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In vitro Evaluation of Heat Stress Tolerance in Some Tomato Cultivars

A. A. Alsadon, M. A. Wahb-allah and S. O. Khalil
Department of Plant Production, College of Food Sciences and Agriculture,
King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

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Abstract. Twenty locally grown tomato cultivars were evaluated for their *in vitro* heat tolerance. Leaf discs were collected during vegetative, flowering and fruiting stages and incubated in water bath at 40°C for 1, 2 and 3 hours. Electrical conductivity (EC) was measured as an indication of cell injury due to electrolytes leakage. The results indicated that cultivars can be classified into three groups according to their heat stress tolerance: (a): heat stress tolerant cultivars (EC ranged from 27.75 to 41.69 µmho/cm) which include Queen, Indian, Super Strain B, Pearson and Strain-B; (b): moderately heat stress tolerant cultivars (EC ranged from 43.80 to 50.70 µmho/cm). This group consisted of nine cultivars Pacmore, Tnshet Star, Rocky, Shohba, Raad VF, VFN-8, Moneymaker, Imperial and Pearson Imperoved; and (c): heat stress sensitive cultivars (EC ranged from 53.12 to 72.20 µmho/cm) and included Malica, Castle Rock, Edkawi, Chico, Pakmore VF and Super Marmand. Correlation coefficients among the three growth stages were positive and significant or highly significant which indicated that this method is effective in selecting genotypes at early growth stages. The incubation period of 2 hours was optimum for this test. This method is a useful tool for plant breeders to screen for heat stress tolerance of genotypes during early growth stages in breeding programs in addition to field evaluation in various agricultural production areas.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop in most regions of the world. Lack of tolerance to high temperature in most tomato cultivars presents a major limitation for growing them in regions where the temperature during part of the growing season, even for short duration, reaches 38°C or higher [1]. Heat stress adversely affects the vegetative and reproductive stages of tomato and ultimately reduces yield and fruit quality [2]. High temperature affects several physiological and biochemical processes leading finally to tomato yield reduction [3]. Progress in developing heat tolerant cultivars has been hindered by the complexity of the trait and its low heritability values [4]. In a genetic analysis of parental lines and their hybrid progenies, Villareal and Lai reported that heritability values for heat tolerant trait ranged from 5% to 19% [5]. In addition, Peter *et al*. recorded low heritability values for the percentage of fruit set and mean fruit number per cluster under conditions of high temperature [6].

The identification of heat tolerance in tomato has been accomplished by evaluating genotypes for flowering and fruit set, because these two processes are sensitive to heat and directly related to yield [2, 7-9]. The impact of high temperature on the plant is not limited to flowering and fruit set. It also affects the subsequent development and maturity of the fruit and thereby further reduce crop yield [10]. Major non-reproductive processes, such as photosynthetic activity [11], and disorganization of cell membranes have been observed [12]. The last effect has been suggested as a possible screening method for heat tolerance by measuring electrolytes leakage from leaves that had been subjected to heat stress [13].

Cellular membrane thermostability, measured as the conductivity of electrolytes leaking from leaf disks at high temperature, has been suggested as a screening technique for heat tolerance in plants [14]. This technique is based on the observation that when leaf tissue is injured by exposure to high temperatures, cellular membrane permeability is increased and cell electrolytes diffuse out into bathing solution. The amount of electrolytes leaking from injured cells can be evaluated by measuring the electrical conductivity of the solution. Effectiveness of this technique in detecting genetic variability in heat tolerance has been reported in warm season crops. Examples include soybean [15], sorghum [16], melon [17], and tomato [12, 13] as well as in cool season crops such as wheat [18] and cowpea [19].

This technique is simple, quick and less expensive than whole plant screening. Plant breeders can apply this technique at an early vegetative stage for plants grown in field nursery environment for selecting plants, advancing generations and accelerating breeding programs. This study is part of a comprehensive breeding program to produce new tomato hybrids tolerant to heat stress under the conditions of the central region of Saudi Arabia. The overall objectives of this study were (a) to utilize a more efficient indirect method in screening tomato genotypes for heat tolerance during different growth stages, and (b) to evaluate and select the parental genotypes for heat stress tolerance for breeding program.

Material and Methods

Plant material

Twenty commercial tomato cultivars were selected for this study (Table 1). Seeds were sown on 12 March 2004 in Jiffy 7 pots and transplanted on 10 April 2004 into 30 cm diameter pots filled with a soil mix (1 peat : 1 sand : 1 vermiculite) in the greenhouse of the Agricultural Research and Experiment Station in Dirab near Riyadh. Three factors experiment in randomized complete block design, with four replicates was applied. Each cultivar was represented by five plants in each replicate. Day/Night air temperatures and relative humidity were maintained at 25/20°C and 65%, respectively. All cultural practices, such as fertilization, irrigation, micronutrients spraying and pest control, were performed as usually recommended for commercial tomato production [20].

Table 1. Tomato cultivars used in heat stress tolerance study

Cultivar*	Origin
Chico	Peto Seed, USA
Edkawi	Nopa Seed, Egypt
Imperial	Atlas Seed, USA
Indian	Pahuja Seed, India
Kastlerock	Peto Seed, USA
Malika	Atlas Seed, USA
Moneymaker	Peto Seed, USA
Pakmore	Top Harvest, Holand
Pakmore VF	Top Harvest, Holand
Pearson	Dessert Call Seeds, USA
Pearson Improved	Dessert Call Seeds, USA
Queen	Top Harvest, Holand
Raad VF	California Vallay Seeds, USA
Rocky	Atlas Seed, USA
Shohba	Genetics international Inc., USA
Strain-B	Atlas Seed, USA
Super Marmend	Popvriend Seeds, USA
Super strain-B	Modesto Seeds, USA
Tnshet Star	Genetics international Inc., USA
VFN-8	Asgrow Seeds, USA

*All cultivars are open pollinated and recommended for commercial tomato production.

Heat tolerance tests

The procedure used in heat tolerance test was similar to that reported by [11, 21]. Leaf disks of tomato cultivars, 13 mm in diameter, were taken at three growth stages (vegetative, flowering and fruiting). Sampling was carried out 40, 55 and 70 days from the sowing date for vegetative, flowering and fruiting growth stages respectively. In each stage, 40 leaf disks were collected from each cultivar (two leaf disks from each plant) from new leaves. Leaf disks were washed with distilled water to remove electrolyte from injured cells at the cut edge and any surface adhering electrolytes. Samples for each cultivar in each replicate were placed in test tube containing 20 ml of distilled water. Each cultivar was represented by four test tubes. Tubes were placed in a water bath at 40°C for one, two and three hours. Electrical conductivity (EC) of the solution was measured using conductivity meter (WTW LF318 model, Weilheim, Germany) as an indication of cell injury due to electrolytes leakage.

Statistical analysis

Data were statistically analyzed using the standard method of the factorial experimental analysis. Means of the different treatments were separated and statistically analyzed according to revised L.S.D. (0.05). Also, Phenotypic correlation coefficients among the growth stages were calculated. The previous statistical analysis was done according to [22, 23].

Results and Discussions

The analysis of variance (Table 2) for heat injury, expressed as EC, showed that the three main studied factors (cultivars, growth stages and incubation periods) were significant. The interactions between cultivars and growth stages, growth stages and incubation period were significant. However, the interactions between cultivars and incubation period, and the second degree of interaction effects between the three studied factors were not significant.

Table 2. Analysis of variance for heat injury, expressed as EC, from 20 tomato cultivars at three growth stages and three incubation periods

Source of variation	d.f	Mean squares
Cultivars (c)	19	3307.896**
Growth stages (s)	2	121027.883**
Incubation periods (p)	2	14884.171**
c*s	38	4141.458**
c*p	38	65.101
s*p	4	2443.544**
c*s*p	76	53.009
Error	537	104.001

** Significant at 0.01 level

Response of cultivars to heat stress at various growth stages

The general performance of the different cultivars over the three growth stages are presented in Table 3. The result illustrated that cultivars can be classified into three groups according to their heat stress tolerance: (a): heat stress tolerant cultivars (EC ranged from 27.75 to 41.69 µm/cm) which include Queen, Indian, Super Strain-B, Pearson, Strain-B; (b): moderately heat stress tolerant cultivars (EC ranged from 43.79 to 50.70 µm/cm), this group consisted of nine cultivars (Pacmore, Tnshet Star, Rocky, Shohba, Raad VF, VFN-8, Moneymaker, Imperial and Pearson Imperoved) and (c): heat stress sensitive cultivars (EC ranged from 53.12 to 72.20 µm/cm) as Malica, Castle Rock, Edkawey, Chico, Pakmore VF and Super Marmand. These findings demonstrate that it may be possible to screen for heat tolerance during different growth stages based on electrolyte leakage from leaf tissue obtained from field nurseries used by breeders to select plants and advance generations.

Significant differences in heat tolerance among tomato cultivars were found at the different growth stages. At the vegetative growth (Fig. 1), the significant highest mean values for electrical conductivity (EC) were found in Edkawi cultivar (63.12 µmho/cm) followed by Pakmore VF, Castle Rock, Chico, Pakmore and Tnshet Star, respectively. This result indicated that these cultivars were more sensitive to heat stress at vegetative growth stage, since the heat injury, expressed as electrical conductivity (EC) from leaf disks, was higher than that from other cultivars. On the other hand, the significant lowest mean value for EC was reported in Pearson, Super Strain-B, Queen, VFN-8 and Strain-B cultivars, respectively indicating that these cultivars have the best performance and were tolerant to heat stress at vegetative growth stage. The other nine cultivars; Moneymaker, Indian, Raad VF, Pearson improved, Rocky, Imperial, Super Marmand, Shohba and

Malika; showed intermediate values of electrolytes leakage (expressed as EC). Therefore, they were considered as moderately heat stress tolerant.

Table 3. Average of heat injury, expressed as EC, from leaf disks of tomato cultivars over growth stages and incubation periods

Cultivars	E C (μmho/cm)
Chico	54.59 cde
Edkawey	54.89 cde
Imperial	50.55 efg
Indian	36.01 l
Kastelrock	61.59 b
Malika	72.20 a
Moneymaker	50.70 efg
Pakmor	45.92 hij
Pakmor VF	55.31 cde
Pearson	41.69 jk
Pearson improved	49.89 e-h
Queen	27.75 m
Raad VF	43.79 ij
Rocky	46.88 ghi
Shohba	44.29 ij
Strain-B	41.67 jk
Supermarmand	53.12 cde
Superstrain-b	38.55 kl
Tanshit star	46.84 ghi
VFN-8	49.78 fgh
$LSD_{0.05}$	4.72

Means values with the same alphabetical letters are not significantly different, using revised LSD test at $P \leq 0.05$.

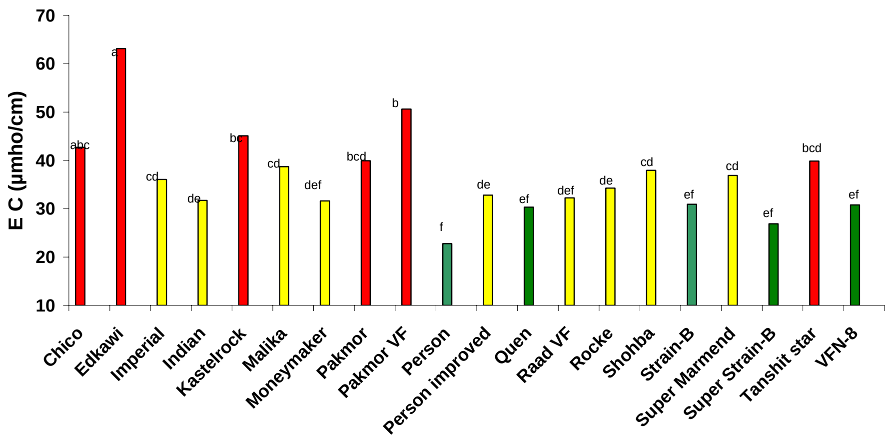

Fig. 1. Heat injury, expressed as EC, from leaf disks of tomato cultivars at the vegetative stage summed over the three incubation periods. Bars sharing the same letter(s) are not significantly different using the revised L.S.D. test at 0.05 level.

In the flowering stage, the cultivars Castle Rock, Malika, Edkawi, Pakmore VF and Super Marmand showed the highest mean values for electrolytes leakage and were considered as heat sensitive at flowering stage (Fig. 2). On contrast, cultivars Queen, Indian, Strain-B, Super Strain-B and Pearson gave the lowest mean values. Therefore, they were considered as having good performance under heat stress conditions. The rest 10 cultivars, which indicated intermediate values for EC, were considered moderately tolerant to heat stress.

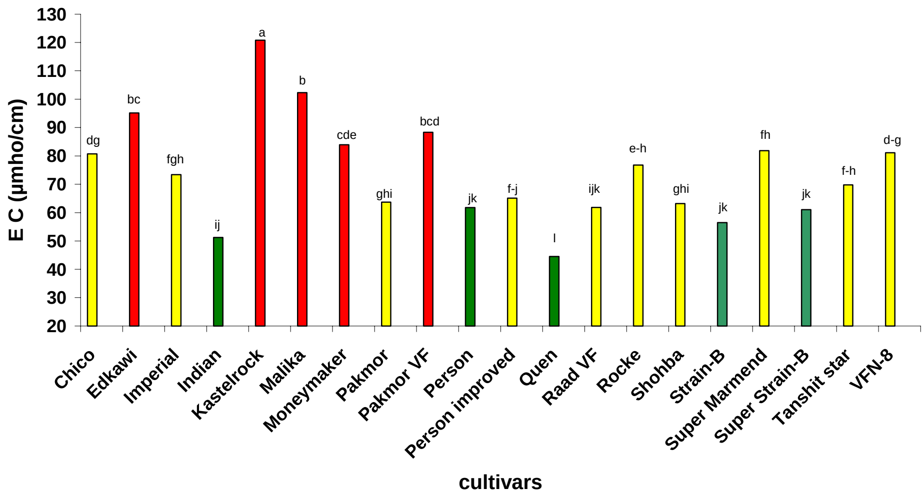

Fig. 2. Heat injury, expressed as EC, from leaf disks of tomato cultivars at the flowering stage summed over the three incubation period. Bars sharing the same letter(s) are not significantly different using the revised L.S.D. test at 0.05 level.

During the fruiting stage (Fig. 3), the cultivars Malika, Pakmore VF, Super Marmand, Chico, Edkawi and Castle Rock had the highest mean EC values with significant differences among them. Therefore, they were found to be more sensitive to heat stress, whereas cultivars Queen, Super Strain-B, Indian, Strain-B and Raad VF gave the lowest EC values and were considered as the best heat tolerant cultivars at the fruiting stage. The rest 10 cultivars, which reflected intermediate values for EC, were considered moderately tolerant to heat stress.

Significant variations in EC values among genotypes indicate that the electrolyte leakage test was sensitive in evaluating heat tolerance and that the trait can be amenable through breeding. Evidence from other crops suggested that tolerant genotypes selected by this test performed well and gave stable yields in hot environments. For instance, Kuo *et al*. showed that vegetable species with low relative injury were more stable in different growing seasons [24]. Ismail and Hall observed consistent genotypic differences among cowpea lines in electrolyte leakage at growth chamber and field environment conditions [19]. Saadalla *et al*. reported that heat tolerant genotypes of wheat determined on the basis of electrolyte leakage, out-yielded sensitive ones by 19% under field conditions [18].

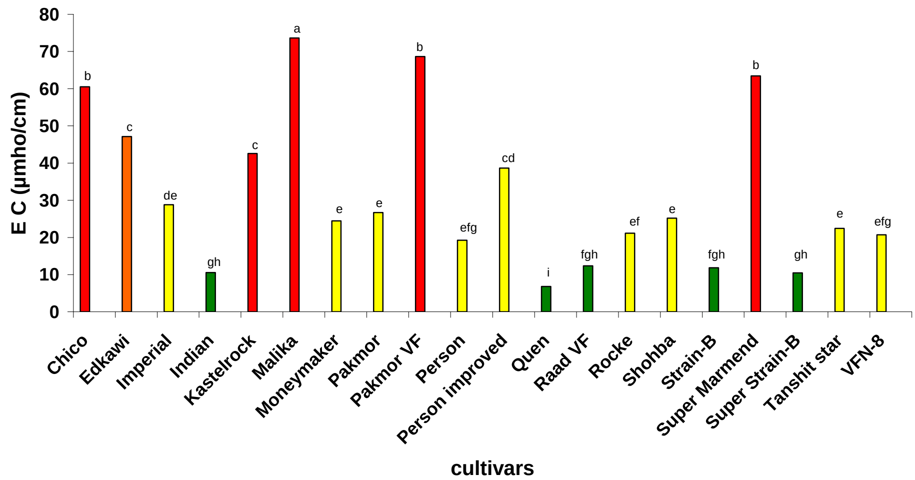

Fig. 3. Heat injury, expressed as EC, from leaf disks of tomato cultivars at the fruiting stage summed over the three incubation period. Bars sharing the same letter(s) are not significantly different using the revised L.S.D. test at 0.05 level.

Effects of growth stage on the response of cultivars to heat stress

The comparison of average EC values among the three growth stages of tomato cultivars were presented in Table 4. EC values were low at the vegetative and fruiting stages (36.85 and 33.92 µm/cm respectively). On contrast, EC values were high at the flowering stage (74.20 µm/cm). The interaction effects between the cultivars and growth stages on EC values are presented in Table 5. Generally, the level of heat tolerance of each cultivar during the three stages was approximately similar. The high EC values from leaves collected at the flowering stage indicated that this stage of growth was more sensitive to heat stress than the other two stages. This finding is in general agreement with that reported by Lahar and Peat suggested that total flower bud and flower production, particularly of the first four trusses, may be a reliable criterion for heat tolerance selection [25]. Also, Abdalmageed *et al*. reported that reproductive processes in tomato were more sensitive to high temperature than the vegetative ones [26]. Kalloo reported that meioses 7-8 days before anthesis is highly sensitive to high temperatures [27]. Thus, earliness could also help to avoid the problems associated with high temperature in an area where the temperature rises gradually with the onset of summer.

Table 4. Effect of growth stage, incubation period and their interactions on heat injury, expressed as EC from leaf disks of tomato cultivars

Growth stages	Incubation period			Means
	1 hour	2 hours	3 hours	
Vegetative stage	26.78 e	40.15 c	43.63 c	36.85 b
Flowering stage	59.66 b	79.12 a	83.82 a	74.20 a
Fruiting stage	31.87 d	34.02 d	35.87 d	33.92 c
Means	39.44 b	51.10 a	54.44 a	

Values with the same alphabetical letters, within a comparable group of means, are not significantly different, using revised LSD test at $P \leq 0.05$.

Table 5. Interaction effects of cultivars and growth stage on heat injury, expressed as EC from leaf disks of tomato plants

Cultivars	E C (µmho/cm)		
	Vegetative stage	Flowering stage	Fruiting stage
Chico	42.68	80.69	60.51
Edkawey	63.11	95.14	47.09
Imperial	36.06	73.38	28.78
Indian	31.71	51.19	10.57
Kastelrock	45.07	120.81	42.56
Malika	38.70	102.32	73.62
Moneymaker	31.61	83.92	24.45
Pakmor	39.94	63.67	26.65
Pakmor VF	50.61	88.30	68.63
Pearson	22.8	61.77	19.25
Pearson improved	32.81	65.06	38.63
Queen	30.32	44.53	06.82
Raad VF	32.27	61.82	12.33
Rocky	34.27	76.78	21.14
Shohba	37.93	63.21	25.16
Strain-B	30.93	56.45	11.84
11.84 Supermarmand	36.87	81.82	63.42
Superstrain-b	26.87	61.02	10.50
Tanshit star	39.86	69.82	22.40
VFN-8	30.78	81.09	20.72
LSD $_{0.05}$		**4.859**	

Correlation coefficients of genotypes mean values of EC among the three growth stages were positive and significant at the different periods of incubation (Table 6). The vegetative growth stage reflected desirable positive correlations with both flowering and fruiting stages at all incubation periods. At the flowering stage, positive and highly significant correlation with the fruiting stage was reported at all incubation times. These results indicated that the selection for heat stress tolerance at the vegetative growth stage would lead, spontaneously, for the improvement of the heat stress tolerance at the the flowering and fruiting stages. In addition, these findings showed that this technique is useful tool for screening for heat tolerance at early growth stage, since the correlation among the three growth stages existed.

Table 6. Correlation coefficients of EC values from the three studied growth stages overall tomato cultivars

Growth stage	Flowering	Fruiting
	After one hour incubation	
Vegetative	0.30*	0.35*
Flowering		0.56**
	After two hours incubation	
Vegetative	0.34*	0.41**
Flowering		0.56**
	After three hours incubation	
Vegetative	0.44**	0.45**
Flowering		0.55**

*,**Significant at 0.05 and 0.01 levels, respectively.

Effect of the incubation period

To determine the optimum incubation period, electrical conductivity (EC) was measured after 1, 2 and 3 hours in all growth stages (Table 4). Differences among cultivars were significant at one hour of incubation and became more apparent with prolonging the incubation period up to 2 or 3 hours. For each cultivar the values at 2 and 3 hours were approximately similar. Therefore, the period of 2 hours was considered the optimum for this test. Such a result seemed to agree with that reached by Florido *et al.,* who established an efficient method for evaluating heat tolerance in tomato [28]. They reported that the best temperature and incubation time were 40°C and 90 minutes, respectively.

Some studies have indicated that the detection of genotypic differences in heat tolerance based on leaf electrolyte leakage might be more effective with plants already subjected to moderately hot temperatures [12, 13]. In this study, leaves from plants that had not been subjected to heat treatments were utilized. Taking leaf samples directly from field-grown plant (or commercial greenhouse) would enable speeding up the screening process in a more efficient manner. Heat acclimation would require putting plants in a controlled environment facility, which would reduce the number of plants that could be processed and increase time and effort of screening. The potential advantages of the screening technique used in this study include its effectiveness in many seasons and parts of the world where very hot field screening environments are not available. The results from this technique can be obtained within short a period of time. Therefore, permitting the selection of potential parental lines that can be used in breeding programs.

In conclusion, this technique can be utilized as an indirect tool for detecting heat stress tolerance of tomato genotypes during different growth stages. The present study has shown that wide genetic variability for leaf heat tolerance exists among tomato cultivars. Electrolyte leakage test is a useful tool for the screening for heat tolerance. Studies under high temperature field condition are in progress to determine the reliability of this technique as a selection tool for heat tolerant in tomato.

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التقييم ا عملي لتحمل الإجهاد ا راري بعض أصناف الطماطم

عبد ا بن عبدالر ن السعدون، و مود عبادي وهب ا ، وصفوت عثمان خليل

قسم الإنتاج النباتي، كلية علوم الأغذية والزراعة، جامعة الملك سعود،

ص.ب ٢٤٦٠ ، الرياض ١١٤٥١ ، المملكة العربية السعودية

(قدم للنشر في ١٤٢٦/٣/١٦هـ؛ وقبل للنشر في ١٤٢٧/٢/١٨هـ)

ملخص البحث. استخدم ٢٠ صنفاً من أصناف الطماطم مفتوحة التلقيح والمنتشرة بالمملكة العربية السعودية وذلك لتقدير تحمل تلك الأصناف للإجهاد الحراري معملياً خلال مراحل النمو المختلفة. أخذت عينات من الأوراق (أقراص) خلال مراحل النمو الخضري والزهري والثمري ووضعت في أنابيب اختبار بها ٢٠ ملم ماء مقطر ثم عرضت لدرجة حرارة ٤٠°م بحمام مائي لثلاث فترات (١ و ٢و ٣ ساعات)، وتم قياس معامل التوصيل الكهربي كمؤشر للضرر الناتج من تسرب الذائبات من الخلايا نتيجة لتعرضها للحرارة المرتفعة.

أوضحت النتائج أنه يمكن تقسيم الأصناف تحت الدراسة إلى ثلاث مجموعات تبعا لتحملها الحراري: (أ) أصناف متحملة للإجهاد الحراري يتراوح معامل التوصيل الكهربائي فيها من ٢٧.٧٥ إلى ٤١.٦٩ ميكروموز/سم وهي كوين، وهندية، وسوبر سترين ب، وبيرسون وسترين ب. (ب) أصناف متوسطة التحمل للإجهاد الحراري يتراوح معامل التوصيل الكهربائي فيها من ٤٣.٨ إلى ٥٠.٧ ميكروموز/سم واشتملت هذه المجموعة على تسعة أصناف هي باكمور، وتنشيط ستار، وروكي، وراد في اف، وشهبا، وفي اف ان ٨، وبيرسون محس، وامبريال، وموني ميكر. (ج) أصناف حساسة للإجهاد الحراري يتراوح معامل التوصيل الكهربائي فيها من ٥٣.١٢ إلى ٧٢.٢ ميكروموز/سم واشتملت هذه المجموعة على ستة أصناف هي ملكه، وكاسل روك، وادكاوي، وشيكو، وباكمور وسوبر مارمند على الترتيب. وكانت فترة الحضانة المثلى لهذا الاختبار هي ساعتان، وكان معامل التلازم بين مراحل النمو الثلاث موجباً وعالي المعنوية مما يوضح إمكانية الاستفادة من هذه الطريقة في انتخاب التراكيب الوراثية خلال المراحل المبكرة من النمو. وتعتبر هذه الطريقة مؤشراً جيداً لمساعدة مربي

النبات في تحديد مدى تحمل الأصناف والسلالات المختلفة للإجهاد الحراري والاستفادة من ذلك في برامج التربية بالإضافة إلى برامج التقييم الحقلي تحت ظروف الحرارة المرتفعة في مناطق الإنتاج الزراعية.