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Sucrose Hydrolyzing Enzyme Activities in Wheat Seedlings Under Heat Stress

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ABSTRACT: *Heat stress affects a wide spectrum of both biochemical and physiological responses within the plants. Crop heat tolerance can be enhanced by preconditioning the plants by exogenous application of osmoprotectants. Acid invertase and Sucrose synthase activity were evaluated under control (25±2°c), heat stress (35±2°c and 40±2°c) and interactive effect of heat stress and trehalose in six wheat (Triticum aestivum L.) genotypes (HD2967, PBW175, C306, PBW343, PBW621 and PBW590). Trehalose at concentration of 1mM and 1.5mM was applied at 7 days after sowing (DAS) followed by heat stress of 35±2°c (moderate) and 40±2°c (severe) on 8DAS for 8 hours. Higher acid invertase activity in shoot of tolerant genotypes appeared to be a characteristic for stress tolerance. Heat stress significantly decreased length and dry matter accumulationin shoot as well as in root. Trehalose application increased the activity of sucrose synthase as it play central role in sink strength and ameliorated the adverse effect of heat stress to some extent. The application of Trehalose @ 1.5mM concentration was found more effective as compared with 1mM concentration.*

Key words: *Heat stress, Triticum aestivum L., Trehalose, Sucrose hydrolyzing enzymes.*

INTRODUCTION

Wheat is an important cereal all over the world and is a staple food used in the form of different products. India, the second largest producer of wheat (*Triticum aestivum* L.) in the world has greatest success stories of Green Revolution and has made rapid progress in food grain production during second half of the 20th century [1]. Wheat is a premier cereal crop of Punjab, cultivated on 35.12 lac hectares in 2012-13 with production of 165.91 lac tonnes. Though Punjab is one of the smallest states of India representing 1.6 per cent of its geographical area and 2.6 percent of cropped area, with a total land area of only 0.33% of the world yet it contributes to 1% of rice and 2% of the wheat in the total world production vis-à-vis 42% rice and 55% wheat production in the country. Globally, wheat is the leading source of vegetable protein in human food, having higher protein content than maize or rice, the other major cereals. Constant efforts are, therefore needed to boost its production, to keep pace with ever increasing population, but these efforts are seriously being hampered by a number of abiotic stresses.

Terminal or late heat stress during the grain filling period of the normal as well as late planted wheat is one of the major environmental factors drastically reducing wheat production [2]. Heat stress due to increased temperature is an agricultural problem in many areas in the world. Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in wheat and other plants, which affect plant growth and development. Heat stress drastically reduces both yield and quality of wheat [3,4,5]. The adverse effects of heat stress can be mitigated by developing crop plants with improved thermotolerance using various genetic approaches.

High temperature regime induces activity of antioxidant enzyme compared to control temperature [6]. Heat shock proteins (HSPs) are synthesized to cope up with the heat stress and thus provide protection and repair to cellular damage caused by heat. Development of heat tolerance is correlated with HSP synthesis. Mutants defective in HSP synthesis are not able to acquire thermotolerance [7].

Trehalose is a soluble, non-reducing disaccharide of glucose. Three isomers exist: α, α -trehalose, α, β-trehalose and β, β- trehalose. Of these, only α, α -trehalose (1-O- (α -Dglucopyranosyl) α -glucopyranoside) is found in biological material. It is present in a large variety of organisms and can serve as reserve of carbohydrate and as a protectant in response to different stress conditions. Trehalose is

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known to protect membranes and macromolecules. Its accumulation has been implicated in allowing plants to tolerate stress, including heat-shock. Though, this role has been largely replaced by sucrose, trehalose does protect against desiccation in certain specialized resurrection plants.

MATERIAL AND METHODS

Plant material

Six genotypes of wheat (*Triticum aestivum* L.) viz. HD 2967, C306, PBW621, PBW590, PBW343 and PBW175 were obtained from Department of Plant Breeding and Genetics (PAU) and used for studies pertaining activity of acid invertase and sucrose synthase under control and different stress conditions.

With a view of evaluate the effect of heat stress on sucrose hydrolyzing enzymes viz acid invertase and sucrose synthase activity, only healthy seeds of six genotypes of wheat were used in experiments. Seeds were surface sterilized with 0.1 per cent mercury chloride for 2-3 min. to avoid any fungal infection during seed germination. Petri dishes were sterilized in oven at 100°C for 1 hour. Ordinary blotting papers were used in Petri dishes and were autoclaved before use. Twenty seeds were sown in each Petri-dish lined with circular blotting paper and incubated at 25±2°C temperature. On seventh DAS trehalose (1mM and 1.5mM) application was given followed by heat stress, incubated at 35°C and 40°C, for 8 hrs. Controlled Petri-dishes were placed in an incubator in which temperature was maintained at 25°C.

Acid Invertase

The enzyme activity of acid invertase was estimated by method as given by [8].

For this plant material was homogenized in extraction buffer (acetate buffer 0.1 M pH 5 containing 1% polyvinylpyrrolodine and 1mM EDTA) with the help of a chilled pestle and mortar by freezing and thawing. After complete homogenization, the supernatant was collected by centrifugation at 10,000 x g for 10-15 min. The resultant supernatant designated as crude enzyme extract, was stored in deep freeze at -10° C till it was further used (within 48 hr). The reaction mixture consisted of 0.6 ml of 0.2 M acetate buffer pH (4.8), 0.3 ml of sucrose 0.4 M and 0.1ml of enzyme extract. In the control tubes, sucrose was added only when enzyme preparation has been inactivated by boiling for 5min after incubation at 30° C for 30 min. Reducing sugars released were measured by dinitrosalicylic acid. One ml of 3, 5-dinitrosalicylic acid reagent was added to the reaction mixture. Tubes were placed in the boiling water bath for 10 min and then cooled at room temperature. Recorded the absorbance at 560 nm. The reference curve was prepared by using standard solution of glucose (100µg/ml). The activity was expressed as µmoles sucrose hydrolysed min^{-1} mg^{-1} protein.

Sucrose synthase

Sucrose synthase was estimated by method as given by [9].

For estimation of sucrose synthase enzyme activity fresh plant tissue (0.5g) was homogenized in a chilled pestle and mortar in 10 ml of 50mM HEPES-NaOH buffer (pH 7.5) containing 5 mM $MgCl_2$, 1 mM sodium EDTA,2.5mM dithiothretiol, 0.5mg/ml BSA and 0.05% (v/v) TritonX-100. It was made sure that no cell was left unbroken during homogenization (15,000 rpm for 20 min.). after centrifugation the supernatants were dialyzed for overnight against 3-4 litres of four times diluted extraction buffer using dialyzing membranes. The dialysate so obtained was used for enzyme assay.

UDP-glucose+fructose ↔ sucrose+UDP

Assay of sucrose synthase

For assaying the activity of this enzyme in sucrose synthesis, the reaction mixture (0.5 ml) contained 0.1 ml of 150mM fructose, 0.1 ml of 20 mM UDP-glucose, 0.2 ml of 50 mM HEPES-NaOH buffer and 0.1ml of enzyme extract. The reaction mixture was incubated at 37 C for 30 min. at the end of incubation period, 0.1 ml of 30% KOH was added. The contents were heated for 30 min in boiling water bath to destroy free fructose. Then cooled the contents to room temperature and added 1ml of resorcinol solution, along with 3ml of 30% HCl. The content were mixed thoroughly on a vortex mixer and incubated at 80°C for 10 min. the intensity of colour developed was read at 490nm against reagent blank. In the blank, UDP-glucose (20mM) was added after boiling reaction mixture for 30 min. the concentration of sucrose formed was calculated from the standard curve prepared by using sucrose standards (10-60µg).

Shoot length and dry matter

Shoot length and dry weight was recorded in centimeters (cms) and milligrams (mg) on 11^{th} day after giving the application of trehalose on 8 DAS at different concentrations (1 and 1.5 mM) in control and heat stressed seedlings.

Root length and dry matter

Root length and dry weight was recorded in centimeters (cms) and milligrams (mg) on 11th day after giving the application of trehalose on 8 DAS at different concentrations (1 and 1.5 mM) in control and heat stressed seedlings.

RESULTS

Acid invertase

The data shown in the Table 1. visualised that the activities of acid invertase enzyme was progressively by increasing the extent of stress so as to reach their maximum activities in leaves of all genotypes. The C306 genotype followed by PBW343 genotype showed maximum invertase activity and PBW175 showed minimum activity of invertase under control conditions. It was recorded in all the genotypes that the activity of enzyme invertase increased with the moderate (35±2°C) and severe (40±2°C) heat stress. The highest activity of invertase was recorded in all the genotypes when they were stressed under the severe heat stress of 8 hours. Such increase in the activity of enzyme due to stress was considered as one of the defence tools against stress. The activity of enzyme was recorded increased with the application of trehalose. The higher concentration (1.5mM) had increased more activity as compared to the lower concentration (1mM).

Sucrose synthase

Carbohydrates serve as a source of energy and act as signalling molecule in regulation of metabolic pathway under normal and stressed conditions. During germination, starch present in the endosperm is hydrolysed to glucose by the amylases and then converted to sucrose by the sucrose phosphate synthase. Sucrose, thus formed is then transported to the growing embryonic axis, where it is hydrolysed and the product so formed is used as energy source for growth of seedlings. Sucrose helps in the osmotic adjustment.

Presently, sucrose synthase activity was studied in the shoots of all the six selected wheat genotypes under control as well as under heat stressed conditions Table 2. The activity of sucrose synthase was recorded highest in HD2967 as compared to other genotypes under the control conditions. It was recorded in all the genotypes that the activity of enzyme sucrose synthase was increased with increase in temperature stress. The maximum activity of sucrose synthase was recorded in all the genotypes when they were stressed under 40±2°C (severe) for 8 hours. The activity of enzyme was increased with the application of trehalose in all the genotypes. But the more percent increase was recorded in C306 in control conditions with 1mM (0.482%) and 1.5mM (0.958%). Under severe heat stress for 8hrs it was more in C306 i.e. (0.712%) for both applications of trehalose.

Sucrose synthase (SS) showed higher catalytic activity and more resistance to heat stress. Biochemical conversion of sucrose to starch is one of the most important components of sink strength and can be determined by the catalytic activities of one or more of the enzymes involved in this pathway.

Shoot length and dry matter

Fig 1. depicts the shoot length of *T. aestivum* genotypes after 8hrs of heat stress along with controls and applications of trehalose (1mM and 1.5mM). Data in Table 3. depicts shoot dry matter after 8hrs of heat stress. In selected genotypes shoot length varied from 11 to 15 cms under controlled conditions. The elongation of shoot was recorded more in C306, PBW343, PBW621, and HD2967 at all temperature regimes i.e 25°C, 35°C and 40°C. The lesser shoot length was recorded in PBW175 and PBW590. The application of trehalose was very effective in different heat stresses. The 1.5 mM showed slight more effective results than 1 mM. Maximum shoot dry matter was recorded in C306 and PBW343 genotypes under control and stress conditions. Minimum shoot dry matter was recorded in PBW175 i.e 32 and 33 under control conditions. Effective increase in shoot dry matter were observed in all genotypes after application of trehalose under severe heat stresses. All the genotypes show maximum shoot length and dry matter at 25°C as compared to severe (40±2°C) heat stress.

Both plant growth and development are effected by temperature [10]. [11] reported that plant height decreased with increasing temperature which was also reported by [12]. [13] reported that high soil temperatures can reduce plant emergence. [14] reported that high temperature stress reduces plant height. [15] reported that both high low temperature decreases the rate of dry matter production an at extremes, can cause production to cease.

Root length and dry matter

The root length of *T. aestivum* genotypes under controlled as well as stressed conditions varied significantly under 8hrs of heat stress fig 2. The root length varied from 5to 7.2 cms under control

conditions. The maximum root length was recorded in C306, PBW621 and HD2967. Minimum root length was recorded in PBW175 and PBW590. With increase in temperature i.e 40°C (severe heat stress) decline in root length was recorded in C306, PBW343, PBW621 and HD2967 genotypes. It was observed that 40°C stress for 8hrs has inhibitory effect on root length in all six genotypes. Data in Table 4. shows root dry matter of different *T. aestivum* genotypes. Under 8hrs of heat stress PBW621 and HD2967 showed maximum accumulation of dry matter and lesser dry matter accumulation was recorded in PBW175 and PBW590. In all the genotypes decline in dry matter with increase in temperature was recorded. There was effective increase in both the root length and dry matter accumulation after the application of trehalose i.e 1mM and 1.5mM. The application of 1.5mM of trehalose was more effective than 1mM in both root length and dry matter accumulation in all the six genotypes.

More trehalose content found in wheat cultivars (*Triticum aestivum* L.) by [16]. Wheat itself also synthesized minor amounts of trehalose, which showed nearly a 150 % increase after heat stress. However, under both control and heat stress conditions trehalose content was higher in trehalose-pretreated leaves showing that exogenously-supplied trehalose was absorbed by wheat roots.

Table 1
Effect of trehalose on Activity of Invertase (μ mol sucrose hydrolysed/min/mg protein) of wheat genotypes under heat stress (8hrs) of 35±2°C and 40±2°C

Treatments	*Genotypes*					
	HD2967	*PBW175*	*C306*	*PBW343*	*PBW621*	*PBW590*
T1-Control at 25°C	0.036	0.032	0.043	0.040	0.034	0.030
T2-T1+(tre-1mM)	0.038	0.035	0.046	0.046	0.038	0.034
T3-T1+(tre-1.5mM)	0.042	0.037	0.051	0.055	0.040	0.036
T4- at 35°C	0.049	0.045	0.052	0.046	0.053	0.048
T5-T4+(tre-1mM)	0.052	0.046	0.056	0.049	0.056	0.050
T6-T4+(tre-1.5mM)	0.054	0.052	0.059	0.053	0.058	0.056
T7- at 40°C	0.055	0.048	0.053	0.058	0.058	0.049
T8-T7+(tre-1mM)	0.056	0.052	0.058	0.059	0.062	0.054
T9-T7+(tre-1.5mM)	0.059	0.056	0.060	0.062	0.066	0.056
CD 5%	V=0.00262, T=0.00321, V×T=0.00786					

Table 2
Effect of trehalose on Activity of Sucrose Synthase (μ mol sucrose Cleavege/min/mg protein) of wheat genotypes under heat stress (8hrs) of 35±2°C and 40±2°C

Treatments	*Genotypes*					
	HD2967	*PBW175*	*C306*	*PBW343*	*PBW621*	*PBW590*
T1-Control at 25°C	13.66	10.42	12.45	11.88	12.93	11.36
T2-T1+(tre-1mM)	13.75(0.655%)	10.66(2.251%)	12.66(1.659%)	11.89(0.084%)	12.95(0.154%)	11.38(0.176%)
T3-T1+(tre-1.5mM)	13.78(0.871%)	10.85(3.963%)	12.82(2.886%)	11.98(0.835%)	12.98(0.385%)	11.48(1.045%)
T4- at 35°C	15.98	13.82	15.45	14.58	15.62	14.11
T5-T4+(tre-1mM)	15.99(0.063%)	13.89(0.505%)	15.52(0.451%)	14.64(0.409%)	15.69(0.446%)	14.14(0.212%)
T6-T4+(tre-1.5mM)	16.06(0.498%)	13.95(0.932%)	15.58(0.834%)	14.72(0.951%)	15.72(0.636%)	14.18(1.269%)
T7- at 40°C	17.95	15.85	16.94	16.48	17.21	15.98
T8-T7+(tre-1mM)	17.98(0.167%)	15.89(0.252%)	16.96(0.118%)	16.52(0.242%)	17.23(0.116%)	16.04(0.374%)
T9-T7+(tre-1.5mM)	18.06(0.609%)	15.95(0.626%)	17.08(0.819%)	16.59(0.663%)	17.34(0.749%)	16.08(0.622%)
CD 5%	V=0.045, T=0.056, V×T=0.137					

Figures in bracket represent percent increase over control.

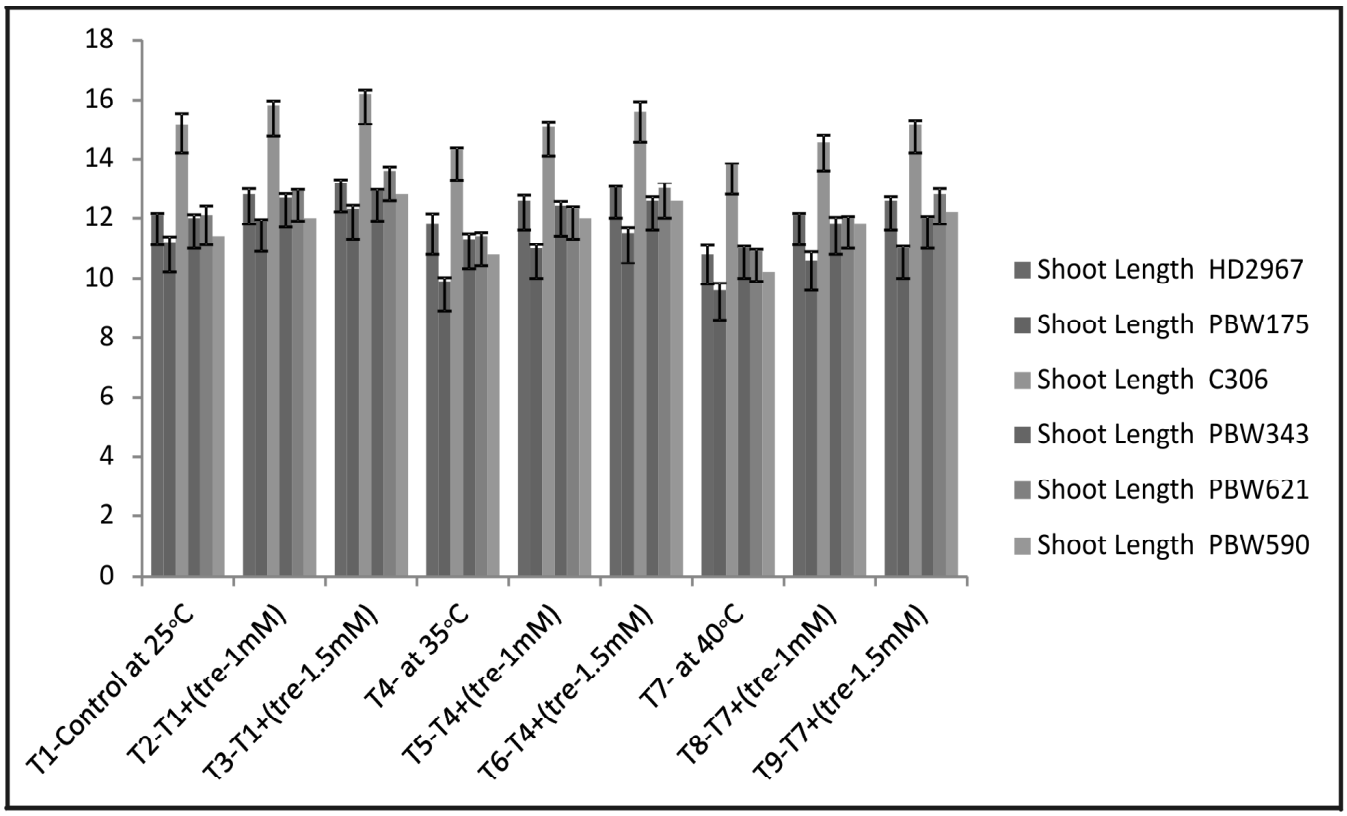

Figure 1: Effect of trehalose on Shoot length (cm) of wheat genotypes under heat stress (8hrs) of 35±2°C and 40±2°C

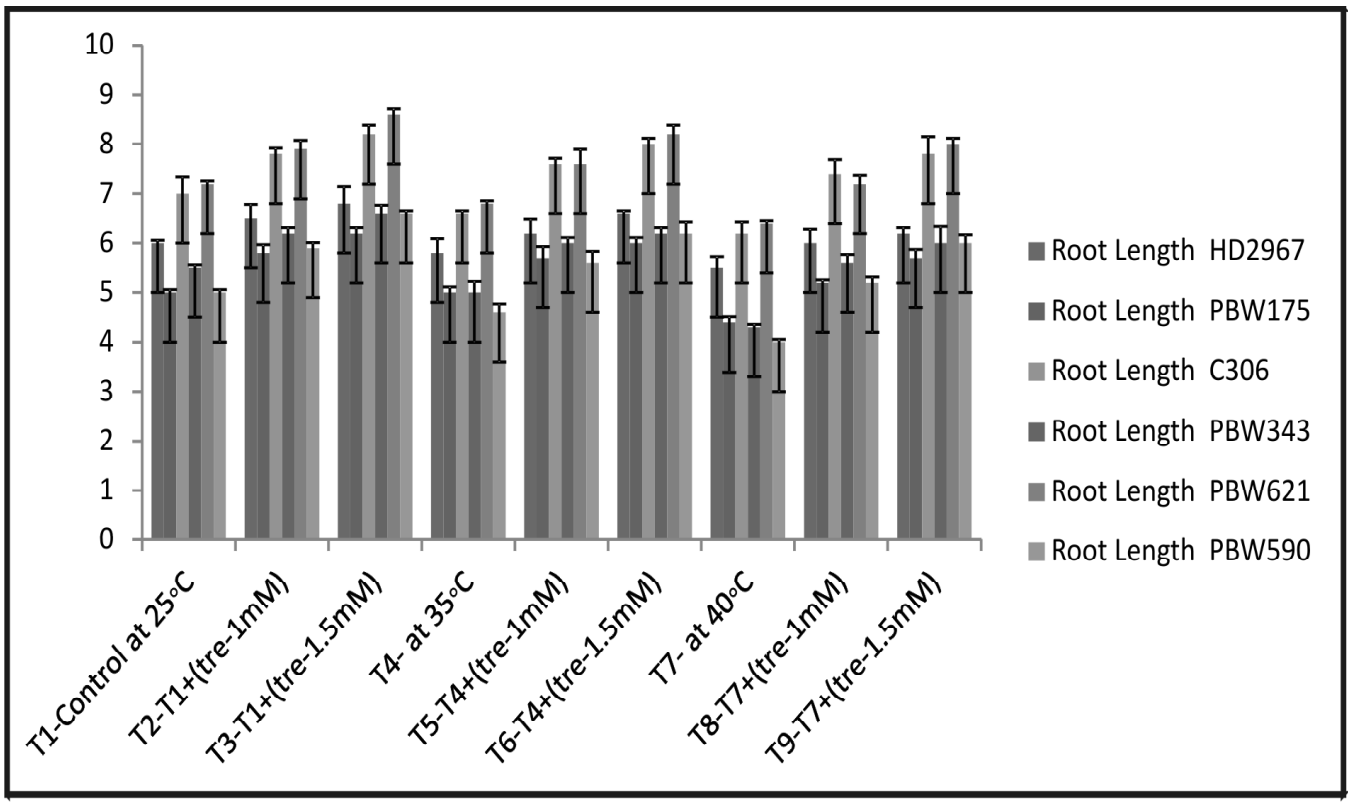

Figure 2: Effect of trehalose on Root length (cm) of wheat genotypes under heat stress (8hrs) of 35±2°C and 40±2°C

Table 3
Effect of trehalose on Shoot dry matter (mg) of wheat genotypes under heat stress (8hrs) of 35±2°C and 40±2°C

Treatments	*Genotypes*					
	HD2967	*PBW175*	*C306*	*PBW343*	*PBW621*	*PBW590*
T1-Control at 25°C	36	33	35	35	36	34
T2-T1+(tre-1mM)	36	34	36	36	36	34
T3-T1+(tre-1.5mM)	37	35	38	38	38	36
T4- at 35°C	34	33	33	34	35	32
T5-T4+(tre-1mM)	36	34	34	35	36	32
T6-T4+(tre-1.5mM)	37	34	35	36	37	33
T7- at 40°C	33	32	32	32	33	30
T8-T7+(tre-1mM)	33	33	32	32	33	30
T9-T7+(tre-1.5mM)	34	34	34	33	35	31
CD 5%	V=2.220, T=1.036, V×T=1.665					

Table 4
Effect of trehalose on Root dry matter (mg) of wheat genotypes under heat stress (8hrs) of 35±2°C and 40±2°C.

Treatments	*Genotypes*					
	HD2967	*PBW175*	*C306*	*PBW343*	*PBW621*	*PBW590*
T1-Control at 25°C	37	36	38	37	38	36
T2-T1+(tre-1mM)	37	36	39	37	39	37
T3-T1+(tre-1.5mM)	38	37	40	38	40	38
T4- at 35°C	35	34	37	36	37	35
T5-T4+(tre-1mM)	36	34	37	36	38	36
T6-T4+(tre-1.5mM)	37	35	38	37	39	37
T7- at 40°C	35	34	35	35	36	35
T8-T7+(tre-1mM)	36	35	36	36	36	35
T9-T7+(tre-1.5mM)	37	36	37	37	37	36
CD 5%	V=0.646, T=0.221, V×T=0.898					

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