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Identification of maize (*Zea mays*) hybrids with enhanced thermotolerance using temperature induction response technique

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ABSTRACT The experiment was conducted to identify thermotolerant single cross maize (*Zea mays* L.) hybrids. The freshly-harvested sun-dried seeds (below 10% moisture content) of 15 maize hybrids were used to determine optimum induction and recovery growth and were further screened for temperature tolerance. Optimum lethal and induction temperatures were assessed for seedling recovery growth (root + shoot). The optimum lethal temperature for 80% reduction in seedling growth was observed as 52°C for 2 hr. The optimum induction temperature was found to be 35°C for 1hr + 40°C for 1hr + 45°C for 2hr, where maximum recovery growth was recorded after induction of hybrids to lethal temperature. Twenty one days old seedlings were assessed for dry weight and chlorophyll stability index (CSI). Based on Z-analysis, Kaveri 50, 30V 92 and RHM 25 were identified as temperature tolerant, whereas KHM218, GHM145, GK3060, and BIO9637 as temperature susceptible genotypes.

Key words: Maize, thermotolerace, growth during recovery, chlorophyll stability index, induction temperature

Maize (*Zea mays* L.) is grown in 8.17 million ha with a total production of 19.73 million tones and productivity of 2.41 tonnes/ha in India [1]. The area under irrigated conditions has increased from ~11% (1950-51) to ~20% (2009-10). Most of the crop remains rainfed and suffers from abiotic stresses. High temperature stress has been reported as the second major abiotic problem after drought, reducing grain yield by > 15%. Thus, to sustain maize production, it has become imperative to breed varieties which tolerate high temperature stress. Single cross hybrids *in lieu* of double or triple cross hybrids in maize, have become popular with farmers and seed industry, due to their cost effectiveness in production [2]. Temperature Induction Response (TIR) technique [3], aids in identification of inbreds/hybrids for their suitability to tolerate high temperature. Therefore, present study was undertaken to identify high temperature tolerant single cross hybrids in maize.

MATERIALS AND METHODS

Fifteen hybrids *viz*. KHM 218, KHM 225, RHM 4, RHM 7, RHM 20, RHM 25, PAC 740, FMH 8899, GK 3060, BIO 9637, 30 V 92, 30B 11, Kaveri 50, Syngenta 1 and GHM 145, were obtained from a private seed industry, Hyderabad, to evaluate temperature tolerance and their response was recorded on two parameters *viz*. growth during recovery and per cent reduction in growth. The moisture content of seeds was estimated by the standard hot air oven method after pre-drying the seeds under sun so as to reduce moisture content to below 10%. Samples of 100 seeds of DHM 117 single cross maize hybrid in three replications were subjected to hydration for 1hr and allowed to germinate using top-paper method by following ISTA rules [4]. The germinated seedlings having minimum growth of 1-15 cm root and shoot length were used for determination of optimum lethal and induction temperatures. These seedlings were subjected to different temperatures ranging from

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50° to 53°C for 1, 2 and 3 hr and were immediately allowed to recover at 30°C for 72 hr in an incubator. At the end of recovery period, temperature treatment at which 80% reduction in growth of seedlings occurred was taken as the challenging temperature.

Seedlings were maintained at 35°C for 3 hr, 40°C for 3 hr and 45°C for 3 hr, 35°C for 1hr + 40°C for 1hr + 45°C for 1hr, 35°C for 2hr + 40°C for 1hr + 45°C for 1hr, 35°C for 1hr + 40°C for 2hr + 45°C for 1 hr and 35°C for 1 hr + 40°C for 1 hr + 45°C for 2 hr and immediately transferred to challenging temperature. Root and shoot lengths were recorded after 72 hr recovery period.

Seedlings subjected to challenging temperatures were allowed to recover at room temperature for 72 hr and observation were recorded. A set of seedlings maintained simultaneously at room temperature throughout the experimental period were considered as the control.

Fifteen hybrids were evaluated for temperature tolerance and response recorded on two parameters *viz*. growth during recovery and per cent reduction of growth in induction temperature over the control. Desirable genotypes were identified by collecting the data on growth during recovery and per cent reduction in growth in induced over the control. Tolerant genotypes were identified by plotting Z-distribution for designated two parameters [5]. Genotypes were categorized into one of the four possible quadrants *viz*. highest per cent reduction over the control and highest growth during recovery (Highly tolerant hybrids), high per cent reduction over the control and high growth during recovery (Moderately tolerant hybrids), low per cent reduction over the control and low growth during recovery (Moderately susceptible), and lowest per cent reduction over the control and lowest growth during recovery (Highly susceptible).

The material was further validated by allowing the seedlings to grow up to 21 days in plastic containers and assessed for their growth. The data for temperature tolerance was recorded in terms of two parameters namely, seedling dry weight and chlorophyll content. The data obtained was analyzed in a completely randomized block design following the procedure of Panse and Sukhatme [6].

RESULTS AND DISCUSSION

Growth during recovery (GDR) in terms of root and shoot growth decreased with increase in temperature (50°-52°C) and increase in duration of exposure (1-3 hr). At 50°, 51°, 52°, 53°C and exposure to 1-3 hr, GDR values decreased from 7.54 to 5.45, 7.10 to 4.51, 4.56 to 1.05 and 0.46 cm. At 52°C, exposure for 2 hr resulted in GDR values of 1.61 cm which corresponded to 80% decrease over the control treatment, where GDR was 8.32 cm. Any increase or decrease in temperature or duration of exposure resulted in change of recovery growth of non-induced seedlings over the control treatments. Hence, 52°C with 2 hr exposure was considered as optimum lethal temperature, where lethal effects on GDR were recorded (Fig. 1). Optimum lethal temperatures reported, to cause a decrease of 80% GDR, were 53°C for 3 hr in groundnut [6], 51°C for 2 hr in sunflower [7] and 49°C for 2hr in castor [8].

The optimum induction temperature on growth was worked out by exposure of seedlings to series of temperature treatments which were given individually or in combination. Root and shoot growth decreased from 4.42 to 3.56 and 3.04 cm with increase in temperature treatments given individually at 35°, 40° and 45°C, respectively. Combination of elevated temperature treatments, on the other hand proved better compared to individual treatment effects. Exposure to 30° + 40°

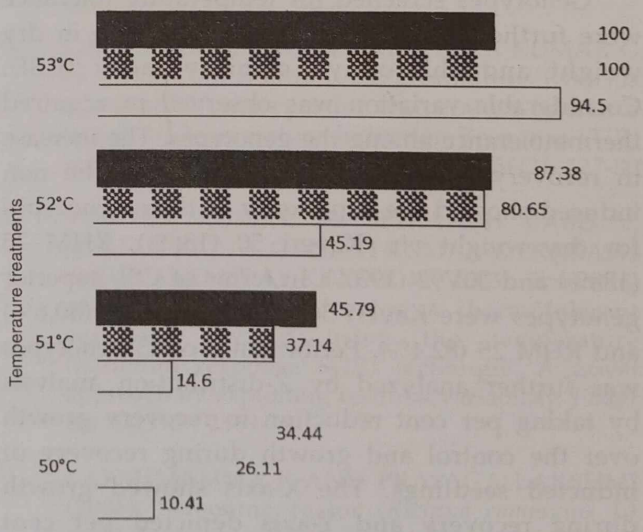

Fig. 1. Standardization of optimum lethal temperature for recovery growth

+ 45°C for 1hr + 1hr + 1hr, 2 hr + 1hr + 1hr, 1hr + 2hr + 1hr and 1hr + 1hr + 2hr showed increased GDR of 5.13, 5.25, 5.36 and 6.02 cm which corresponded to 37, 36, 34 and 25% reduction in recovery growth, respectively. The control and non-induced treatments recorded a mean growth during recovery of 8.24 cm (100%) and a minimum of 1.52 cm (81.52%). Thus, optimum induction temperatures were fixed at 30° + 40° + 45°C for 1hr + 1hr + 2hr duration, where minimum reduction in growth of 26.94% was recorded (Table 1). Induction temperatures of 30°C + 40°C + 45°C with an exposure period of 2hr + 2hr + 1hr was reported as optimum induction temperature in castor [9]. The higher recovery growth in induced seedlings has been attributed to enhanced expression of genes during induction [10]. Other studies also clearly showed genetic variability for the stress response upon exposure to an induction stress [11].

The per cent seedling growth was recorded at the end of recovery period. Among genotypes, 255% increase in recovery growth, of induced over non-induced, was recorded in Kaveri 50, followed by 30V92 (221%) and RHM 25 (210%). These three genotypes simultaneously showed less reduction in seedling recovery growth in induced treatment over the control *i.e.* Kaveri 50 (32%), 30V92 (36%) and RHM 25 (37%). The reduction in recovery growth in all other genotypes varied from 42% to 73% (Table 2).

Genotypes screened for temperature tolerance were further assessed in terms of increase in dry weight and chlorophyll stability index (CSI). Considerable variation was observed in acquired thermotolerance among the genotypes. The increase in recovery growth of individuals over the non induced showed the superiority of three genotypes for dry weight *viz.* Kaveri 50 (186%), RHM 25 (188%) and 30V92 (193%). In terms of CSI, superior genotypes were Kaveri 50 (78%), RHM 20 (80.6%) and RHM 25 (82.4%). Performance of 15 genotypes was further analyzed by Z-distribution analysis by taking per cent reduction in recovery growth over the control and growth during recovery of inducted seedlings. The X-axis showed growth during recovery and Y-axis depicted per cent reduction over the control. The genotypes tested were distributed mainly into two quadrants. Based

Table 1. Standardization of optimum induction protocol in maize for recovery growth

Optimum induction temperature (°C)	Growth during recovery root + shoot (cm)	Reduction in recovery growth of induced over the control (%)
Non-induced	1.52	81.52
35°C 3 hr	4.42	46.3
40°C 3 hr	3.56	56.7
45°C 3 hr	3.04	63.03
30°+40°+45°C (1hr+1hr+1hr)	5.13	37.74
30°+40°+45°C (2hr+1hr+1hr)	5.25	36.28
30°+40°+45°C (1hr+2hr+1hr)	5.36	34.95
30°+40°+45°C (1hr+1hr+2hr)	6.02	26.94
Control	8.24	-
SEm±	0.17	2.33
CD(5%)	0.37	5.01

on Z-distribution analysis maize genotypes can be grouped into four categories. They are:

- Highly tolerant types (three) like Kaveri 50, RHM25, 30V02
- Moderately tolerant (four) types like 30B11, Syngenta 1, RHM20, and PAC740
- Moderately susceptible (four) types like RHM7, FMH8899, KHM225, RHM4
- Highly susceptible types (four) like KHM218, GHM145, GK3060, ad BIO9637.

Temperature induction response (TIR) technique had shown the existence of significant genetic variability across the genotypes of pea [12], and thermotolerant lines from sunflower open-pollinated population cv. Morden [13]. The TIR was found to be a potential technique to identify

Table 2. Genetic variability for temperature tolerance among 15 hybrids of maize

Hybrid	Growth during recovery (root + shoot)		Dry weight CSI(%)	
	Increase in recovery growth of induced over the control (%)	Reduction in recovery growth of induced over the control (%)	Increase in recovery growth of induced over the control (%)	Induced
KHM 18	118	61	96	71.60
KHM 225	138	54	102	62.64
RHM 4	207	52	128	62.83
RHM 7	183	50	142	78.63
RHM 20	177	47	149	80.59
RHM 25	210	37	188	82.43
PAC 740	192	46	122	76.87
FMH 8899	173	50	136	73.85
GK 3060	94	69	82	64.32
BIO 9637	94	73	73	74.72
30V92	221	36	193	73.41
30B11	195	42	139	76.89
Kaveri 50	255	32	186	78.16
Syngenta I	199	42	138	69.60
GHM 145	147	52	107	71.93
SEm±	5.69	2.32	4.15	2.01
CD(5%)	11.64	5.59	8.50	5.81

thermotolerant hybrids. Thus, it is clear that there is variation among genotypes for temperature tolerance. The plant characters that showed consistency for temperature tolerance encompassed recovery growth, dry weight and chlorophyll stability index. Among 15 genotypes studied, RHM25, Kaveri 50 ad 30V92, showed acquired tolerance. These temperature tolerant hybrids indicate possible sources of thermotolerance to be explored, identified and used in breeding programmes.

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