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## The Effect of Silicon and The Status of Phenolic Compounds On The Germination of Cowpea (*Vigna unguiculata* L. Walp.) Under Temperature Stress

### Abstract

The aim of the study was to evaluate the elevated temperature on germination and seedling growth and the ameliorative effect of silicon on the phenolic compounds of black-eyed cowpea. The seeds were allowed to germinate at 30 (control), 36 and 44°C temperatures, under controlled conditions, silicones were with concentrations of 0.0 (control) and 1.5 mM. In the second phase of the experiment, some of the seeds were grown in plastic cups with sieved soil at 30 °C, 36 °C and 44 °C for phenolic compounds. Among the temperature treatments, plant deaths occurred at 44 °C. The raise in temperature importantly reduced germination and related traits. Except for SL, the seeds showed good germination at 30 °C after Si application. In high temperatures, silicon applications had a positive effect on germination and seedling growth, except for the germination rate. Results showed that an increase in GA, PA, Q, CAM, CA, PCA, SA, VA, CAF as temperatures increased. But chlorogenic acid and Q values were higher at 30 °C. This study clearly observed that the growth of cowpea seedlings decreased with temperature increase, but silicon attenuated these effects.

### Keywords

Germination, phenolic compounds, silicon, temperature stress, *Vigna unguiculata*

## INTRODUCTION

Temperature is the primary factor affecting plant growth. Due to climate change, minimum air temperatures are expected to increase, affected by changes in atmospheric water vapor content. Maximum temperatures will be affected by factors such as soil water and heat loss. Therefore, it has been emphasized that climate change will cause an increase in precipitation or that the probability of significant increases in maximum temperatures in irrigated lands will be lower (Hatfield and Prueger, 2015). Expected high temperatures will further affect plant yield (Meehl et al., 2007). Minimum air temperatures will reduce plant biomass and yield (Hatfield et al., 2011). Climate change resulting from the release of CO<sub>2</sub> and different greenhouse gases can raise temperatures between 2.5-4.5 °C (IPCC 2007). It is thought that this increase in global temperatures will affect the distribution of plants and the survival of the species (Dove, 2010). Maximum temperatures will be affected by factors such as soil water and heat loss. Therefore, it has been emphasized that climate change will cause an increase in precipitation or that significant increases in maximum temperatures will be less likely in irrigated lands. It has been observed that plants exposed to high temperatures grow faster and mature faster. In the short term, temperature increases may occur a few degrees above the control temperatures (Hatfield and Prueger, 2015). In nature, plants are often exposed to a variety of abiotic stresses, such as salt, drought, and heat. Light, heat stress or both cause the formation of Reactive Oxygen Species (ROS) that induce oxidative stress, and high temperatures also cause irreversible damage to plant growth, physiological mechanisms such as photosynthesis and respiration (Essemene et al., 2010). Temperature is an important limiting factor affecting germination in arid and semi-arid regions (Iloh et al., 2014). High temperatures are very detrimental to seed viability,

germination and seedling formation, and significantly affect plant phenology and yield (Bhandari et al., 2017). Therefore, high temperature is one of the most important constraints in agriculture (Harsh et al., 2016). Increases in temperature reduce metabolic activity in plants, increase respiration, which directly affects plant growth and development. High temperatures also cause an imbalance in CO<sub>2</sub> absorption and elimination by plants. Changes in day and night temperatures have an impact on stomatal behavior, enzymatic activity, flowering, photosynthesis and senescence (Angelotti et al., 2020). High temperatures disrupted the photosynthetic machinery in bean genotypes, significantly reducing yields (Omae et al., 2006). Short-term exposure to high temperatures impairs the function of photosystem II, both the structure and function of proteins and enzymes (Bhandari et al., 2017). Since high temperature increases membrane fluidity and affects membrane structure and function, the first damaged areas are membranes. In legume plants exposed to heat stress, germination (%), seedling emergence, abnormal seedlings are seen, root and plumule development are weakened (Sita et al., 2017). During seed growth, prolonged exposure to high temperatures delays germination, reduces viability and dry matter production (Iloh et al., 2014). Temperature also affects the rate and percentage of germination, biochemical reactions, and physiological processes that determine germination. Germination takes place within a certain temperature range and germination does not occur above or below these limits. Temperature is also important for the development of seedlings and primary roots, and insufficient temperatures directly affect root development (De Oliveira et al., 2013). Germination is particularly affected by temperature, and it is a physiological process that increases the digestibility, nutrition and content of some bioactive substances in legumes. In beans, temperatures of 40 °C and above significantly inhibited germination

(Machado et al., 2006; Televičiūtė et al., 2020). Since germination is vital to the plant, any change at this stage will affect the growth and development of plants (Iloh et al., 2014). In germination, changes are observed in phenolic compounds depending on factors such as species, light and germination time. Therefore, the biochemical content of sprouted legumes changes with stress conditions. Phytochemicals, acting as natural phytoalexins, in protecting plants against stress factors, respond to biotic and abiotic stresses (Televičiūtė et al., 2020). Legumes are plants grown in low-input growing systems. (Raveneau et al., 2011). Considering that the population will increase rapidly until 2050, the need for food will increase significantly and it will be difficult to meet the food needs of the increasing population (Bhandari et al., 2017). Therefore, it is thought that edible legumes with high protein content, such as cowpea, resistant to high temperature and drought, will gain more importance in the future (Carvalho et al., 2019). Silicon (Si) applications are applied both under various abiotic stress conditions, including high temperature stress, as well as under normal conditions. Thus, metabolic, physiological and structural properties of the plant are regulated. In high temperature conditions, silicon (Si) application also increases the temperature tolerance of plants by improving germination, root and shoot development, photosynthesis, membrane stability, nutrient uptake, secondary metabolites and yield (Saha et al., 2021). There are limited studies on the effect of silicon application on high temperature stress compared to drought and salinity conditions. And these studies suggest that Si application increases ROS accumulation, activates antioxidant defense systems and alleviates oxidative damage in heat stress (Muneer et al., 2017; Younis et al., 2020; Khan et al., 2020a, Khan et al., 2020b).

## MATERIAL and METHODS

The black-eyed cowpea seeds (*Vigna unguiculata* L. Walp.) were disinfected by immersion in 70% (v/v) alcohol solution, and agitated for 30 seconds, followed by two washes with distilled water. They were then immersed in 2.5% (v/v) NaClO solution for 2 minutes, washed with distilled water and dried. The seeds were placed in petri dishes with a double layer of filter paper moistened with 10 ml distilled water, and placed in a germination chamber at temperatures of 30, 36 and 44°C. Silicon applications were with concentrations of 0.0 (control) and 1.5 mM. The experiment was conducted in a completely randomized design with, in a  $2 \times 2$  scheme (silicon doses and temperature degrees) of subdivided plots, five replicate per treatment. The petri dishes were controlled for germination every two days, and study was ended two weeks after. Expansion of the radicle was considered as germination. The variables under analysis were FGP, SVI, RL, SL, RFW, RSW, SFW, SDW and GR.

**Final germination percentage (FGP) (%)**  
The total seeds germinated at the end of experiment /number of initial seeds used.

### Seedling Vigor Index (SVI)

SVI was calculated according to formula;  

$$(SVI) = (\text{Average SL (cm)} + \text{Average RL (cm)}) \times \text{Germination percentage (\%)}$$

### Root length (RL) (cm)

The length of the five seedling of the each replication from the seed to the tip of the root were measured and stated in cm.

### Shoot length (SL) (cm)

The length of the five seedling of the each replicate from the seed to the tip of the leaf blade were measured and stated in cm.

### Root fresh weight (RFW) (g)

The weight of 10 seedling of roots was recorded and stated in g.

### Root dry weight (RDW) (g)

The weight of 10 seedling roots was recorded and oven drying at 105 °C at 24 h.

**Shoot fresh weight (SFW) (g)**

The weight of 10 seedling of shoots was recorded and stated in g.

**Shoot dry weight (SDW) (g)**

The weight of 10 seedling shoots was recorded and oven drying at 105 °C at 24 h.

**Germination rate (GR)**

$\Sigma Ni/\Sigma TiNi$ , Where; Ni = the number of germinated seeds at the time of Ti. =  $(a/1)+(b-a/2)+(c-b/3)+...+(n-n-1/N)$

The second experimentation was performed in plastic cups with sieved soil. First of all 120 g of soil was filled in all plastic cups with 8 cm diameter. Ten seeds per cups were propagated at 1 cm depth at equal distance. And then, 10 ml distilled water was dispensed with pipette for moisture. 10 plastic cups of each temperature degrees were placed in a germination chamber. Temperature applications were 30, 36 ve 44 °C. 5 ml sterile distilled water was added with pipette in each cup every other day to maintain water content. Plant deaths occurred at 44 °C within the heat treatments. After fourteen days, the experiments were finished and phenolic compounds of seedlings were measured and calculated. Analysis of gallic acid (GA), protocatechuic acid (PA), catechin (C), chlorogenic acid (CA), vanillic acid (VA), caffeic acid (CAF), syringic acid (SA), P-cumaric acid (PCA), cinnamic acid (CA), quercetin (Q) and camphorol (CAM) components were conducted in Aydin Adnan Menderes University, TARBIYOMER (Agricultural Biotechnology and Food Safety Application and Research Center). For this analysis, COI / T.20 / Doc. No 29 / Rev.1 2017 standard method has been used in a modified manner. This study was carried out with Agilent brand, UV / DAD detector, high pressure, liquid chromatography device (HPLC).

**RESULTS and DISCUSSION**

The results showed that the interaction between temperature and silicone treatments was significant for all characters except germination rate, furthermore, FGP

decreased with increasing temperature. The highest FGP was recorded at 30 °C and 1.5 mM silicon dose and the lowest was found at 36 °C and without silicon. The cowpea had significantly higher seedling vigor index at 30 °C than 36 °C. Silicon increased the SVI. The application of Si caused an increase in SVI of temperature application-plants. Highest RL produced from the 30 °C and 1.5 mM silicon dose, which was 89.96 cm, 30 °C and without silicon which was 88.65 cm, respectively. It could be stated that raising temperature levels from 30 °C to 36 °C reduced RL by 1520.46 and 1361.92 respectively. Low temperature increased the SL. The lowest SL was found in plants supplied without silicon at 36 °C (Table 1). Seed germination is temperature dependent as temperature is one of the essential requirements at this stage (Rainey and Griffiths, 2005). While it is observed that the germination of warm season legumes tends to reach a maximum at 25 °C, the germination of cold season legumes varies between 10-25 °C (Butler et al., 2014). When the pea seedlings are exposed to temperature stress (45 °C, 50 °C), the growth of the plants is negatively affected (Shereena and Salim 2006). 8-day 30 °C / 30 °C day / night temperature application to soybean and bean plants resulted in non-irrigated conditions with 50.4% and 36.2% dead seeds, respectively, while under well-watered conditions 87.6% and 36.8% were obtained. Root length has also decreased significantly (Nemeskéri, 2004). In mungbean plants, exposure to 50 °C for 2 hours adversely affected the seedlings (Mansoor and Naqvi, 2011). In addition, under temperature stress (35-40 °C) conditions, germination in lentil seeds decreased and seedling growth was delayed (Chakraborty and Pradhan, 2011). High temperatures include soybeans (Ortiz and Cardemil, 2001; Ren et al., 2009), peas (Nemeskéri, 2004; Ren et al., 2009), lentils (Chakraborty and Pradhan, 2011) and chickpea (Kaushal et al., 2011; Piramila et al., 2012) decreased germination.

**Table 1.** Effects of temperature and silicon application on FGP, SVI, RL and SL of black-eyed cowpea

Treatments	FGP	SVI	RL (cm)	SL (cm)
30 °C + 0.0 mM silicon	88.65 aB	1520.46 aB	88.65 aB	10.87 aA
30 °C + 1.5 mM silicon	89.96 aA	1650.00 aA	89.96 aA	10.85 aA
36 °C + 0.0 mM silicon	82.20 bB	1361.92 bB	82.20 bB	9.77 bB
36 °C + 1.5 mM silicon	84.95 bA	1421.88 bA	84.95 bA	10.21 bA
Temperature (A)	**	**	**	**
Treatment (B)	**	**	**	*
Temperature x Treatment (AxB)	**	**	**	*
LSD (AxB)	0.37	0.68	0.37	0.23

The sources of variance were as follows: two temperatures, two silicon applications and interaction between temperature and silicon. Different lower case letters in the same column indicate that the difference between temperatures and upper case letters indicate that the difference between silicone applications is significant. Least significant difference (LSD) of the Temperature x Treatment interaction \* and \*\*, significant at 5 % and 1% levels of probability respectively.

The results in Table 2 showed that silicon application significantly increased SFW, SDW, RFW, and RDW in heat-treated cowpea plants. RFW (g) decreased significantly in untreated plants at 36 °C, but increased at low temperature. A strong

decrease was observed at 36 °C compared to 30 °C. The data show that the silicon treatment improves the SFW. Similarly, Si treatments were found to be effective in overcoming the detrimental effect of temperature on SDW.

**Table 2.** Effects of temperature and silicon application on RFW, RDW, SFW and SDW of black-eyed cowpea

Treatments	RFW (g)	RDW (g)	SFW (g)	SDW (g)
30 °C + 0.0 mM silicon	1520.46 aB	0.19 aB	0.19 aB	0.65 aB
30 °C + 1.5 mM silicon	1650.00 aA	0.24 aA	0.24 aA	0.72 aA
36 °C + 0.0 mM silicon	1361.92 bB	0.17 bB	0.17 bB	0.55 bB
36 °C + 1.5 mM silicon	1421.88 bA	0.18 bA	0.18 bA	0.60 bA
Temperature (A)	**	**	**	**
Treatment (B)	**	**	**	**
Temperature x Treatment (AxB)	**	**	**	**
LSD (AxB)	0.681	0.001	0.001	0.003

The sources of variance were as follows: two temperatures, two silicon applications and interaction between temperature and silicon. Different lower case letters in the same column indicate that the difference between temperatures and upper case letters indicate that the difference between silicon applications is significant. Least significant difference (LSD) of the Temperature x Treatment interaction \*\*, significant at 1% levels of probability.

Exposure of 42 °C for 1 day during the vegetative period in the broad bean plant negatively affected the development and caused a decrease in photosynthesis (Hamada, 2001). High temperature (45 °C) is physiologically accepted as a lethal and it is emphasized that it causes the death of cells and tissues in the seed. On the other hand high temperature (45 °C) biochemically affects reserve mobilization to the embryo. At the molecular level, a decrease in total protein amount was observed after 22 hours of germination at 45

°C (Essemine et al., 2007). Membrane damage was observed at 40/30 °C in sensitive chickpea genotypes, and the damage was exacerbated by the increase in temperature to 45/35 °C (Kumar et al., 2013). Increases in temperature significantly increased the properties related to germination and germination in wheat. Root length, shoot and root fresh weight, root dry weight and seed viability index increased with increasing temperatures (Burio et al., 2010). The effect of high temperature (37, 40, 42, 45

and 50 °C) on the germination and seedling growth of corn, rice and sorghum plants was examined and it was observed that the germination rate decreased as the temperature increased. It was revealed that corn plants exposed to 37 and 40 °C for 96 hours showed significant increases in shoot length. However, it was determined that there was a serious decrease in root and shoot lengths at 42, 45 and 50 °C. A similar situation was observed in sorghum plants. In rice plants, a decrease in SL and RL was determined only at 50 °C (Iloh et al., 2014). In wheat, high temperatures during seed germination caused a decrease in the dry matter of the plant (Cargnini et al., 2006). Exposure of grass plants to very high temperatures reduced shoot and root growth, root number and diameter (Xu et al., 2000). Temperatures 5-6 °C above ambient temperature preserved the structural integrity of *Trichilia emetica*, so germination and seedling growth were not compromised (Sershen et al., 2014). Similarly, it has been observed that photosynthesis decreases significantly in cotton starting from 40 °C (Demirel, 2008).

Liu et al. (2008) emphasized that temperature has a significant effect on growth, yield and quality of soybean. It was clear that, germination rate of 30 °C and 36 °C treated plants was not change with silicon application. However, increase in germination rate was observed only at temperature applications. The highest value was observed at 30 °C and without silicon (Table 3). Temperatures had a negative influence on germination percentage and germination rate coefficient. Significant differences were observed between cultivars in germination and germination rate coefficient at all 3 temperatures. Plants with high germination or germination rate coefficient at medium temperature, but it didn't show high germination at low or high temperature. Species that germinated more at high temperatures showed lower germination ability at low temperatures (Islam et al., 2006). *Diptychandra aurantiaca* seeds showed 97 % germination at 25 °C and 87 % at 30 °C. Temperatures not only stimulated germination but also increased germination rate (De Oliveira et al., 2013).

**Table 3.** Effects of temperature and silicon application on germination rate of black-eyed cowpea

Treatments	GR (%)
30 °C	99.88 a
36 °C	98.98 b
0.0 mM silicon	99.51 a
1.5 mM silicon	99.31 a
Temperature (A)	**
Treatment (B)	ns
Temperature x Treatment (Ax B)	ns
LSD (A): 0.60; LSD (B): 0.48	

The sources of variance were as follows: two temperatures, two silicon applications and interaction between temperature and silicon. Different lowercase letters in the same column indicate that the difference between temperatures are important. Least significant difference (LSD) of the Temperature x Treatment interaction ns and \*\* non-significant and significant at 1% levels of probability respectively.

Eleven phenolic acids were found in germinating cowpea seeds: GA, PA, C, CHA, VA, CAF, SA, PCA, CA, Q and CAM (Table 4 and Table 5). During germination, the content of these acids increased with the increase of temperature, except for chlorogenic acid. Germinated

cowpea contained the highest concentrations in the two phenolic compounds, which constituted 39.57 mg.g<sup>-1</sup> and 32.44 mg.g<sup>-1</sup>, respectively at 36 °C. Under elevated temperature (36 °C), a rapid increase in catechin in germinated seeds was noticed.

**Table 4.** The effect of temperature on different phenolic compounds (mg.g<sup>-1</sup> dry weight) of black-eyed cowpea

Temperatures (°C)	GA	PA	C	CHA	VA	CAF
30 °C	13.15 b	18.51 b	8.78 b	4.30 a	9.82 b	2.60 b
36 °C	32.44 a	29.91 a	39.57 a	0.22 b	19.85 a	7.92 a
Temperature (A)	**	**	**	**	**	**
LSD (A)	4.12	3.88	1.25	0.24	3.49	0.71

\*\*, significant at 1% levels of probability respectively.

During the experiment, the content of these acids increased with the increase of temperature, except for quercetin. The highest concentration in the analyzed seed extracts was achieved by catechin at 36 °C. Statistically differences were verified between temperatures. Among the 11 identified phenolic compounds, caffeic acid and chlorogenic acid were characterized by the lowest concentrations. The amounts of 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, coumaric acid, GA, CA, benzoic acid and VA were observed in hardy fescue plants under 21-day heat stress conditions. On the other hand, while

salicylic acid and SA contents remained unchanged, significant decreases occurred in homovanilic acid, CAF and ferulic acid contents (Wang et al., 2019). In tomato and melon plants, thermal stress caused the accumulation of soluble phenolics (Riviero et al., 2001). Similarly, as the heat stress increased, the polyphenol content in eggplant increased (Helyes et al., 2015). It was also observed that phenolic compounds increased with temperature increases (Wang and Zheng, 2001; Swigonska et al., 2014). However, sinapinic, PCA and ferulic acid contents increased significantly with stress conditions (Swigonska et al., 2014).

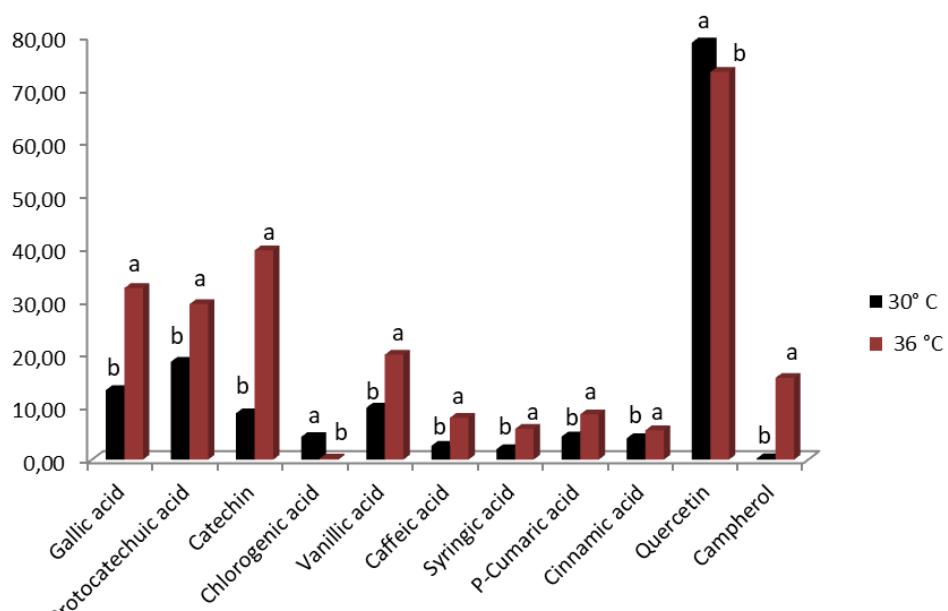
**Table 5.** The effect of temperature on different phenolic compounds (mg.g<sup>-1</sup> dry weight) of black-eyed cowpea

Temperatures (°C)	SA	PCA	CA	Q	CAM
30 °C	1.99 b	4.37 b	4.06 b	78.86 a	0.26 b
36 °C	5.81 a	8.56 a	5.54 a	73.29 b	15.47 a
Temperature (A)	**	**	**	*	**
LSD (A)	0.81	0.60	0.66	4.89	0.86

\* and \*\*, significant at 5 % and 1% levels of probability respectively.

Figure 1 summarizes the result of the phenolics at 30 °C and 36 °C. Among the phenolic compounds, quercetin was

characterized by the highest concentrations with 78.86 and 73.29 mg.g<sup>-1</sup> at 30 °C and 36 °C, respectively



**Figure 1.** The effect of temperatures (30 °C and 36 °C) on phenolic compounds

## CONCLUSION

Temperature is an important factor affecting seed germination and plant yield, causing physiological and biochemical changes that affect plant growth and development. Cowpea seeds germinated well with silicon application at 30 °C, except for shoot length. Results from the experiments showed a significant decrease in germination parameters tested at 36 °C. Secondary metabolites, including phenolic acids, play an important role in plant defense against abiotic stress. In black-eyed cowpea seeds, temperatures of 36°C-44°C were above the optimum germination temperature, resulting in higher content of some phenolic acids, other than chlorogenic acid and quercetin, in the germinated seeds. Considering that temperatures will increase as a result of global climate change, it has been understood that the germination and growth of plants will be affected significantly, whereas silicon application will alleviate this effect.

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