Wheat Special Report No. 20

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Preface

The production of wheat germplasm with resistance to high temperature increases in importance with world wheat demand. Temperatures considered above optimal for wheat growth and development of presently grown varieties are usually found in most tropical and subtropical environments during the growing season or at least part of it. In this Special Report, two complementary approaches are presented that are shown to help in adaptation and grain yield of wheat under high temperatures. One looks at membrane thermostability as a heat resistance trait. The other examines the role of evaporative cooling through transpiration, a particularly useful mechanism in hot and dry environments. Practical screening methodologies are provided in both cases.

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Note on Citing this Wheat Special Report
The information in this wheat special report is shared with the understanding that it is not published in the sense of a refereed journal. Therefore, this report should not be cited in other publications without the specific consent of E. Acevedo, CMP Subprogram Leader.

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Introduction

While wheat currently is a minor crop in the tropics, its consumption is increasing rapidly causing a large drain on foreign exchange of these countries. Over 60% of the wheat consumed in more than 50 tropical countries is imported (Fischer and Byerlee 1990). Many governments have decided that it would be better to conduct research necessary to overcome the different production constraints facing wheat in the tropics.

Spring wheat production is affected by high temperature stress in these nontraditional wheat growing areas (Mann 1985). However, terminal heat stress can also be a problem even in irrigated environments where 42% of the spring wheat of developing countries is produced. Identification of heat-tolerant genotypes and their use in breeding programs are, therefore, important objectives for CIMMYT.

The prerequisites of a genetic improvement program for heat tolerance are the identification of heat tolerance mechanisms with high heritabilities and the development of suitable methodologies for identifying the traits in large breeding populations showing genetic variability for the trait (Acevedo and Ceccarelli 1990).

Two traits investigated in separate studies reported on here are membrane thermostabilty and canopy temperature depression.

Membrane Thermostability

According to Blum (1988), although resistance to high temperatures involves several complex tolerance and avoidance mechanisms (Acevedo and Fereres 1993, Acevedo et al. 1991), sites of primary physiological heat injury are the membranes. Therefore, a cell membrane system that remains functional during heat stress appears central to environmentally fitted plants at high temperature (Raison et al. 1980). Heat damage to the plasma membranes can be estimated by conductometric measurement of solute leakage from the cells.

Genetic variation in membrane thermostability (MT) has been found using conductometric measurements in field-grown sorghum by Sullivan (1972) and Sullivan and Ross (1979), in soybean by Martineau et al. (1979), in pearl millet by Blum and Sullivan (1986), and in spring wheat by Blum and Ebercon (1981). Positive relationships between membrane thermostability and yield performance were shown. Shanahan et al. (1990) obtained a significant positive increase of yield of spring wheat in hot locations by selection of membrane thermostabile lines, determined by MT measurements on flag leaves at anthesis. Li et al. (1991) obtained positive correlations between membrane thermostability of 1-month-old acclimated leaves and plant dry-weight, pod set, pod weight, and yield, among common bean genotypes grown in controlled environments under heat stress. Applying the membrane thermostability test on winter wheat seedlings, Saadalla et al. (1990) found a high correlation in membrane thermostability between seedlings and flag leaves at anthesis of genotypes under controlled environmental conditions.

The main objective of the study was to evaluate a number of alternative strategies for measuring MT in spring wheat so as to identify those procedures which would enable effective discrimination of MT among genotypes.

Materials and methods

Germplasm and growth conditions--Sixteen genotypes of spring wheat were used for conductometric determinations of MT. The selected genotypes were chosen because they comprise the material being studied as part of a collaborative experiment being conducted between CIMMYT and the national agricultural research programs in eight international hot locations (Reynolds et al. 1992). Measurements were made on leaf tissue taken from plants at three stages of phenological development, 10-day-old seedlings, plants at the 5-7 leaf stage, and plants at heading/anthesis.

Field-grown plants were raised with good agronomic management (fertility, irrigation and pest and disease control) at CIMMYT's Tlaltizapan station (18°N, 99°W, 940 masl) during the 1992 spring growing cycle, during which the mean maximum and minimum temperatures were 34/12°C, and relative humidity between 20 and 30%. The controlled environment experiments were conducted at El Batan, CIMMYT headquarters in Mexico between May and August 1992.

For greenhouse-raised plants, approximately 50 fungicide-treated seeds of each of the 16 genotypes were sown directly in soil, in 15-cm pots. Ten plants were kept in each pot and four replications were arranged in an RCB design. The plants were grown as close as possible to 21/14°C day/night temperature regime. Pots were irrigated as needed to keep the soil moisture and under high fertility. The MT was determined on the youngest fully expanded leaf of 5-7 leaf stage plants. For MT measurements on seedlings, fungicide-treated seeds of the 16 genotypes were wrapped in moistened germination paper and allowed to germinate and grow in an environmental growth chamber with a 16-hour photoperiod and a light intensity of 200 $\mu E/m^2/s$, and 17°C temperature regime day and night. The oldest leaves of 10-day-old seedlings were used for MT measurements, these were the first leaves drawing at least 5 cm long.

Heat acclimation and sampling procedures--In all experiments, plants were heat-acclimated before MT measurements were taken. For field-grown plants, heat acclimation took place *in situ*, where a 35/15°C max/min temperature regime prevailed for at least 2 days before the MT test. Ten flag leaves were sampled from each field replication. The leaves were randomly collected and placed with their cut ends immersed in water in stoppered glass jars. All jars were placed in a cold box for transportation from field to the laboratory. In the laboratory, the middle portions of the leaves were isolated, quickly washed with deionized water, and completely re-hydrated by keeping them in deionized water over night in a refrigerator. For the MT measurements, 1-cm sections of each leaf were cut for both control and heat shock treatment. An acclimation temperature of 35°C was chosen in subsequent experiments to parallel the mean maximum air temperature experienced by field-acclimated plants.

For the greenhouse-raised plants, half of the pots were placed at 35°C for 48 hours in a temperature-controlled growth chamber with a 16-hour photoperiod, so as to heat-acclimate whole plants. During acclimation, the pots were irrigated as needed to prevent water stress and to maintain a high relative humidity inside the chamber. For the rest of the pots, the fully expanded youngest leaves were cut and placed with their base in water in open glass jars. All jars were placed in a temperature-controlled water bath at 35°C for 48 hours to acclimate leaves. The water bath was covered with a plastic cover to maintain a high relative humidity inside the bath. The MT test was performed using 1-cm sections of the middle portions of the leaves. The youngest fully expanded leaf was also used for the MT test in the pot-acclimated plants.

For the seedling treatment, acclimation was imposed 10 days after germination when the first leaf attained approximately 5 cm in length. Ten seedlings for each replication were placed in the covered water bath, as described above, with their roots immersed in water maintained at 35°C for 48 hours. For the MT test, the upper portion of the leaf was used for the heat shock treatment and the base was used as the control.

Heat treatments and conductometric measurements--Once acclimated, the plant material was washed with deionized water and divided into vials containing either 17 ml or 2 ml of deionized water. Half of the vials were maintained at 46.5 or 49°C for 40, 60, or 80 minutes in the water bath. Experimental details are summarized in **Table 1**. The second set of vials were used as controls and maintained at room temperature for the same time periods. After the treatment periods, treatment and controls were held at 6°C in refrigeration over night. A first conductometric reading was made at 25°C and a second (also at 25°C) was made after autoclavation for 20 minutes at 120°C and 0.10 MPa. Membrane thermostability was expressed as relative injury (RI) using the following calculation:

$$RI\% = (1-(1-T1/T2)/(1-C1/C2)) \times 100:$$

where T is the treatment, C is the control, and 1 and 2 are the first and second readings of conductance, i.e., before and after the autoclaving.

Statistical procedures--Analysis was performed after arcsine transformation on the relative injury (RI) values, an appropriate transformation for data expressed as a percentage (Little and Hills 1972). The analysis of variance was used to determine variation for RI among genotypes for all methodologies used. Analysis of covariance was used to correct RI values from flag leaves using the number of days between measurement and anthesis date as the covariate. For 5 to 7 leaf stage plants their growth stage was used as a covariate (Haun 1973). Linear correlation analysis was used to determine the relationship between RI evaluated by different methodologies involving field-grown and controlled environment grown plants and to determine the relationship between MT and yield in the heat-stressed locations of the international experiment.

Results and discussion

According to previous studies, a mean RI of approximately 50% across all genotypes is the optimum level at which differences between genotypes can be most easily detected (Martineau et al. 1979, Saadalla et al. 1990). Furthermore, the point at which heat- or drought-induced membrane injury results in 50% electrolyte loss is considered to be the point at which tissue death occurs (Li et al. 1991, Chen et al. 1982). One of the specific aims was to see how different procedures compared in the mean level of RI they caused across genotypes. Mean values of RI ranged from 18.7 to 50.6% (Table 1), depending on the methodology used. The methodological variables used in this study, stage of phenological development, and heat acclimation procedure, intensity of heat shock (determined by volume of the heat shock medium and the duration and absolute temperature of heat shock treatment used) were not compared in a complete factorial manner. Nonetheless, the results (Table 1) show that most treatments gave a mean relative injury approaching the optimum of 50%. The two main exceptions were for 5-7 leaf stage plants acclimated as whole plants, and for 10-day-old seedlings where the heat shock treatment of 40 minutes was apparently not long enough to allow a higher RI level.

One of the experimental variables examined in this study were two methods of imposing heat shock: by exposing leaf samples to a minimal amount of water (2 ml) or to an increased amount to water (17 ml) during the procedure. The effect of increasing the amount of water is to slow the onset of the heat injury since a larger volume will heat up more slowly upon immersion in the water bath. The results obtained from conductometric tests on the flag leaves showed a highly significant correlation (r=0.81) between RI values obtained by both methodologies (**Table 2**) and a consistent relative ranking of genotypes for the MT trait (**Table 3**). Since the volume factor seemed to have

Table 1. Summary of experimental conditions and mean relative injury values and range of relative injury for 16 genotypes used to compare different methodologies for measuring membrane thermal stability.

Place of experiment	Plant tissue	Water volume (ml) for heat shock	Growth Temp. (°C)	Acclimation Temp. (°C)	Acclimation Time (h)	Heat shock Temp. (°C)	Heat shock Time (min.)	Mean relative injury(%) (n=16)	LSD (0.05)
Field, Tlaltizapan (1992)	Flag leaf, at anthesis	2	34/12	35/15	field	46.5	60	49.3	1.3
	Flag leaf, at anthesis	17	34/12	35/15	field	46.5	60	42.8	1.4
Greenhouse (El Batan, 1992)	5-7 leaf stage plants	17	21/14	35	48	49	60	42.7	0.73
	-acclimated as detached leaves	17	21/14	35	48	49	80	47.9	2.9
	-acclimated as pot-grown plants	17	21/14	35	48	49	60	18.7	0.34
		17	21/14	35	48	49	80	38.3	1.4
Growth chamber	Seedlings, 10 days old	17	17	35	48	49	40	27.5	4.2
	Seedlings, 10 days old	17	17	35	48	49	60	50.6	2.9

Table 2. **Relative injury as determined by MT test on flag leaves for the 16 genotypes of spring wheat and their criteria for choice in the International Heat Stress Genotype Experiment.**

Genotype	Relative injury (%) 2-ml water for heat shock	Relative injury (%) 17-ml water for heat shock	Criteria for choice[a]
Glenson	27.9	18.9	Heat-tolerant check
Debeira	32.1	18.3	Grown in Sudan
Bacanora	34.6	44.9	High-yielding Mexican variety
Seri 82	40.9	29.1	Broadly adapted variety
Fang 60	44.9	29.4	Grown in Thailand
Nesser	48.5	39.1	Heat tolerant
Anza	49.1	38.3	Grown in Sudan
Nacozari	49.7	36.1	Heat-sensitive check
Genaro 81	53.8	59.6	Widely grown standard check
Sonora 64	54.2	40.7	Widely grown early variety
Pavon	54.4	60.3	Heat-sensitive check
Trigo 3	57.7	60.4	Developed in the Philippines
Siete Cerros	61.2	53.3	Heat-sensitive check
Kanchan	62.7	54.9	New variety from Bangladesh
Ciano 79	63.2	48.8	High-yielding Mexican variety
IP 4	68.0	58.6	Old variety from Myanmar
Mean	49.3	42.8	
LSD 0.01	2.3	2.6	

Correlation coefficient between values of relative injury obtained by 2-ml and 17-ml methods on flag leaves = 0.81***

[a] Source: Reynolds et al. (1992).

no significant effect, all other experiments were conducted with the larger volume of 17 ml.

Comparison of seedlings versus field-grown plants--The correlation coefficient for RI between leaf tissue of seedlings and flag leaf tissue from plants at approximately anthesis was 0.77 (**Figure 1**), indicating that RI determined at the two developmental stages was reasonably well associated. This supports the idea that the screening procedure using seedlings raised under artificial conditions gives MT values comparable with those coming from plants growing in a realistic target environment, i.e., the heat-stressed field environment. Further support for this comes from the comparison of correlations between yield in the heat-stressed environment and MT values measured on seedlings and field-grown plants, respectively (**Table 4**). This is an important result for breeding programs wishing to screen large numbers of genotypes for MT. The seedling methodology is favorable logistically since the conditions of plant acclimation can be controlled, while this is not possible in the field. The importance of this point was illustrated indirectly by

Table 3. Correlation coefficients between membrane relative injury using two different methodologies, and yield and biomass of 16 wheat genotypes grown in nine heat stressed locations.

Country and cycle	Flag leaf (2-ml) covariated[a]		Seedlings 60, heat shock	
	Yield	Biomass	Yield	Biomass
Sudan, 1990-91	-.58	-.63	-.42	-.64
Central Mexico, 1990-91	-.51	-.54	-.27	-.46
Thailand, 1990-91	.53	.23	.38	.16
India, 1990-91	-.66	-.75	-.63	-.70
Brazil, 1991	-.52	-.86	-.32	-.77
Bangladesh, 1990-91	-.07	-.55	-.13	-.58
Syria, 1991	-.09		-.16	
N.W. Mexico, 1990 (Feb. sown)	-.41		-.28	
Central Mexico, 1991-92	-.75	-.79	-.38	-.62
Mean across locations[b]	-.52*	-.69**	-.37	-.63**

[a] Covariated with number of days to anthesis.

[b] Mean of seven locations excluding Bangladesh and Thailand.

Correlation coefficient significant 0.497.

Table 4. Analysis of variance for an experiment on wheat seedlings evaluating the effects of replication day, heat shock treatment, and genotype on the degree of temperature-induced injury.

Source of variation	**d.f.**	**Mean squares**	**Significance at P 0.01**
Replication (time)	2	2897.299	**
Genotype	15	254.151	**
Heat shock treatment	1	4498.892	**
Genotype*treatment	15	27.927	NS
Error	62	49.042	
Genotype*replication	30	42.501	NS
Error	47	46.479	
			P 0.01

the data. In the case of the seedling procedure, the three repetitions of the experiment were measured for MT on 3 subsequent days. While the interaction of genotype with repetition was not significant, the main effect of repetition (i.e., day of experiment) was highly significant (Table 4). Even under controlled conditions, unintentional discrepancies, either in procedure or day-to-day variability of conditions, influenced absolute values of RI. Since it would not be practical for a breeding program to assess RI

Table 5. Analysis of covariance for an experiment involving 5-7 leaf stage plants and evaluating the effect of cultivars, acclimation, treatment, and growth stage (Haun stage 5-7) on relative injury of plasma membranes.

Source of variation	d.f.	Mean squares	Significance at P 0.01
Replication	1	0.031	
Genotype	15	117.844	**
Heat shock treatment	2	1939.755	**
Genotype*treatment (H.S.)	15	90.176	**
Acclimation	1	3442.976	**
Genotype*acclimation	15	146.486	**
Genotype*treatment(HS)*acclimation	15	93.567	**
Covariate (stage)	1	2.892	NS
Error	62	9.503	

on all germplasm of interest in one experimental run, a methodology involving controlled conditions would seem to be favored. In addition to standardizing procedures as far as possible, quantitative assessment of MT would be more reliable if a range of standard genotypes, i.e., of known MT, were included every time the procedure were run by which to calibrate the results (Balota and Saulescu 1992).

Comparison of acclimation of attached versus detached leaves--The greenhouse experiments were conducted mainly to compare the effect of acclimating detached leaves as opposed to whole plants, thermal acclimation being an important prerequisite to heat stress tolerance (McWilliams 1980). This would be useful, for example, if one wished to evaluate field-grown material for MT when weather conditions do not permit acclimation of whole plants ***in situ*** (Li et al. 1991). Our data showed a highly significant genotype x acclimation interaction (**Table 5**), suggesting that acclimation of detached tissue would probably not be recommendable. However, the fact that there was a three-way interaction for the greenhouse experiment (genotype x heat shock treatment x acclimation) made it difficult to interpret these results with much confidence, especially as the covariate for stage of development was nonsignificant (Table 5), which might otherwise have explained some of the variability in the data.

Correlation of MT with performance in target environments--To give an idea of the degree to which our MT measurements predicted performance of genotypes under a range of heat-stressed environments, the RI values for flag leaves and seedlings were compared with yield and biomass (Table 3) of 16 genotypes grown in eight heat-stressed wheat growing environments (Reynolds et al. 1992). In most locations, MT values were significantly correlated with yield and biomass (Table 3).

Canopy Temperature Depression

This study focused on the possibility of using canopy temperature depression (CTd) as a screening tool for heat tolerance in wheat. Canopy temperature (CT) was first used to estimate water stress for different species (Jackson et al. 1981). For example, water stress, as measured in terms of plant water potential in wheat, could be identified when canopy temperature-air temperature (Tc-Ta) was positive (Idso et al. 1981). Sharratt et al. (1983) found that under different irrigation treatments CTd showed a qualitative relationship with plant water stress in alfalfa. Clowson and Blad (1982) showed similar results in maize. CT was also found to be negatively related to yield in maize (Gardener et al. 1981), spring wheat (Diaz et al. 1983), soybean (Harris et al. 1984), and in pearl millet (Singh and Kanematsu 1983) under a range of water-stressed conditions.

Since CTd correlated with crop yield and moisture stress, it was speculated that CTd could be used to screen genotypes of a species for drought tolerance (Singh and Kanematsu 1983, Harris et al. 1984). Differences among species for CT have been reported (Blad and Rosenberg 1976a,b; Erhler 1973), but still little is known about the variation among genotypes of a species. Carlson et al. (1972) found differences for CTd between two soybean cultivars under some, but not all conditions, varying in vapor pressure deficit (VPD). Significant differences among lines of soybean were reported by Harris et al. (1984), but change of ranking occurred from year to year. Singh and Kanematsu (1983) found differences in CTd among 10 pearl millet lines under well watered conditions, but not under water stress. McKinney et al. (1989) conducted a divergent selection study to characterize genetic variation for CTd in six populations of soybean and to assess the effectiveness of selection within the populations for CT. The mean CTd of hot vs cool selections was significantly different in three out of the six populations.

If the breeders are to use CTd variation as an effective screening tool for heat and drought tolerance, more information is needed about the conditions under which it would be most reliable. For example; at what time of day and stage of development is the genetic variability best correlated with yield? Are there significant interactions between genotype and time of the day or stage of development for CTd? What is the effect of irrigation on this variability? In this study, CT measurements were made on a set of genotypes currently being grown as part of an international collaborative experiment in 10 heat stressed environments worldwide. Previous work in one of these environments (Mexico) has shown significant variability in CTd among these genotypes, which correlated well with yield (Reynolds 1994). One of the main focuses of this study was to observe the interaction between CTd and stage of development, time of the day, irrigation status, and sowing date. An additional objective was to investigate the physiological relationship between CTd and stomatal conductance and frequency.

Materials and methods

Growing conditions--During the 1992-93 growing season, two cycles of the experiment were conducted at the CIMMYT experimental station in Tlaltizapan, state of Morelos, Mexico (18^{o}N, 99^{o}W, 940 masl). Cycle I had an average daily maximum and minimum temperatures of 32.7 and 11.1^{o}C, respectively, with slightly higher mean max/min temperatures, 35.2/13.0^{o}C, for Cycle II. Average relative humidity was 29% for cycle I and 20% for cycle II. The soil is a calcareous vertisol (Isothermic Udic Pellustert) 1.3-1.8 m in depth, with a pH of 7.6.

Each genotype was sown in an eight-row, 6-m long plot, with 15 cm between rows at a seeding rate of 400 seeds/m^2. The experiment was highly managed to avoid factors that may interact with the thermal effects. The crop was irrigated when soil water potential was approximately 65 mb at a 30-cm depth. High levels of chemical fertilizer were applied (200-50-0 N-P-K). Sulfur was applied at a rate of 2 t/ha as a soil fungicide and to compensate for the soil pH. Pesticides were applied regularly to prevent diseases and insect infestation.

Twenty-three genotypes were chosen based on the fact they are either grown or were bred in warm areas, broadly adapted, high yielding under irrigation, or heat-sensitive. A brief description of the 23 genotypes is given in **Table 6.**

Table 6. Description of the 23 genotypes used in the Membrane Thermostability Experiment.

No.	Genotype	Descriptions
1	Anza	Grown in Sudan, USA
2	Bacanora	High-yielding Mexican variety
3	CIANO79	High-yielding Mexican variety
4	Debeira	Grown in Sudan
5	Fang 60	Grown in Thailand
6	Genaro 81	Widely grown check
7	Glennson	Heat-tolerant check
8	IP4	Old variety from Myanmar
9	Kanchan	New variety in Bangladesh
10	Nacozari	Heat-sensitive check
11	Nesser	Heat-tolerant
12	Pavon	Heat-sensitive check
13	Seri 82	Broadly adapted variety
14	Siete Cerros	Standard from earlier studies
15	Sonora 64	Widely grown early variety
16	Trigo 3	Developed in the Philippines
17	Kauz	Heat-tolerant in Tlaltizapan
18	CNO*2/HE1	Heat-tolerant in Tlaltizapan
19	THB/CEP 77	Heat-tolerant in Tlaltizapan
20	Tepoca	Heat-tolerant in Tlaltizapan
21	Chuanma 18/Baus	*Helminthosporium* resistant
22	Sonalika	Resistant to bird damage
23	Peas	*Helminthosporium* resistant

Canopy temperature measurements--CTs were measured using a hand-held infrared thermometer (IRT) (Model AG-42, Telatemp Crop, Fullerto, CA) having a field view of 2.5°. Measurements started when the IRT could view 100% of the canopy cover when held at an appropriate angle and distance from the plot, thus avoiding the effect of soil temperature. Four measurements were taken per plot with the IRT held 1 m away from the edge of the plot and approximately 50 cm above the plants. Two measurements were made at the east end of the plot and two at the west end. Measurements were made 1 day before irrigation when soil water potential (SWP) measured tensiometrically was approximately 60-70 mb at 30 cm; and after irrigation when most of the soil profile was still at field capacity; at six times during the day (8:00,10:00, 12:00,14:00,16:00, and 18:00 h), to observe the behavior of CTd in response to irrigation status and to the diurnal microclimatic variations. Dry bulb and wet bulb temperatures were measured with an aspirated psychrometer.

VPD was calculated by the subtraction of actual vapor pressure of the air from the saturated vapor pressure for that occasional temperature. Both can be looked up in tables that show vapor pressure (VP) for any given values of wet and dry bulb temperatures (i.e., the relation between VP and relative humidity (RH%) at any given dry bulb temperature when measured with an aspirated psychrometer).

Readings were divided into three groups according to three categories of development: preheading (group 1), heading/anthesis (group 2), and grain-filling (group 3). In order to ensure that a sufficient number of readings were available for the same number of genotypes in any given developmental category, it was necessary to eliminate from the analysis the two earliest genotypes from group 1, the two latest from group 3, and all four genotypes from group 2. CTd values at each level of all treatment factors represent the mean of two to four separate sets of readings.

Stomatal conductance--Stomatal conductance was measured using a Delta-T devices AP4 cyclic porometer (Delta-T Devices, Burwell, Cambridge, UK). The instrument works by measuring the time it takes for a leaf to release sufficient water vapor to change the relative humidity in a small chamber by a fixed amount. This is compared with a calibration plate of known resistances in order to calculate stomatal conductance of the leaf. Measurements were made mainly during the early afternoon on two plants per plot. Conductance through the adaxial and abaxial sides of the flag leaf were measured in the central part of the flag leaf. Measurements were also made at different times of the day (10:00, 16:00, and 18:00 h) on one replication to give a general idea of how the stomatal diffusion varies during the day.

Stomatal density--Stomatal density was measured in 10 genotypes contrasting in CTd; using the nail polish procedure (LeCain et al. 1989, Parkhust 1982). Stomata were counted in both the adaxial and the abaxial sides of the flag leaf in the same part of the leaf as used for the measurements of the stomatal diffusion.

Statistics--The field plot design for genotypes was a randomized complete block design (RCBD) with three replications for each genotypes. Temperature data were analyzed as the differential of leaf temperature from that of the air (CTd) using the statistical program Mstatc. CTd was analyzed using a split-split RCBD with three factors (Irrigation as main plot, time of day as split plot, and genotype as split-split plot). This was done for each of the three designated stages of development in the two cycles. Covariance analysis was done to see the effect of phenology on CTd, using days to anthesis and maturity as covariates. The RCBD design was used to analyze stomatal diffusion and density. Measurements were related to dry grain yield at maturity of the crop.

Results

Physical environment--The range of environmental conditions under which the experiments were conducted are summarized in **Table 7** in addition to yield and phenological data for the two sowing dates.

Canopy temperature--The study shows that genotype, irrigation status and time of day were significantly affecting CTd at the three different developmental stages (preheading, heading/anthesis, and post anthesis) (**Tables 8** and **9**). CTd averages across the three developmental stages, for times 12, 14, and 16 h, in both cycles; ranged between 8.1°C for the two coolest genotypes and 5.7°C for the hottest ones. Mean CTd for all genotypes averaged across all other factors was 6.4°C under wet conditions and 5.8°C for dry ones (p 0.001). Mean CTd for different times of the day; averaged across all other factors; were 3.4, 4.7, 6.2, 7.0, 7.8, and 7.0°C for 8, 10, 12. 14, 16, and 18 hours, respectively. Interactions between genotype*irrigation, genotype*time of the day, and

Table 7. Environmental and growth parameters for 23 genotypes of bread wheat grown at two sowing dates on which canopy temperatures were measured (Tlaltizapan, Mexico 1992-93).

Sowing date	November	February
Avg. temp. (°C)	21.9	24.1
Avg. max. temp. (°C)	32.7	35.2
Avg. min. temp. (°C)	11.1	13.0
Evap. (mm)	4.6	7.0
Avg. CT (°C)	22.6	28.8
d/anth.	63	50
d/mat.	107	81
Yield (t/ha)	4.7	3.7

genotype*irrigation*time were significant for canopy temperature depression as shown in Tables and 8 and 9).

Generally, significant correlations were found between yield and CT for readings taken between noon and 16:00 h in all three described developmental categories, under both irrigation conditions for both growing cycles (**Tables 10** and **11**).

Stomatal conductance--It was found that stomatal conductance (SC) was significantly different among genotypes (P=0.05). A higher conductance was observed at the adaxial side of the flag leaf than that of the abaxial one. The average value for the adaxial side was 1.06 cm/s (SE=0.133) and for the abaxial side 0.47 cm/s (SE=0.074).

A significant correlation was found between SC of the abaxial side of the leaf and CTd (r=0.65) when measured for the 23 genotypes during late vegetative to grain-filling stage between noon and 16:00 h. The correlation with the adaxial side of the leaf was weaker and outside statistical significance (r=0.37). Diurnal changes in SC seemed to be paralleled by diurnal changes in CTd (**Figure 2**).

Stomatal density--Stomatal frequency (SF) varied significantly with genotype (P 0.001) with a mean frequency of 4170 stomata/cm^2. The abaxial side had a SF that was approximately 30% lower than that of the adaxial side of the leaf and they were significantly correlated to each other (r=0.699). No correlation was found for average stomatal frequency (SF) with stomatal conductance (r=-0.24), CTd (r=-0.40), nor with yield (r=-0.31).

Discussion

This study confirmed the genetic variability for CTd among wheat genotypes under heat-stressed conditions. CTd ranged from 5.4-8.2°C across both cycles, for the two hottest to the two coolest genotypes, when measured under well watered conditions, in mid-afternoon between heading and anthesis. CTd correlated significantly with yield under most treatment levels of the factors: stage of development, irrigation, and sowing date, to varying degrees. With respect to time of the day, CTd was correlated better with yield between noon and 16:00 h than it was in the morning or late afternoon.

Stage of development--Stage of development was studied as a factor to see if canopy structure and source and sink relationship would affect relative ranking of genotypes for

Table 8. Analysis of variance, means of different treatment factors for CTD in November's sowing date for the three developmental stages (pre-heading, heading/anthesis, and post-anthesis). Tlaltizapan, Mexico 1992-93.

Treatment	Degree of freedom	Mean square	F-value	Prob.	Mean	Stand. Error
Pre-heading						
Genotype	20	6.572	5.118	***	6.176	0.189
Irrigation	1	11.342	9.651	*(0.003)	6.177	0.056
Hour	5	757.177	928.476	***	6.176	0.081
G*I[a]	20	1.783	1.517	NS	6.164	0.256
G*H[b]	100	1.157	1.418	*(0.01)	6.185	0.369
G*I*H[c]	100	0.556	0.682	NS	6.178	0.521
Heading/anthesis						
Genotype	18	25.49	10.012	***	6.533	0.266
Irrigation	1	51.015	67.326	***	6.533	0.047
Hour	5	1112.382	1260.272	***	6.532	0.088
G*I	18	2.336	3.083	**(0.002)	6.534	0.205
G*H	90	1.891	2.142	***	6.530	0.384
G*I*H	90	0.598	0.678	NS	6.535	0.542
Post-anthesis						
Genotype	20	9.587	39.458	***	4.585	0.082
Irrigation	1	31.656	136.194	***	4.585	0.025
Hour	5	341.764	1461.308	***	4.584	0.043
G*I	20	1.153	4.962	***	4.585	0.114
G*H	100	1.413	6.040	***	4.585	0.197
G*I*H	100	1.35	5.771	***	4.586	0.279

[a] Interaction of genotype by irrigation condition.

[b] Interaction of genotype by hour (time of the day).

[c] Interaction of genotype by irrigation by hour.

Table 9. Analysis of variance, means of different treatment factors, and standard errors for CTd in February sowing date for the three developmental stages (pre-heading, heading anthesis and post-anthesis). Tlaltizapan, Mexico, 1992-93.

Treatment	Degree of freedom	Mean square	F-value	Prob.	Mean	Stand. Error
Pre-heading						
Genotype	20	14.414	7.220	***	6.5	0.236
Irrigation	1	606.972	259.327	***	6.5	0.079
Hour	5	213.678	231.7246	***	6.5	0.086
G*I[a]	20	1.661	0.710	NS	6.5	0.361
G*H[b]	100	1.379	1.495	*(0.004)	6.5	0.392
G*I*H[c]	100	0.670	0.727	NS	6.5	0.554
Heading/anthesis						
Genotype	18	14.157	4.900	***	6.61	0.283
Irrigation	1	25.783	8.653	*(0.006)	6.61	0.093
Hour	5	252.534	183.745	***	6.61	0.110
G*I	18	5.51	1.849	*(0.05)	6.61	0.407
G*H	90	2.624	1.909	***	6.61	0.479
G*I*H	90	5.166	3.759	***	6.61	0.677
Post-anthesis						
Genotype	20	26.646	16.729	***	6.04	0.210
Irrigation	1	28.467	28.342	***	6.04	0.052
Hour	5	167.591	237.325	***	6.04	0.075
G*I	20	3.020	3.007	**(0.001)	6.04	0.236
G*H	100	2.974	4.211	***	6.04	0.343
G*I*H	100	6.015	8.518	***	6.04	0.485

[a] Interaction of genotype by irrigation.

[b] Interaction of genotype by hour (time of the day).

[c] Interaction of genotype by irrigation by hour.

Table 10. Correlation coefficients of yield with CTd for 23 genotypes measured at different time of the day (8, 10, 12, 14, 16, and 18 h), under different irrigation conditions for the three developmental stages (pre-heading, heading/anthesis and post-anthesis), averaged across two-four locations. November sowing date, Tlaltizapan, Mexico, 1992-93.

		8:00	10:00	12:00	14:00	16:00	18:00	Mean of (12,14,16h)
Pre-heading	wet	0.114	0.732	0.646	0.630	0.749	0.632	0.675
	dry	0.078	-0.107	0.347	0.570	0.501	0.561	0.473
Heading/ anthesis	wet	0.347	0.740	0.740	0.73	0.730	0.653	0.730
	dry	0.279	0.680	0.640	0.64	0.710	0.669	0.663
Post-anthesis	wet	0.097	0.469	0.547	0.480	0.471	0.506	0.500
	dry	0.099	0.123	0.495	0.539	0.189	0.285	0.408
Mean		0.170	0.440	0.570	0.600	0.56	0.55	

Table 11. Correlation coefficients of yield with CTd for 23 genotypes measured at different times of the day (8, 12, 14, 16, and 18 h), under different irrigation conditions of the three developmental stages (pre-heading, heading/anthesis and post-anthesis) averaged across two-four locations. February sowing date. Tlaltizapan, Mexico, 1992-93.

		8:00	10:00	12:00	14:00	16:00	18:00	Mean of (12,14,16 h)
Pre-heading	wet	0.096	0.676	0.806	0.707	0.591	0.700	0.701
	dry	0.560	0.608	0.729	0.632	0.720	0.649	0.694
Heading/	wet	0.182	0.476	0.571	0.647	0.429	-0.300	0.549
	dry	0.298	0.450	0.782	0.372	0.783	0.298	0.646
Post-anthesis	wet	-0.001	0.813	0.574	0.558	0.429	0.102	0.520
	dry	0.465	0.737	0.812	0.767	0.783	0.632	0.787
Mean		0.150	0.630	0.710	0.610	0.620	0.35	

CTd. During the preheading stage of development (vegetative), the IRT was viewing only leaves in stage two (heading/anthesis); the IRT would view leaves as well as spikes but grain-filling had not yet started (low sink). The post-anthesis stage represents the grain-filling phase when spikes were the major sink, which might be driving conductivity (R.A. Fischer, pers. comm.). Average CTd for the three developmental stages in both growing cycles are presented in **Table 12**. It seems that CTd decreased with later developmental stages, suggesting that the source-sink relationship was not a major factor effecting CTd. This change in CTd in the later developmental stage was not related to a lower VPD despite the general relationship between VPD and CTd, which is discussed later. Correlations of CTd with yield were significant at all stages of development (Tables 10 and 11).

Since we eliminated very early and very late genotypes from the analysis of variance to ensure a sufficient number of complete sets of readings within each developmental category, we ran a covariance analysis to include all genotypes even though the extremes may not have been in the appropriate developmental category. For groups 1 and 2 (preheading and heading/anthesis), the covariate was days to anthesis; for group 3, it was days to maturity (grain-filling). Results showed no significant effect of the covariates, which further support the robustness of CTd across developmental stages. Also, when corrected means were included in correlations between yield and CTd, values were not significantly altered.

Time of day--VPD was assumed to be an important variable that would effect evapotranspiration (ET). For this reason, effect of time of the day on CTd was one of the major factors investigated in this study for a better understanding of the effect of diurnal microclimatic variation on CTd. We found that CT varied during the day in relation to VPD (**Figure 3**). The dependence of CTd on VPD has been described empirically by Idso et al. (1981). **Figure 4** presents a non-water stress baseline for data collected during the study. It has a different intercept from that of Idso's. This may simply be because we did not have enough readings at lower VPDs, although the same type of negative intercept has been reported for spring wheat under high-yielding, temperate conditions (K.D. Sayre, pers. comm.).

Irrigation--Water availability is considered to be one of the major factors that may limit evapotranspiration (ET), and hence evaporative cooling. For this reason, irrigation status was studied among the other factors that effect CTd. Results showed a significant effect of irrigation status on CTd (p 0.001). Means of CTd for the two different irrigation conditions, averaged over all other factors, ranged between 5.8^{o}C for dry conditions and 6.4^{o}C for well irrigated ones. Correlations of CTd with yield were not affected by irrigation conditions (Tables 10 and 11).

Interactions among factors--One important question raised by this study is how consistent was CTd data among genotypes at different levels of each treatment factor. For example, would the relative estimates of CTd among genotypes be consistent at different irrigation levels, different times of the day, or even with different sowing dates? One way of addressing this question was to compare the correlation of CTd with yield in all of these treatments (Table 10 and 11). It is clear that, in general, CTd significantly correlated with yield between noon and 16:00 h in all three defined developmental categories, under both irrigation conditions, and both cycles. Alternatively, we can consider the interactions between treatments for CTd. Despite the fact that most interactions were statistically significant (Tables 8 and 9), the correlation of CTd among various levels of each factor revealed a high degree of consistency between sets of readings. For example, when comparing CTd at different hours (12, 14, and 16), the average correlation among sets of readings was 64% in cycle I and 58% in cycle II,

Table 12. Mean CTd for three developmental stages (pre-heading, heading/anthesis and post-anthesis) for both growing cycles averaged across all times of the day (8:00-18:00 h) and irrigation conditions. Tlaltizapan, Mexico, 1992-93.

Stage	Pre-heading	Heading/anthesis	Post-anthesis
Cycle I	7.5	7.1	5.7
Cycle II	7.1	7.7	6.8

Table 13. Correlation coefficients for relative CTd compared among times of the day (12, 14, and 16 h) averaged across irrigation conditions for the three developmental stages (pre-heading, heading/anthesis, and post-anthesis) with 23 genotypes. November's sowing in Tlaltizapan, Mexico 1992-93.

		Pre-heading			Heading/anthesis			Post-anthesis	
		12:00	14:00	16:00	12:00	14:00	16:00	12:00	14:00
Pre-heading	14:00	0.86							
	16:00	0.83	0.79						
Heading/ anthesis	12:00	0.73	0.77	0.83					
	14:00	0.63	0.70	0.82	0.97				
	16:00	0.74	0.74	0.91	0.92	0.94			
Post-anthesis	12:00	0.55	0.64	0.69	0.86	0.88	0.76		
	14:00	0.56	0.67	0.75	0.91	0.95	0.87	0.88	
	16:00	0.28	0.35	0.49	0.66	0.70	0.68	0.49	0.49

Table 14. Correlation coefficients for relative CTd compared among developmental stages (pre-heading, heading/anthesis, and post-anthesis) of each growing cycle (November and February sowing dates); and among the two cycles, averaged across irrigation conditions (wet and dry) and time of the day (12, 14, and 16 h) with 23 genotypes. Tlaltizapan, Mexico, 1992-93.

		Growing cycle I[a]		Growing cycle II[b]		
		Pre-heading	Heading/ anthesis	Post-anthesis	Pre-heading	Heading/ anthesis
Growing cycle I	Heading/ anthesis	0.85				
	Post-anthesis	0.66	0.92			
Growing cycle II	Pre-heading	0.69	0.89	0.83		
	Heading/ anthesis	0.77	0.87	0.78	0.89	
	Post-anthesis	0.70	0.71	0.88	0.81	0.85

[a] Growing cycle sown in November 1992.
[b] Growing cycle sown in February 1992.

averaged over both levels of irrigation and all three stages of development (**Table 13**). When comparing CTd of different developmental groups the average correlation among stages was 71% averaged across three different times of the day (12, 14, and 16 h), both irrigation conditions, and cycles (**Table 14**). Similarly, CTd measurements under different irrigation regimes averaged across all other factors were well correlated (67%). It is also of interest whether this consistency would hold out under different heat stress regimes as induced by, for example, late sowing. This was addressed by comparing CTd across the two cycles. The comparison was favorable (Table 14), especially when comparing growth stage 2 for both cycles when the correlation was 76% averaged across all other factors.

Using the information discussed, we are in a position to specify a range across which the breeders can take measurements. Based on correlation with yield (Tables 10 and 11) and data consistence (Tables 13 and 14), our data suggest that during the heading/anthesis stage, between solar noon and 16:00 h would be the best for measuring CT and that irrigation status, if well watered, is not a confounding factor. Our findings would seem to support the assumptions of McKinney et al. (1989) with soybean when he measured CT during the late vegetative and early reproductive stages, within 2 hours after solar noon under minimal water stress. The higher correlation of CTd with yield during the afternoon is supported by the trend for better correlation of CTd with yield at higher VPDs, which tend to occur late in the day (**Figure 5**).

Physiological basis--Another objective of this study was to understand the physiological basis that underlays CTd variation. One of the factors that may have affected CTd is SC. Results showed significant genetic variability of SC and a reasonable correlation between abaxial conductance and CTd (r=0.65). This and the relation between CTd and SC during the day (Figure 2) suggest that SC has a role in CTd variability and may be a mechanism determining the degree of evaporative cooling.

The apparent contradiction we saw when the SC of abaxial side of the leaf correlates with CTd better than that of the adaxial, despite the fact that it has the lower SC, is probably explained by the observation that the wheat flag leaf is twisted with abaxial side being the surface where we measured temperature.

Another factor that may affect variability is the number of stomata per unit leaf area (i.e., stomatal frequency or SF). It was found to be genotypically variable, which agrees with other studies like that of Miller (1938), who stated that the number of stomata per unit leaf surface in barley is, to some extent, a characteristic of a particular plant species or variety. Wood (1934) showed that variations in SF were connected more closely with generic and family characters than with environmental conditions. Teare et al. (1971), who showed wheat cultivars were variable in SF, also reported that the stomatal frequency was greater on the adaxial than on the abaxial surface, which agrees with the findings of this study.

SF of the abaxial side of the leaf was found to correlate negatively with conductance (r=-0.52), while no relation was reported between the adaxial side SF and conductance. Though hard to explain, this would seem to be consistent with the findings of Heichel (1971) when he reported that a maize cultivar with lower SF had a faster net photosynthesis than a cultivar with greater SF. Miskin (1970) reported that SF did not influence rate of photosynthesis in barley, but did influence transpiration and stomatal diffusion resistance. In this study, we have no measure of stomatal size or potential for diffusion and since the maximum rate of conductance is dependent on stomatal size as well as frequency, this may be the reason that no relationship was found between grain yield and SF. This agrees with the work of Teare et al. (1971) who found no relationship between grain yield and the number of stomata/cm^2 of leaf area, in addition to no relationship between grain yield and the product of stomatal number*stomatal length. All this supports the conclusion that SF alone is not influencing conductivity.

Conclusions

The first study supports the usefulness of membrane thermostability as a heat tolerance trait, which may be used to enhance yield selection of spring wheat for hot environments. Furthermore, the findings suggest that using heat-acclimated seedlings to estimate MT is a viable alternative to measuring MT of heat-stressed, field-grown plants.

Results of the second study allow us to advise breeders that canopy temperature depression, measured early in the reproductive phase within 2 to 3 hours after solar noon, would be effective for selecting for heat tolerance under different irrigated, heat-stressed conditions.

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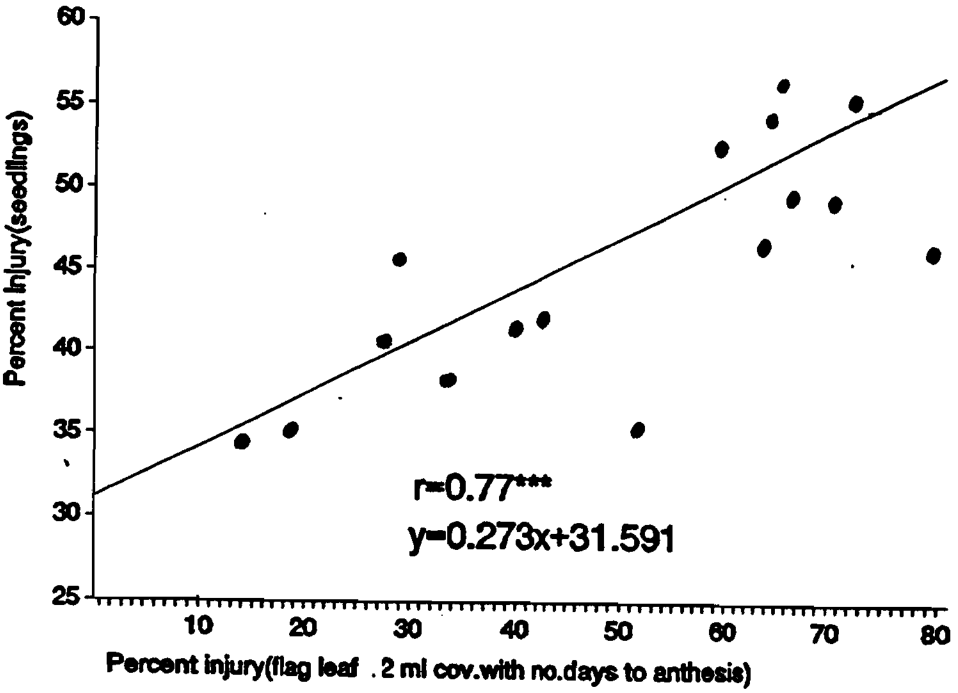

Figure 1. Percent injury by heat for 16 genotypes in control-grown and acclimated seedlings compared with field-grown and acclimated mature plants.

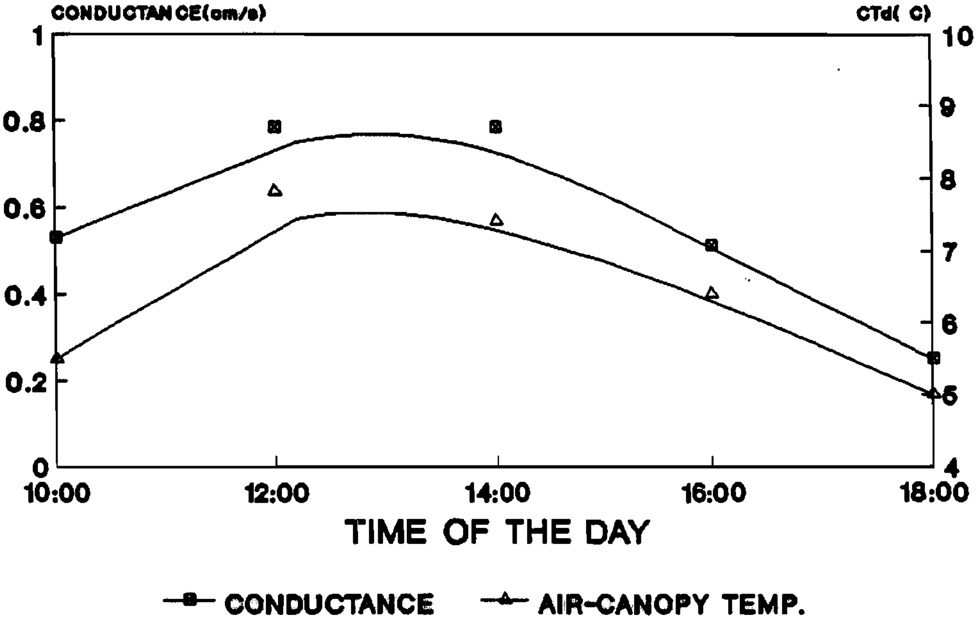

Figure 2. Diurnal variation of stomatal conductance in relation to CTd measured on the same days, averaged across 23 genotypes. Tlaltizapan, Mexico, 1992-93.

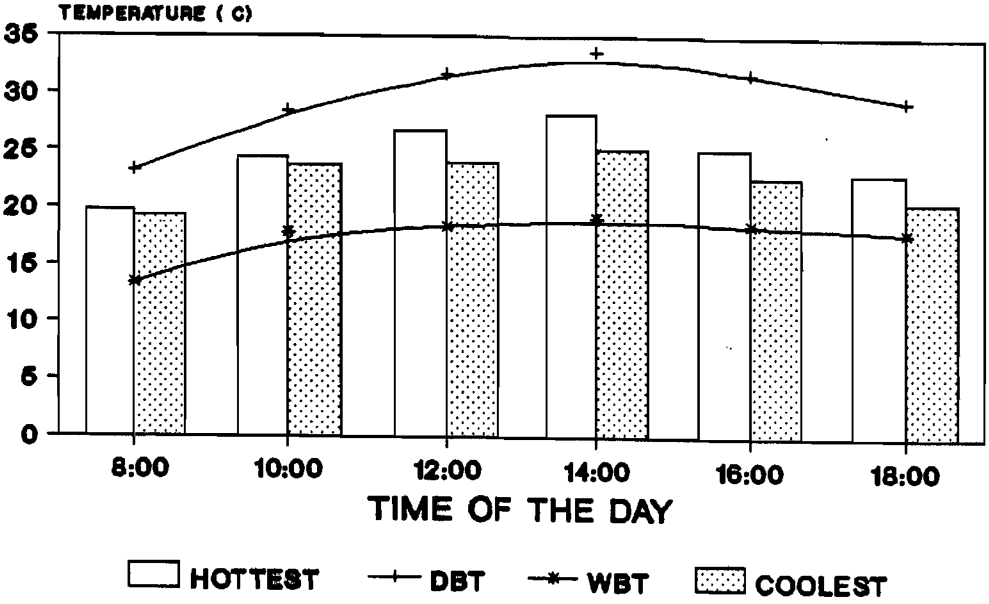

Figure 3. Diurnal variation of CT of two of the coolest genotypes and two of the hottest ones in relation to dry and wet bulb temperatures averaged for stage two (heading/anthesis) across the two growing cycles. Tlaltizapan, Mexico, 1992-93.

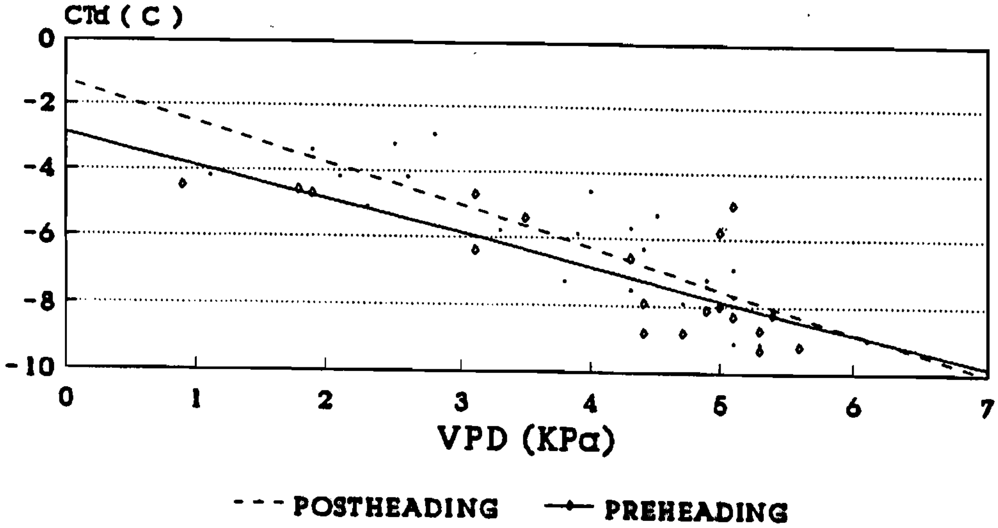

Figure 4. A nonwater stress baseline for CTd averaged across 23 spring wheat genotypes vs the corresponding VPD for each occasion. It presents date measured pre-heading and post-heading with different lines. Tlaltizapan, Mexico 1992-93.

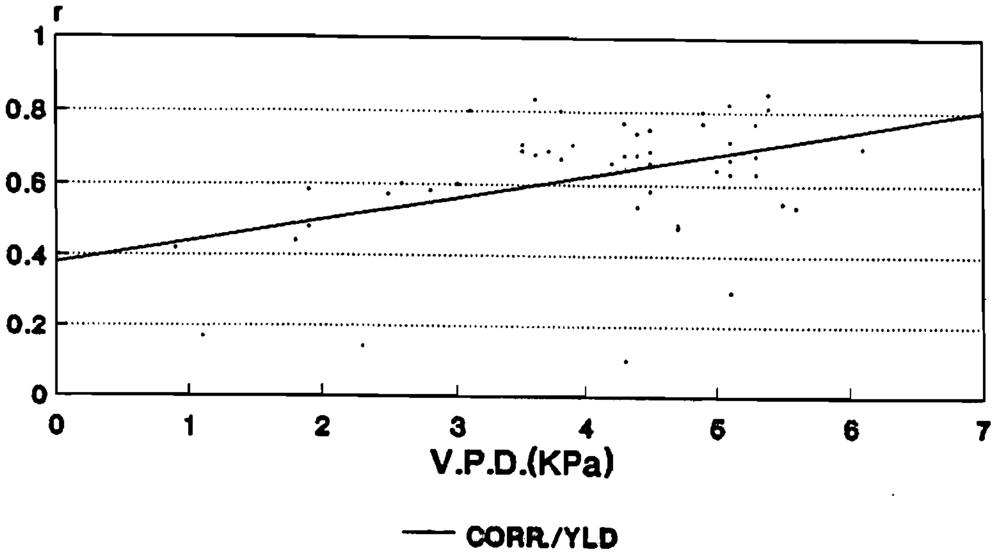

Figure 5. Effect of VPD on yield correlation with canopy temperature differential (r) presented for different occasions measured in Tlaltizapan, Mexico, 1992-93.

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