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Studies on Biochemical Basis of Heat Tolerance in Sunflower (*Helianthus annus* L.)

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Abstract: A pot culture experiment was conducted in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore in summer viz., April – June, 2004 to identify parameters closely related to heat stress tolerance by induction response technique. Studies on physiological, biochemical and molecular basis of thermotolerance were carried out under pot culture condition. Based on the results of laboratory screening methods, selected tolerant (Morden and CO 4) and susceptible (EC 68415 and ARM 242) sunflower genotypes were used. The plants experienced mean temperature range of 27-33°C with maximum temperature ranging from 33 to 39° C in glasshouse conditions. Induction treatment significantly enhanced the soluble protein and NRase activity in all the four genotypes. Genotype differences in proline accumulation were observed in plants subjected to heat stress. The response of the genotypes to induction treatment was high showing three fold increase in proline content over absolute control. The non induction treatment however showed only two fold increase over control. Lipid peroxidation was significantly enhanced by temperature treatments. Compared to tolerant genotypes, the susceptible genotypes, ARM 242 and EC 68415 recorded increased per cent (82 and 75 µmoles respectively) of MDA content. CO 4 and Morden, however, showed lesser per cent of 22 and 36 respectively.

Key words: *Lipid peroxidation, soluble protein, Proline, Genotypes*

INDRODUCTION

Stress is the result of the sum of damages in all cellular components (lipids, proteins and nucleic acids). Each kind of stress induces production of reactive oxygen species, especially singlet oxygen and other free radicals, which are known to alter DNA, destroy the functions of proteins and induce lipid peroxidation. Plants have evolved diverse strategies of acclimatization and tolerance to cope with adverse environmental conditions. This includes accumulation of compatible solutes like glycinebetaine, proline and sugar alcohols. It is well known that the concentration of proline increases in a large variety of plants under stress, upto 100 times the normal level, which makes upto 80 per cent of the total amino acid pool. The function of proline in stressed plants is often explained by its property as an osmolyte, to balance the tissue water status. Due to its action as a quencher of singlet oxygen and scavenger of OH^- radicals, proline is able to stabilize proteins, DNA and membranes. Accumulation of proline rich proteins and particularly proline residues in protein provides additional protection against oxidative stress. With these ideas in view a pot culture experiment was conducted in sunflower to find out the parameters closely related to heat stress tolerance by induction response technique.

MATERIALS AND METHODS

A pot culture experiment was conducted at the glasshouse of Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore in summer viz., April – June, 2004 to identify parameters closely related to heat stress tolerance by induction response technique. Studies on physiological, biochemical and molecular basis of thermotolerance were carried out under pot culture condition. Based on the results of laboratory screening methods, selected tolerant (Morden and CO 4) and susceptible (EC 68415 and ARM 242) sunflower genotypes were used. The plants experienced mean temperature range of 27-33°C with maximum temperature ranging from 33 to 39° C in glasshouse conditions.

The sprouted seeds were subjected to gradual increased temperature induction. After gradual induction, lethal temperature of 53°C was given and the seedlings were transplanted in pots. The experiment was laid out in FCRD design replicated five times. The treatments included were absolute control, Induced - 35°C-1h → 40°C-1h → 45°C-2h (sub lethal) → 53°C - 1h (lethal) and non induced - direct exposure of sprouted seeds to 53°C.

Pot mixture was prepared by mixing thoroughly two parts of soil and one part each of well decomposed farmyard manure and sand and filled in pots. Seedlings of sunflower genotypes were

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transplanted at five seedlings per pot and later were thinned to a uniform population of three plants per pot. A basal dose of N, P and K were applied at the rate of 200, 100 and 100g per pot in the form of urea, super phosphate and potash respectively. Normal plant protection measures were adopted throughout the crop growth period.

Soluble protein content of leaf was estimated following the method and expressed in mg g^{-1} fresh weight [12]. NRase activity of leaf was estimated as per the method described and expressed in µ g NO_2 g^{-1} fresh weight h^{-1} [13]. The leaf portion was used for estimation of proline and the content was expressed in µg g^{-1} fresh weight [2]. The ECW was estimated by using potassium dichromate and expressed in µ g per dm^2 [6]. Lipid peroxidation was estimated by thiobarbituric acid (TBA) –trichloroacetic acid (TCA) colour reaction [5].

RESULTS AND DISCUSSIONS

The levels of total as well as soluble proteins are altered in plants growing under water stress environments compared with plants growing under non stress conditions. Rubisco accounts for about 50 per cent of soluble protein content. In the present study, high temperature stress significantly reduced the soluble protein content in all the genotypes. The genotypes CO 4 and Morden recorded higher soluble protein under both the situations (Table 1). Poor performance was observed in ARM 242 under non induction stress. These results corroborated the reports and it was revealed that soluble protein contents were more affected in susceptible than in tolerant genotypes [9]. A decreased level of the total as well as the soluble protein contents in water stressed tissue was due to more degradation of proteins as well as the overall inhibition in protein synthesis under water stress [11].

N Rase Activity: In the present investigations, NRase activity (Table 2.) was monitored to understand the relative adaptability of sunflower genotypes under induction and non induction stress conditions. Both the treatments affected NRase activity. The effect was more pronounced in susceptible genotypes, ARM 242 and EC 68415 with per cent reductions of 28.23 and 26.55 respectively. The tolerant genotypes CO 4 and Morden, however, showed only 13.31 and 14.24 per cent reductions over control under non induction stress treatment. NRase activity was significantly enhanced by induction treatments. The genotype CO 4 again showed higher activity (0.850) followed by Morden (0.828). The genotype ARM 242 recorded lower activity of 0.754 than the others. Nitrogen assimilation and photosynthetic efficiency were reduced in water stressed plants mainly owing to the decreased activities of the key enzymes involved in these processes. NRase, the prime enzyme in the N assimilation process, is markedly inhibited by water stress. The reduction in activity might be either due to reduction in enzyme level [1] or due to inaction of enzyme dictated by stress conditions [13]. The reduced NRase activity was due to decrease in nitrate content caused by reduced nutrient uptake under stress condition in chickpea. The decrease in the enzyme activity might have resulted in changes in protein structure and synthesis under stress condition.

Proline Content: In present investigation, non induction temperature stress caused higher Proline accumulation (Table 3) which was in the range of 150.4 µg (ARM 242) to 300.6 µg (CO 4). The increase over control was 49, 66, 106 and 110 per cent in ARM 242, EC 68415, Morden and CO 4, respectively. The response of the genotypes to induction treatment was very high showing three fold increase in proline content. Similar results in *Vigna radiata* (L.) seedlings subjected to heat shock

Table 1: Pretreatment of sprouted seeds with induction and non induction temperature stress on soluble protein content (mg g^{-1} fr. wt.) of sunflower genotypes (10 days old).

	Soluble protein (mg g^{-1} fr.wt.)			
Genotypes / treatments	Control	Induced	Non induced	Mean
Morden	6.28	5.80 (7.60)	5.33 (15.12)	5.80
CO 4	6.73	6.43 (4.45)	5.85 (13.08)	6.33
EC 68415	6.19	5.46 (11.79)	4.65 (24.87)	5.43
ARM 242	5.95	5.22 (12.26)	4.23 (28.91)	5.13
Mean	6.28	5.72	5.01	
		SEd		CD(0.05)
Genotypes		0.006		0.014
Treatments		0.006		0.012
Genotypes x treatments		0.012		0.024

Table 2: Pretreatment of sprouted seeds with induction and non induction temperature stress on NRase activity (µ moles NO_2 g^{-1} h^{-1}) of sunflower genotypes (10 days old).

	NRase activity (µ moles NO_2 g^{-1} h^{-1})			
Genotypes / treatments	Control	Induced	Non induced	Mean
Morden	0.928	0.825 (11.10)	0.796 (14.24)	0.849
CO 4	0.946	0.850 (10.15)	0.820 (13.31)	0.872
EC 68415	0.885	0.773 (12.46)	0.650 (26.55)	0.769
ARM 242	0.875	0.754 (13.82)	0.628 (28.23)	0.752
Mean	0.908	0.801	0.723	
		SEd		CD(0.05)
Genotypes		0.0009		0.002
Treatments		0.0008		0.002
Genotypes x treatments		0.001		0.003

(Values in the parentheses indicate per cent reduction over control)

Table 3: Pretreatment of sprouted seeds with induction and non induction temperature stress on proline content (µg g^{-1} fr. wt.) of sunflower genotypes (10 days old).

	Proline content (µg g^{-1} fr.wt.)			
Genotypes / treatments	Control	Induced	Non induced	Mean
Morden	125.30	368.58 (194)	259.30 (106)	251.06
CO 4	143.16	428.97 (199)	300.65 (110)	290.92
EC 68415	118.56	250.34 (111)	197.52 (66)	188.80
ARM 242	100.78	210.53 (108)	150.43 (49)	153.91
Mean	121.95	314.60	226.97	
		SEd		CD(0.05)
Genotypes		0.98		2.03
Treatments		0.85		1.76
Genotypes x treatments		1.70		3.52

(Values in the parentheses indicate per cent increase over control)

Table 4: Pretreatment of sprouted seeds with induction and non induction temperature stress on epicuticular wax content (µg cm^{-2}) of sunflower genotypes (10 days old).

	Epicuticular wax content (µg cm^{-2})			
Genotypes / treatments	Control	Induced	Non induced	Mean
Morden	0.82	0.92 (12.19)	0.87 (6.09)	0.87
CO 4	0.85	0.97 (14.11)	0.93 (9.42)	0.91
EC 68415	0.83	0.88 (6.02)	0.85 (2.41)	0.85
ARM 242	0.79	0.82 (3.79)	0.80 (1.27)	0.80
Mean	0.82	0.89	0.86	
		SEd		CD(0.05)
Genotypes		0.0005		0.001
Treatments		0.0004		0.0009
Genotypes x treatments		0.0009		0.0018

(Values in the parentheses indicate per cent increase over control)

Table 5: Pretreatment of sprouted seeds with induction and non induction temperature stress on lipid peroxidation (µ moles MDA g^{-1} fr.wt.) of sunflower genotypes (10 days old).

	Lipid peroxidation (µ moles MDA g^{-1} fr.wt.)			
Genotypes / treatments	Control	Induced	Non induced	Mean
Morden	1.36	1.56(14.27)	1.85(36.03)	1.59
CO 4	1.26	1.40(11.11)	1.54 (22.22)	1.4
EC 68415	1.44	2.00 (36.00)	2.53 (75.65)	1.99
ARM 242	1.5	2.10 (40.00)	2.74 (82.66)	2.11
Mean	1.39	1.76	2.16	
		SEd		CD(0.05)
Genotypes		0.004		0.009
Treatments		0.003		0.008
Genotypes x treatments		0.007		0.016

(Values in the parentheses indicate per cent increase over control)

at 40°C, 50°C and 60°C was reported and the reason attributed that increase in sugar content was by activation of starch degrading enzymes and that the increase in proline may be due to temperature stress [4]. Synthesis of proline and its enormous accumulation in plants resulting in high temperature may be due to stimulation of proline synthesis from glutamate by loss of feed back inhibition, decline in proline oxidation or due to decrease of its incorporation into protein [7,10].

Epicuticular Wax (EW): Genotypic differences in epicuticular wax (EW) were significant (Table 4). Both high (CO 4 and Morden) and low EW (ARM 242 and EC 68415) among genotypes could be identified. Non induction temperature stress caused a slight increase in EW with significant differences among the genotypes. Induction treatment, however, resulted in a remarkable increase in EW in all the genotypes. The size of wax deposit on plants grown at high temperature was positively correlated with temperature acclimatization in *Eragrostis* [8]. Cuticular transpiration is the major source of water loss from the plant when stomata are closed during stress. Upon heat stress, the EW content increased in all the genotypes but the increase was higher in CO 4 (0.93) than in ARM 242 (0.80) under non induction stress.

Lipid Peroxidation: Lipid peroxidation was significantly enhanced (Table 5) under non induction temperature stress condition. Compared to tolerant genotypes, susceptible genotypes ARM 242 and EC 68415 recorded higher MDA content of 2.74 and 2.53 µ moles of MDA g^{-1} with an increase of 82 per cent and 75 per cent respectively over control. The genotype, CO 4, however, showed lesser per cent increase of 22 over control than the others. Induction treatment reduced the effect of lipid peroxidation in all the genotypes. High MDA content indicates membrane lipid peroxidation [14]. Under optimum temperature conditions, plants maintain a balance between producing and scavenging active oxygen species [3]. Heat stress may disturb this balance and promote lipid peroxidation, either by increasing the production of active oxygen or by decreasing the O_2 radical scavenging ability in cell [3].

Conclusion: Induction treatment significantly enhanced the soluble protein and NRase activity in all the four genotypes. Genotype differences in proline accumulation were observed in plants subjected to heat stress. The response of the genotypes to induction treatment was high showing three fold increase in proline content over absolute control. The non induction treatment however showed only two fold increase over control. Lipid peroxidation was significantly enhanced by temperature treatments. Compared to tolerant genotypes, the susceptible genotypes, ARM 242 and EC 68415 recorded increased per cent (82 and 75 µmoles respectively) of MDA content. CO 4 and Morden, however, showed lesser per cent of 22 and 36 respectively.

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