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HEAT AVOIDANCE: A NEW APPROACH FOR BREEDING FOR HEAT RESISTANCE

Final Report
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3. EXECUTIVE SUMMARY

Breeding for resistance to an environmental stress in general and to heat or drought in particular has been one of the most difficult problems faced by plant breeders. While progress in heat resistance (and drought) for yield and quality has been made in a few crops, overall progress has been slow. The primary cause has been the inability to identify efficient, reliable and rapid selection criteria for heat resistance. Until such criteria can be identified, breeding for heat resistance will be extremely difficult and effectively a serendipitous exercise.

In this program we examined an alternative approach, heat avoidance, for improved heat resistance. As models we used two crops important to Kazakhstan and Israel, tomatoes and wheat. The traits examined include those which allow plants to avoid heat stress by: (a) reducing the amount of energy absorbed via solar radiation; and/or (b) increasing cooling efficiency. The traits include stomatal conductance, leaf area, leaf curling, leaf rolling, leaf rotation, plant architecture and fruit quality. (Leaf curling occurs in dicotyledons and is the movement of both leaf sides upwards and then inward forming a heart shape leaf; leaf rolling occurs in monocotyledons and is the movement of one leaf side to produce a tight cylinder shaped leaf; leaf rotation is the twisting of the leaf petiole so that the abaxial side faces the sun).

In Kazakhstan they examined heat and drought avoidance traits in selected monosomic wheat lines. They identified leaf rolling as a wheat leaf trait that is associated with heat and drought resistant cultivars. They produced and tested a series of monosomic lines for leaf rolling for genetic relationships and for testing for heat and drought resistance. They concluded that leaf rolling may potentially be a useful selection criteria for these stresses.

The Israelis focused their program on heat avoidance in tomatoes. After screening an array of cultivars for the presence or absence of potentially useful heat avoidance traits, they identified two cultivars, 'd' and 'g', with the former having poor heat avoidance traits (low stomatal conductance, large leaves, low leaf curling and rotation) and the latter being good (high stomatal conductance, small leaves, high leaf curling and rotation). After crossing, they produced F2s which were found to have a high correlation between heat avoidance traits and leaf temperature; high stomatal conductance, low leaf area and high leaf curling and rotation were correlated with low leaf temperature. They produced a series of F6 generation isogenic line to examine each trait separately; this will be done in the future.

The results of both the Kazakh and Israeli research programs have confirmed the potential of heat avoidance as an alternative to the traditional heat tolerance approach for breeding heat resistance. For international development this approach is extremely important. The traditional approach, heat tolerance, often requires a large scale, expensive project to screen, identify and utilize a physiological or biochemical trait. Usually the utilized trait is initially very promising but during the progress of the breeding program the trait fails to meet this promise. Most heat avoidance traits are morphological which makes them easy and inexpensive to identify and use. This is very important for developing countries were money and quality technical help for research is limited. A researcher can utilize our method in a simple and inexpensive program with at least, if not better, chance of improving heat resistance than traditional heat tolerance programs.

4. RESEARCH OBJECTIVES

Heat stress caused by high temperatures is one of the more costly serious environmental stresses in much of the developing world. Heat stress causes reduced yields, poor quality and increased pest and diseases. The need to breed for improved heat resistance varieties of our important crops is self evident. The traditional approach for improving heat resistance has been to work toward heat tolerance; i.e. to identify and utilize traits (usually biochemical or physiological) that allow a plant to tolerate high temperatures before suffering from heat stress. These programs are usually quite costly to conduct and require sophisticated equipment and highly trained manpower. In developed countries these are not serious problems.

Unfortunately, in the developing world, money, equipment and well trained technicians and students are limiting. What is needed is a methodology for improving heat resistance that is reliable, relatively parsimonious and easy to train people to use. Heat avoidance is one possible solution. In heat avoidance rather than identify traits that allow a plant to survive high temperatures, we use traits that either prevent or slow down a plant from reaching high leaf temperatures and thus reduced the time that a plant is stressed. If avoidance occurs then a plant would be in a more optimum temperature range for a longer period during the day. Heat avoidance traits can be placed into two groups:

(a) active cooling - active cooling requires an increase in stomatal conductance and thus sufficient water for this to occur;

(b) passive cooling - these are systems that reduce or retard solar absorption and thus reduce leaf temperature. These include reduced leaf area, increased leaf curling in dicots and leaf rolling in monocots and a more compact, shade producing plant.

Specifically, in this project we had two research objectives:

(a) to determine the potential of an array of physiological and morphological heat avoidance traits as rapid, reliable, inexpensive and independent selection criteria for future breeding programs for heat resistance;

(b) to begin to determine the genetic control of the more promising heat avoidance traits.

5. RESEARCH METHODS AND RESULTS

1. GENERAL INTRODUCTION

The continuous improvement of yield and product quality in most crops to meet the increasing demand of the world's population is an important, if not central, goal for agricultural researchers. An important limiting factor in maximizing yield and quality is the relatively poor resistance of present cultivars to heat stress. Plants grown under high stress inducing temperatures usually have significantly reduced yields and/or quality then when grown under more optimum temperatures. Thus while breeding programs have lead to dramatic increases in yield/quality in almost all crops when grown under environmentally non-stressed conditions, they have usually failed to improve heat resistance. This is primarily due to two reasons:

1. the absence of rapid and reliable selection criteria - the large number of studies that have been conducted to improve heat resistance, whose two components are heat tolerance (the ability of metabolism and structures to function normally or nearly so under high temperatures) and heat avoidance (the ability of a plant to prevent the buildup of high plant temperatures when grown at high temperatures). To date, most studies examined traits associated with heat tolerance and very few with heat avoidance (Lu et al., 1998; Blum, 1988; Shapelier, 1986). While these studies have added to our knowledge of the effects of higher temperatures on plant physiology and biochemistry, they have not provided breeders with any reliable selection criterion;
2. little information on the genetic control of heat resistance traits - while some information is known for a few trait, it is still not sufficient for a breeder to use *a priori* for a specific character (Zeng and Khan, 1984; Lu and Zeiger, 1994; Schaff, 1985; Wessel-Beaver and Scott, 1992; Abdul-Baki and Stommal, 1995).

Heat avoidance as an alternative approach to heat tolerance for improved heat resistance has recently been proposed (Lu and Zeiger, 1994; Radin et al., 1994; Lu et al., 1998). Two characters, increased stomatal conductance and reduced leaf size, were suggested. The Israeli team examined these characters plus other leaf and plant characters in a historic collection of five elite cultivars of tomatoes, one from each decade from 1940-1980, which were bred for improved quality, yield and disease resistance under the high temperatures of central California. They identified two cultivars, one with an array of heat avoidance traits and the other without. They were crossed and a series of lines were bred into order to produce isogenic lines that different in a heat avoidance traits. In 199 they examined 22 F4 generation lines. The Kazakh team examined a series of monosomic lines in wheat with the same aim as the tomato project. Among the traits that they examined was leaf rolling.

2. *KAZAKHSTAN*

A. Tomatoes

Five lines of tomatoes, provided by the Israeli partners, were tested in an experimental plot 70 km west from Almaty. The area is semi-arid with light soil. Plants were grown under furrow irrigation with supplementary organic fertilizer applied before seeding. Tomato seeds were sown in April and transplanted into he field as seedlings in May. Anthesis was completed by the end of June.

Line number	Time of anthesis
1940-c	26.06.1999
1950-d	23.06.1999
1960-e	23.05.1999
1970-f	19.06.1999
1980-g	17.06.1999

Line 1940-c was found to have four distinct plant phenotypes. They were:
a. a small round-shaped fruit, which was the predominant type;
b. small round fruits (max. 15 mm in cross section);
c. bigger round grape-like (compound) fruits (20-25 mm in cross section);
d. average, plane fruits.

Line 1950 represents intermediate-ripening miscellaneous plants with extended, ellipse, or plum-like fruits with average to small fruits suitable for storage. Lines 1970 and 1980 had higher yield under our conditions with yields reaching 8.0-8.6.kg per each plant. Despite early frosts, fruit formation continued until October-November. Line 1950 exhibited little leaf curling abilities.

Line 1960 represents early-ripening plants with hard fruits that are of the average size and oval shape with a characteristic sharp tip. The fruits may be stored for months. Yield is not high with 3.5-3.8 kg per plant.

Line 1970 was also found to be early-ripening. Fruits were oval and suitable for storage. Yield was 5.5-5.9 kg per plant. Leaf curling was minor.

Line 1980 represented an intermediate-ripening plant which had hard, ellipse-like fruits narrowing to the fruit stalk. Fruits are suitable for storage and mechanical harvesting. Productivity was about 4.0 kg per plant. It had high leaf curling.

Our main conclusion is that the tomatoes tested may be used as a model for the study on leaf curling or other heat avoidance markers, but one should also count tomato line variability observed in our experiments, along with seasonal peculiarities influencing on the phenotype expression each year.

B. Wheat

The second part of our investigation was examining 21 monosomic lines of wheat (*Triticum aestivum* L.) variety Kazakshtanskaya 126, a heat resistant variety, for genetic analysis. Physiological parameters were compared to wheat varieties Dneprovskaya 521 and Grecum 476 which were used as controls. Under high temperatures it has been demonstrated that leaf rolling ability is found in hybrids of Grecum 476 with Kazakhstanskaya 126 as a monosomic line series. The genetic dominance of this trait in F1 plants was shown in field trials. Comparison of F2 disomic (control) with monosomic plants has revealed splitting between leaf rolling (RL) and normal plants in the ratio of 15:1, indicating two genes controlling leaf rolling; i.e. the ratio between RL and normal F2 plants (15:1) points to the existence of two dominant, RL -coding genes. They were designated as RL1 and RL2, respectively. Relevant genetic combinations are shown in Table 1.

Physiological parameters were estimated at stooling (emergence of the second upper leaf) and flag leaf development stages. Such parameters as ABA concentration in leaf and overall water supply were examined. Genetic analysis and harvest dynamics were also examined after ripening.

Cvs. Kazakhstanskaya 126 and Dneprovskaya 521 had normal leaf characteristic of bread wheats. Beginning at heading, the leaves were found to be orientated toward sun light. Increasing insulation caused the leaf edge to be move down. Significant decreases in leaf humidity may lead to leaf rolling, unless two cylinders along the central lamina vein are not 'built up'. Such a reaction to water stress is typical of almost all cultivated varieties. At anthesis, especially in dry seasons, the basal part of the sheath may be burned by the sun which produces early senescence and consequently 'switch off ' of the leaves from seed formation.

F2 plants obtained from the 6A and 4D lines revealed a normal leaf ultrastructure. At the same time, the x2 value for these hybrids was determined as reaching a maximum.

From these results it appears that RL- controlling genes of cv. Grecum 476 are located in chromosomes 6A and 4D. Significant CHI-^{2} values for 3A, 4A, 6B, 5D, 6D and 7D monosomics were found which might reflect the presence of genes impeding leaf rolling, when located in the RL-gene containing chromosome.

At the beginning of vegetation all genotypes in this study were found to have normal leaf phenotypes. Diverse leaf shapes, areas and water supply parameters were found at stooling and continued until the end of vegetation.

Comparison of the transpiration intensities (Table 2) among tested varieties has indicated differences, exemplified by Grecum 476 plants, during the day. At stooling stage in May plant leaf laminae were rolled between 11.00 a.m. to 2.00 p.m. in order to reduce water transpiration; i.e. to save water. After 2:00 p.m. water transpiration increased.

In May the average intensity of transpiration at stooling is higher for Grecum 476 than for Dneprovskaya 521. In June, at heading, leaves of Grecum-476 rolled between 10.00 a.m. until 6.00 p.m. Besides rolling, the leaves also change their orientation. At this time the transpiration intensity for Grecum 476 was quite small. Seed maturation, which occurs by the end of June, is usually the hottest period in the region. While unfolding in the afternoon, the laminae never completely open. Drastic temperature increase determines exceptional leaf rolling which may produce leaves that are almost needle-like. The rates correlate to the transpiration intensities depicted in Table 2, where transpiration rates for Dneprovskaya 521 at heading or seed maturation are higher than those for Grecum 476.

Leaf ABA (abscisic acid) content throughout vegetation has demonstrated that Grecum 476 accumulates higher ABA concentrations comparing to Dneprovskaya 521 (Table 3). ABA involvement in the hormonal regulation of water supply is of special significance under water deficit as ABA simultaneously controls reduced transpiration and a dry bulk deposits. In addition to the regulation of water supply by intracellular agents, the RL attribute, as lamina re-orientation in response to sun radiation, may play an essential part in optimizing optic conditions over the plant plot. Lower leaf densities among RL plants may enable the leaf to act longer than normal. This gain might weaken the competition of individual plants in the phytocenoses, thus leading to better yields.

Grecum 476 is distinguished by large seed. Under irrigation the 1000 grains weight is 62 g compared to only 53 g under dry land conditions. Related records for Dneprovskaya 521 in similar field trials are 46 and 43 g, respectively. Ultrastructurally contrasting plant genotypes, usually differing for lamina orientation, show that water sufficiency in Grecum 476 plant tissues has varied within ranges that would cause minor effects of living functions in Dneprovskaya 521 plants under described conditions. Being environmentally adaptive, the distinct feature of Grecum 476, the RL trait has been stressed to decrease the water flow in transpiration and thereby exercising strict control over the water equilibrium. Needle-like leaves do facilitate optimal self-cooling during hot hours of the day. Lamina re-orientation provides such a disposition of the leaves that sun rays fall at a sharp angle offering an efficient mechanism to prevent excessive heating by means of leaf avoidance of the sun. Therefore, the RL attribute may serve as a marker of 6A and 4D monosomics. Insertion of RL1 and RL2 genes in genotypes harvested under different climate and ecological conditions may enrich their drought resistance capacities apart from significant reduction of the underground water consumption.

Back-cross breeding is used for the transfer of one or several traits to the recurrent parent to avoid undesirable changes happening to the parental genotype. No more than 3-5 back-cross breeding operations under reliable control of the breeding material is needed for the preservation of marker trait that should remain unchanged throughout phenotypically identical recurrent variety. Under long-term back-crosses the attribute may be lost because of active genes modifications. This is the second reason for field trials of this material together with the study on a strict preference of genes carriers suitable for the subsequent back-cross manipulations. The scheme proposed for the transfer of the P gene of variety B into recurrent variety A has been employed to this study. It is an appropriate design for transferring one or two genes. Initially in studying RL1 and RL2 genes the main task was to ascertain principal monosomic variations by analyzing the plants carrying transferable genes, and remaining however phenotypically identical to the recurrent cultivar. Unexpectedly, growing plant diversity has compelled us to find auxiliary phenotypes displaying the RL attribute that is controlled by RL1 and RL2 genes. Control over the induced genes has been completed by picking out hybrids for seed quality, yield, disease and vermin resistance. Such a complex approach has allowed us to breed new spring wheats.

Table 4 updates harvest composition for tested lines, as compared to the recurrent parent, Omskaya 9. Hybrids analyzed under water deficit surpassed the parent in stem height, ear length, numbers of spikelets and seeds. Noteworthy, for certain hybrids larger seed numbers attributed to the ear were negatively correlated to the 1000 grains weight values.

The average yield of all examined hybrids was higher than the control and substantially exceeded that of the recurrent Omskaya-9. Table 5 summarizes seed quality data for hybrids in comparison to the controls.

By flour capacity (W.e.a.) Alba is similar to Albidum and is the best. Cv. Alba was obtained by triple back-breeding of Omskaya 9 (spring wheat) with Grecum 476 (winter wheat contributing RL1 and RL2 genes). Alba is characterized by downy blades and intensive anthocyan-mediated color of the coleoptiles, stems and anthers. Its developmental mode it is similar to Omskaya 9. Under precipitation deficiency at the end of jointing it continues to steadily tiller without further heading. Water sufficiency during this period may hasten development, and Alba plants may head 2-3 days earlier than those of the parental stock (Omskaya 9). RL1 and RL2 gene activities after their transfer to Alba could be followed due to the ability of the plants to change their lamina shape and allocation in reply to growing air temperature , changing insulation or water deficit.

Cv. Otan derived from cv. Lutescens has been shown to be advantageous by its higher seed protein, an important flour capacity. This variety has originated as BC5- F1 breed combination between Omskaya 9 and Grecum 476. It is known for slightly downy leaves and ascribed to carry one of the RL genes (RL1 or RL2). The new line is proposed for sowing in semi-arid areas.

The study on leaf rolling in tomatoes and wheat in response to heat and drought thus found:

1). leaf rolling is one of the main and ubiquitous marker traits for both heat and drought avoidance in monocotyledonous crops. This trait is worth further analysis of its role in plant viability and productivity under arid conditions.

2). hybrids carrying the trait may be used in breeding programs. Genetic combinations already received on this basis have been shown to successfully withstand drought and the effects of high temperatures and rapid heating. The experiments were conducted in accord to the joint program with Israel.

Table 1. Genetic segregation among F2 plants of monosomic combinations between cvs. Kazakhstanskaya 126 and Grecum 476.

List of mono- and disomic F2 hybrids	Number of Plants		Total Number of Plants	CHI-2 (15:1)
	RL	normal		
Control (Kazakhstanskaya 126 x Grecum 476 (2n=42)	196	14	210	0.06
Mono:				
1A	176	14	190	0.41
2A	188	14	202	0.16
3A	151	3	154	4.86
4A	170	20	190	5.93
5A	192	18	210	1.93
6A	252	2 *	254	12.94
7A	176	16	192	1.42
1B	136	4	140	2.75
2B	190	14	204	0.13
3B	216	13	218	0.38
4B	205	10	215	0.94
5B	136	14	150	2.43
6B	224	26	250	7.35
7B	158	12	170	0.19
1D	173	15	188	0.96
2D	143	16	159	3.95
3D	200	20	220	3.03
4D	280	4 *	284	11.36
5D	164	20	184	6.70
6D	131	15	146	4.04
7D	174	20	194	5.46

* Nullisomic-like phenotypes

Table 2. The rate of transpiration (mg/gxhr) at different life stages

Phase of development and hybrid description	Hours of the day 8	12	15	18	Daily mean
Stooling					
Dneprovskaya 521	319+11.6	1025+22.1	2132+20.0	1057+22.1	4533
Grecum 476	521+23.8	690+23.5	2037+20.9	1538+24.1	4786
Heading					
Dneprovskaya 521	1646+37.8	2850+42.3	1460+26.1	866+29.0	6922
Grecum 476	935+29.6	1870+28.5	985+31.9	548+37.8	4338
Seed maturation					
Dneprovskaya 521	769+26.1	1335+40.1	1546+36.5	657+42.7	4307
Grecum 476	561+24.4	873+36.0	951+49.4	532+41.8	2917

Table 3. ABA content in wheat leaves (in micrograms/gram).

Wheat cultivar	Stage of development: Tillering (Intensive leaf formation)	Stem formation	Ear formation (Flowering)
Dneprovskaya 521	0.15	0.12	0.15
Grecum 476	0.40	0.39	0.30

Table 4. Spring wheat harvest compositions and yields in competitive trials under semi-arid conditions.

Wheat line	Length		Number of		Weight of 1000 grains	Harvest 100 kg/ha
	stem	ear	spikelets	seeds		
Kaz3 stand.	63.1+0.44	9.8+0.12	15.2+0.31	41.0+0.53	40.0	34.6
Oms9 stand.	80.5+1.40	7.7+0.58	14.6+0.15	33.0+0.95	38.5	25.9
Otan	89.0+0.54	10.8+0.15	18.8+0.21	53.9+1.20	36.2	42.5
Alba	90.7+0.72	10.9+0.10	17.9+0.21	42.9+0.46	42.2	41.6
Mirrass	87.8+1.18	11.8+0.12	16.2+0.12	53.2+0.86	34.0	41.2
Albidum-9	81.2+0.97	11.3+0.12	18.3+0.24	53.1+1.28	41.6	39.9
Lutescens-19	94.5+0.92	11.4+0.19	17.7+0.15	50.6+1.26	41.4	39.9
Lutescens-27	88.9+0.56	11.7+0.40	20.1+0.18	63.0+0.69	37.4	40.4
Lutescens-17	83.8+0.78	9.5+0.23	16.1+0.45	46.3+1.64	39.7	39.5
Lutescens-11	92.3+0.78	11.1+0.26	16.5+0.30	45.1+1.27	35.2	42.5

Table 5. Seed quality of the spring wheat hybrids.

Cultivar	Seed protein (%)	Flour rough gluten content (%)	W.e.a.	Bread Volume (ml) 100 g flour	Final bakery evaluation (marks)
Oms9	15.2	38.5	304	1020	3.7
Otan	15.7	39.8	340	1020	4.2
Alba	14.4	33.3	379	1150	4.3
Mirrass	13.6	29.0	300	960	3.9

3. *ISRAEL*

A. Introduction

From the results of the first two years (Mendlinger et al., 1998, 1999) we identified both an array of traits that were associated with heat avoidance a well as two cultivars to use in producing isogenic lines. The traits included:

a. stomatal conductance - we found that stomatal conductance had significant negative correlation with leaf temperature;

b. leaf architecture - plants with smaller leaf area (both individual leaves as well as total leaf area) and less biomass had significant negative correlation with leaf temperature;

c. leaf curling and rotation - cultivars with leaves that curled, i.e. the movement of both leaf edges upwards and then inwards forming a heart shaped leave, and leaves which rotate at the petiole which results in the abaxial side facing upward were found to be negatively correlated with lower leaf temperature.

Two cultivars were identified for producing isogenic lines, 'd' and 'g', the former having poor heat avoidance traits and the latter having very good heat avoidance traits. After crossing the two, last year we examined their F1 and F2 generations. We selected 22 F2 lines for further selfing in order to produce F6 isogenic lines. We plan on producing a series of isogenic lines with each pair from each line being genetically identical except for one heat avoidance trait with one of the pair being good and the other poor for the trait. Comparing the pairs of each line will enable us to understand the importance of the examined trait for heat avoidance and lower leaf temperature.

In the third year, after producing the F3 generation in the winter of 1998/9, we examined the 22 F4 lines for the array of heat avoidance traits listed above in order to select the lines to continue to the F6 generation. The plants were grown in the same net houses as were used in the previous years.

B. Materials and Methods

On May 13, we sowed in seedling trays about 25 F3 seeds from each of the 22 selected lines plus cultivars 'd' and 'g'. On June 8, when most seedlings were at the three leaf stage, they were transplanted into 10 liter buckets, one plant/bucket, filled with an appropriate commercial potting mixture for tomatoes. Three identical nethouses, each covered with a fine-mesh net which prevented insects from entering, were used in this experiment. Each nethouse was divided into 2 blocks with 2 plants/line/block for a total of 12 plants/line. Two minimum/maximum thermometers were placed in each nethouse, one at 25 cm and the other at 150 cm above ground level and the temperatures recorded daily.

Fertigation was via drippers with two 2-liter/hour drippers per bucket. The irrigations began with 1 per day and increased with increasing temperature and plant size to 5 per day with each irrigation giving 2 liters/bucket. The amount of irrigation needed was determined by measuring the outflow of water from 9 randomly chosen buckets. Our target was a daily outflow of 25% which prevents salt buildup while maintaining water saturation of the potting mixture. Fertilizer was given in every irrigation. The plants were periodically examined for diseases and insect infestation and prophylactic fungicide treatment was performed during the growing season. At no tine during the growing season was there any evidence of a lack of any nutrient or a folial disease.

Each plant was examined for an array of traits. These included:

1. stomatal conductance and leaf temperature - stomatal conductance and leaf temperature were measured on the youngest fully developed leaf using a Licor 1600 steady state porometer in each plant at five life stages: beginning of flowering, at flowering, beginning of fruit set, fruit filling and beginning of fruit coloring. Each stage was one week apart;

2. leaf curling - the amount of leaf curling was scored the same day as stomatal conductance and leaf temperature using a scale from 1-5 with 1 being no curling and 5 a complete heart shape leaf;

3. leaf area - leaf area was determined three times during the growing season;

4. number of leaflets - the number of leaflets were counted when leaf area was determined;

5. leaf, petiole and total dry weight - these were determined when we measured leaf area;

6. fruit quality - from each plant we chose 5 fruits at random in the middle of the harvest period and examined then for TSS (total soluble solids), acidity, pH and EC. The fruits re still being analyzed and will not be presented in this report.

C. Results and Discussion

Stomatal conductance was significantly different both among the lines and between the five growth stages (Table 1). For most lines (20 out of 24) their highest stomatal conductance was recorded at the beginning of flowering. This was the opposite of what we found in previous years when almost all lines had their lowest stomatal conductance at the beginning of flowering (Mendlinger et al., 1998, 1999). We could find no explanation for this except that June was the hottest month of this summer, a rare occurrence (data not presented). Nevertheless, significant differences were found among the lines with lines d1, d3, g5 and g21 had high stomatal conductance whereas lines d36, g5, g43, g41 were low. No differences were found between the lines which had maternal cytoplasm 'd' or 'g' respectively.

Leaf temperatures were also found to be significantly different among the lines and among the dates (Table 2). Lines d3, g21, g28, g16 and g23 had the lowest leaf temperature while lines d26, d36, g10 and g5 had the highest. A significant negative correlation was found between stomatal conductance and leaf temperature at each of and pooled over the five dates ($r=-0.843$, $p<0.001$). This is what we previously found and is strong evidence indicating that high stomatal conductance reduces leaf temperature. However, the differences among the lines for leaf temperature, especially the parental lines 'd' and 'g' were less then what we found in previous years. This may have been because this summer was milder than the previous years (average day time temperatures, except for June, were 2-3^{0}C lower than previous years, data not shown).

Leaf curling was found to be significantly different among the lines and increased over the five dates (Table 3). Among the lines d15, d16, g5 and g16 had the highest amount of leaf curling whereas lines d45, g14, g28 and g43 the least. The increase of leaf curling over time or with the age of the plant, was what we had found in previous years (Mendlinger et al., 1998, 1999). However, unlike in previous years, cultivar 'g' did not achieve complete curling and only one line, 'd16', had complete curling. This is further evidence of the mild summer in 1999. Lines 'd3' and 'd1', which had high stomatal conductance, had only moderate leaf curling whereas g5 was high for both traits.

Leaf area was found to be significantly different among lines and dates (Table 4). When the plants were young, leaf area was larger than in the same plants when they became older, larger and with more leaves ($r=-0.96$, $p<0.01$). Thus when the plant has relatively few leaves but requires a large amount of photosynthates, it produces larger leaves; as fruits become larger. The number of leaflets/plants followed leaf area with fewer leaflets/plants produced in the older plants (Table 5).

Leaf dry matter also was reduced with time but to a smaller extent than leaf area (Table 6). Petiole dry weight was not found to be different over time (Table 7).

Total dry matter at the end of the growing season was significantly different among the lines with the difference from the smallest plants, 'g25' and 'g41', having less than half the biomass of the larger plants, 'g1' and 'd36' (Table 8).

For further selection for producing isogenic lines we needed to identify lines that were already fixed for either high or low for an important heat avoidance traits and intermediate in others. Towards this goal the ranking over all traits of all 22 F4 lines are presented in Table 9. We ranked each line for each trait in respect to being good for heat avoidance (ranked 1-5), poor for heat avoidance (ranked 20-24) and intermediate (ranked 10-15). If the ranking was high or low for heat avoidance we assumed that the trait was fixed in the line (as it is in 'd' and 'g') and if it was intermediate that we could still select for high and low. Using the results we selected for producing the F6 generation:

1. d1- high stomatal conductance, low leaf area and biomass but moderate leaf temperature;
2. d3 - high stomatal conductance, low leaf area and biomass, moderate curling and low leaf temperature;
3. d15 - moderate stomatal conductance, high curling;
4. d16 - high curling, low leaf area, moderate leaf temperature, good stomatal conductance;
5. d36 and g10 - most traits were moderate;
6. d37 - all traits are moderate;
7. g5 - a curiosity as it has high stomatal conductance and curling but high leaf temperature;
8. g16 - stomatal conductance is moderate, leaf curling is high and leaf area is low;
9. g41 - stomatal conductance is low, curling is moderate and leaf temperature is high.

Table 4: Leaf area (cm^2) at three growing stages in two tomato lines (heat sensitive and heat tolerant) and 22 lines of their F4 generation.

Line	leaf area beg. of flowering	leaf area beg. of fruit set	leaf area beg. of fruit coloring
d	223.1±16.5	173.2±13.4	119.5± 7.6
g	147.5±13.9	115.5± 7.5	86.0± 6.3
d1	106.3± 5.8	113.9± 9.4	87.6± 7.5
d3	119.4± 6.2	123.6± 9.9	83.3± 6.9
d7	118.4±13.6	145.5±14.6	111.9± 9.6
d15	136.0±14.8	132.0±11.6	90.8± 7.0
d16	112.9± 9.3	106.0± 7.5	82.7± 7.4
d26	241.4±16.0	198.7±17.0	125.3±10.9
d28	166.1± 8.2	168.3± 9.8	103.0± 4.0
d36	175.8±11.0	161.3± 9.4	104.5± 5.2
d37	166.4±13.9	122.6± 7.5	108.4± 5.4
d45	135.2± 8.9	154.6± 9.0	114.4± 9.1
g1	143.4±13.2	166.0± 7.1	109.7± 6.1
g5	127.3± 9.8	137.1± 9.1	100.9± 7.0
g10	123.2±13.9	145.5± 7.1	130.0±11.4
g14	154.9±10.5	148.9±12.0	103.0± 5.1
g16	116.4± 5.1	98.3± 8.8	83.6± 4.2
g21	137.5± 6.1	126.7± 5.7	98.3± 5.6
g23	177.7±12.0	126.6± 6.2	115.6± 4.6
g25	100.3± 8.8	99.9± 9.5	96.2±10.0
g28	135.7±13.7	125.5± 8.4	96.0± 7.1
g30	214.4±18.5	148.9±10.2	115.4± 5.7
g41	148.3± 8.6	130.6± 8.5	95.8± 6.4
g43	131.0± 7.5	127.6± 8.4	99.5± 6.5
critical range*	58.5	47.6	37.0

* according to Tukey procedure, $p<0.05$

Table 5: No of leaflets at three growing stages in two tomato lines (heat sensitive and heat tolerant) and 22 lines of their F4 generation.

Line	No of leaflets beg. of flowering	No of leaflets beg. of fruit set	No of leaflets beg. of fruit coloring
d	15.5±0.8	13.5±0.9	11.1±0.5
g	10.2±06	10.5±0.9	9.1±0.6
d1	9.8±0.3	9.3±0.5	8.4±0.5
d3	10.7±0.4	9.1±0.5	7.8±0.6
d7	9.3±0.5	9.8±0.8	8.8±0.6
d15	10.0±0.7	12.1±0.9	10.2±0.4
d16	9.8±0.5	10.7±0.5	9.8±0.5
d26	12.3±0.9	14.8±0.9	12.3±0.5
d28	10.5±0.5	10.7±0.4	9.4±0.4
d36	11.8±0.6	12.1±0.6	10.6±0.4
d37	13.2±0.7	11.9±0.5	11.1±0.4
d45	10.7±0.4	11.7±0.6	11.8±0.3
g1	11.5±0.7	11.0±0.7	10.6±0.4
g5	9.2±0.3	9.4±0.7	8.7±0.5
g10	10.9±0.8	10.8±0.5	10.2±0.6
g14	12.6±0.6	14.1±0.6	10.8±0.4
g16	10.8±0.3	10.8±0.5	10.2±0.3
g21	11.8±0.4	10.7±0.4	9.8±0.6
g23	11.8±0.5	10.9±0.4	10.2±0.3
g25	9.5±0.5	8.3±0.1	7.8±0.6
g28	11.4±0.5	11.8±0.4	8.8±0.4
g30	13.6±0.5	13.1±0.7	10.7±0.4
g41	10.6±0.5	11.3±0.4	10.3±0.2
g43	11.9±0.7	10.3±0.5	9.7±0.4
critical range*	2.7	3.2	2.4

* according to Tukey procedure, $p<0.05$

Table 6: Leaf dry weight (g) at three growing stages in two tomato lines (heat sensitive and heat tolerant) and 22 lines of their F4 generation.

Line	leaf dry weight beg. of flowering	leaf dry weight beg. of fruit set	leaf dry weight beg. of fruit coloring
d	3.68±0.08	3.59±0.06	3.36±0.07
g	3.32±0.08	3.23±0.06	3.02±0.03
d1	3.08±0.03	3.13±0.04	3.02±0.04
d3	3.20±0.03	3.22±0.06	3.04±0.06
d7	3.18±0.07	3.36±0.08	3.27±0.07
d15	3.25±0.07	3.23±0.06	3.06±0.06
d16	3.20±0.06	3.09±0.04	3.07±0.06
d26	3.81±0.08	3.80±0.10	3.38±0.07
d28	3.53±0.06	3.72±0.06	3.24±0.05
d36	3.65±0.08	3.60±0.19	3.37±0.05
d37	3.44±0.07	3.20±0.04	3.17±0.04
d45	3.29±0.06	3.35±0.11	3.30±0.07
g1	3.30±0.09	3.16±0.05	3.16±0.04
g5	3.22±0.06	3.27±0.05	3.13±0.05
g10	3.28±0.08	3.58±0.07	3.63±0.08
g14	3.34±0.05	3.41±0.07	3.14±0.04
g16	3.24±0.04	3.13±0.07	3.08±0.05
g21	3.30±0.03	3.23±0.04	3.17±0.05
g23	3.53±0.05	3.27±0.05	3.17±0.05
g25	3.15±0.06	3.13±0.07	3.13±0.07
g28	3.30±0.08	3.23±0.06	3.14±0.05
g30	3.71±0.10	3.42±0.08	3.24±0.06
g41	3.36±0.05	3.23±0.15	3.18±0.05
g43	3.21±0.04	3.30±0.04	3.23±0.06
critical range*	0.32	0.40	0.28

* according to Tukey procedure, $p<0.05$

Table 7: Leaf stem dry weight (g) at three growing stages in two tomato lines (heat sensitive and heat tolerant) and 22 lines of their F4 generation.

Line	Leaf stem DW beg. of flowering	Leaf stem DW beg. of fruit set	Leaf stem DW beg. of fruit coloring
d	1.79±0.02	1.76±0.02	1.72±0.02
g	1.70±0.02	1.71±0.02	1.65±0.02
d1	1.62±0.01	1.63±0.01	1.60±0.01
d3	1.65±0.01	1.64±0.01	1.61±0.02
d7	1.66±0.02	1.70±0.02	1.70±0.02
d15	1.69±0.02	1.71±0.02	1.68±0.02
d16	1.68±0.02	1.67±0.01	1.68±0.02
d26	1.75±0.02	1.83±0.02	1.74±0.02
d28	1.77±0.02	1.82±0.02	1.73±0.02
d36	1.80±0.02	1.81±0.01	1.77±0.02
d37	1.71±0.01	1.67±0.01	1.65±0.01
d45	1.67±0.01	1.68±0.01	1.65±0.01
g1	1.68±0.02	1.65±0.01	1.66±0.01
g5	1.67±0.02	1.69±0.02	1.67±0.02
g10	1.70±0.02	1.76±0.02	1.79±0.02
g14	1.71±0.02	1.72±0.02	1.66±0.01
g16	1.68±0.02	1.66±0.02	1.66±0.01
g21	1.69±0.01	1.70±0.01	1.64±0.01
g23	1.74±0.01	1.67±0.01	1.65±0.04
g25	1.66±0.02	1.63±0.03	1.64±0.02
g28	1.69±0.02	1.67±0.01	1.65±0.01
g30	1.78±0.03	1.73±0.02	1.71±0.02
g41	1.74±0.02	1.77±0.02	1.69±0.02
g43	1.68±0.01	1.70±0.01	1.70±0.02
critical range*	0.09	0.09	0.09

* according to Tukey procedure, $p<0.05$

Table 8: Total dry weight (g) at the end of the growing season in two tomato lines (heat sensitive and heat tolerant) and 22 lines of their F4 generation.

Line	total DW
d	170.6±10.1
g	126.7±10.4
d1	132.7±13.4
d3	118.8±11.6
d7	128.8±15.2
d15	106.1± 7.9
d16	85.1± 6.4
d26	149.9±10.7
d28	123.8± 3.4
d36	184.0±13.6
d37	124.0±11.9
d45	145.1± 9.0
g1	205.4±13.1
g5	119.7±16.1
g10	170.2±19.0
g14	143.3±11.0
g16	105.3± 9.7
g21	161.4±10.7
g23	163.7± 4.6
g25	70.5± 7.8
g28	158.2±10.5
g30	171.3±10.1
g41	84.5± 6.5
g43	158.7± 8.7
critical range*	50.2

* according to Tukey procedure, $p<0.05$

6. IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

The results of this program are extremely useful for developing countries. We found two important things. First, heat avoidance does appear to be a promising rapid, parsimonious and easily mastered alternative to heat tolerance for heat resistance. Out heat avoidance traits, especially stomatal conductance, leaf area and leaf curling and rolling, were significantly related to lower leaf temperature in tomato and wheat. More conclusive proof requires more direct cause and effect evidence which we hope to obtain in the coming few years after we examine our F6 isogenic lines (which is outside the scope of this project as we only planned within the time frame of this project to test only to the F4 generation). Nevertheless, the results are extremely hopeful and could be used in other research programs.

Second, the passive cooling traits, which are morphological and are simple to examine and use, were negatively correlated to leaf temperature. Thus they are suitable for use in breeding programs for heat resistance.

The program has been important for both laboratories. The Kazakh laboratory, under Prof. Polimbetova, has been for the past twenty years conducting research and breeding programs for heat and drought resistance in wheat. These results have provided them with a rapid and parsimonious selection trait for heat resistance. Her laboratory has been able to purchase necessary equipment. Several junior Kazakh scientists and graduate students have obtained training in Kazakhstan. These include:

1. Dr. E.D. Bogdanova
2. Dr. I.S. Shayakhmetova
3. Dr. R.N. Tormanova
4. Mrs. K. Makhmutova (Ph.D.)
5. Mr. K. Gastenko (Ph.D.)
6. Mr. D.D. Satybaldin (Ph.D.)

In addition, the following Kazakh scientists obtained training through the project in Dr. Mendlinger's laboratory in Israel:

1. Dr. Irina Shulgina
2. Dr. Elizabetha Bogdanova
3. Ms. Irina Pirogova
4. Ms. Anal Tormanova

Overall the Institute for Plant Physiology, Genetics and Bioengineering has been improved in three ways: (a) improvement in laboratory equipment and infrastructure; (b) improvement in manpower via training; and (c) new research directions.

In Israel, Dr. Mendlinger's laboratory has been improved by; (a) purchase of needed equipment; (b) several students were trained; and (c) new directions for breeding programs.

In Israel the tomato program will continue by examining the F6 isogenic lines. If the results are positive they will initiate a breeding program. The Israeli are now in the process of writing their results in a series of papers. The Kazakh team are using their results in a breeding program for improving the heat resistance in winter wheat.

7. PROJECT ACTIVITIES

see part 6.

8. PROJECT PRODUCTIVITY

All of our major goals have been achieved. We demonstrated not only the potential utility of heat avoidance traits but also the ease or simplicity of using them in a breeding program.

9. FUTURE WORK

As stated in section 6, the Israelis are continuing their research by examining the isogenic F6 lines that they produced. The Kazakhs are in the middle of a breeding program.

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