26.2. HEAT STRESS

The adverse effects on plants of temperatures higher than the optimal is considered as *heat stress*. Heat would affect (1) survival, (2) growth and development and (3) the physiological processes, the nature and extent of the effects depending mainly on the temperature, the plant species and the process in question.

26.2.1. Cell and Tissue Survival

Cell and tissue death usually occurs in such organs that lack transpirational cooling, *e.g.*, twigs, trunks, fruits, etc., and when their surface temperatures reach 48-50°C. Seedlings are more prone to such injuries as they are exposed to and are in contact with soil surface. Reproductive processes are especially sensitive to heat, which causes flower abscission, pollen death and poor fruit set. However, such near lethal temperatures would occur for brief periods, when surviving the heat stress becomes paramount.

26.2.2. Growth and Development

Phenological events are accelerated by the cumulative effect of temperature, which is commonly referred to as ***heat units*** or ***heat sums***. Therefore, the extent of detrimental effects of heat depends both on the temperature and the duration of exposure at that temperature, *i.e.*, on temperature × time interaction. As a general rule, *the acceleration of plant development by heat reduces yield, organ size and even total biomass yield; it also reduces the duration of grain filling and, as a result grain size.* Sometimes, accelerated development may lead to premature senescence.

The different stages of growth and development differ in their sensitivity to heat stress. For example, the period from onset of spike initiation to flowering in wheat is very sensitive to temperature, and acceleration of this phase seems to be the main cause of yield reduction under hot conditions. Wheat variety 'Kalyan Sona' is very heat stable in this respect, which may partly explain its stable performance in India. The acceleration of flower formation is a general stress factor for all crop plants where yield is proportional to the number of flowers per plant. In some cases, *e.g.*, in potato and wheat, this period may also be sensitive to photoperiod; in such cases, photoperiod may modify the temperature effect.

26.2.3. Physiological Effects

Temperature affects a host of physiological processes, the most relevant of which are as follows: (1) respiration, (2) photosynthesis, (3) photosynthate translocation, (4) protein denaturation, (5) membrane composition and stability, and (6) heat shock proteins.

26.2.3.1. Respiration. Total respiration increases with temperature, and it is more resistant to heat than photosynthesis. Therefore, heat may lead to the utilization of a greater amount of photosynthates than that produced by photosynthesis, resulting in a progressive depletion of photosynthates. This effect is of great relevance to crop yields. It may be pointed out that a portion of respiration is associated with growth, while the other part is concerned with the maintenance of life processes.

26.2.3.2. Photosynthesis. Photosynthetic processes are extremely sensitive to heat. Photosystem II, *viz.*, photolysis of water and reduction of CO_2, is far more readily inactivated by heat than is photosystem I. The various enzymes located outside the thylakoid membranes of chloroplasts may be destabilized by heat and, thereby, result in inhibition of photosynthesis.

26.2.3.3. Photosynthate Translocation. Grain growth, especially in cereals, is reduced by heat. This effect may be the result of reduced photosynthate translocation to the grain as a consequence of heat. Alternatively, heat itself may reduce grain growth, which, in turn, reduces photosynthate translocation to the grain (due to a reduced sink size).

26.2.3.4. Protein Denaturation. It has been proposed that heat-induced conformational changes in proteins (which may affect their functions, may lead to their denaturation and enhance their susceptibility to proteolytic enzymes) is central to heat-induced injuries. But protein denaturation occurs at nearly or exactly lethal temperatures. Therefore, heat stability of proteins is a questionable index of heat resistance.

26.2.3.5. Membrane Composition and Stability. Heat may affect membrane composition and stability, which is a function of the water, the protein and the lipid components of the membrane as well as their interactions with each other. As the temperature rises, the lipids become increasingly liquid; this may affect the membrane function and even membrane protein stability. The fatty acid composition of membranes changes with temperature. Further, the fluidity of bulk cellular lipids decreases in plants prehardened at high temperature; this may be associated with a decrease in unsaturated fatty acid content of the membranes. It appears that the loss of cellular-membrane function with increasing temperature is gradual; this loss seems to be reversible so long as death is not reached; irreversible damage occurs at near-lethal temperatures. But *membranes (cellular and chloroplast) are central to heat injury and heat tolerance.*

26.2.3.6. Heat Shock Proteins (HSP). It is a group of about a dozen or so proteins that normally exist in cells, but their synthesis is accelerated by heat. The acceleration phase is of about 20-30 min in bacteria and several hours in plants; after this phase, a new steady state is reached. In case of *E. coli,* Hsp constitutes about 25% of the total cellular proteins at the upper range of its growth temperature. A heat shock enhances the level of Hsp by 10 to 50-fold. Hsp are diverse in molecular mass, and are also produced in response to other stresses like UV radiation, ethanol, virus infections, etc. Hsp seem to be essential at all the temperatures, although at higher temperatures they are needed in much larger quantities. Some of the Hsp may condition heat tolerance. Some Hsp, *e.g.*, Hsp70, function as ***chaperononins,*** which are a class of proteins that associate with unfolded proteins and, thereby, prevent their improper folding or denaturation. The chaperononin function may be involved in heat tolerance and tolerance to other stresses that may induce protein unfolding.

26.3. HEAT STRESS RESISTANCE

Heat stress resistance may be defined as the ability of some genotypes to perform better than others when they are subjected to the same level of heat stress. The various mechanisms of heat resistance may be grouped into two categories: (1) heat avoidance and (2) heat tolerance (Table 26.1).

TABLE 26.1

Different mechanisms of heat stress resistance in plants

Mechanism	*Contributory processes*	*Consequences/remarks*
Heat avoidance	Transpiration	Cooling
	Leaf reflectance due to 1. Pubescence 2. Glaucousness	Reduces light interception by leaves
	Insulation by bark	Reduces heating
Heat tolerance	Membrane stability Stability of photosystem II Photosynthate translocation Stem-reserve mobilization Osmoregulation	Associated with heat hardening and possibly Hsp synthesis

26.3.1. Heat Avoidance

The ability of a genotype to dissipate the radiation energy and, thereby, to avoid a rise in plant temperature to a stress level is called ***heat avoidance***. The primary mechanism of energy dissipation is ***transpiration***. *Transpirational cooling is the link between dehydration and heat avoidance so that under conditions of high solar radiation and water stress the effects of heat avoidance become inseparable from those of dehydration avoidance.* The ***reflective properties*** of leaves determine the proportion of incident solar radiation absorbed by leaves. Therefore, leaf surface attributes like pubescence, glaucousness, etc. are a component of heat avoidance. In addition, ***bark*** may be involved in reducing organ and twig temperatures by insulating these organs from heat.

26.3.2. Heat Tolerance

Ability of some genotypes to withstand/perform better than others when their internal temperatures are comparable and in the realm of heat stress is called ***heat tolerance***. Heat tolerance is largely associated with the cellular and subcellular components, and its expression is highly dependent on heat hardening or heat acclimation. ***Heat hardening*** may be defined as an improved ability of a genotype to withstand a period of high temperature as a consequence of an earlier exposure to high temperatures for a given period of time. The exposure time may vary from 20 sec at 50°C to 4-6 hr, < 1 hr being the most common. In general, *the lower the hardening temperature, the lower is the level and slower is the process of hardening*. When heat hardened plants are subjected to lower temperatures, they lose their hardening; this is called ***dehardening***. Generally, *dehardening is slower with higher hardening temperatures and with lower dehardening temperatures*. Heat shock proteins most likely form an important component of heat hardening induced by a heat shock. The hardening induced by a prolonged (3 days) exposure to moderately high temperatures, however, most likely involves some other mechanism.

Heat tolerance may involve the following components: (1) membrane stability, (2) reduced heat sensitivity of photosystem II, (3) photosynthate translocation, (4) stem-reserve mobilization and (5) osmoregulation. These effects are all integrated into growth under stress and recovery after stress.

26.3.2.1. Membrane Stability. Heat tolerance usually improves ***membrane stability*** under heat stress. Membrane stability may be determined as (1) lipid fluidity or (2) electrolyte leakage. *A given state of lipid fluidity in heat-hardened plants reaches at a temperature ~10°C higher than that in case of unhardened plants.* Solute leakage may be measured conductometrically from leaf tissue segments taken from properly heat-hardened plants subjected to a period of heat exposure, *e.g.*, 50 min at 50°C, and compared with a proper control. The ***control*** consists of leaf tissue segments of similarly heat exposed non-hardened plants. In general, *cell membrane thermostability is a fair index of genetic variation in heat tolerance that bears a reasonable relationship with plant performance under stress.*

26.3.2.2. Thermosensitivity of Photosystem II. Effect of heat stress on photosynthesis and chloroplast damage can be assayed as variable chlorophyll fluorescence at 685 nm. In some cases, genotypes adapted to hot climates, *e.g.*, in case of potato, show reduced sensitivity of photosystem II to heat stress. But this was not found in some other cases, *e.g.*, in maize. Different plant parts may show differential tolerance to heat stress in terms of photosynthesis. For example, photosynthesis in the awns of cereals is more heat tolerant (optimum temperature 32°C) than that in leaves and glumes (optimum temperature 25°C). This adaptation to heat is essential for productivity of cereals since *awns are a xerophytic organ and lack transpirational cooling.*

26.3.2.3. Photosynthate Translocation. *Callose formation in the phloem sieve tubes is the major cause for inhibition of translocation by heat in tomato.* Tomato varieties were found to differ in the amount of callose formation and photosynthate translocation when subjected to a 72 hr heat stress; the heat-susceptible variety was more affected than the heat tolerant variety.

26.3.2.4. Stem-Reserve Mobilization. In cereals, this seems to be an important component of grain yield under heat stress. For example, the yield advantage of Pitic 62 wheat variety at three temperatures could be partly due to a better mobilization of stem reserves at all the three temperatures.

26.3.2.5. Osmoregulation. Osmoregulators like proline and glycine-betaine may have a protective role in heat stress. It is not clear if proline accumulates in response to heat stress. However, it does accumulate in response to drought stress, which is associated with heat stress as a component. Proline and glycine-betaine protect several enzymes from heat inactivation *in vitro*. Exogenous glycine-betaine also protects cellular membranes against heat damage in beet root disks.

There is evidence that *the importance of a given component of heat tolerance may vary according to the intensity of heat stress.* Therefore, when specific components of tolerance are used as selection criteria, the intensity and duration of heat stress assume great significance. However, *all the tolerance components are ultimately integrated into growth under heat stress and recovery after heat stress. It should, however, be kept in mind that growth under heat stress and recovery after heat stress may have totally different physiological bases and backgrounds.*

26.4. GENETICS OF HEAT TOLERANCE

Genetic variation in heat tolerance is known for germination, growth during heat stress, growth recovery after heat stress, photosynthesis, photosynthate translocation, flowering and fruit/seed set, and stability of cellular membranes. The limited information on genetics of traits concerned with heat tolerance reveals a polygenic control. The only report of oligogenic control relates to number of pods/plant under heat stress in snap bean; one dominant gene governed higher pod number in one heat tolerant line, while in another line the trait was conditioned by 2 dominant genes showing epistatic interaction (Table 26.2).

TABLE 26.2

Genetic control of characteristics involved in heat tolerance in crop plants

Characteristic	*Crop*	*Genetic control*	*Remarks*
*Recovery after heat shock	Maize	Partial dominance	—
**Tuber yield	Potato	—	Recurrent selection (1 cycle) enhanced yield by 27%
**Per cent fruit set[†]	Tomato	Polygenic; additive gene action	Moderate heritability
**Flowers/plant[†]	Tomato	Polygenic; high heritability	High flower number due to recessive genes
**Seed set	Tomato	Polygenic; dominance effects more important	Epistasis also involved
**Stima exsertion	Tomato	Polygenic; partially dominant genes	High heritability
††Number of pods/plant	Snap bean	1 or 2 dominant genes	Epistasis present
+Per cent seed set	Rice	Both GCA and SCA highly significant; heritability ~ 70%	Positively correlated with the amount of pollen shed on stigma
Thermostability of cellular membranes (conductivity method)	Soybean	Polygenic; mainly additive effect	Heritability high; selection effective in one cross (out of 2 crosses)
	Rajma (*P. vulgaris*)	Polygenic; additive effects predominant	Epistasis in some crosses; heritability ~60%

* A 6 hr heat shock at 52°C.

** Under high temperature.

† The two traits are independent of each other.

†† Day time temperature 38-43°C in a glasshouse; combined with drought stress.

\+ Anthesis at 38/27°C.

In general, additive as well as dominance gene actions were involved: in most cases, additive effects were more important than dominance effects, while in some others, *e.g.*, seed set under heat stress in tomato, dominance effects were predominant. Heritability estimates ranged from moderate to high depending on the crop and the material used for the study. Recurrent selection for yield under high temperature was carried out for one cycle in potato. This resulted in a 27% improvement in yield, 15% increase in tuberization and a 3% enhancement in survival. In case of rice, per cent seed set under high temperature was positively correlated with the amount of pollen shed on stigma. Selection for enhanced membrane thermostability (measured by the conductivity method) was successful in one soybean cross but was ineffective in another (Table 26.2).

26.5. SOURCES OF HEAT TOLERANCE

In most crops, sources of heat tolerance are available within the breeding germplasm, the only condition being that the materials should have been properly evaluated for the concerned traits. In general, *use of exotic genetic resources is not essential for attaining progress in heat tolerance*. But when search for exotic germplasm becomes necessary, heat tolerant collections are likely to come from the margins of the geographical distribution for each crop since such areas would represent the thermal limits of adaptation of the concerned crops.

26.6. SELECTION ENVIRONMENT

Selection for heat tolerance can be carried out under the following four types of environment: (1) normal field environment, (2) 'abnormal' field environment, (3) programmed environments, and (4) *in vitro* environments.

26.6.1. Normal Field Environment

The natural field environment is the simplest and the cheapest to use. But its effectiveness depends mainly on the repeatability of the heat-stress profile over years, and on the nature of heat tolerance being selected for. It is unsuitable for the selection of such traits, which require a critical temperature during a specific stage of development since the same can not be ensured particularly over the years. This problem will be aggravated by phenological variation among different genotypes so that they will reach the specified stage of development at different points of time. This problem could be resolved as follows. When the critical temperature is reached in the field, all the plants at the specific developmental stage, *i.e.*, anthesis, may be marked by, say, a paint spray. Only the marked plants are evaluated at maturity, and the remaining population is regarded as escapes.

26.6.2. 'Abnormal' Field Environments

When normal field environment does not provide the suitable heat stress conditions, 'abnormal' field environments available at certain locations or during the off-season may be used. This practice is followed in several crops, *e.g.*, for wheat in Israel. (Wheat is grown during summer, which is the off-season).

26.6.3. Programmed Environments

Such environments are available either in growth chambers or in greenhouses. The temperature programme should be such that the plants are subjected to appropriate heat hardening before their heat tolerance is evaluated. In case of bean, potato, soybean and tomato, hardening was the maximum with a 24 hr exposure to 35-37°C. There is considerable evidence that the extent of hardening may be affected by plant/organ (*e.g.*, leaf) age; this should be accounted for in the tolerance test. Obviously, such environments are costly to create and use, and require considerable expertise and effort.

Usually, it is desirable to avoid water stress during evaluation of heat tolerance. Therefore, adequate irrigation regime must be provided both under the field and programmed environments. In addition, a high relative humidity should also be maintained by using humidifiers (in growth chambers) or misting/fogging facilities (in green-houses).

26.6.4. *In Vitro* Environments

Certain assays for heat tolerance can be performed in test tubes, *e.g.*, membrane thermostability by the electroconductivity method. Small pieces, *e.g.*, 12 mm leaf disks, are collected from field/greenhouse/growth chamber-grown and heat-hardened plants. Care should be taken to collect samples from leaves of comparable age. The tissue samples are washed 2-3 times with deionized water before they are kept in test tubes/glass vials. For each genotype, a total of 10 such vials are prepared. The vials are loosely capped and 5 vials for each genotype are kept in a water bath at 42-54°C for 1 hr (treatment); the remaining 5 vials for each genotype are kept at room temperature and serve as control. Finally, 10-20 ml deionized water is added into each vial, and the vials are stoppered and incubated at 10°C for 24 hr. The conductivity of the water (which will contain the solutes leaked from the leaf discs) is measured by inserting a conductivity electrode into each vial after its temperature is

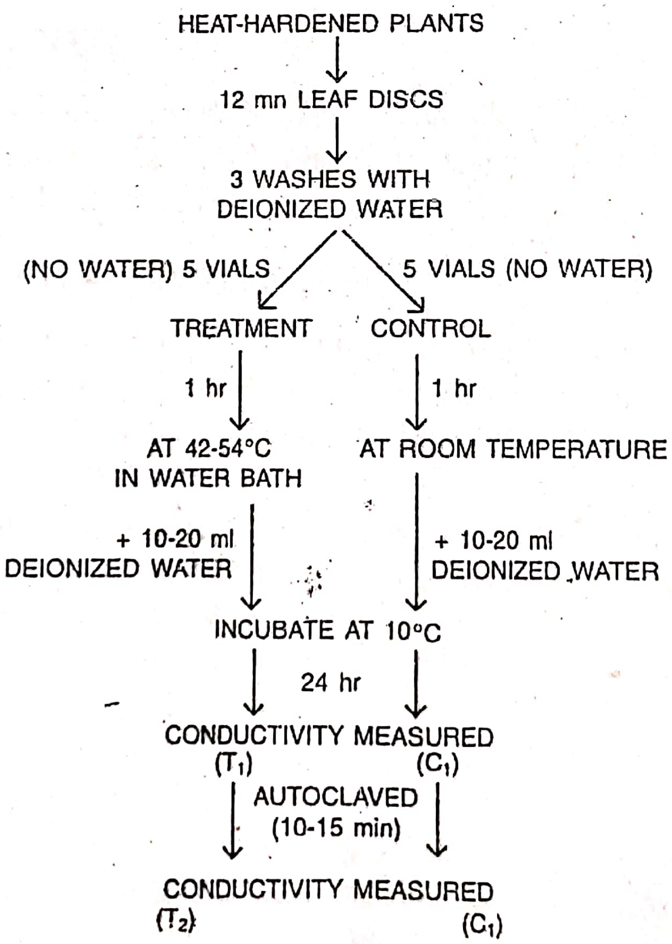

Fig. 26.1: A schematic representation of the protocol followed for electroconductimetric assay of membrane stability under heat stress.

brought up to 20°C. All the vials are then autoclaved for 10-15 min, and the conductivity of their medium is estimated after their temperature is brought down to 20°C (Fig. 26.1). The extent of heat injury is calculated as follows.

$$\text{Heat injury (\%)} = \left[1 - \left\{\frac{(T_1/T_2)}{(C_1/C_2)}\right\}\right] \times 100$$

where, T_1 and T_2 are the mean conductivities of treatment vials for a genotype before and after autoclaving, respectively, while C_1 and C_2 represent the mean conductivities of the control vials of the same genotype before and after autoclaving, respectively.

26.7. SELECTION CRITERIA FOR HEAT RESISTANCE

Heat tolerance can be determined in terms of several features (Table 26.3). When selection is carried out in the target environment (1) ***growth under heat stress*** is almost always used as selection criterion. Growth would reflect the integrated consequences of various cellular components involved in heat tolerance. (2) ***Yield under heat stress*** would also reflect the effects of heat on reproductive processes like flower development, pod/fruit/seed set, pollen fertility, grain filling (especially in cereals), etc. When heat stress either occurs or can be created during the reproductive phase, the various traits related to this phase, *e.g.*, (3) ***flower, fruit, seed, etc. formation and pollen fertility,*** etc., may be used as selection criteria for heat tolerance. In some situations, germination may be adversely affected by soil heating, *e.g.*, in case of sorghum and pearl millet in India. In such cases, (4) ***seed germination under heat stress*** should form an important component of the selection criterion. It should, however, be kept in mind that the environment under which the seed samples were produced would considerably affect their germination under heat stress. Therefore, as far as possible, *all the seed samples being compared should have been produced under comparable temperature regimes.*

TABLE 26.3

Different selection criteria for heat tolerance in crop plants

Characteristic	*Measured as*	*Usefulness as selection criterion*
Germination	Per cent germination under stress	Useful when crop faces heat stress at germination, *e.g.*, in case of sorghum and pearlmillet (in India)
Growth during heat stress	Yield, biomass	Most commonly (almost always) used selection criterion
Membrane stability	Solute leakage (conductivity test)	Reasonable correlation ($r = -0.7$) with yield under heat stress or with drought resistance
Photosynthesis sensitivity	Chlorophyll fluorescence at 685nm	Becoming increasingly important: difficult to assay and, especially, interpret
Recovery after heat stress	Yield, biomass etc.	Used whenever relevant for the target environment
Sensitivity of reproductive phase	Flower/pod/fruit/seed production; pollen fertility	Useful selection criterion; accounted for in selection based on yield under heat stress

In certain heat stress environments, crops may be subjected to brief periods of extreme heat stress. In such conditions, (5) ***recovery after heat stress*** would be a more relevant selection criterion than tolerance to prolonged moderate heat stresses. (6) ***Sensitivity of the photosynthetic process*** (photosystem II) and of chloroplasts to heat stress can be assayed as variable chlorophyll fluorescence at 685nm. It may develop as a tool for selection provided the associated difficulties are resolved. (7) ***Membrane stability following heat shock*** is readily measured by the electrical conductivity test (Section 26.6.4). It is a fair index of plant performance under heat stress; in case of sorghum it showed a reasonable (r = 0.69**) correlation with yield under heat and water stress conditions. In case of soybean, it was also associated (r = 0.78**) with drought resistance. Membrane thermostability may offer an advantage to growth at defined stress temperatures, and would be of considerable value when selection is carried out under 'abnormal' field or programmed environments because in such cases yield/biomass production would not be a suitable selection criterion.