Genotypic responses to seedling stage heat stress in *Brassica napus* and its mitigation through pretreatment with salicylic acid and abscisic acids

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ABSTRACT

Heat stress is most often associated with the synthesis of a highly constitutive family of stress response proteins known as heat shock proteins (HSPs) and accumulation of reactive oxygen species (ROS). The present communication reports our attempt to screen *Brassica napus* germplasm for heat tolerance and to analyze the impact of salicylic acid (SA) and abscisic acid (ABA) pretreatment on three week old seedlings of *B.napus*. Pretreatment of SA and ABA markedly increased percent survival. Paired T-test analysis showed that SA contributed more towards heat tolerance than ABA. The protein profile by SDS-PAGE showed intensification of bands under heat stress and appearance of some new bands after SA pretreatment.

INTRODUCTION

Agricultural yield potential is under continuous threat due to projected rise in global temperature beyond threshold. Transient elevation in temperature 10-15^{o}C above ambient is considered heat stress or heat shock. The deleterious effects of heat stress are associated with decline in percent survival and disturbances in metabolic cascade of plants in spite of existences of various defense mechanisms. Survival under stress conditions is assessed by the capability of plant to instigate biochemical changes for adaptation. In addition, application of some phenolic compounds (SA) and hormones (ABA) are reported to play a pivitol role in enhancing heat tolerance. Heat stress is most often associated with the synthesis of a highly constitutive family of stress response proteins known as HSPs. The role of antioxidant enzymes viz. peroxidase enzyme (POD) and catalase (CAT) has been well documented in adaptation of plants to such environmental stress. Thorough knowledge regarding biochemical reactions viz. photosynthesis and lipid peroxidation via production of ROS and changes in antioxidant enzymes, is required for development of thermotolerant varieties. The present study was thus undertaken to screen *Brassica* germplasm for heat tolerance and to reveal the impact of SA and ABA pretreatment on three week old seedlings of *B.napus*.

MATERIALS AND METHODS

Growth conditions and determination of LT_{50}

Three weeks old seedlings raised under 16 hours light and 8 hours dark period at 25±2^{o}C in temperature control room were used for heat stress treatments. LT_{50} determination was carried out on standard cultivar, GSL-1 by exposure to different temperature range (40^{o}C -50^{o}C) for different time durations (5-10 hrs). Twenty six genotypes were evaluated for thermotolerance at LT_{50} (48^{o}C for 5.5 hours). Seedling survival (regrowth) was assessed on the basis of number of seedlings survived 24 hours after heat shock. Seedlings were subjected to SA @ 50µM/L and ABA @ 50µM/L pretreatments (spray 24 hours before heat shock). Control plants were sprayed with distilled water. Sampling for biochemical analysis was done 24 hours after pretreatment followed by heat shock treatment and after 24 hour of recovery.

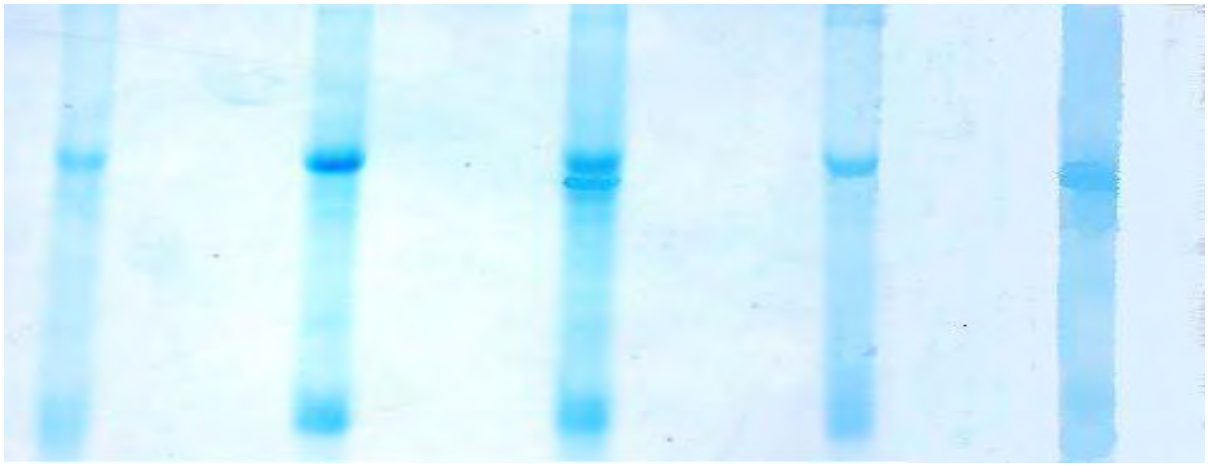
For extraction of chlorophyll, seedlings (0.1g) were homogenized in 2 ml of 80% acetone following standard method. Lipid peroxidation was estimated in terms of malondialdehyde (MDA) formed using its extinction coefficient of 155mM^{-1}cm^{-1} following method of Dhindsa *et al* (1981).Peroxidase and CAT activity activity was assayed by method of Claiborne & Fridovich (1979) and Aebi (1983) respectively. Proteins and sugars were estimated following standard methods. Protein profile analyses was carried out by SDS-PAGE. Out of three temperatures selected for acclimation i.e. 40^{0}C, 42^{0}C and 44^{0}C, 40^{o}C gave the best results and was considered for acclimation studies. GSL-1 genotype was acclimated at 40^{o}C for 2 hours prior to heat shock and then evaluated for the protein profile by SDS-PAGE.Tissue (0.25 g) was

extracted in phosphate buffer pH 8.0 having 0.4% NaCl and then centrifuged for 30 mins at 10,000 rpm. The supernatant was used for performing SDS-PAGE.

RESULTS AND DISCUSSION

The genotypes were classified mainly into 2 classes: Resistant (>60% survival) and susceptible (<50% survival) on the basis of regrowth potential. The mean percent survival value of resistant genotypes was about 4 fold greater than the susceptible genotypes.High percent survival and comparative rise in chlorophyll content as observed in the present study could be expected due to presence of HSPs that are protecting cellular membrane from heat damage (Hartl 1996). Decrease in chlorophyll content in susceptible genotypes could be due to damage to photosynthetic machinery. This parameter is an indicator of free radical damage to cell membranes under stress conditions which leads to decreased cell membrane stability. Lipid peroxidation of plasma membrane of plant cells was estimated in terms of MDA formed. After heat stress, MDA concentration increased in both sensitive and resistant genotypes, though increase in MDA was more pronounced in sensitive as compare to resistant genotypes. Induction in POD activity and a trend of decreased CAT activity after heat stress was observed as compared to control. A similar trend following heat stress exposure has been reported in *Cicer arietinum* (Chakroborty and Tongden 2005).The increase in POD activity suggests an accelerated production of activated O_2 species in tissue. High POD activity could be associated with chloroplast degeneration induced by increase of superoxide radical resulting from decline CAT activities. The decrease in CAT activities would results in H_2O_2 accumulation, which can react with O^{2-} to produce hydroxyl-free radical. The hydroxyl free radicals can directly attack unsaturated fatty acids of membrane lipids to induce lipid peroxidation in the cell. The decrease in CAT and increase in POD indicates that the scavenger ability in the cells of leaves was inhibited under heat stress conditions.Decrease in total proteins and increase in total soluble sugars under heat stress was observed. Protein content varied with types of cultivar and ranged from 16.00 - 49.18 mg/g fresh wt. seedlings but following heat stress ranged from 9.44 - 36.76 mg/g fresh wt. seedlings. Increased sugar accumulation in seedlings conveys that the carbon supply is probably not a limiting factor under stress. Salicylic acid pretreatment @ 50µM/L resulted in antagonizing the effects of heat stress as evidenced by increase in survival, chlorophyll content, proteins, sugars, POD activity and decrease in lipid peroxidation and CAT activity. Increased POD activity is related to decrease in oxidative damage caused by ROS which leads to increased thermotolerance of the plant. Pretreatment of ABA @ 50µM/L was found to be most effective. Abscisic acid was comparatively less effective against heat stress as compare to SA, although it has similar effect on biochemical parameters except on antioxidant enzymes. The activities of POD was unaffected whereas by ABA pretreatment that of CAT increased.Genotypes showed 18% and 14% increase in % survival by SA and ABA pretreatment respectively. Chlorophyll data supported the plant regrowth data i.e. increase in % survival was associated with less decrease of chlorophyll content. Increase in protein content by SA and ABA was 18% and 15% respectively. Both SA and ABA pretreatment elevated the sugar level by 10% and 8%.

Electrophoretic pattern of heat stress, SA, ABA and heat acclimation pretreatment showed the intensification of bands after heat stress and appearance of additional bands after SA pretreatment as compared to control. This could be expected due to induction of HSPs after heat stress and enhancement of HSPs induction after SA and heat acclimation pretreatment. The involvement of SA in induction of HSPs under stress conditions has also been documented in tobacco plants (Burkhanova *et al* 1999). However, no change in protein banding pattern was observed on ABA pretreatment.



I II III IV V

Fig1.

I Control II Heat stressed III SA pretreated IV ABA pretreated V Heat acclimated

Paired T-test values for various biochemical parameters between HS-SA, HS-ABA and SA-ABA were also calculated (Table 1). SA pretreatment values were higher than ABA values although both SA and ABA were effective (significant at α =1% and α = 5%). The results indicated that SA contributes more towards heat stress tolerance than ABA.

Table 1: Paired T-test values for *B.napus*

	B.napus		
	HS-SA	HS-ABA	SA-ABA
Percent survival	8.696**	7.535**	0.853
Lipid peroxidation	5.066**	4.908**	0.717
Chlorophyll content	6.258**	5.424**	0.658
Peroxidase activity	7.268**	1.938	6.557**
Catalase activity	0.404	9.955**	4.579**
Total protein content	12.594**	10.345**	1.750
Total sugar content	14.788**	8.533**	3.089**

** Significant at α = 1%

* Significant at α = 5%

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