Singh et al., Vegetos 2016, 29:3
DOI: 10.5958/2229-4473.2016.00060.4

Vegetos- An International Journal of Plant Research

Research Article

A SCITECHNOL JOURNAL

Effect of Elevated Temperature on *In Vitro* Microtuberization of Potato Genotypes with Different Thermotolerance Levels

Anupama Singh*, Nimisha Kaushal, Reena Sharma, Vinay Bhardwaj, Brajesh Singh and Rajinder Singh

Abstract

Extension of potato cultivation to tropical areas has been constrained by the thermosensitivity of most cultivars which require low temperature during induction of and their subsequent development. Development of culivars for heat tolerance requires robust phenotyping methods for evaluating germplasm which is generally maintained as *in vitro* plant cultures. Microtuber production behaviour of five potato genotypes with contrasting themotolerance levels was studied to develop an *in vitro* assay system for screening potato genotypes for tolerance to high temperatures. Significant differences were observed with respect to tuber number, tuber weight and plant weight in all the genotypes at ambient (18°C) and elevated temperature (25°C). Kufri Himalini and CP4054 produced highest tuber number at 18°C and 24°C respectively. Although plant weight increased with an increase in temperature in all genotypes, total biomass increased only in tolerant genotypes. Heat tolerant genotypes exhibited comparable tuberization at both the temperatures whereas tuber formation was reduced in heat sensitive genotypes at elevated temperature. All genotypes formed micro tubers *in vitro* but only thermotolerant genotypes formed tubers in soil at elevated temperature, suggesting that mixotropic culture media overcome the effect of high temperature in the inhibition of tuberization indicating the need to use culture media devoid of hormones for developing a reliable *in vitro* assay system.

Keywords

Potato; Microtuberization; Thermotolerance

Introduction

Tuberization in potato is affected by several factors including light intensity, photoperiod, temperature and genotype [1]. However one of the major constraints in the tuberization process is its thermosensitivity in most potato cultivars limiting the extension of potato cultivation to tropical areas.

Under field conditions tuberization is reduced when night temperatures are above 20°C and there may not be any tuberization at 25°C or above [2]. To extend potato cultivation to relatively warmer areas where night temperatures are usually considerably higher than 20°C and in view of global climate warming, development of heat tolerant potato varieties needs urgent attention. Since there is considerable evidence for strong and consistent correlation between the formation of micro tubers under *in vitro* conditions and tuber formation under field conditions, for their structure as well as starch and protein composition [3], development of an *in vitro* assay system to evaluate the effect of high temperature on micro tuber formation will be highly desirable to facilitate early clone selection and considerably lessen the need for field evaluation of large number of genotypes under high night temperature conditions, thus accelerating breeding programs.

Under *in vitro* conditions, a temperature range of 20-25°C is optimum for plantlet growth whereas for micro tuber induction, the temperatures required are generally lower (15-18°C) [4,5]. Although much work has been done on the effect of day and night temperatures on the developmental physiology and growth of the potato crop, relatively little is known about the effects of temperature on micro tuber production in different potato cultivars.

In the present study we evaluated the effect of two temperature regimes on micro tuber formation in five heat tolerant and heat sensitive genotypes of potato to standardise an *in vitro* system for early selection of heat tolerant clones.

Material and Methods

Plant material

Tuber samples of three Indian cultivars; Kufri Surya (KS), Kufri Himalini (KH) and Kufri Chandramukhi (KCM) and two advanced heat tolerant clones from CIP (Lima, Peru); CP 4398 and CP 4054, were drawn from the National Potato Breeding Program at the Central Potato Research Institute, for the present study (Table 1).

Well sprouted tubers of similar size were planted in potting mixture in 15 cm pots with five replications per genotype. Plants were grown for a month under non tuberizing conditions at 24°C under continuous light (600 $\mu Es^{-1}m^{-2}$) in a controlled environment chamber (Conviron, Model E-15, Canada). After 30 days, the plants were subjected to day/ night temperature treatments of 18/18°C and 24 /24°C with 12 h photoperiod.

Establishment of axenic cultures

Single nodal cuttings, 0.5 to 1.0 cm long, were obtained from etiolated sprouts of each cultivar/clone. These were disinfected with a mixture of 0.1% $HgCl_2$ and 0.1% sodium lauryl sulphate for 5 min, washed thrice with sterile water and cultured aseptically in test tubes with one segment per tube [1]. Each tube contained approximately 15 ml of semisolid (7 g agar L^{-1}) MS basal medium [6] with 3% sucrose. The cultures were incubated under 16 h/ day photoperiod of 38 µmol m^{-2} s^{-1} and at day and night temperatures of 22 ± 2°C.

Microtuberization

Eight week old axenic plantlets were sub cultured aseptically in 250 ml Erlenmeyer flasks containing 25 mL liquid MS medium with 3% sucrose, each flask containing 10 nodal cuttings. The cultures were incubated under 16 h/ day photoperiod of 38 µmol m^{-2} $s^{-1}$and at day and night temperatures of 22 ± 2°C for 30 days. For tuber induction, the culture medium was replaced with MS medium containing 8% sucrose and 10 mg L^{-1} BAP. Flasks were incubated in the dark at

***Corresponding author:** Anupama Singh, Central Potato Research Institute, Shimla, H.P.171001, India, Tel: 91 177 262 5073; E-mail: anupama_s8@yahoo.co.in

Received: July 05, 2016 **Accepted:** September 08, 2016 **Published:** September 11, 2016

two temperatures: 18°C and 25°C The experiment was conducted in a randomized complete block design (5 genotypes × 2 temperatures) with 4 replications. Each replication consisted of one flask with 10 nodal sections. Thus there were 40 plantlets per genotype per culture condition.

Data recording and statistical analysis

After sixty days of incubation in tuber induction media plantlets were taken out of the culture vessels, the medium was rinsed off and data were recorded for number, weight and total biomass of micro tubers. As the aim of the study was to compare the effect of temperature on microtuberization, the fixed effect model was used (for what?)

Results and Discussion

Microtuber formation occurred in all the genotypes at both the temperatures. The time required for micro tuber induction was almost the same for all genotypes but differed at two temperature regimes (Figure 1). Tuber formation was induced after 5 days at 25°C and after 8 days at 18°C. However, only three genotypes formed tubers when potted plants were given heat stress at 24°C with 12 h photoperiod (Figure 2).

Analysis of variance showed that mean squares due to genotypes for all parameters were significant at $p \leq 0.05$, indicating genetic variability among the genotypes for micro tuber number, weight, plantlet weight and total biomass. However, mean squares due to treatment were significant only for tuber number and average tuber weight, suggesting that the temperature influenced only these two attributes. These results are similar to those observed under field conditions, where moderately elevated temperature affects tuberization only and not the haulm growth [7]. The significant effect of genotype x treatment interactions for all the characters indicated differential response of genotypes to low and elevated temperatures (Table 2).

KH produced maximum number of tubers at lower temperature followed by KCM (Figure 3). At elevated temperature, tuber number was highest for CP 4054. Number of tubers was reduced significantly at elevated temperature in KH, KCM and KS. At 18°C KH had the highest average tuber weight and total tuber weight per flask, which was reduced significantly at elevated temperature. The tolerant genotypes showed an increase in tuber weight per flask at 25°C as compared to 18°C, with CP 4054 recording the highest tuber weight per flask as well as average tuber weight. These findings suggest

Table 1: List of potato clones used for in vitro heat tolerance assay.

Clone selection code	Variety/ CPRI Accession No.	Pedigree/Parentage	Heat tolerance
HT/92-621	Kufri Surya	Kufri Laukar x LT-1	Tolerant
SM/91-1515	Kufri Himalini	I-1062(E) X Bulk pollen	Sensitive
A-2708	Kufri Chandramukhi	Seedling 4485 X Kufri Kuber	Sensitive
CIP-397068.28	CP 4054	C90.266 X C93.154	Tolerant
CIP 304394.56	CP 4398	Shepody X LR 93.050	Tolerant

Figure 1: Tuber formation in potted plants 21 d after heat stress at 24°C in controlled environment chambers. (a) CP 4054 (b) CP 4398 (c) Kufri Surya (d) Kufri Chandramukhi (e) Kufri Himalini. Well sprouted tubers of similar size were planted in potting mixture in 15 cm pots with five replications per cultivar for each experiment. Plants were grown under non tuberizing conditions at 24°C under continuous light (600 $\mu Es^{-1}m^{-2}$) in a controlled environment chamber (Conviron, Model E-15, Canada). After 30 days, the plants were subjected to heat stress treatment of (24 /24 °C) with 12 h photoperiod. Tuber formation was observed 21 days after stress.

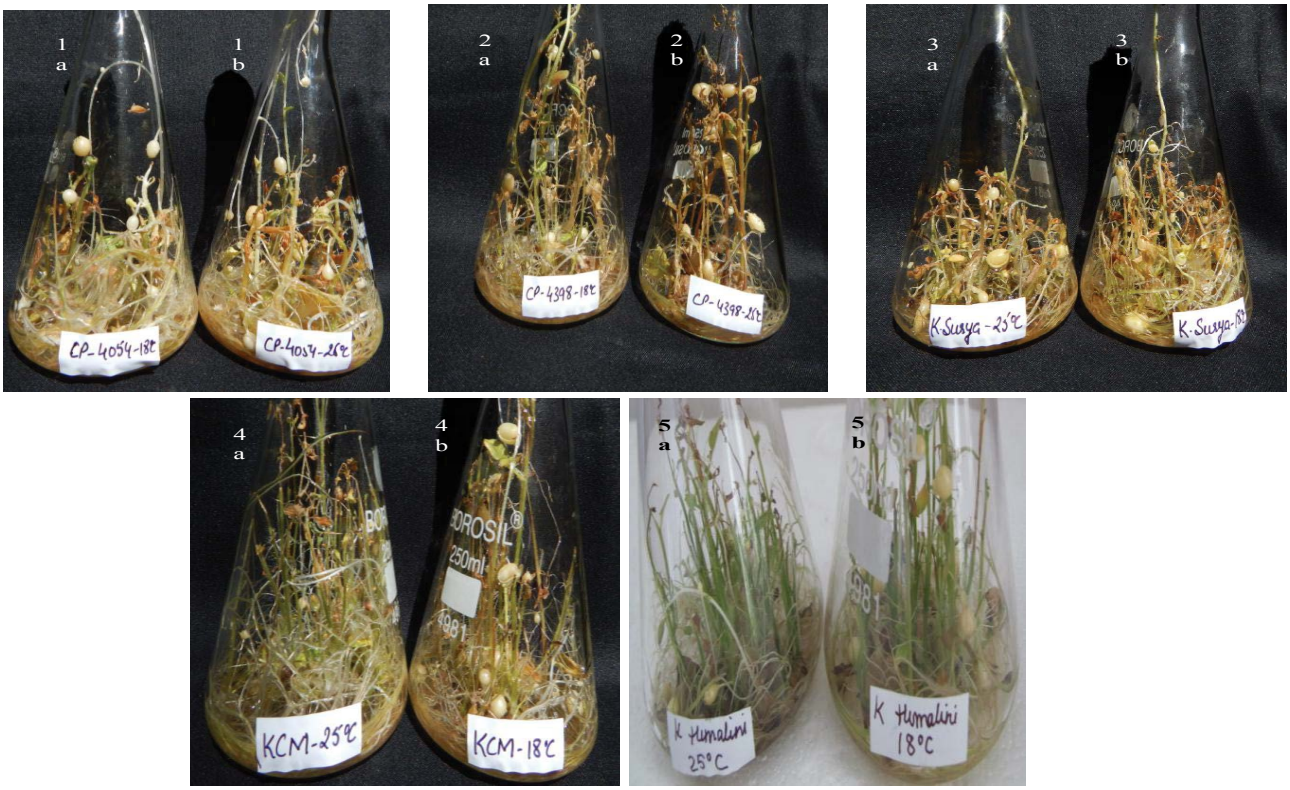

Figure 2: Microtuberization in heat tolerant and susceptible potato genotypes. 1. CP 4054 at (a) 18°C (b) 25 °C. 2. CP 4398 at (a) 18°C (b) 25°C .3. Kufri Surya at (a) 25°C and (b) 18 °C. 4. Kufri Chandramukhi at (a) 25°C and (b) 18 °C. 5. Kufri Himalini at (a) 25°C and (b) 18 °C.

Table 2: Analysis of variance of *in vitro* performance of 5 genotypes for various characters.

		Tuber number (per flask)	Tuber yield (mg per flask)	Plant weight (mg)	Total biomass (mg)	Average tuber weight (mg)
Source of Variation	df	MS				
Genotypes	4	23.65*	*0.86	10.289*	15.95*	*0.0039
Treatments	1	6.40*	0.009	0.139	0.223353	*0.0019
Interaction	4	24.27*	*1.44	1.635*	4.77*	*0.0041
Error	30	1.3	0.009	0.171	0.15629	0.0002
Total	39					
CD(G)		1.162	0.096	0.422	0.403242	0.013
CD(t)		0.735	0.061	0.267	0.255033	0.008
CD(GXT)		1.644	0.136	0.596	0.570271	0.018

* Significant at P<0.05

that the *in vitro* plantlets exhibited a similar level of tolerance to elevated temperature with respect to tuber induction as under natural conditions. Similar results were reported by Khan et al. [8] where *in vitro* assay of heat tolerant breeding population positively correlated to tuber family evaluation in field conditions in a semiarid tropical environment.

CP 4054 exhibited maximum fresh plant weight and total biomass both at 18°C and 25°C. Plant weight was higher at elevated temperature in all the genotypes except KCM. However, total biomass increased only in the tolerant genotypes KS, CP 4054 and CP4398 at 25°C. These results indicate that higher temperature has a greater influence on substrate partitioning in sensitive genotypes than in tolerant genotypes. Induction of tuberization even in heat sensitive genotypes at elevated temperature may be due to externally supplied BAP. Promotion of tuberization on cultured shoots by cytokinin as been demonstrated by many workers [1,5,9]. According to Garner and Blake [10] innate physiological responses may be evaluated best in growth regulator free culture media, when culture conditions mimic the major components of field environment. Therefore, standardizing *in vitro*

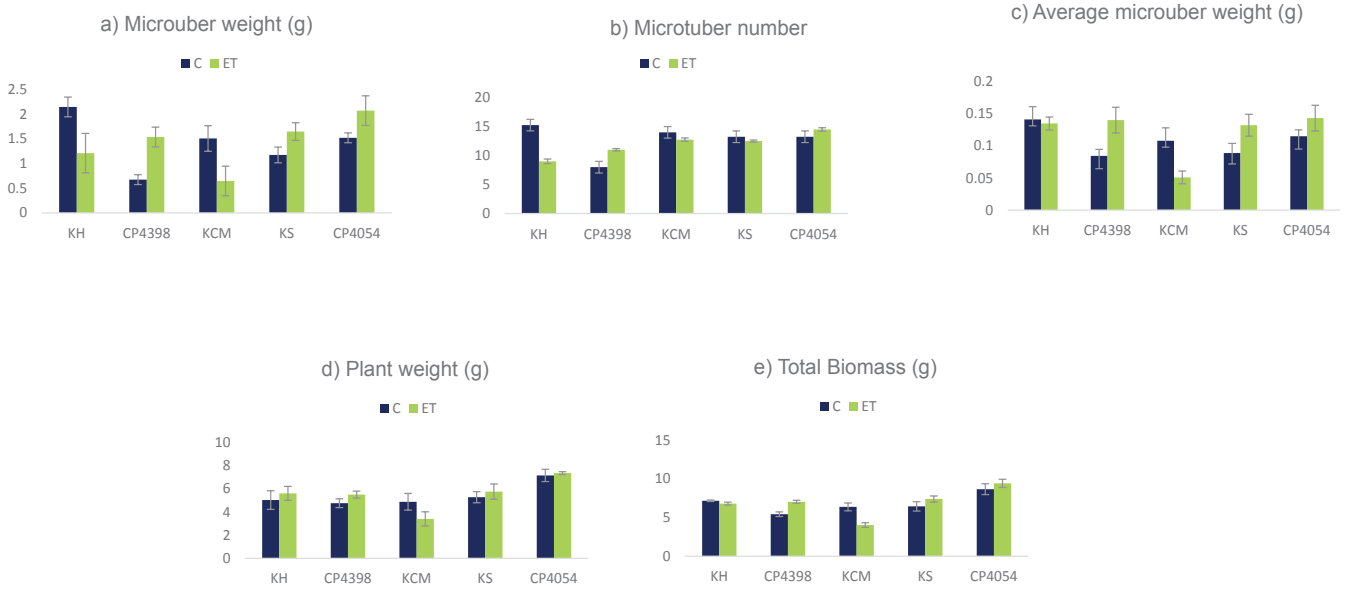

Figure 3: Effect of temperature on a) microtuber weight b) microtuber number c) average microtuber weight d) microplant weight e) total biomass in five potato genotypes.

assays for tuber induction in hormone free media may better reflect actual differences in thermotolerance in various genotypes [11].

Acknowledgements

The present study was supported by DST (WOS-A) grant no SR/WOS-A/LS-166/2012 and DST (SERB) grant no. YSS/2015/001995 from Department of Science and Technology, Government of India, to AS. The overall financial assistance and infrastructure provided by Indian Council of Agricultural Research is duly acknowledged.

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Author Affiliations

Top

Central Potato Research Institute, Shimla, H.P.171001 India