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Heat treatment affects regeneration, protein expression and genetic make-up of *Vigna aconitifolia* (Jacq.) Marechal

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ABSTRACT

High temperature affects diverse physiological, biochemical and molecular processes including gene expression and genetic stability. To demostrate this, primary leaves of moth bean were harvested from eight days old seedlings followed by heat shock treatment at 37, 42, 47 and 52 °C for 10, 20 and 30 min. The response of tissue for callusing was comparatively early in explants treated at 37, 42 and 47 °C for 10 min. However, regeneration was negatively affected by most of the heat treatments. A few polypeptides were found to be up regulated as well as down regulated with heat shock treatments. Some proteins were specifically regulated at higher temperatures of 42° and 47 °C. Two polypeptides were also up regulated in the protein profiling of callus; however, these were different than the once observed in protein profiling of leaf explant immediately after heat treatment. Moreover, these bands were found in only one treatment each, *viz*. one in 20 min of 47 °C (100 kD) and another in 30 min of 47 °C (36.7 kD). These may be expected to be consequence of genetic change (mutation). RAPD analysis further revealed that plantlets obtained at 47 °C generated a novel band indicating mutagenic effect of heat shock treatment.

Introduction

Climate change in the last years resulted in global average temperature increases between about 0.15 and 0.3 °C per decade from 1990 to 2005 with a mean of 0.2 °C per decade. A transient elevation in temperature, usually 10–15 °C above ambient, is considered heat shock or heat stress [1]. At the cellular and molecular level, heat shock, transitory or constantly, leads to adverse outcomes in plant cell functions, including alterations in cellular composition of membrane fluidity and permeability, enzyme activity, metabolism, production of reactive oxygen species, and gene expression [2]. Moreover, it can alter cellular homeostasis by causing protein denaturation and lipid peroxidation [3]. It is envisaged that heat stress may destabilize bio-molecules including DNA hence induce DNA lesions. A massive data dealing with heat stress in plants has been generated but the information on mutation through high temperature induced DNA damage is infrequent. However, stress-induced directed or adaptive mutations have been documented in many organisms like *Escherichia coli* and *Saccharomyces cerevisiae*.

Being a native crop of hot arid region moth bean [*Vigna aconitifolia* (Jacq.) Marechal] present a very efficient system to study the effect of temperatures on both gene action and DNA stability. Globally, it grows throughout the tropic, sub-tropics and warm areas. It is endowed with few morphological and physiological features and has evolved into one of the extremely drought and heat tolerant crop [4]. Thus, it constitutes an important food legume of sustainable economic importance in hot regions. It is a good source of lysine and leucine amino acids. In moth bean, mutation breeding in addition to local selection is only way to create variability and to develop new genotypes. However, procedures available for mutagenic treatment to explant like gamma rays or chemicals warrant availability of gamma chamber or handling of hazardous chemicals, respectively.

Therefore, search for simpler mutation methods is needed to make the induced mutagenesis procedure easy and available to vide range of laboratories. Heat shock treatment is one such option

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that not only influences regeneration mostly enhancing it also destabilizes genome. Keeping this in view, the present investigation was carried out to determine the effect of heat treatment on regeneration potential, gene expression and genetic stability in moth bean, a drought tolerant pulse crop amenable to various tissue culture techniques. The present study is expected to provide information to be used in manipulating the regeneration of *in vitro* culture of the moth bean, provide alternative method to study effect of high temperature and for mutation induction.

Materials and methods

Plant material, seedling growth and heat shock treatments

Seeds of moth bean cv. RMB – 225 were surface sterilized by 0.1% $HgCl_2$ and grown in test tubes on filter paper bridges in a growth chamber at 27 °C and a 12-h light cycle. Heat treatments were given to the aseptically grown fully expanded primary leaves (explants) harvested from 8 d old seedlings. Before heat treatment proximal and distal ends of harvested leaves were cut to avoid preexiting meristem. For heat treatment, harvested leaves were placed in autoclaved deionised water maintained at respective temperature (37, 42, 47 and 52 °C) in a thermo shaker. The leaf explants were collected from heat shock treated (37, 42, 47 and 52 °C for 10, 20 and 30 min) and control plantlets (27 °C). Treated and control explants were cultured at temperature of 27 ± 1 °C and 14-h photoperiod (fluorescent tubes, the average irradiance of 68 μmol m^{-2} s^{-1} at bench level) on MS medium supplemented with 3 mg dm^{-3} benzylaminopurine (BAP) and 1 mg dm^{-3} indoleacetic acid (IAA). Survival percentage of explants, number of days to callus induction, shoot emergence and number of shoots produced per explant were recorded periodically. Finally, only the number of explants surviving after the heat shock treatments was considered for recording of observations and further analysis. Any of the cultures destroyed by contamination was supplemented by additionally maintained cultures for each of the treatment.

Protein profiling

Total proteins were extracted from explants immediately after heat shock treatment and after callus induction (45 d). A total of 500 mg of tissue was ground in 0.8 cm^3 of buffer [50 mM Tris-HCl, urea (8%), sodiumdodecyl sulfate (SDS; 2.0%), glycerol (10%), β-mercaptoethanol (5%)] using chilled mortar and pestle. The ground material was collected in Eppendorf tube (2 ml) and was centrifuged at 25 000 *g* for 10 min. Samples denatured by boiling for 5 min were loaded in 12% polyacrylamide gel. The gels were stained by Coomassie brilliant blue R250 and silver [5].

Table 1
Mutagenic effect of heat treatment demonstrated by appearance of novel band in regenerated plantlets from treated explants in month bean cv. RMB – 225.

Primer	Sequence (5′-3′)	Total no. of bands	Presence of novel band
OPG-2	GGCACTGAGG	3	0
OPG-6	GTGCCTAACC	3	0
OPG-8	TCACGTCCAC	8	0
OPG-9	CTGACGTCAC	4	0
OPG-11	CAGCTCACGA	2	0
OPG-12	CAGCTCACGA	5	0
OPG-17	ACGACCGACA	5	1

DNA isolation and RAPD

Random amplification of polymorphic DNA (RAPD) analysis was conducted using purified DNA from nine plantlets one each representing all the eight treatments (37 °C-10, 20 and 30 min, 42 °C-10, 20 and 30 min and 47 °C-10 and 20 min) and control. Genomic DNA of nine plantlets was isolated by the method of Doyle and Doyle [6]. The conditions of RAPD were set according to Jangid et al. [7] for moth bean with 7 decamer primers (Operon Technology, USA; Table 1) selected based on earlier studies [8,9]. The PCR was carried out in *Biometra* (Germany) thermocycler with cycling parameters: initial denaturation at 94 °C for 5 min, 42 cycles of denaturation at 94 °C for 1 min, primer annealing at 38 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 5 min. After separation of amplicons in 1.5% agarose electrophoresis with a 200 bp ladder, the gel was viewed under UV radiation, photographed and the appearance of novel bands was recorded.

Results and discussion

The effect of heat shock treatments on regeneration

The explants responded in the form of swelling after 24-h of

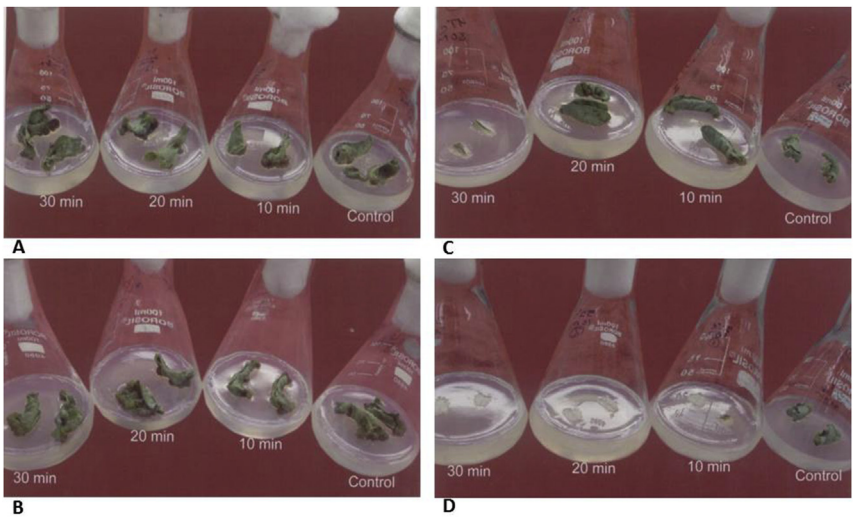

Fig. 1. Effect of heat treatment on callus induction in moth bean A) explant treated at 37 °C for 10, 20 and 30 min B) explant treated at 42 °C for 10, 20 and 30 min C) explant treated at 47 °C for 10, 20 and 30 min D) explant treated at 52 °C for 10, 20 and 30 min.

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