ORIGINAL RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

Changes in seed germination, and some physiological and ultra structural aspects of *Calotropis procera* seedlings under heat stress

Ali Ibrahim[1*], Ateeq Al-Zahrani[2], Zakria Baka[3], Hussain Almalki[2]
[1]Botany Department, Faculty of Science, Port Said University, Port Said, Egypt.
[2]Biology and Chemistry Department, University College at Al-Qunfudah, Umm Al-Qura University, Al-Qunfudah, Saudi Arabia.
[3]Botany Department, Faculty of Science, Damietta University, New Damietta, Egypt.

Received: July 7, 2016; **Revised:** July 25, 2016; **Accepted**: August 13, 2016
Available online: 1st September 2016

Abstract: Global warming is a problematic for many plants and understanding how the plants cope with temperature stress is an important objective. In this study, two experiments were conducted; the first one explored the effect of different temperatures on *Calotropis procera* seed germination, and the second disclosed the physiological and ultrastructural aspects of plant seedling. A temperature rise from 25°C to 30°C increased seed germination enormously, whereas seed incubation at 35°C significantly decreased its germination. The treatment of 40°C drastically inhibited seed germination. During the experimental periods, the seeds did not germinate at all at 45°C. Seed incubation at 30°C for 5 days notably enhanced seedling growth. This effect was accompanied by accelerated reserve mobilization and enzymes activity. A mild increase in lipid peroxidation (30% increase) and electrolyte leakage (45% increase) was also observed in response to these temperatures. Additionally, the plasma membrane moved away from the cell wall and became thicker in response to this temperature. Seeds exposure to 35°C for 5 days significantly reduced seedling growth. This adverse effect was accompanied with an increase in lipid peroxidation and electrolyte leakage by 100 and 170%, respectively over the values of 25°C treatment. Furthermore, the plasma membrane was damaged and double stranded with the 35°C application. Cytoplasmic vesiculation appeared in response to 30°C and 35°C treatments. Except for total soluble sugars which was decreased, all other biochemical changes in response to 35°C were comparable with those observed at 30°C treatment.

Key words: Cell Membrane; Cytoplasm Vesiculation; Electrolyte Leakage; Enzymes; Germination; Heat Stress; Ultrastructure.

Introduction

Plant growth and productivity is adversely affected by global warming due to the devastating effects of high temperature stress. At the end of the 21st century, the world air temperature will increase by 2°C to 4°C, which could result in widespread famine [1,2]. The responses of plants to high temperatures depend on the level of the temperature increase, its duration, and the plant type. The adverse impacts of this stress on plants include protein denaturation, fluidity of membranes, induced sterility, enzyme inactivation, protein synthesis inhibition and impairment of chlorophyll biosynthesis [3,4].

Exposure to high temperatures leads to oxidative stress via induction of reactive oxygen species (ROS) accumulation in plant tissues. These ROS are the main factor that causes lipid peroxidation and protein denaturation under stress conditions and consequent membrane disruption [5,6]. In this regard, Savicka and Škute [7] found that high temperature stress increased O^{-}_{2} production, which led to lipid peroxidation, as indicated by the malondialdehyde content, which was 40% over the control level in wheat seedlings. Plant anatomy and cell ultrastructure were also modified in response to different environmental stresses [8,9]. Pareek *et al.,* [10] noticed that salinity and high temperature stresses caused an impairment in the plasma membrane, mitochondrial membranes, endoplasmic reticulum and dictyosomes, and cytoplasmic lysis and enhanced association of ribosomes with the endoplasmic reticulum in young rice leaves also occurred.

The structure of cell membranes is dynamic, which supports numerous biochemical and biophysical reactions. Membrane injury, which is accompanied by an increase in membrane permeability, is a hallmark of plant responses to different stresses, such as salinity, drought and extreme temperatures [4,11]. Leakage points in cellular membranes may be due to the appearance of domains with different configurations or lipid damage[12]. Consequently, membrane stability has been considered to be a primary component of stress tolerance in plants. Yeh and Lin[13] screened twenty-one chrysanthemum cultivars for heat tolerance depending on cell membrane thermo-stability. These researchers observed that the cultivars with low relative injury values were those with high membrane thermo-stability and shorter heat induced delay to flowering. Usually, membrane injury is evaluated indirectly by estimation of

***Corresponding Author:**
Dr. Ali Hassan Ibrahim,
Associate Professor of Plant Physiology,
Botany Department, Faculty of Science,
Port Said University, Egypt.
E-mail: Ibrahim2910@yahoo.com

http://dx.doi.org/10.21746/ijbio.2016.09.006

electrolyte leakage in plant tissues [11,14,15]. Although this phenomenon has been successfully used to assess membrane stability under different stressors, the relationship between the increase in electrolyte leakage and cellular membrane deterioration has recently been questioned [16].

In the past decades, a large amount of data have documented the heat stress impact on plants; however, the ultrastructural and physiological markers for high temperature tolerance have rarely been reported in the literature and are not fully understood. *Calotropis procera* (Asclepiadaceae) is an evergreen xerophytic shrub known as giant milkweed or rubber tree and can withstand different adverse environmental conditions [17,18]. This species has the potential for medicinal and economic uses [19]. Temperature was found to be a critical factor limiting *C. procera* seed germination. In this regard, Galal *et al.,* [20] observed that *C. procera* seed germination was inhibited by the temperature rising from 30°C to 35°C. Understanding the mechanism of *C. procera* performance under stress conditions would help for extending its cultivation in hard environment [21]. To our knowledge, no study has addressed the physiological and ultrastructural changes in *C. procera* seedlings under high temperature stress. Therefore, this work was undertaken to explore the effect of different high temperatures on seed germination and seedling growth in *C. procera*. Additionally, the changes in lipid peroxidation, protein levels, enzyme activity, electrolyte leakage and hypocotyl ultrastructure were investigated.

Materials and Methods

Seed collection

Seeds of naturally opened fruits of *C. procera* shrubs were collected from the Makah region, Saudi Arabia in September. Seed viability (based on a germination test) was approximately 90%. These seeds were used in two germination experiments.

Seed germination experiment

This experiment was performed to explore the effect of different temperatures on seed germination. The seeds were surface sterilized with 2.5 g L^{-1} sodium hypochlorite solution for 5 min and later rinsed with sterile distilled water. The sterilized seeds were soaked in distilled water for 6 h and sown in plastic boxes (20 seeds/box; 12 × 20 cm) lined with filter papers. Next, the seeds were moistened with distilled water. The boxes were divided into five groups with four replicates for each group. All groups were incubated in the dark in Wise incubators for five days. The first group was incubated at 25°C, the second group at 30°C, the third group at 35°C, the fourth group at 40°C, whereas the fifth group was incubated at 45°C. Seeds were scored as germinated when the emerged radicle was visible. The number of germinating seeds were recorded every day and the seed germination percentage was calculated.

Seedling growth experiment

In this experiment, the treatments of 25°C, 30°C and 35°C were repeated under the same previous conditions to disclose the changes in seedling growth, sugar content, enzyme activity, lipid peroxidation, electrolyte leakage, membrane injury and hypocotyl ultrastructure at 3 days and 5 days germination periods.

Determination of total soluble sugars:

Soluble sugars in a known dry seedlings mass were extracted by immersion in 80% ethanol overnight with periodic shaking. Next, the extract was filtered and total soluble sugars in the filtrate were determined spectrophotometrically with a phenol-sulfuric acid method as described by Sadasivam and Manickam [22].

Extraction and assay of total amylase activity:

A known fresh weight of seedlings was homogenized in cold tris-maleate buffer (0.05 M; pH 7.0). The homogenate was centrifuged for 25 min at 5,000 g, and the supernatant was used to assay total amylase activity with a spectorphotopmetric method, as described by de Morais and Takaki [23].

Extraction and assay of guaiacol peroxidase activity:

Peroxidase extraction was based on the method adopted by Bhardwaj *et al.,* [24]. Fresh seedlings were macerated in cold potassium phosphate buffer (pH 7.0) containing 0.2 mmol L^{-1} ascorbate. The homogenate was centrifuged at 10,000 g for 30 min. Peroxidase (EC1.11.1.7) activity in the supernatant was determined spectrophoto-metrically by the guaiacol oxidation method [22].

Determination of soluble and insoluble proteins:

Based on the method adopted by Khademi *et al.,* [25], a known seedling's fresh weight was homogenized in 5 mM Tris-buffer (pH 8.0). The extract was centrifuged at 6,000 g for 45 min, and the supernatant was collected and used for determination of total soluble proteins. The pellet was frozen overnight and then dissolved in a solution of 5% SDS and 0.5 N NaOH (1:1 ratio). This protein extract was allowed to stand for 1 h on ice with periodical shaking and then was centrifuged at 6,000 g for 45 min. The supernatant was used for measuring the insoluble protein. The soluble and insoluble protein concentrations were measured according to the dye binding assay method of Bradford [26].

Measurement of lipid peroxidation:
Seedlings were ground in 80% ethanol and the supernatant was centrifuged at 5,000 g for 10 min. The lipid peroxidation product; i.e., malondialdehyde (MDA), in the supernatant was measured by the thiobarbituric acid (TBA) method of Heath and Packer [27]. Briefly, the sample was heated with TBA solution at 95°C for 24 min and cooled on ice. The developed colour was read at 532 nm and 600 nm using an UV-Vis spectrophotometer (APEL, PD-303 UV).

Measurement of electrolyte leakage:
As described by Bajji *et al.,* [11], hypocotyl mid-segments (approximately 5 mm) were washed quickly with deionised water and placed in sugar tubes containing 10 ml of deionised water. Next, the initial electrical conductivity (ECi) was measured. After this, the tubes were incubated in dark at the corresponding experimental temperature and a subsequent measurement (ECf) was obtained after 6 h. Following these readings, samples were autoclaved, cooled at 25°C and the total electrical conductivity (ECt) was measured. Electrolyte leakage was expressed as: (ECf-ECi)/(ECt-ECi) × 100.

Sample processing for transmission electron microscopy:
Segments (2–3 mm) were cut from the middle region of seedling hypocotyl and fixed for 2 h with 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). Fixed samples were washed in 0.1 M sodium cacodylate buffer and post-fixed in 1% osmium tetroxide in the same buffer. Subsequently, samples were gradient dehydrated in a series of ethanol solutions. The dehydrated samples were embedded in Spurr resin and ultrathin sections were prepared following the procedure of Xing *et al.,* [28]. The sections were stained with uranyl acetate followed by lead citrate and examined by a Jeol 1011 Transmission Electron Microscope (90 kV) at King Abdul-Aziz University, Saudi Arabia.

Statistical analysis
The data are presented as the mean from 3 to 6 replicates. The results were subjected to an analysis of variance (one-way ANOVA) and the least significant difference between means (LSD) at $P < 0.05$ was also evaluated. All statistical analyses were carried out with SPSS software version 19.

Results

Changes in germination percentage
This experiment was conducted to evaluate the effects of different constant temperatures on the germination percentage of *C. procera* seeds and the results are presented in Table 1. It can be observed from the obtained data that the temperature rising from 25°C to 30°C extremely accelerated seed germination. After one day from seed sowing, the germination percentage was approximately 0.0% and 60% at 25°C at 30°C, respectively. After two days of incubation, seed germination in response to 30°C was two-fold higher than that of 25°C. This effect continued until 5th day, but with a low level. The effect of seed exposure to 35°C and 40°C treatment was not regular and appeared to depend on the exposure period. Constant exposure to 35°C markedly enhanced the germination% in relation with 25°C until the 3rd day. After that, this temperature significantly decreases seed germination. Application of 40°C increased seed germination by 6% after one day of incubation only, but seed germination was greatly reduced with other incubation periods in comparison with 25°C. At an extreme temperature; i.e., 45°C, the seeds did not germinate at all within the experimental periods. It can be seen from table 1 that the optimum temperature for seed germination in *C. procera* was 30°C. Some rotting seeds appeared as a result of the 40°C and 45°C treatments. Therefore, the temperature treatments at 25°C, 30°C and 35°C were used for further growth, physiological and ultrastructural studies.

Table 1: Effect of high temperature stress on seed germination in *Calotropis procera*. Values are means ±SE.

Temperature	Germination%				
	1st day	2nd day	3rd day	4th day	5th day
25°C	0.00c	42.3±2.9c	70.3±2.5b	82.6±3.17b	85.6±2.6b
30°C	60.0±1.33a	85.0±2.6a	89.6±2.6a	93.0±2.3a	93.7±2.6a
35°C	58.7±1.85a	70.3±2.02b	74.33±3.4b	75.0±2.9c	75.3±2.7c
40°C	6.3±2.0b	7.3±1.3d	13±1.5c	13.3±1.6d	13.7±1.9d
45°C	0.00d	0.00e	0.00d	0.00e	0.00e

Values in each column with the same letter(s) are not significantly different at $P > 0.05$.

Change in seedling growth parameters
It can be seen from figure 1A that seedling length, after 3 days exposure period, was approximately 75% higher in response to 30°C and 35°C than at 25°C. This pattern of change was observed only for 30°C after 5 days from seed sowing. Conversely, the seedling length was significantly reduced in response to 35°C after 5 days exposure period. After 3 days from seed sowing, increase in the incubation temperature from 25°C to 30°C increased seedling fresh mass by approximately 90% (Fig 1B). This rate of increase appeared to decrease with time, whereas after a 5-day germination period, the 30°C treatment increased the seedling fresh mass only by approximately 35%. After 3 days exposure period, the effect of

35°C treatment on seedling fresh mass was not significantly different from that observed at 30°C. However, this treatment (35°C) significantly reduced the seedling fresh mass after 5 days exposure periods in relation with 30°C. The changes in the fresh/dry mass ratio were comparable with those for seedling fresh mass (Fig. 1C). These data revealed that seedling water content was enormously enhanced in response to temperature increases from 25°C to 30°C and sometimes at 35°C treatment.

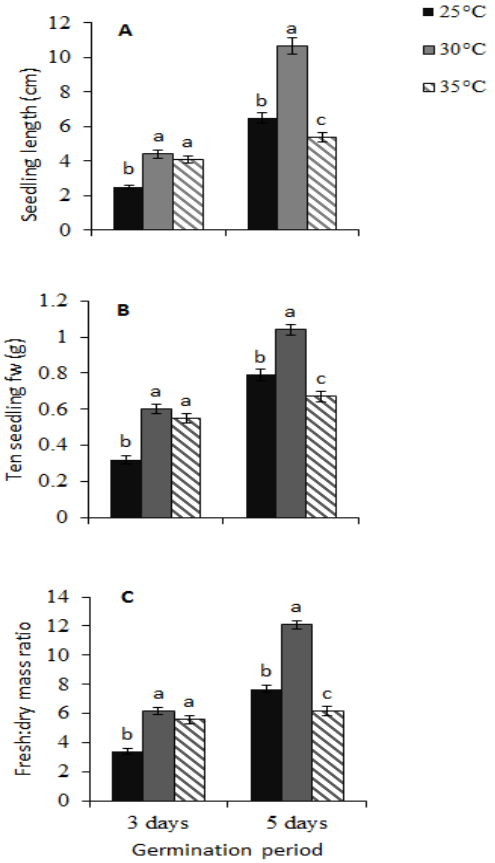

Figure 1: Effect of temperature increase on some growth parameters of *C. procera* seedlings. Values are mean± SE. Values in a group with the same letter (s) were not significantly different at P≤0.05.

Changes in total soluble sugars and enzyme activity

Results for total soluble sugars content (TSS) and enzymes activity are presented in figure 2. After 3 days, both 30°C and 35°C incubation temperatures increased the total soluble sugars content by approximately 20% in relation to 25°C treatment (Fig. 2A). After 5 days exposure period, the 30°C treatment increased TSS by about 50%, whereas the 35°C decreased it by 30% in comparison with 25°C treatment. After 3 days incubation period, the patterns of change in total amylase activity in response to temperature rise were comparable with those of total soluble sugars. After 5 days exposure period, both 30°C and 35°C treatments significantly enhanced the amylase activity. Peroxidase activity was also increased in response to temperature increases but the degree of enhancement depended on the length of the incubation period. Germination at 30°C or 35°C caused a 25% and 100% increase in peroxidase activity after 3 days and 5 days of incubation, respectively (Fig. 2C). It can be also noted from the recorded results that TSS content was increased in *C. procera* seedlings with the increase in the developmental period and vice versa was true for amylase and peroxidase activity.

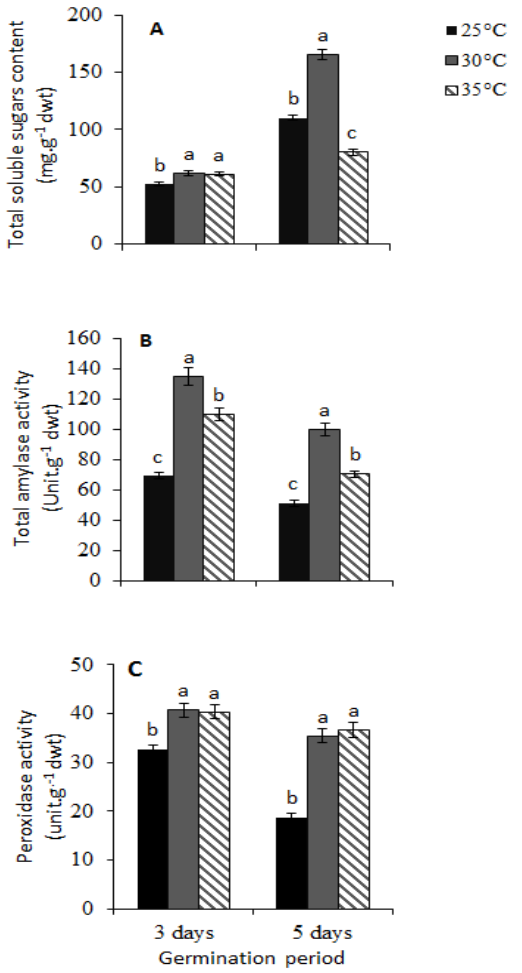

Figure 2: Effect of temperature increase on total soluble sugar content and some enzyme activity in *C. procera* seedlings. Values are means ±SE. Value in a group with the same letter (s) are not significantly different at P≤0.05.

Changes in protein content and lipid peroxidation

The results for protein content and lipid peroxidation are shown in figure 3. Temperature increases from 25°C to 30°C or 35°C increased the total soluble protein by about 45%, after a 3-day germination period (Fig. 3A). The same effect was also observed at 5 days after seed sowing but the difference between treatments was lower than that recorded after 3 days. On the other hand, exposure

to 30°C and 35°C significantly decreased the insoluble protein content in comparison with 25°C treatment (Fig. 3B). It can be observed from the results that soluble and insoluble proteins decreased in *C. procera* seedlings with progression in time. Exposure to 30°C and 35°C during seed germination increased lipid peroxidation (MDA content) by approximately 70% and 100% over the values at 25°C, respectively, after a 3 day period (Fig. 3C). A longer exposure (5 days) to 30°C elevated the MDA content by only about 30%. At that time, the 35°C treatment increased the lipids peroxidation by 110% over the values at 25°C.

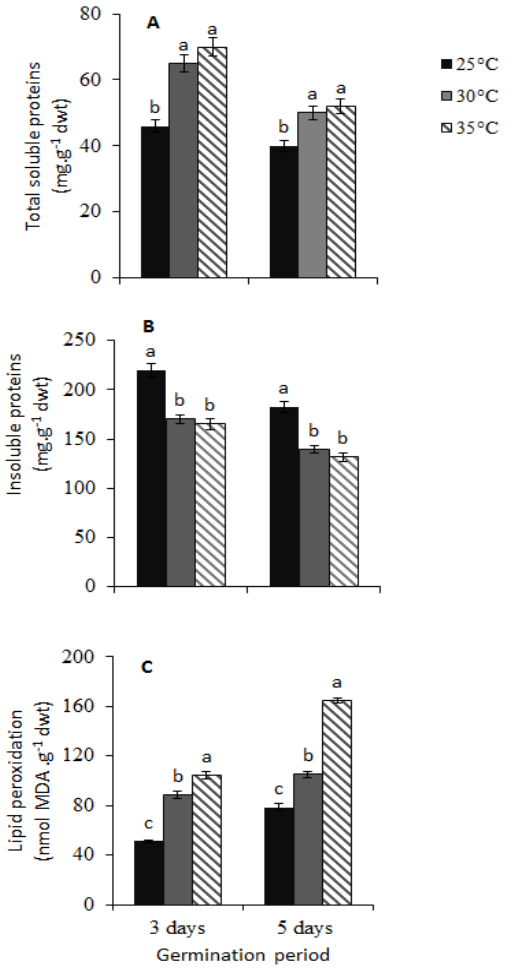

Figure 3: Effect of temperature increase on protein content and lipid peroxidation in *C. procera* seedlings. Values are mean± SE. Values in a group with the same letter (s) are not significantly different at P≤0.05.

It can be seen also that the lipid peroxidation, under all temperatures, increased in *C. procera* seedlings with progress in time.

Changes in electrolyte leakage (EL)

It can be observed from Figure 4 that increasing the incubation temperature from 25°C to 30°C or 35°C increased hypocotyl electrolyte leakage by about 50%, after 3 days germination period. The same effect of 30°C treatment continued to 5 days after seed germination. Seedling growth at 35°C induced more increase in the hypocotyl EL. After 5 days germination period, this temperature stress increased the EL by 170% over the values of 25°C treatment. It is worth mentioning that the recorded values of electrolyte leakage at 25°C and 30°C were decreased with increases in the germination period and the vice versa was true for 35°C.

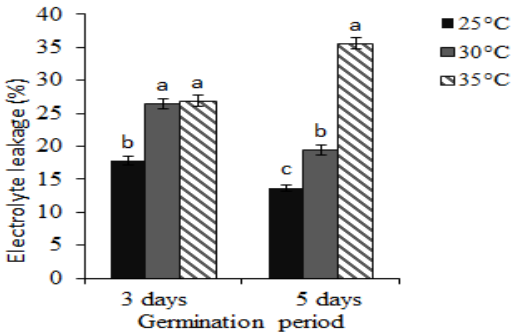

Figure 4: Effect of temperature increase on electrolyte leakage in *C. procera* seedlings. Values are mean± SE. Values in a group with the same letter (s) are not significantly different at P≤0.05.

Changes in ultrastructural aspects

TEM micrographs of the *C. procera* hypocotyl after a 5-day germination period at 25°C revealed the presence of a normal intact plasma membrane. In addition, an elongated normal nucleus in a thin cytoplasm was also observed (Fig. 5). Increasing the germination temperature to 30°C caused some changes in the ultrastructure of hypocotyl cells, such as the plasma membrane being moved away from a damaged cell wall and becoming thicker. The cytoplasm was more electron-dense, vacuolated, and vesiculated (Figs. 6 A-C).

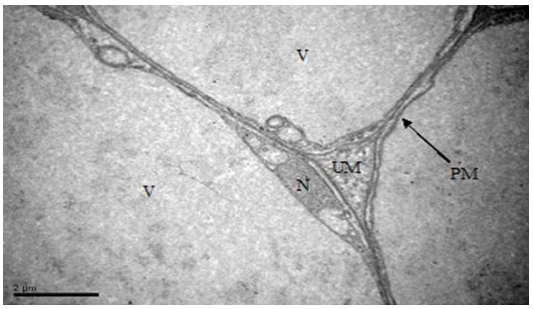

Figure 5: TEM micrograph of *Calotropis procera* hypocotyl after 5 days of germination at 25°C. An intact normal plasma membrane (PM) was observed. Note: unknown materials (UM) are present in the intercellular space and the nucleus (N) was in a thin cytoplasm. Bar = 2.0 µm.

After 5 days of germination at 35°C, TEM micrographs of the *C. procera* hypocotyl revealed the presence of a damaged plasma membrane, which became doubled stranded and moved away

from a split cell wall. An intact proplastid, vesicles and an unknown electron-dense body were also detected in the cytoplasm (Figs. 7 A-C).

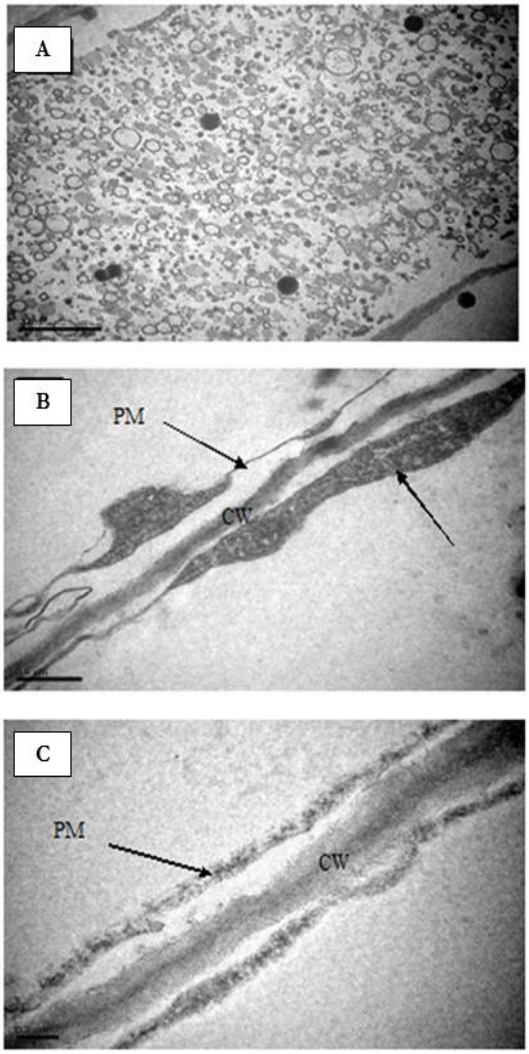

Figure 6: TEM micrographs of *Calotropis procera* hypocotyl after 5 days of germination at 30°C. (A) A vacuolated and vesiculated cytoplasm was observed. Bar = 2.0 µm**.** (B) The plasma membrane (PM) was moved away from cell wall (CW). Note: an electron-dense granulated cytoplasm (arrow). Bar = 0.5 µm. (C) A thick and electron-dense plasma membrane (PM) was observed. The membrane was moved away from a damaged cell wall (CW). Bar = 0.2 µm.

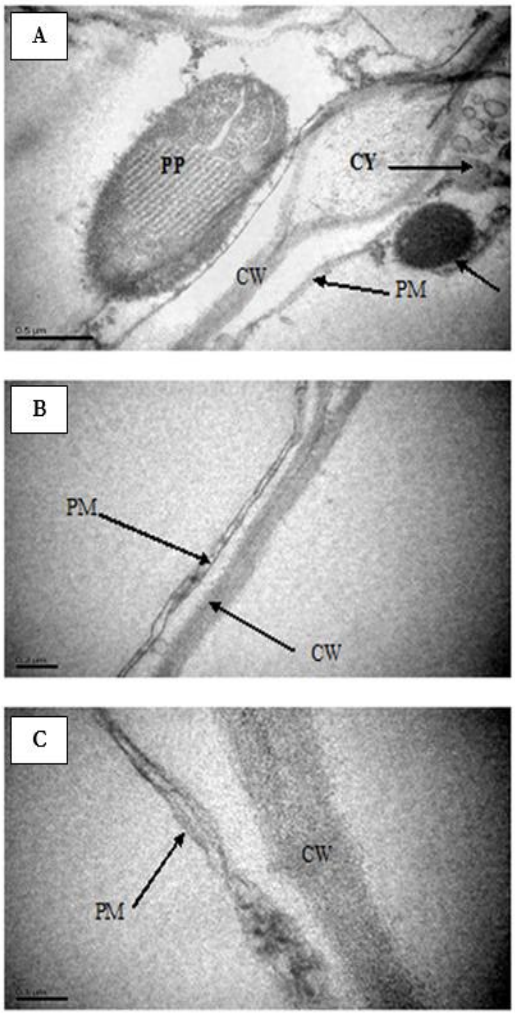

Figure 7: TEM micrographs of *Calotropis procera* hypocotyl after 5 days of germination at 35°C. (A) The plasma membrane (PM) was moved away from a split cell wall (CW). Note: There was also a proplastid (PP) and a vesiculated cytoplasm (CY). An unknown electron-dense body (arrow) can also be observed. Bar = 0.5 µm. (B). A double strand plasma membrane (PM) was moved away from the cell wall (CW). Bar = 0.2 µm. (C). A double strand damaged plasma membrane (PM) was moved away from an electron-dense granulated cell wall (CW). Bar = 0.1 µm.

Discussion

High temperature stress is one of the major constraints to plant growth and development worldwide [3, 29]. In this study, the extreme reduction in germination percentages due to the temperature's increasing to 40°C and the inability of germination at 45°C clarified that these temperatures were lethal and caused great injury to *C. procera* at this stage. Consequently, these temperatures (40°C and 45°C) were considered as distressful agents for *C. procera* germination. The adverse effects of high temperature stress included reserve immobilization, protein denaturation, respiratory disturbances, protoplasmic coagulation,

disintegration of the plasma membrane and accumulation of ROS [6,30]. In support of our results, Essemine *et al.,* [31] reported that exposure of two wheat varieties to 10°C or 15°C above the optimum temperature produced accumulated toxic ROS and damaged reserve mobilization by affecting the enzymes involved in starch hydrolysis, which consequently retarded embryo growth and seedling establishment.

The negative impact of 35°C treatment, after 5 days exposure period, on seed germination and seedling growth was consistent with the notable increase in lipid peroxidation as quantified by a thiobarbituric acid test and electrolyte leakage. These changes could be attributed to the accumulation of ROS [32] and the observed damage of cellular membrane which became double stranded and moved away from a cracked cell wall. This membrane disruption leads to nutrient escape and reduction of cell growth [4, 15]. The noted increase in enzymes activity and soluble proteins, and decrease in insoluble proteins after 5 days exposure period to 35°C clarified that this treatment increased reserve mobilization. The contradictory results of total soluble sugars and amylase activity in the *C. procera* seedlings may be due to increased respiration and/ or sugars leakage which resulted from the membrane damage.

One of the primary findings in this study was the enhanced germination and seedling growth parameters of *C. procera* at 30°C in relation to 25°C treatment. This increase appeared to be related to the increase in total amylase activity and total soluble sugars, and a decrease in insoluble protein, indicating a high mobilization rate due to temperature increase. Furthermore, this temperature as well as 35°C increased the activity of the antioxidant enzyme peroxidase, which is a part of the enzymatic defence system in plant cells and functions as a scavenger of free oxygen species by catalyse the conversion of H_2O_2 to water and oxygen [33]. Others attributed this improvement in seedling growth, under a high temperature, to the increases in free auxin levels that resulted from the regulation of auxin biosynthesis or catabolism [34]. The results of this study clarified that the optimum temperature for *C. procera* germination and seedling growth was 30°C and this finding was compatible in a broad sense with those of Galal *et al.,* [20] and Menge *et al.,* [35].

The mild increase in electrolyte leakage (EL) in response to temperature increases from 25°C to 30°C was compatible with the findings of many authors [5,15]. This moderate change in membrane permeability with temperature increases could be related to the observed increase in lipid peroxidation (LPO). The increase in these stress markers (EL and LPO) indicated that the seedlings survived with a mild internal oxidative stress which did not interfere with the enhanced seedling growth at 30°C treatment. This moderate increase in LPO was not lethal and could enhance the plant tolerance to oxidative damage via regulation of antioxidant enzymes and compounds [32]. The results on electrolyte leakage and lipids peroxidation at 30°C may be unexpected and elusive because it was accompanied with enhanced germination and seedling growth, but the ultrastructural study with transmission electron microscopy confirmed that temperature increases from 25°C to 30°C caused some averse changes in the plasma membrane and cell wall structure. These changes included movement of the plasma membrane away from the damaged cell wall and vesiculation of the cytoplasm. However, the observed continuity of the plasma membrane and integrity of tonoplasts revealed that these negative changes were not lethal and could be reversible. Cytoplasm vesiculation may be an adaptive mechanism by which *C. procera* seedlings compensated the mild disturbance of the cellular membrane. In support of our results, Sergio *et al.,* [36] found that low levels of heavy metal lead (Pb) caused some modification in the *Elodea canadensis* cell ultrastructure such as deep alteration in the cell wall structure where the regular arrangement of microfibrils was lost, and there was an appearance of many cytoplasmic vesicles. However, these effects were not lethal for *Elodea* plants. Biotic stresses were also found to induce the formation of cytoplasmic vesicles [37].

This study clearly revealed that mild changes in cellular membrane stability due to temperature rise from 25°C to 30°C did not interfere with the enhancement of *C. procera* seed germination and seedling growth, which resulted from accelerated metabolism and stability of cytoplasmic organelles in short-term experiments. The adverse effect of temperature rise to 35°C on seedling growth was mainly related to the notable damage of cellular membrane and cell wall as appeared from physiological and ultrastructural results.

Authors' contribution

AHI conceived and designed the experiments. AHI and AAAl-Z performed the physiological analyses. HAD, ZAB and AHI performed the ultrastructural study. AHI, ZAB and AAAl-Z contributed to the writing of the manuscript. All authors read and approved the final manuscript.

References

1. Intergovernmental panel on climate change (IPCC). The Physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Solomon S., Qin D., Manning M., Chen Z., Marquis M., Averyt K.B, Tignor M., and Miller H.L. (eds.), Cambridge University Press, Cambridge, U K and New York, USA, 2007.

2. Jha U.C., Bohra A. and Singh N.P. "Review: Heat stress in crop plants: its nature, impacts and integrated breeding strategies to improve heat tolerance." Plant Breeding 133 (2014): 679–701.

3. Wahid A., Gelani S., Ashraf M. and Foolad M.R. "Heat tolerance in plants: An overview." Environ. Exp. Bot. 61 (2007): 199–223.

4. Demidchik V., Straltsova D., Medvedev S.S., Pozhvanov G.A., Sokolik A. and Yurin V. "Review paper: Stress-induced electrolyte leakage: the role of K^+-permeable channels and involvement programmed cell death and metabolic adjustment." J. Exp. Bot. 65 (2014): 1259-70.

5. Bavita A., Shashi B. and Navtej S.B. "Nitric oxide alleviates oxidative damage induced by high temperature stress in wheat." Ind. J. Exp. Biol. 50 (2012): 372-378.

6. Hasanuzzaman M., Nahar K., Alam M., Roychowdhury R. and Fujita M. "Review: Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants". Int. J. Mol. Sci. 14 (2013): 9643-9684.

7. Savicka M. and Škute N. "Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.)." Ekologija 56 (2010): 26–33.

8. Aldesuquy H., Baka Z. and Mickky B. "Kinetin and spermine mediated induction of salt tolerance in wheat plants: Leaf area, photosynthesis and chloroplast ultrastructure of flag leaf at ear emergence." Egypt. J. Basic. Appl. Sci. I (2014): 77-87.

9. Eleftheriou E.P., Adamakis I-D.S., Panteris E., and Fatsiou M. "Chromium-Induced ultrastructural changes and oxidative stress in roots of *Arabidopsis thaliana*." Int. J. Mol. Med. 16 (2015): 15852-15871.

10. Pareek A.N., Singla S.L. and Grover A. "Short-term salinity and high temperature stress - associated ultrastructural alterations in young leaf cells of *Oryza sativa* L." Ann. Bot. 80 (1997) 80: 629-639.

11. Bajji M., Kinet J.M. and Lutts S. "The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat." Plant Growth Regul. 36 (2002): 61–70.

12. Campos P.S., Quartin V., Ramalho J.C. and Nunes M.A. "Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. plants." J. Plant Physiol. 160 (2003): 283–292.

13. Yeh D.M. and Lin H.F. "Thermostability of cell membranes as a measure of heat tolerance and relationship to flowering delay in chrysanthemum." J. Amer. Soc. Hort. Sci. 128 (2003): 656- 660.

14. Premchandra G.S., Saneoka H. and Ogata S. "Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean." J. Agri. Sci. 115 (1990): 63-66.

15. Wang L.C., Tsai M.C., Chang K.Y., Fan Y.S., Yeh C.H. and Wu, S.J. "Involvement of the Arabidopsis HIT1/AtVPS53 tethering protein homologue in the acclimation of the plasma membrane to heat stress." J. Exp. Bot. 62 (2011): 3609–3620.

16. Rolny N., Costaa L., Carrióna C. and Guiamet J. "Is the electrolyte leakage assay an unequivocal test of membrane deterioration during leaf senescence?." Plant. Physiol. Biochem. 49 (2011): 1220–122.

17. Hassan L.M., Galal T.M., Farahat E.A. and El-Midan M.M. "The biology of *Calotropis procera* (Aiton) W.T." Trees 29 (2015):311–320.

18. Ibrahim A.H. "Tolerance and avoidance responses to salinity and water stresses in *Calotropis procera* and *Suaeda aegyptiaca*." Turk. J. Agri. For. 37 (2013): 352-360.

19. Erdman M.D. and Erdman B.A. "*Calotropis procera* as a source of plant hydrocarbons." Econ. Bot. 35 (1981): 467–472.

20. Galal T.M., Farahat E.A., El-midany M.M. and Hassan L.M. "Effect of temperature, salinity, light and time of dehiscence on seed germination and seedling morphology of *Calotropis procera* from urban habitats". Afr. J . Biotechn . 14 (2015): 1275-1282.

21. Boutraa T. "Effects of water stress on root growth, water use efficiency, leaf area and chlorophyll content in the desert Shrub *Calotropis procera*." J. Int. Environ. Appl. Sci. 5 (2013): 124-132.

22. Sadasivam S. and Manickam A. "Biochemical methods." New age international limited publishers, 2nd edition, New Delhi 1996.

23. de Morais G.A. and Takaki M. "Determination of amylase activity in cotyledons of *Phaseolus vulgaris* L. cv. Carioca." Brazil. Arch. Biol. Techn. 41 (1998): 7-25.

24. Bhardwaj J., Anand A. and Nagarajan S. "Biochemical and biophysical changes associated with magneto priming in germinating cucumber seeds." Plant Physiol. Biochem. 57: (2012): 67-73.

25. Khademi M., Koranski D.S., Hannapel D.J., Knapp A.D. and Gladon R.J. "Water stress and storage-protein degradation during germination of impatiens seed." J. Amer. Soc. Hort. Sci. 116 (1991): 302-306.

26. Bradford M.M. "A dye binding assay for protein." Anal. Bioch.72 (1976): 248-254.

27. Heath R.L. and Packer L. "Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry

of fatty acid peroxidation." Arch. Biochem. Biophys. 125 (1968):189-198.

28. Xing W., Wang J., Liu H., Zou D. and Zhao H. "Influence of natural saline-alkali stress on chlorophyll content and chloroplast ultrastructure of two contrasting rice (*Oryza sativa* L. japonica) cultivars." Aust. J. Crop. Sci. 7 (2013): 289-292.

29. Siddiqui M.H., Al-Khaishany M.Y., Al-Qutami M.A., Al-Whaibi M.H., Grover A., Ali H.M. and Al-Wahibi M.S. "Morphological and physiological characterization of different genotypes of faba bean under heat stress." Saudi. J. Biol. Sci. 22 (2015): 656–663.

30. Levitt J. "Responses of plants to environmental stress. Vol. I. Chilling, freezing and high temperature stresses." Academic Press New York 1980.

31. Essemine J, Ammar S. and Bouzid S. "Impact of heat stress on germination and growth of higher plants: Physiological, biochemical and molecular repercussions and mechanisms of defence." J. Biol. Sci. 10 (2010): 565-572.

32. Niki E. "Lipid peroxidation: physiological levels and dual biological effects." Free Radic. Biol. Med. 47 (2009):469-484.

33. Gulen H. and Eris A. Effect of heat stress on peroxidase activity and total protein content in strawberry plants. Plant Sci. 166 (2004): 739–744.

34. Gray WM, Ostin A, Sandberg GR, Romano CP and Estelle M. "High temperature promotes auxin-mediated hypocotyl elongation in Arabidopsis." Proc. Natl. Acad. Sci. USA 95 (1998): 7197–7202.

35. Menge E. O., Bellairs S. M. and Lawes M. J. "Seed-germination responses of *Calotropis procera* (Asclepiadaceae) to temperature and water stress in northern Aust." Aust. J. Bot. 64 (2016): 441-450.

36. Sergio E., Cobianchi R.C., Sorbo S., Conte B. and Basile A. "Ultrastructural alterations and HSP 70 induction in *Elodea Canadensis* Michx. exposed to heavy metals." Caryologia 60 (2007): 115-120.

37. Wei T. and Wang A. "Biogenesis of cytoplasmic membranous vesicles for plant potyvirus replication occurs at endoplasmic reticulum exit sites in a COPI- and COPII-dependent manner." J. Virol. 82 (2008): 12252-12264.

Cite this article as:
Ibrahim A.H., Al-Zahrani A.A., Baka Z.A., Almalki H.D. Changes in seed germination, and some physiological and ultra structural aspects of *Calotropis procera* seedlings under heat stress. *International Journal of Bioassays* 5.9 (2016): 4815-4823.
DOI: http://dx.doi.org/10.21746/ijbio.2016.09.006

Source of support: Nil
Conflict of interest: None Declared