



Impact of Heat Stress on Agronomic and Physiological Parameters of Different Potato (*Solanum tuberosum* L.) Cultivars

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ARTICLE INFO

Keywords: Heat Stress, Potato Plant, Growth, Physiological Parameters, Chlorophyll Contents, Proline and Sugar Concentrations.

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Declaration

Authors' Contribution

All authors equally contributed to the study and approved the final manuscript

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History

Received: 24-06-2025 Revised: 28-08-2025

Accepted: 08-09-2025 Published: 15-09-2025

ABSTRACT

Globally, the climate change is expected to upsurge further. An increase will be noted in the future in the occurrence and sternness of life-threatening measures as extreme temperatures, heat waves and drought. This slight rise in temperature turns as stress and employ an adverse effect on different morpho-physiological attributes of plants as phenology, anatomy and gene expression. Also, the speedy rise in population and the drastic climatic changes severely affect global food security. Thus, how the plant responds to high temperatures and drought has become a key research topic. Heat stress causes a drastic effect on the growth and development of plants by bringing a series of morpho-physiological and biochemical changes. Our study aimed to address the impacts of heat stress on agronomic and physiological parameters in three different potatoes (*Solanum tuberosum* L.) cultivars, i.e. Diamant, Multa, and Ajax. Heat stress of 42 °C was applied to the plants at 30 days after their germination. Increased heat stress for about seven days considerably affected the growth as well as physiological parameters in potato plants. The leaf relative water contents and chlorophyll contents resulted in decrease at high temperatures. An increase in plant height was detected for all the cultivars. Increase in proline and soluble sugars contents have also been observed. Our study suggested that heat stress has a significant effect on potato plants. To summarize, this research will subsidize to an inclusive understanding of the past, present, and future research on plant responses to high temperatures.

INTRODUCTION

Food security of the world is threatened by the instant growth in population and extreme changes in climate (1). Temperature is the main crucial factor that determines the vegetation of a specific region. A proper balance between an optimal temperature and time of occurrence is very important to standardize plant growth (2).

A report submitted by IPCC (3) suggested that the injurious effects of climate change on yields of the crops are higher as compare to their beneficial effects. The temperature of the global average surface has been risen up by 1.1 °C and is expected to rise by 2-5 °C since the industrial revolution. This increase will lead to severe meteorological event in the form of high temperature and drought and these events will continue to increase in most of the land areas in the future (4). A bit of change in the atmospheric temperature can lead to abnormal biological, morphological and molecular changes in the plants that in turn, can cause stunted growth and reduction in crop yield (2). Scientists have predicted a double increase in the current ambient CO₂ concentration from 380 ppm to about

700 ppm till the end of 21st century. This increase will also be coupled with an increase in air temperature from 0.3 to 4.8°C (5). Various studies have been reported that high temperatures and drought stress badly affect plant growth and physiology (6, 7, 8). Studies suggested that the plants in future will be exposed to both mean temperature of the heat and to the extreme heat stress (HS). As extreme temperatures often have been found to adversely affect plant growth and development (9).

Potatoes are amongst the four primary foods which is broadly cultivated and consumed in most parts of the world (10). Potato belongs to the family Solanaceae, genus *Solanum* and species *S. tuberosum* (11). Potato is referred to as both the plant and also to the edible tuber (12). Amongst the most cultivated crops of the world following maize, wheat and rice, potato is number four (13, 10). It is amongst the most domestic consumption food that has added significant support to the food needs (14).

In Pakistan, there is a significant rise in the potato production. In 2020, potato was cultivated on an area of about 234, 400 hectares with an estimated production of 4.55 million tons. In 2021-22, the production has been



increased to 7.74 million tons showing almost 50% hike (15). From the past decade research has been focused of the plant responses to a single stress. Though, many of the abiotic stresses occur simultaneously and the crops are always exposed to different abiotic stresses (16, 17). Regarding the present research work, it is important to understand the physiochemical responses of potato plants to high temperature. It is thought that by the end of 21st century, plant will be much more susceptible to HS (18). In this respect, the mechanism of heat responses in plants and the use of heat resistant plants have got much attention. Therefore, the negative and damaging effects of HS can be overcome by developing heat resistant crops through genetic engineering, tissue culture and biotechnology. The Crop Wild Relatives pre-breeding project encourages using wild varieties to enrich and preserve the gene bank collection and to make potatoes adaptable to the harsh environment as it is the most demanding and challenging issue because of the climatic change (19).

The main objective of our work was to assess the response of different potato cultivars under heat stress and to understand the response of these cultivars to heat stress.

MATERIALS AND METHODS

Tested Cultivars and Experimental Setup

Three different potato varieties named Diamant, Multa and Ajax were obtained from National Agricultural Research Centre, Islamabad. The experimental work was carried out in the growth chamber and Green house in the Department of Botany, University of Science and Technology Bannu. Wisd laboratory growth chamber (WiseCube WGC-1000) was used for the experiments.

Growth Conditions and Treatments

Potato tubers were sown in pots (16×20 cm) filled with a mixture of clay and sand (1:1). The tubers were sown in the second week of February. Tubers were first pre-sprouted for three weeks. The pots were irrigated three times a week to avoid any water deficiency. The level of temperature for experiment was established at the growth chamber at 25°C with 16/8 hours light/dark periods as previously described by (20) with a little modification. The humidity was set at 70%. All the varieties, except control, were exposed to heat stress at 42°C at 30 days after germination (DAG). The data was taken one week after the heat stress was given.

Parameters studied

Determination of Relative Water Contents (RWC)

RWC was calculated as described by (21). Fresh weight (FW) of leaves of all varieties was taken. After FW, leaves were placed in water at 4°C for 4 hours to record the turgid weight (TW). For the dry weight (DW), the leaves were then kept in oven at 80°C for 2 days. The RWC measurement was taken using the formula;

$$RWC (\%) = (FW - DW) / (TW - DW) * 100$$

Plant Height, Leaf Length and Leaf Length and Width

Parameters like plant height were recorded using an inch tape. The data for plant height was taken from the bottom

of the plant at the ground surface to the top. Leaf length and width were measured using an inch tap.

Determination of Plant Fresh (FW) and Dry Weight (DW)

Plant fresh (FW) and dry weights (DW) were calculated in grams using an electrical balance. Only the upper part of the plant, starting at the bottom, was taken for fresh and dry weights. The FW was calculated immediately when the plants were removed from the soil. The plants were shade dried for few days to get the dry weight. The FW and DW data was collected at 60 days after germination (DAG).

Determination of Photosynthetic Pigments

Chlorophyll contents were assessed as described by (22). Fresh leaves from both control and HS plants were taken and ground into fine powder with liquid nitrogen. Around 25mg of the sample were weighed from control and HS plants materials. About 750µl of 80% acetone solution was added to the samples. Mixture was vortex for 15 minutes and then centrifuged at 4°C for 10 min at 1400rpm. Absorbance was determined on 645nm and 663nm as,

$$\text{Chlorophyll } a = 15.65 A_{666} - 7.340 A_{653}$$

$$\text{Chlorophyll } b = 27.05 A_{653} - 11.21 A_{666}$$

Proline Contents

Method of (23) was used for the determination of proline contents. Fresh samples of the leaves (0.5g) were mixed with 10ml of 3% sulfosalicylic acid. Mixture was ground by pestle and mortar and then filtered into test tube, added 2ml of glacial acetic acid ninhydrin reagent. The sample was boiled using water bath at 100°C for an hour and then was allowed to cool down on ice. 4ml of toluene was mixed with shaking to make two layers. Absorbance of the upper layer was measured at 520nm wavelength using spectrophotometer (24).

Soluble Sugar Concentration

For soluble sugars, method of (25) was used. 0.5g of fresh leaf samples was taken in a test tube. 10 ml of 80% ethanol was added to the sample. The sample was heated for an hour at 80°C using water bath. Half of the sample was then added to another test tube containing 0.5ml of dH₂O and 1ml of 18% phenol. The sample was kept for an hour. At the end, 2.5ml of sulphuric acid was added to the sample. The mixture was shaking and its optical density was measured at 490nm using spectrophotometer. Lastly, the total soluble sugar content was calculated using the standard equation:

$$\text{Soluble sugar content } (\%) = (C \times 7.5) / (W \times 104)$$

Statistical Analysis

The experiment was designed in randomized complete block design. Three replicates per treatment were considered. The data was noted and analyzed using Analysis of Variance (ANOVA) and Least Significant Difference (LSD) Test (26).

RESULTS

Relative water contents

In the present study, leaf RWC was greatly reduced by heat stress. Significant results were found for RWCs as presented in (Figure 1). All the potato varieties showed about 50% decrease in their RWCs. Of all the three

varieties, Ajax showed considerable reduction of 52% in RWCs as compared to control, followed by Diamant (50%) and Multa (46%).

Plant Height, Leaf Length and Leaf Length and Width

Increase plant height has been recorded for all the treatments when compared to control (Figure 2). Maximum plant height was noted for Diamant (97cm) followed by Multa (96cm) and Ajax (90cm) compared to control height 71cm. A maximum 37% increase in plant height was noted for Diamant under high heat stress. Leaf length also showed significant results. All the treatments showed increase in leaf length when compared to control (Figure 3).

Figure 1

Determination of leaf RWCs in different potato varieties

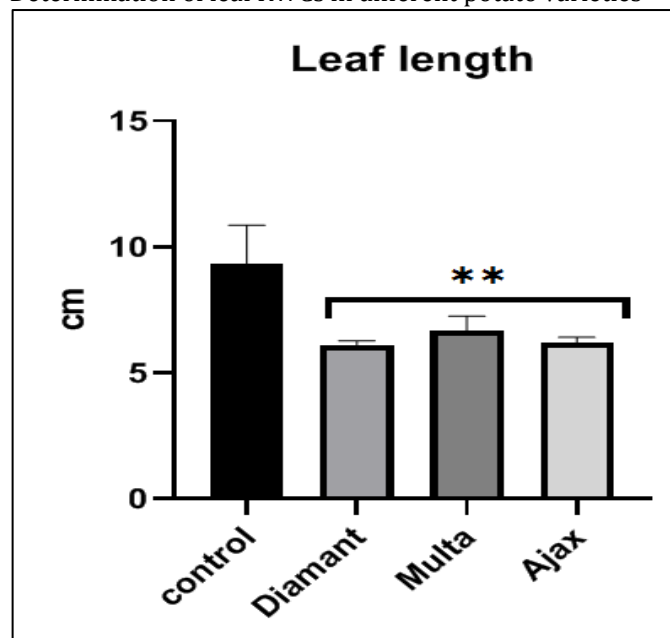


Figure 2

Impact of heat stress on plant height in different potato cultivars.

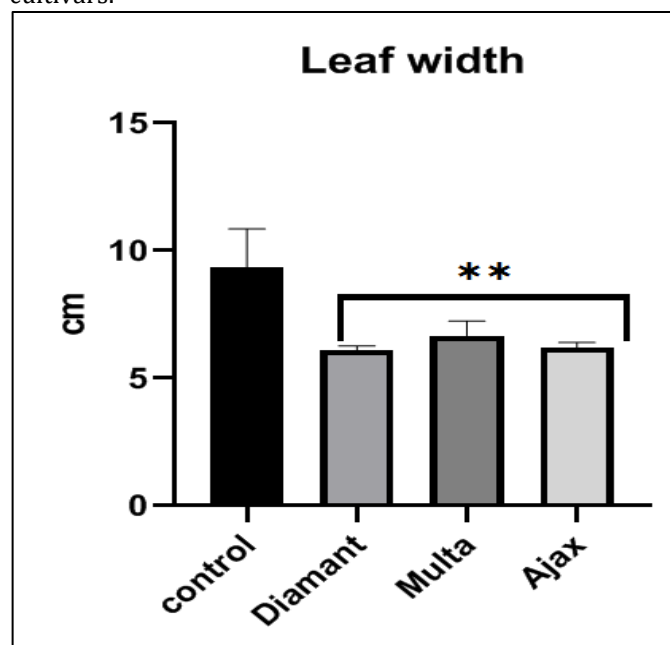
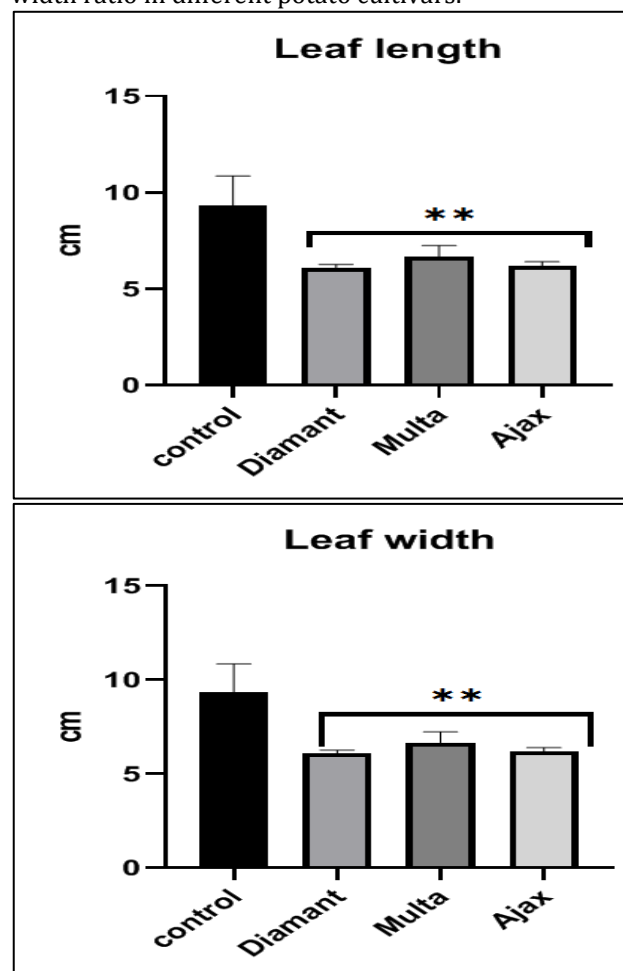


Figure 3-4

Impact of heat stress on leaf length and leaf length and width ratio in different potato cultivars.

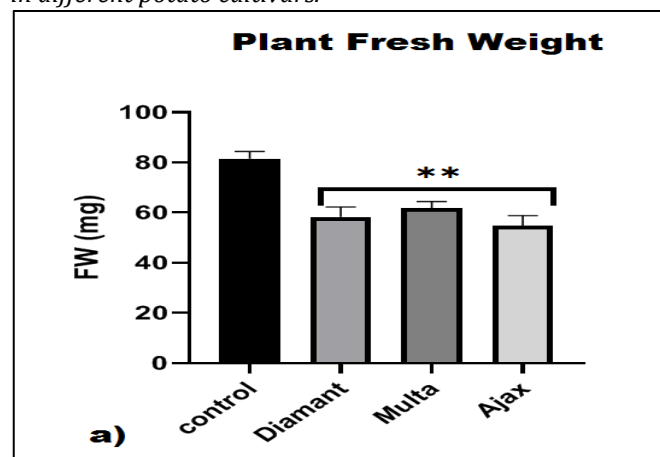


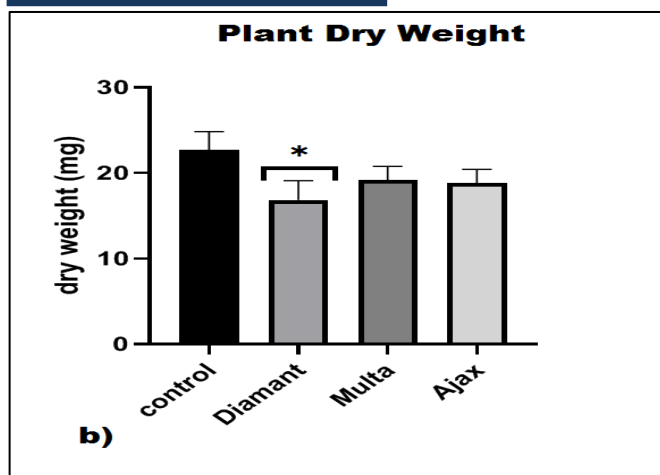
Plant FW and DW

The FW and DW of three potato cultivars have shown a significant decrease under heat stress when compared to control (Figure 4). Diamant showed a reduction of 13.46% in fresh weight and about 25% in dry mass. A reduction of 9% in fresh weight and 15% in dry weight was recorded for Multa and Ajax showed a reduction of 17% and 23% for fresh and dry weight respectively.

Figure 4

Impact of heat stress on plant fresh (a) and dry weights (b) in different potato cultivars.



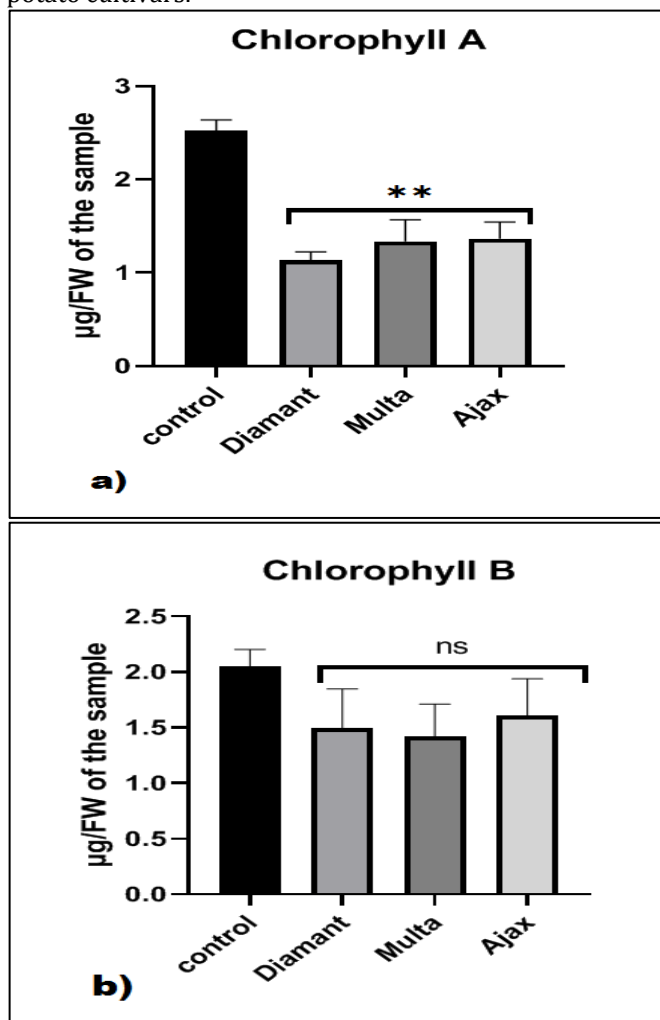


Chlorophyll contents

The photosynthetic pigments were significantly decreased by HS at 42°C (Figure 5). This might be due to the damage caused by HS to chloroplast structure. Diamant showed a major decline of about 55.15% in chlorophyll a when compared to control. The decrease in chlorophyll a contents was followed by Multa (47.22%) and Ajax (46.03%). The reduction in chlorophyll b contents for all the cultivars were Diamant (26.82%), Multa (30.7%) and Ajax (21.9%) respectively.

Figure 5

Effect of heat stress on Chlorophyll contents in different potato cultivars.

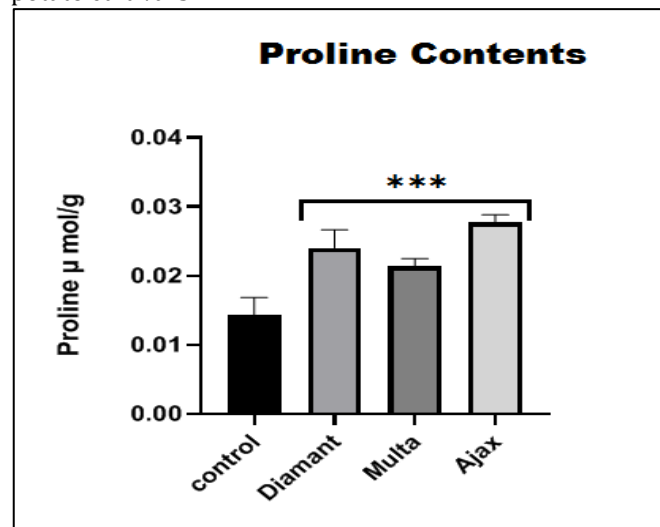


Proline contents

High proline contents were recorded as compared to control (Figure 6). The maximum proline contents detected in Ajax 0.027 (µmol.g-1FW). The lowest value was noticed for control (0.014 µmol.g-1FW).

Figure 6

Effect of heat stress on Proline contents in different potato cultivars.



Soluble Sugars

Results in Figure 7 signified that sugar contents are significantly elevated at high temperatures as compared to control. High sugar concentrations have been listed for Diamant which has about 50% increased value.

Figure 7

Determination of soluble sugars in different potato cultivars.



DISCUSSION

Plant functions are directly and indirectly effected by heat stress causing various morphological and physiological changes, hindering various growth parameters as well as metabolic activities (27). The present study signifies some of the negative as well as positive effect observed on different parameters of potato plant. Literature claimed that heat stress causes wilting, curling and yellowing of

leaves. Further, reduction in shoot and root growth, root number and diameter, plant height, and biomass have also been shown due to heat stress. Our findings are in confirmation with the previous reports of, who reported decreased plant height and reduction in leaf size in different potato cultivars under heat stress. High temperature affected dry matter contents as previously reported by (28). (29) also reported reduction in cell water contents, cell and plant size, reduction in growth and biomass and also net assimilation rate and relative growth rate. This may be because of the reason that the plants were thinner as compared to normal control plants with thicker leaves. As normally, the plant height is proportional to the mass of the plant but in our study plant height is increased with a decrease in plant fresh and dry masses. Both high temperature and drought are correlated under climatic change (30). Alternatively, plants often close their stomata in order to minimize the loss of water at the expense of photosynthesis (31). Plant growth is also affected by high temperature that reduces net assimilation rates of the shoot thus affecting the total dry mass of the plant (32). In our studies, the leaf water contents were reduced that add up to the plant mass as well. HS has also been found to blight photosynthetic apparatus and decrease photochemistry activities which result in decreased photosynthesis and related physiological processes i.e. reduction in chlorophyll contents and increased proline concentration) (33, 34, 35). The damage to the photosynthetic pigments clearly showed sensitivity of potato plants to HS because of high temperatures. High temperature along with drought stress has been witnessed to decrease photosynthetic rate and also the efficiency of PSII photochemical to a larger extent than individual stress (36, 37, 38). However, contrast data has also been found where (39) recorded an increase for chlorophyll contents in potato cultivars when exposed to high heat stress for prolong period. The results showed that increase in proline activity helps the plants to cope with the damage caused by ROS under severe stress. Primary metabolites like proline, betaine, glycine and soluble sugars are shown to accumulate during HS (29). The proline under HS serves as an antioxidant to keep the cell and other protective enzymes safe from damage. Previous reports are

consistent with the present study. High proline contents have also been reported in potato by (23) with increased heat stress. Heat stress is found to enhance the conversion of starch to reducing sugars (40, 28). Plants have a defensive mechanism against extreme temperatures by accumulating osmo-protectants such as accumulating proline, betaine and soluble sugars are important in regulating osmotic activities (41). Similar increase in soluble proteins was also listed by (23) under heat stress.

CONCLUSION

In our present study, potato plants treated with HS expressed a clear increase in plant height, proline contents and soluble sugar concentrations. However, a decrease in fresh and dry mass of the plant, leaf length and leaf length to width ratio was noted. A clear reduction in photosynthetic pigments has been observed by increase HS. However, proline activity was enhanced in HS. Potato plant is highly susceptible to HS. To overcome this problem, various approaches are under considerations to minimize the adverse effects of HS and other stresses. These approaches include plant breeding and genetic methodologies or induction of stress resistant varieties. Certain approaches can be made to identify the stress related traits at molecular, biochemical and physiological level. The new "omics-driven" techniques maybe used for advance screening to breed new potato cultivars and to improve the plant adaptability and productivity in response to HS.

Acknowledgement

The author is thankful to all the coauthors for their contribution in reviewing, designing the manuscript. Specially thanks for the valuable lab and technical assistance provided by the Department of Botany, UST Bannu and all the teachers who supported me during the whole period of practical research work.

Ethical Statement

This research was conducted in accordance with ethical standards and approval was obtained from the relevant ethics committee and institutional review board (IRB). Any use of wrong information can lead to the rejection of article.

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