decrease in A can be attributed to the direct effect of stress on chloroplast functioning. But in a similar study significant decrease in carbon assimilation was reported only when tissue water status decreased to below 30 per cent RWC (Kaiser, 1987). Both at 80 and 70 per cent of tissue water status, the carbon fixation ability was high in Pusa Ruby and 987 compared to 990 and 1301.

It can be concluded that, maintenance of chloroplast integrity was high in Pusa Ruby and moderate in 987 and relatively low in 990 and 1301. Therefore the carbon assimilation efficiency by the chloroplast under stress did not show any relationship to the observed desiccation tolerance arrived at by empirical assessment. Genotype Pusa ruby which showed relatively less tolerance both in rapid as well as severe stress conditions showed higher mesophyll stability and carbon assimilation.

Maintenance of carbon assimilation under extreme desiccation has been shown to be mainly by better osmotic adjustment or by altered protoplast volume (Kaiser, 1987; Gupta and Berkowitz, 1987). Plants, grown at higher potassium levels shown to

maintain low osmotic potentials and also high carbon assimilation rates (Gupta et al., 1989). Therefore the maintenance of chloroplast integrity depends more on cell water relations.

The extent of osmotic adjustment was less in Pusa Ruby compared to 987 (Fig.9a, b; Table 31). Both species with distinct differences in osmotic adjustment, showed higher carbon fixation in this experiment. In tissues subjected to live wilting which decrease tissue water potential rapidly, there may not be any significant osmotic adjustment. Hence, there was no relation between the relative osmotic adjustment ability of 987 and the maintenance of chloroplast carbon fixation ability under stress. But the interesting aspect here is, maintenance of higher carbon gain in Pusa Ruby than in other genotypes at a comparable tissue water status.

Protein synthesis under stress and its relevance to desiccation tolerance

Protein synthesis and turnover have been shown to be affected even at mild stress. Hence, can be considered as an important parameter reflecting the desiccation tolerance.

The differential survival ability at low tissue water potentials has been attributed to the ability to continue the protein synthesis at moderate

or even at fairly severe water deficits. The ability to continue the protein synthesis in hardened seeds, in tissue subjected to slow wilting and in desiccation tolerant mosses has been shown to be the major factor for their desiccation tolerance (Henkel, 1979; Gwozdz and Bewley, 1975; Dhindsa and Bewley, 1977). In plants which can not survive under severe desiccation, protein synthesis does not resume on stress alleviation. Therefore resumption of protein synthesis on stress alleviation seems to be more important and relevant.

Differences in ^{14}C leucine incorporation during dehydration after subjecting the tissue to osmotic stress was studied in these four distinct genotypes differing in their desiccation tolerance. During the stress alleviation even when adequate rehydration occurred in the tissues, the total uptake of ^{14}C leucine was significantly reduced in all the species. However, at mild stress reasonable uptake was maintained in 987 compared to other genotypes. The decrease in incorporation in to the protein could be due to inadequate uptake or due to the direct stress effect on protein synthesizing machinery. Apart from the uptake of the labelled leucine, the protein content also reduced in all the genotypes significantly. Therefore, it is more appropriate to compare the incorporation per unit protein content. Hence, the ^{14}C

leucine incorporation per unit protein content alone was used for comparing the stress effect on protein synthesis.

Both under moderate and severe stress condition, the per cent reduction in protein synthesis was less in 987. For instance in tissue subjected to -1.5 MPa of osmoticum (PEG), reduction in incorporation of label per mg of protein was only 3.4 in 987 whereas the corresponding values for Pusa Ruby, 1301 and 990 were 20.51, 33.1 and 48.9 respectively. Similar trend was observed in tissue subjected to -3.0 MPa osmotic stress.

Based on the uptake of labelled leucine, per cent decrease in protein content and per cent incorporation per unit protein it can be concluded that 987 showed higher protein synthesizing ability on stress alleviation. When the leaf tissue was subjected to moderate stress, apart from 987, in Pusa Ruby also protein synthesis was maintained on stress alleviation.

Studies on membrane integrity

Attempts were made to look into desiccation tolerance based on the ability to repair and regenerate cellular organelle membranes on stress alleviation. This is often referred to as membrane integrity characteristics of tissues and widely used as criteria to identify resistant genotypes to

desiccation. Dhindsa and Bewley (1977) showed that leakage of solutes from the drought sensitive mass was more than drought tolerant mass. The greater tolerance observed in Gossypium annamoli has also been attributed to its ability to maintain membrane characteristics under desiccation (Vieira De Silva, 1976). Since, the primary stress injury is on membrane systems, the desiccation tolerance differences has also been assessed based on the extent of leakage of solutes in rehydration medium, often in excised tissues subjected to rapid wilting.

The variation in the extent of leakage has been shown in different crops like soybean and finger millet (Marathe, 1989).

The Sullivan's leaf disc test developed to identify heat tolerant sorghum lines is also based on membrane integrity of leaf tissues subjected to high temperature (Sullivan and Eastin, 1974).

Although loss of membrane integrity is considered as primary stress effect, very often measurable differences in extent of leakage of solutes were not noticed in tissues subjected to mild stress i.e., upto 60 to 70 per cent of loss in fresh weight (Marathe, 1989). Always differences between the treatments or amongst genotypes could not be noticed upto 50 per cent loss in fresh weight. Even in this

study, measurable differences between these four distinct types were seen only beyond 50 per cent loss in fresh weight.

With regards to the genotypic variation in this character, there was no consistent trend between the experiments. The lack of consistency could be due to difference in the time duration between experiments to decrease the tissue water status to 50 per cent or 75 per cent fresh weight. The other reason could be that, the stress imposed was too rapid to observe any genotypic variation in this character.

Several dehydration tolerant characteristics, which have been often used to explain the survival ability of plants exposed to extreme stress have been examined in the present investigation. We examined the differences in proline accumulation ability, proline synthesis, osmoregulation, membrane integrity and chloroplast integrity for carbon fixation. In several of these characteristics studied, a marked variation was noticed between the four distinct species differing in their survival ability. However, both susceptible and tolerant types showed higher ability of proline accumulation, chloroplast integrity which was assessed by carbon fixation and membrane stability. It therefore appears that the differential ability of these four species under stress may be due to their

intrinsic protein turnover characteristics osmotic adjustment and may be other biochemical characteristics which were not studied in the present investigation.

SUMMARY

VI. SUMMARY

In view of importance of both avoidance and tolerance mechanisms under drought prone situations, an attempt was made in the present investigation, to study the variability in a few important avoidance and tolerance mechanisms amongst a few *Lycopersicon* species and *Lycopersicum esculentum* genotypes with diverse genetic back ground. The major aspects studied were;

1. Differences in WUE under varying environmental conditions.
2. Differences in root characters as an important avoidance mechanism to maintain higher tissue water status.
3. Differences in cuticular transpiration, as an avoidance mechanism under severe stress conditions.
4. Differences in desiccation tolerance, based on empirical assessment (difference in leaf survival, seedling survival and recovery rating) and to understand biochemical basis for the observed differences of tolerance.

To meet these objectives, several pot culture, laboratory and small plot experiments were conducted

with the following seven distinct *Lycopersicon* species and *Lycopersicum esculentum* genotypes.

1. 990 - *L. peruvianum*
2. 1301 - *L. pimpinillifolium*
3. 1086 - *L. aufretut*
4. 987 - *L. esculentum* var. *cerasiforme*
5. 1048 - *L. esculentum*
6. 564 - *L. esculentum*
7. Pusa Ruby - *L. esculentum*

Water use efficiency (WUE)

Differences in WUE were determined at the whole plant level in container studies by determining cumulative water transpired (CWT) and dry matter production (DMP) at a particular growth period varying in duration from 20-35 days. In some experiments, differences in WUE were assessed at adequate and inadequate water input but similar to all the species. At the whole plant level there were significant variations in WUE amongst these genotypes. However, considerable environmental influence was noticed in both light and moisture stress, which can be summarised as follows.

- (1) When plants were grown under high light and adequate input situation (non-stress), *Lycopersicum esculentum* genotypes, 1048, 564

and Pusa Ruby showed higher WUE compared to the other Lycopersicon genotypes 1301, 1086 and 987.

The differences in WUE were mainly due to variations in mean transpirational water loss for the growth period and therefore a significant negative relationship was observed between these two parameters.

(2) Under stress situations, both low light and moisture stress, the behaviour of species was different with regards to WUE. One group (I) of genotypes 1048, 564 and Pusa Ruby which showed high intrinsic WUE under non-limited water and light, did not show any substantial increase in WUE under these two abiotic stresses. Whereas in the second group, 1301, 1086 and 987, with intrinsically low WUE, a substantial increase in WUE was observed both under low light as well as moisture stress conditions.

(3) Under low light stress the WUE increased by 46 per cent in the second group of genotypes. Whereas, in the first group the WUE decreased by 1.5 per cent. Although the mean assimilation rate and rate of water loss decreased under low light in all genotypes, a

greater decrease in this parameter in genotypes belonging to group II resulted in higher WUE. Two of these genotypes in group II namely 1301 and 1086 also maintained high growth rates under low light because of higher leaf expansion rates. Under low light conditions the assimilation rate per unit stomatal conductance (A/g_s) which is a measure of intrinsic WUE at single leaf level, was high in light adapted species like 1301.

- (4) When plants were subjected to moisture stress (limited water input), the increase in WUE observed in genotypes of group II, was mainly due to maintenance of a greater stability of mean assimilation rate under stress. Although a significant decrease in biomass was seen due to reduction in leaf area, the marginal decrease in mean assimilation rate (4%) in the II group resulted in a 17 per cent increase in WUE. But in the I group of genotypes, mean assimilation rate decreased. Therefore no decrease in WUE, was observed.
- (5) Based on the genotypic and environmental interaction with regards to WUE, the genotypes were categorised as follows;

1048, 564 - High intrinsic WUE, no adaptation to low light and water stress.

1301, 1086, - Low intrinsic WUE, greater
987 adaptation to low light and moisture stress.

Pusa Ruby - Moderately high intrinsic WUE and moderate adaptation to low light and moisture stress.

Differences in root characters

The root/shoot ratio increased from 0.23 in control to 0.34 under moisture stress. In some Lycopersicum esculentum genotypes 990, 987 and Pusa Ruby, there was an absolute increase in root biomass under stress which was also associated with significant increase in root/shoot ratio.

The water extraction efficiency of the roots was more in 987 and 564 both in stressed and non-stressed conditions and it was not only associated with higher root biomass but also with other intrinsic root extraction characters. Total leaf area or root to leaf area ratios were not related to water extraction efficiency of genotypes.

Non-stomatal rate of water loss

The cuticular transpiration, a desiccation avoidance character measured in excised leaves subjected for rapid wilting showed consistent and distinct variation among the Lycopersicon genotypes. The cuticular transpiration rates were high in 990, moderate in Pusa Ruby, 1301 and 987 and low in the remaining three, 1048, 1086 and 564.

Desiccation tolerance

Emperical assessment: Based on leaf survival to rapid desiccation at a comparable leaf water status, the relative ranking was (1) high survival 1048, 1086 and 564, (2) moderate survival - 987, (3) low survival - 990, Pusa Ruby and 1301.

Based on seedling survival, extent of leaf desiccation and recovery growth rates under slow but severe stress conditions, the relative ranking was (1) Tolerant - 987, 1086 and 1301, (2) Moderately tolerant - 1048, 564 and 990, (3) Susceptible - Pusa Ruby.

The comparative emperical assessment of the four selected genotypes based on several experiments showed high tolerance ability in 987, moderate in 990 and 1301 and low in Pusa Ruby.

Proline accumulation

At a comparable tissue water status, the amount of proline accumulated was different indicating differences in proline synthesizing ability of the *Lycopersicon* genotypes. Genotypes 1048, 987 and 1086 were high proline accumulators. However, the intrinsic desiccation tolerance (leaf and seedling survival) was not related to extent of proline accumulation. But recovery growth rates were related.

Osmoregulation

In 987 compared to the Pusa Ruby, there was higher solute accumulation and lower solute potentials under stress. Where active OA was delineated from passive concentration, the duration and degree of OA was longer in 997 than in Pusa Ruby. However, under severe stress (d14) OA was significantly higher in 987. Therefore a better and a longer duration of active OA possibly contributed to the greater desiccation tolerance of 987. This greater OA in 987 could be attributed to a two fold increaseⁱⁿ soluble sugars (hexoses, pentoses) on all days of stress. Similarly the sucrose and amino acid content were also significantly higher. Therefore a better osmotic adjustment could be one of the factors for higher desiccation tolerance observed in this species.

Protein synthesis

In excised leaf tissues subjected to osmotic stress (PEG), per cent decrease in protein content was less in 987 followed by Pusa Ruby, 1031 and 990 indicating a higher turnover or less degradation. ¹⁴C leucine incorporation per unit protein was also high in 987 followed by Pusa Ruby and least in 990. Maintenance of high protein turnover could be one of the reasons for the observed tolerance in 987.

Potential photosynthetic rate

Potential photosynthetic rate at saturated carbon dioxide concentrations studied as a measure of mesophyll stability under stress, showed lesser decline in Pusa Ruby and 987. But under severe tissue desiccation, the differences amongst the four genotypes were marginal. Both tolerant (987) and susceptible (Pusa Ruby) types showed higher potential photosynthetic rates under stress, indicating that an assessment of this character in rapidly desiccated tissue may not reflect the desiccation tolerance arrived at by empirical screening.

Membrane integrity

Although with increasing tissue desiccation, loss in membrane integrity was noticed, a consistent trend in this character was not observed between the

experiments. At very rapid desiccation rates, species variations in this character seems to be marginal.

Distinct genotypic differences in dehydration tolerance could be arrived at based on several empirical assessment approaches. A few important physiological traits which were studied to relate the intrinsic desiccation tolerance, did not show any relationship. However, osmotic adjustment, maintenance of high protein turnover seem to be important traits for desiccation survival.

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VII. REFERENCES

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APPENDICES

Appendix-1: Meteorological data for I summer experiment 1988.

Weeks after sowing	Mean temperature(°C)		Mean RH(%)		Light intensity cal cm ² day
	Maximum	Minimum	7.30 AM	2.30 PM	
1	27	13	78	52	590
2	29	14	18	23	560
3	30	14	64	22	603
4	31	16	77	33	567
5	32	17	72	37	548
6	32	19	88	45	482
7	32	19	85	46	475
8	33	13	31	46	530
9	34	13	73	42	493.5
10	34	19	75	26	522.3

Date of sowing 10th January 1988.

Appendix-2: Meteorological data for monsoon experiment 1988.

Weeks after sowing	Mean temperature(°C)		Mean RH(%)		Light intensity cal cm2 day
	Maximum	Minimum	7.30 AM	2.30 PM	
1	30	18	88	52	500.0
2	30	19	81	38	502.0
3	34	21	78	39	503.0
4	33	21	76	46	509.0
5	32	20	79	49	406.0
6	32	21	79	49	504.0
7	31	21	79	98	453.9
8	29	20	79	44	415.3
9	31	20	32	45	436.7
10	30	19	34	43	476.0

Date of sowing 15th April 1988.

Appendix-3: Meteorological data for summer 1989.

Weeks after sowing	Mean temperature(°C)		Mean RH		Light intensity cal cm ² day
	Maximum	Minimum	7.30 AM	2.30 PM	
1	26.1	14.0	71.6	41.3	525.6
2	26.2	15.5	86.4	46.6	521.9
3	25.1	14.4	94.9	55.7	456.3
4	25.3	12.9	88.0	39.3	437.9
5	26.0	14.0	85.0	46.0	437.7
6	26.0	12.0	82.0	37.0	493.2
7	27.0	13.0	81.0	39.0	436.6
8	27.0	14.0	80.0	37.0	479.4

Date of sowing 15th November 1988.

Appendix-4: Rate of water loss Experiment-I.

Genotypes	H o u r s											
	1/2	1	2	3	4	5	6	7	8	10	12	15
Pusa Ruby	5	8	12	15	18	19	21	20	25	28	30	31
1048	8	9	11	13	17	18	20	22	24	27	28	30
990	8	12	18	22	29	31	36	39	46	47	48	54
564	5	6	10	14	22	24	27	29	33	34	37	38
1086	10	10	11	14	19	20	22	24	27	29	30	32
1301	4	6	11	16	22	24	27	29	34	35	37	38
987	4	6	10	13	18	19	20	23	26	28	30	32
CD at 5%	2.6	2.9	4.2	3.7	4.5	3.3	5.5	4.2	5.1	4.8	4.9	5.5

Appendix-5: Rate of water loss Experiment-II.

Genotypes	1	2	3	4	5	6	7	8	9	10	13
Pusa Ruby	12	19	25	30	33	35	38	41	46	48	52
1048	8	17	19	24	26	28	31	33	36	39	43
990	12	22	28	34	37	43	47	48	58	62	65
564	5	20	21	26	28	30	35	36	40	42	47
1086	9	10	14	19	21	24	26	31	33	37	39
1301	11	19	22	25	30	32	33	36	43	47	49
987	12	22	25	31	33	36	40	42	46	49	54

CD at 5%	1.1	2.2	3.0	3.0	3.3	2.6	3.1	3.0	4.1	2.6	-

Appendix-6: Rate of water loss Experiment-III

Genotypes	1	2	3	4	5	6	7	8	9	10	13
Pusa Ruby	15	21	26	31	35	41	44	50	53	54	58
1048	13	17	20	24	26	33	35	39	42	41	45
564	12	14	19	23	24	27	32	34	38	40	43
1086	13	16	19	25	26	32	35	38	39	41	44
1301	15	20	25	28	32	41	42	49	50	53	58
987	13	18	21	25	29	34	37	52	44	46	54
CD at 5%	2.3	2.3	2.7	3.1	4.0	3.6	3.2	5.5	3.0	3.1	5.2

Appendix-7: Proline content ug/9 dry weight small plot experiment

Genotypes	T1	T2	T3	T4	T5	T6
Pusa Ruby	1465	2713bc	2946cd	2976de	3715c	-
1086	2795	4103bc	4237	6594e	6832d	7283c
990	2014	2071a	2093	2355a	2693a	3233a
564	1219	1719c	2692de	3367cd	4309bc	4393bc
1086	2208	3151ab	3723ab	4195	4396	4756b
1301	2255	2663b	3321bcd	4127b	4764b	4859b
987	2809	3281ab	3362bc	4040bc	4754b	5307b

Mean	2109	2814	3196	3951	4477	4972
CD at 5%	1775.1	1259.7	680.4	750.2	628.3	1325.3

Appendix-8: Per cent leakage of solutes in four Lycopersicon species subjected to live wilting.

Genotypes		I Expt.	II Expt.	III Expt.
Pusa Ruby	50%	45	70	39
	75%	48	80	63
990	50%	22	52	75
	75	22	55	77
987	50%	45	13	39
	75%	52	40	66
1301	50%	40	20	40
	75%	64	48	66

CD at 5%	G	2.29	3.31	3.42
	S	1.62	2.34	2.42
	GxS	3.23	4.67	4.84

Appendix-9: Water content, total osmotica, sucrose, total amino acids, hexoses and pentoses of two Lycopersicon genotypes under stress situation.

	Pusa Ruby				987			
	do	d4	d9	d14	do	d4	d9	d14
Water content (ul/4 leaf)	163	86	64	52	163	152	146	94
Sucrose (mg/45 leaf discs)	2.72	1.98	2.60	2.46	4.16	6.57	7.80	5.43
Sucrose (mM)	49	68	120	139	75	127	157	170
Total amino acids (mg/45 leaf discs)	1.65	1.17	1.05	1.15	2.53	4.49	5.81	5.23
Total amino acids (mM)	78	105	126	270	119	228	306	429
Hexoses (mg/45 leaf discs)	0.42	0.30	0.36	0.62	0.80	0.94	1.24	1.70
Hexoses (mM)	20	28	43	92	38	48	65	140
Pentoses (mg/45 leaf discs)	0.57	0.43	0.56	0.76	1.12	1.46	1.78	2.34
Pentoses (mM)	23	39	58	98	46	64	81	166

Each value represent a mean of 8 samples.

Appendix-10: Characterization of various Lycopersicon genotypes to a few stress avoidance and tolerance mechanisms.

Resistance mechanisms	Genotypes
I. Avoidance mechanisms:	
a) WUE	1301, 1086, 987 low WUE but greater adaptation to light and moisture stress.
b) Root characteristics	990, 987 high root/shoot ratio high WEE index
c) Cuticular transpiration	Low in 1048, 1086, 564
II. Tolerance mechanisms:	
a) Empirical assessment	
1) Based on leaf survival	High in 1086, 1048, 564 Moderate in 987 Low in 990, Pusa Ruby
ii) Based on seedling survival	Tolerant in 987, 1086, 1301 Moderate in 1048, 564 Susceptible in Pusa Ruby
b) Osmotic adjustment	High in 987 Low in Pusa Ruby
c) Proline	High in 987, 1086
d) Protein synthesis	High in 987
e) Potential photosynthesis	Maintenance potential photosynthesis under stress, high in 987 and Pusa Ruby whereas less in 990, 1301.

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