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Impact of heat stress on germination and seedling growth of chili pepper (*Capsicum annuum* L.)

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Impact of heat stress on germination and seedling growth of chili pepper (*Capsicum annuum* L.)

Rosmaina*, D Utami, E Aryanti and Zulfahmi

Department of Agrotechnology, Faculty of Agriculture and Animal Science, Universitas Islam Negeri Sultan Syarif Kasim Riau, Panam Campus-Pekanbaru 28293, Riau, Indonesia.

Corresponding author: rosmaina@uin-suska.ac.id

Abstract. Temperature plays an important role in plant growth and development. The increased temperature suddenly may cause severe consequences. This study aimed to observe the impact of temperature, exposure of duration, and the interaction between temperature and exposure duration at the germination phase and seedling growth of chili pepper. The research design used Factorial Completely Randomized Design. The first factor was the temperature consisted of five-level of temperature i.e: 35°C, 37°C, 39°C, and 41°C. The second factor was the exposure duration consisted of four hours, eight hours, and twelve hours. The Parameters observed were the percentage of seed germination, shoot length, root length, number of roots, seedling height, number of leaves, fresh weight of seedling, dry weight of seedling, and chlorophyll content. The results of this study showed that there is an interaction between temperature and duration of exposure, the rise in the temperature and duration of exposure decrease shoot length 35.15%, root length (23.23%), number of roots (29.82)%, fresh weight of seedling (40%), number of leaves (30%), chlorophyll content. As a result, the growth of Chili seedling showed a drastic reduction in shoot development, root development, and physiological parameters tested, the germination stage is very susceptible to raise the temperatures.

1. Introduction

Abiotic stress such as high temperature, drought, and salinity is one of the limiting factors for plant growth and development. High-temperature stress in plants occurs when the environmental temperature exceeds the optimum temperature required by plants [1]. The temperature of the earth's surface is predicted to increase for 1.5°C in the year 2030 [2]. This means that the plant will expose to higher temperatures than normal. Plants exposed to high-temperature stress will experience disturbances in plant growth and development, such as shorter plant size, smaller leaf length, and width, a faster flowering, increase the flower abortion, degrade pollen viability, reduce fruit sizes, and a significant loss in production. [3, 4, 5]. High-temperature stress causes an imbalance between photosynthesis and respiration so that it disrupts the plant's metabolic system [6, 7, 8]. Chilli (*Capsicum annuum*) belongs to the Solanaceae family and is one of the horticultures crop important economically. Chili is widely cultivated in the world and is used as fresh food and processed products [9] (FAO, 2018). Chili is a plant sensitive to high-temperature stress [4, 10, 11, 12, 13]. High-temperature stress affects growth and development at various stages of growth, even in the early stages of plant development.

The response of plants to high-temperature stress varies widely, depending on temperature, species, age, phase of plant growth, and the length of temperature exposure. Different species tend to show different responses, even within the same species often show different responses [3, 5, 14, 15]. High-temperature stress affects all phases of plant growth, including the germination phase. The high-temperature stress in the seed can delay the germination process [16]. Several reports related to the effect of high temperature on the germination phase have been reported in rice [17] on wheat [18,19], maize, and sorghum [20]. So far, no reports regarding the effect of high-temperature stress on the germination phase of chili. Therefore, the study on the impact of high temperature on the germination phase of chili is needed to be carried out. As we know, the germination stage and seedling development have an important role in cropping sustainability, temperature stress at this stage has a negative impact on plant growth and development, so it directly affects productivity. The success of plants to grow and develop is strongly influenced by optimum germination conditions. This study aims to know the effect of temperature stress on shoot growth, root growth, and physiological characters of chili plants due to temperature stress at the germination and seedling stages. The result of this study is expected useful to a fast method of screening at the germination level of the chili plant.

2. Materials and methods

The research was conducted in the greenhouse of Genetic and Breeding Laboratory, Faculty of Agriculture and Animal Sciences, Universitas Islam Negeri Sultan Syarif Kasim, Riau, Indonesia. The experiment was laid out following Factorial Completely Randomized Design (CRD) consisting of two factors. The first factor was temperature (control, 35^{o}C, 37^{o}C, 39^{o}C, and 41^{o}C). The second factor was the high-temperature stress duration (4, 8 and 12 hours), and 12 of total treatments. Each treatment is consisted of 100 seeds. The materials used in this research were Kopay variety curly red chili pepper, seedling trays, topsoil, and 80% acetone for analysis of chlorophyll contents. The tools used in this research were water bath, Petri dish, and UV-Vis spectrophotometer. The seed is soaked in the water bath according to temperature and exposure duration treatments. Furthermore, the seeds are transferred to the seedling medium with daily temperature. After seven days old, the sprouts were transferred to seedling tray. The Parameters observed were percentage of germination, shoot length, root length, the number of roots, fresh weight, dry weight, seedling height, the number of leaf, and chlorophyll content. The measurement of chlorophyll content was carried out destructively following the method by [21].

3. Results and discussion

3.1. Shoot development

3.1.1. Percentage of germination. High-temperature stress treatment with various durations of exposure resulted in a seed germination percentage of 65–98% at five days after sowing. Temperature stress of 39^{o}C and 41^{o}C resulted in a higher percentage of germination, which is 81–98%, while control plants only produced an average germination percentage of 63.33% (Table 1). The results of this study indicated that the lower temperature decreased the percentage of germination and caused a delay in germination. Delaying of germination at low temperatures has also been reported in wheat [22], furthermore, [23] explained that at low temperatures, the process of imbibition and protein synthesis in the embryo also occur slowly and gradually. Increasing the temperature up to 41^{o}C increases the germination percentage of chili. Enhancement germination due to temperature increases were also reported in rice [17], wheat, rice, and maize [20], and *Arabidopsis* [24]. Temperature stress in short-term heat stress conditions can increase the germination percentage of rice up to 30% because in short-term heat stress occur an increase in gibberellin (GA), starch content, and decreases ABA, but higher increases temperature above 39^{o}C and exposure time of 72 hours declined the germination rate of rice and only 30% that is able to germinate. This is closely correlated with a reduction in starch, a decrease of GA, and an increase in ABA [17, 24]. Gibberellin acid (GA) and ABA are the two main phytohormones that act as antagonists in stressful conditions, especially in the germination phase [25]. ABA has an important role as a regulator of HSPs activation in tolerance mechanism for high-temperature stress, but extreme temperatures of 45°C are not good for germination because it causes embryo damage, cell death, and increased ABA [17, 20, 26].

3.1.2. Shoot length, number of leaves and seedling height. High-temperature stress, exposure time, and both interaction significantly difference the length of shoot, number of leaves, and height of seedlings. The highest shoot length was found in the control, which ranged from 2.36–2.39 cm and was significantly different from others, while the temperature at 35°C after 8 hours exposure produced the shortest shoot, 1.55 cm, or reduction of 35.15% (Table 1, Figure 1A). The rise in temperature significantly reduced the shoot length, ranged from 15.90–35.15% compared to the control. The highest number of leaves obtained at the temperature of 37°C with four hours exposure duration was 6.02 leaves per plant, while the lowest number of leaves was obtained in the 41°C and 35°C with 12 hours of exposure and both were not significantly different from the others (Table 1). The decreased in the number of leaves ranged from 4.78–30.00% compared to the control, except for 37°C, the number of leaves increased of 4.78% (Figure 1B). Seedling height ranged from 6.61-4.89 cm, the temperature of 37°C produced the highest seedlings (6.61 cm), and it was significantly different from the temperature of 41°C which resulted in 4.89 cm (Figure 4A). The 12 hours exposure time resulted in an average seed height of 5.1 cm, which was significantly from the 4 hours which resulted in seed height average of 6.18 cm (Figure 4B). Reductions of the shoot length, leaf number, and seedling height were also reported in rice [17, 20, 27], maize [20, 28], Chili [11], *arabidopsis* [29], wheat and cotton [30].

Table 1. Germination rate (%), shoot length (cm) and the number of leaves on germination and seedling growth of chili exposed to high temperatures with different levels of temperature and exposure time

Exposure duration (hours)	Temperature (°C)					Average
	Control	35	37	39	41	
			Germination rate (%)			
4	65.00	76.00	76.00	98.00	97.00	82.40
8	69.00	55.00	56.00	82.00	92.00	74.80
12	65.00	63.00	66.00	94.00	86.00	70.80
Average	66.33	64.66	66.00	91.33	91.67	
			Shoot length (cm)			
4	2.39^{a}	1.68^{de}	1.70^{de}	1.92^{bc}	1.84^{bcd}	1.90
8	2.37^{a}	1.55^{e}	1.75^{cd}	1.84^{bcd}	1.65^{de}	1.83
12	2.36^{a}	1.80^{cd}	2.01^{b}	1.69^{de}	1.67^{de}	1.91
Average	2.37^{a}	1.68^{c}	1.82^{b}	1.81^{b}	1.72^{bc}	
			Number of leaves (cm)			
4	5.32^{bcd}	5.07^{cde}	6.02^{a}	4.82^{defg}	4.55^{efghi}	5.16^{a}
8	5.27^{bcd}	4.42^{fghi}	5.47^{bc}	4.62^{efgh}	4.20^{hi}	4.80^{b}
12	5.75^{ab}	4.02^{i}	4.92^{cdef}	4.25^{ghi}	4.02^{i}	4.59^{b}
Average	5.45^{a}	4.50^{b}	5.47^{a}	4.56^{b}	4.25^{b}	

The magnitude of the reduction due to high-temperature stress is strongly influenced by temperature, exposure duration, species, and the genotype tested. In Maize, it was reported that increased of the shoot length still occurred at 37°C and 40°C with exposure duration of 48 hours, and then sharply dropped at a temperature of 42°C–50°C [20]. In rice, stress at 35°C for 24 hours raise shoot length compared to control, but the enhanced exposure duration to 48–72 hours significantly decreased the length of the shoot [17]. In chili, the reduction in shoot length occurred up to 9.12–54.05%, and is strongly influenced by the genotype used [11]. The leaf growth rate, leaf number, leaf

Length continued to increase linearly until the optimum temperature limit [29]. The optimum temperature for growth and ideal development of each plant is different [3, 30]. High-temperature stress triggers Ca^{2+} fluxes, kinase cascade, and ABA accumulation [31], that matter cause mitotic cell division delay [32], and even lead to cell death [33]. Furthermore, in conditions of heat stress, there is a reduction in metabolism and an imbalance in the mobilization of nutrients. Moreover, the disintegration of membrane structures, chlorophyll and protein molecules are also found, as well as low nutrient uptake [28, 34]. Morphological changes in plants due to high-temperature stress in plants were also reported by [35, 36] which was directly effected of high-temperature stress on plants. In tomatoes, high-temperature stress reduces canopy width, and stem diameter [37], inhibits the growth of twigs and branches and the number of leaves [38, 39]. High-temperature stress causes denaturation and aggregation of proteins, reduce cell function, loss of function several enzymes that play an important role in cell division, and even causes cell death so that it affects plant growth and development [31, 40]. The inference that high-temperature stress discourages plant growth and development predictably is closely related to the disruption of physiological processes in plants.

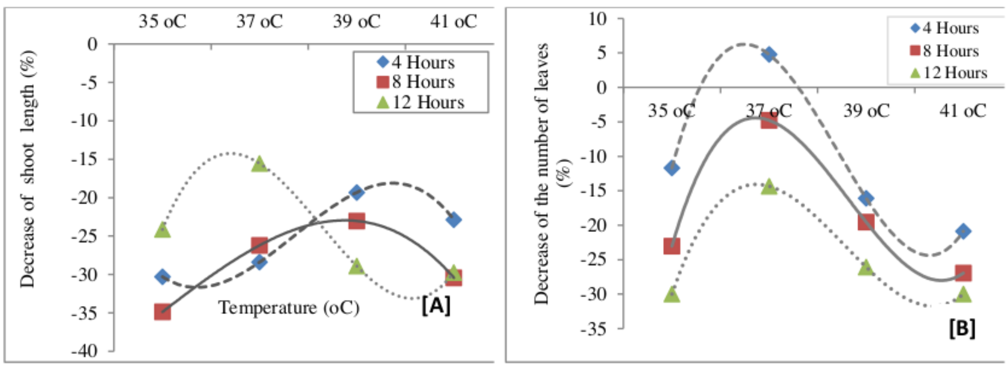

Figure 1. The decreased percentages of shoot length [a], and the number of leaves [b] on germination and seedling growth of chili exposed to high temperatures with different levels of temperature and exposure time

3.2. Root development

3.2.1. Root length and number of roots. The root length and number of roots were significantly affected by temperature, exposure time, and both interactions. The highest root length was obtained in control plants, namely 2.54 cm and was significantly different from other treatment, the smallest root length was obtained at the temperature of 39°C with 8 hours of exposure of 1.95 cm or lower of 23.23% but it was no significant difference with the treatment of 41°C and an exposure duration of 8-12 hours (Figure 2A). High temperature stress increased the number of roots, especially at 39°C for 12 hours and 41°C for 8 hours, with an average of 5.42 and 5.14 roots per shoot, respectively (Table 2) or increase of 19.80% and 13.47%, respectively and both were higher than the control (Figure 2B).

The results of this study indicate that an increase of the number of roots only occurs up to a certain temperature, while an increase of higher temperatures will decrease the number of roots. The similar results were reported for wheat, in which elevated temperatures of 37°C and 40°C were still an increase in root length, but an increase in temperature and duration of exposure were significantly decreased root length [20]. In Arabidopsis, high temperatures of 29°C tend to an increase in root length compared to control plants (23°C) through increased cell division and increased auxin content in the roots [41]. Decrease of the root length and an increase in root numbers under high-temperature stress were also reported in rice [42], sunflower [43], wheat [18], and cotton [44]. Increasing the number of roots under stress is closely related to the reduction of carbohydrates in the endosperm [44], and activates root function in balancing respiration and nutrient uptake [45, 46]. Different varieties showed different responses, generally, varieties with longer roots are considered having a higher tolerance to stress [47]. This is related to the ability of plants to absorb water under conditions of temperature stress and drought. In wheat, the reduction in root length reached 78% at 34°C [44] and 12-32% at 36°C [19], and in this study, the reduction in root length was 23.23%. This data confirms that the magnitudes of the reduction in the root length and the number of root are influenced by temperature, length of exposure, the type and genotype of plants used.

Table 2. Average of root length (cm) and the number of roots on germination and seedling growth of chili exposed to high temperatures with different levels of temperature and exposure time

Time expose (hours)	Temperature (°C)					Average
	Control	35	37	39	41	
	Root length (cm)					
4	2.54^{a}	2.09^{def}	2.09^{def}	2.15^{cd}	2.26^{c}	2.22^{a}
8	2.43^{ab}	2.09^{def}	2.09^{def}	1.95^{f}	2.15^{cd}	2.14^{b}
12	2.45^{ab}	2.14^{cde}	2.39^{b}	1.99^{ef}	2.14^{cd}	2.22^{a}
Average	2.47^{a}	2.11^{c}	2.18^{b}	2.03^{c}	2.18^{b}	
	Number of roots					
4	4.66^{abc}	3.56^{ef}	4.54^{abcd}	4.30^{bcde}	3.76^{cdef}	4.16ab
8	4.36^{bcde}	3.18^{f}	$3.68d^{ef}$	3.84^{def}	5.14^{ab}	4.04b
12	4.58^{abcd}	4.14^{cde}	3.68^{def}	5.42^{a}	4.6^{abcd}	4.48a
Average	4.53^{a}	3.63^{b}	3.97^{b}	4.52^{a}	4.50^{a}	

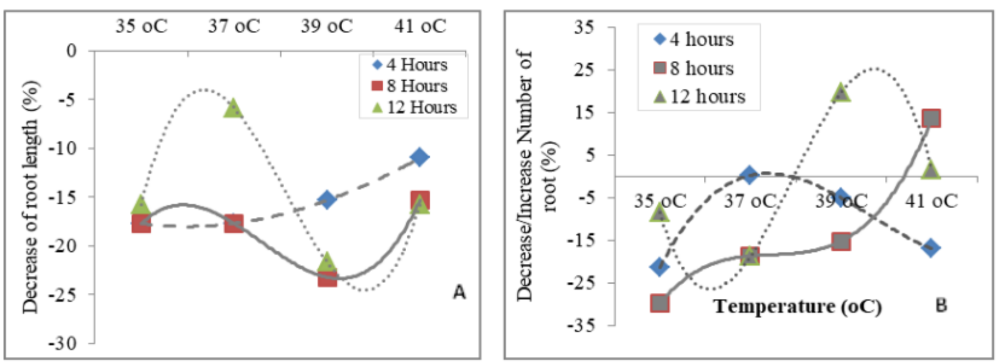

Figure 2. The decreased percentages of the root length [a] and the number of roots [b] on germination and seedling growth of chili exposed to high temperatures with different levels of temperature and exposure time.

3.3. Physiology response

3.3.1. Fresh weight, dry weight and chlorophyll content. The result of analysis of variance showed that fresh weight was influenced by high-temperature stress, exposure time, and both interactions, the highest shoot weight was obtained in the control with an average of 0.40 g, which was significantly difference from others. The lowest fresh weight was obtained in the treatment of 35°C with an eight hours of exposure time, that was 0.24 g and no significantly from the temperature of 37°C and 39°C (Table 3). The high-temperature stress that was given reduced the fresh weight of the shoot up to 40% compared to the control. The magnitude of the reduction due to high-temperature stress is strongly influenced by the temperature and length of exposure given (Figure 3A). High-temperature stress reduced fresh weight, dry weight, chlorophyll content and photosynthetic rate [28, 48]. The dry weight of shoots was significantly affected by the temperature and duration of exposure, while the interaction between temperature and exposure time did not significantly difference. Dry weight ranged from 0.034g–0.026g, which the highest dry weight was found in the control and the lowest dry weight was found in temperature of 39°C (Figure 4A). The high-temperature stress in this study reduced the dry weight of 17.65–23.53%. The exposure duration of 12 hours showed the highest of dry weight (0.031 g) and the exposure duration of 8 hours displayed the lowest of dry weight (0.026 g) (Figure 4B). A decrease of the dry weight and dry root due to high-temperature stress was also reported by [19] on wheat 38–41% and up to 70%, respectively, and rice [27].

Table 3. Average fresh weight of sprouts (g) and chlorophyll a (mg/l) on germination and seedling growth of chili exposed to high temperatures with different levels of temperature and exposure time

Time expose (hours)	Temperature (°C)					Average
	Control	35	37	39	41	
			Fresh weight of sprouts (g)			
4	0.41^{a}	0.28^{cd}	0.26^{d}	0.31^{c}	0.33^{bc}	0.27^{a}
8	0.39^{a}	0.24^{d}	0.25^{d}	0.28^{cd}	0.33^{bc}	0.30^{b}
12	0.40^{a}	0.33^{b}	0.38^{ab}	0.28^{cd}	0.32^{c}	0.34^{a}
Average	0.40^{a}	0.28^{c}	0.29^{c}	0.29^{c}	0.33^{b}	
			Chlorophyll a (mg/l)			
4	2.06^{ab}	1.78^{c}	2.13^{a}	1.91^{abc}	1.95^{abc}	1.97
8	1.87^{bc}	1.99^{abc}	2.17^{a}	1.92^{abc}	1.84^{bc}	1.96
12	1.80^{c}	1.99^{abc}	1.93^{abc}	2.01^{abc}	1.91^{abc}	1.93
Average	1.91	1.92	2.08	1.95	1.90	

The total chlorophyll content was not affected by temperature, exposure duration, and both interactions, but chlorophyll a was significantly influenced by the temperature and interactions between temperature and exposure duration, while chlorophyll b was significantly influenced by the temperature tested. The average value of chlorophyll b content ranged from 0.86–0.98 mg/l, the highest chlorophyll b was observed at 37°C and the lowest chlorophyll b was found at 39°C. In general, chlorophyll a and chlorophyll b decreased due to high-temperature stress. The amount of reduction in chlorophyll a content ranged from 4.19–13.61%, but several treatments exhibited an increase in chlorophyll a, such as treatment of 39°C for 4–8 hours of exposure (Table 3, Figure 3B). The reduction of chlorophyll a and chlorophyll b content in this study were lower than those reported by [49] in which of 46.10% and chlorophyll b 21.05%, respectively, when strees is given in juvenile stage. Furthermore, [49] explained that the decrease of chlorophyll a and chlorophyll b in chili pepper is closely related to stomata damage. Other study also reported that high-temperature stress decrease in chlorophyll content in sorghum plants (28%) [50], in Thalassia hemprichii (58.20%) [51], and wheat (>75%) [52].

The reduction of chlorophyll content has a significant correlation with degradation in the rate of photosynthesis [28, 53, 54] and has an impact on lost of yield and biomass [55]. [11] reported that decrease of chlorophyll content from 2.61–8.40% will reduce the photosynthesis rate of 20.42–38.42%. Increased of temperature reduces photosynthetic activity through decreasing chloroplast pigments in leaves [56], structural damage of pigment-protein complexes and activity on PS2 [57,58], interference with chlorophyll biosynthesis [59,60, 61, 62], an inhibition of the electron transfer process and some of the enzymatic activities required for photosynthesis.

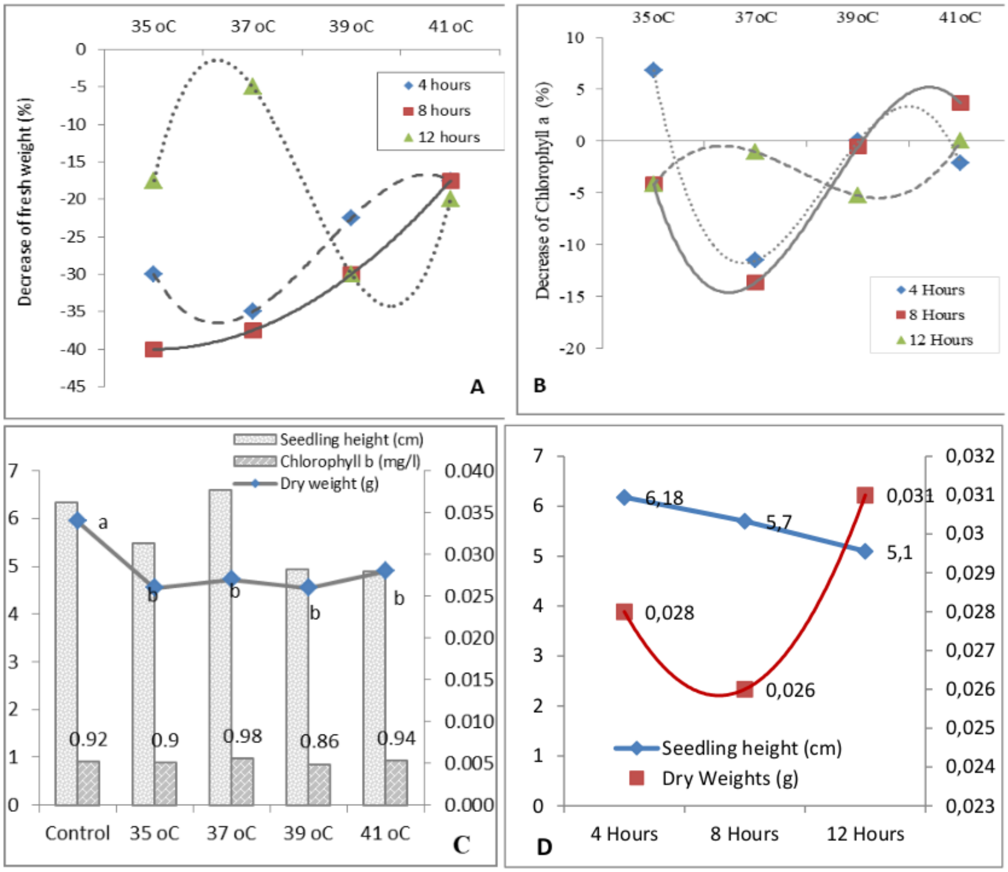

Figure 3. Decrease of fresh weight percentage [A] and chlorophyll a [B], the impact of temperature on seedling height, chlorophyll b, and dry weight [C], effect of temperature on seedling height and dry weight [D] on Germination and Seedling Growth of Chili exposed to high temperatures with different levels of temperature and exposure duration

In addition, the accumulation of reactive oxygen species (ROS) causes damage to thylakoid structures in chloroplasts, and stomatal damage [49, 50, 52, 60, 63]. Based on the results of study [52], of the 12 genotypes tested, there were several genotypes that did not experience a significant reduction in chlorophyll content, so it was concluded that changes in chlorophyll content could be used as an indicator of high-temperature tolerant plant selection. This is a line with those reported by [52].

4. Conclusion

Based on the results of this study, we concluded that germination stage is susceptible to high temperature stress due to reducing in shoot development, root development and physiology characters of chili pepper.

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