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Effects of temperature treatment on seed germination, root development and seedling growth of *Citrullus lanatus* (watermelon)

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Abstract

Khandaker, M. M., Fazil, R. Alam, M. A., Saifuddin, M. & Zakaria, A. J. (2020). Effects of temperature treatment on seed germination, root development and seedling growth of *Citrullus lanatus* (watermelon). *Bulg. J. Agric. Sci., 26 (3)*, 558–566

The objective of the present study is to investigate the effects of temperature on the seed germination, root development and seedling growth of watermelon. Yellow flesh watermelon seeds were incubated with control (normal temperature 25°C, 20°C, 30°C, 35°C and 40°C under lab condition. This experiment was carried out with Completely Randomized Design (CRD) with five treatments and each treatment consists of five replications. The morphological and physiological parameters were measured once a week for five weeks during the germination and early seedling establishment. The results showed that seed treated under 35°C treatment produced the best results for the days require to germination, germination percentage, root development, vine and root length, leaf expansion and seedling growth of watermelon. In addition, chlorophyll content, carotenoid content, chlorophyll fluorescence, photosynthetic yield and stomatal conductance also affected positively with the temperature treatments. Fresh and dry biomass accumulation in the seedlings of watermelon was also the highest in 35°C treatment. It can be concluded that seeds incubated with 35°C during the germination increased the germination rate, root development and seedlings growth of watermelon.

Keywords: germination; temperature; growth; development; watermelon; seedling

Introduction

Citrullus lanatus (watermelon) is a member in the family Cucurbitaceae, kingdom Plantae, order Cucurbitales, genus *Citrullus* and species *lanatus*. It refers to vine-like (scrambler and trailer) flowering plant. Watermelon fruit are generally believed to have originated from sub-Saharan Africa several thousand years ago and to have travelled over time from Africa to Asia to Europe to North America. Their arrival in Asia and the Middle East is believed to have dated back to approximately 900 – 1000 A. D., and their arrival in Europe is estimated to have occurred in 1300 – 1400 A.D (Worlds Healthiest Food Organization, 2013). Watermelon is one of the most important and demanding widely cultivated crops in the world and its global consumption is greater than that of any other cucurbit (Gichimu et al., 2008; Dalorima et al., 2018). It accounts for 6.8% of the world area devoted to vegetable production (Goreta et al., 2005). According to Ding & Syazwani (2012), watermelons ranks top in Malaysia fruit export with 43,489 tons of watermelon which is worth USD

11 962.00. Watermelons are ultimate source of water and as such are helpful in preventing dehydration (Dalorima et al., 2019). The low calories content of the watermelon fruits makes it the best choice for diet-conscious people. Watermelon fruits contain water around 93%, plenty amount of sugar and many vitamins like as thiamin, riboflavin and niacin. Watermelon is the ultimate potassium rich fruits, which is considered to help in the control of high blood pressure and perhaps avert strokes (IITA, 2013).

Citrullus lanatus is a popular fruit in Malaysia and Malaysia exports watermelons to Singapore, Hong Kong, Middle East and Europe (Yau et al., 2010). The demand for tropical fruits is on the increase, not only for watermelons but also other tropical fruits. It is an important cucurbitaceous creeper crop originally from Southern Africa. The production and consumption of this fruit are greater than that of any other species in the Cucurbitaceae family (Adeoye et al., 2007). Germination of watermelon seed and seedlings growth after germination depends on soil temperature. Sometimes seed germination percentage and initial seedlings growth is very low due to inadequate soil temperature. Farmers sometimes get problem to germinate seed and they may need to buy low quality seedling from others. The need of different temperature treatments is to solve the problem of seed germination percentages, which helps farmers to increase the more number of seedlings and for that requiring less number of seeds. Slow seedling growth and poor root development occur due to lack of optimum temperature. In this study, we investigated the effects of different temperatures on the germination, growth and development of watermelon seedlings.

Materials and Methods

Plant Materials

The experiment was carried out at the plant physiology laboratory and green house of the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia. Yellow flesh watermelon (*Citrullus lanatus* (Thunb) Matsum and Nakai) seeds were purchased from Bumi Maju Agro, an authorized seed dealer of Terengganu and used for germination and growth and developmental study. The mixture of cocopeat and soil as the growing media were prepared in the germination tray. The mixture of cocopeat and BRIS soil were prepared and added in 16 × 16 inches poly bags for the planting of watermelon seeds.

Seed Sowing and treatment application

The germination trays were prepared by filling the holes with growing media mixture of cocopeat and soil. Then, the growing media was moistened with water prior to sowing the watermelon seeds to provide moisture to the media. After that, each hole of the tray was sown with one seed only. All the planted seeds in the tray were placed in the germinator. The seeds were treated with five different temperatures including control (normal temperature 25°C), 20°C, 30°C, 35°C and 40°C. All the seeds were treated with different temperature regimes only two days. After treatment application all the seedlings were kept at normal condition. The growing media regularly moistened by applying water to ensure saturation throughout the germination period.

Transplanting in polybags

After 3 weeks, all the seedlings were transplanted into the poly bags containing the planting media which are the mixture of coco peat and BRIS soil with ratio 1:1. Each poly bag contained about 1 kg of growing media. The growing media in the germination tray was sprayed with water to make it easy to pull out the seedling from the tray. A small hole was dug at the center of the poly bag. After that, the seedlings were transplanted in the 16 × 16 inches poly bags. Each poly bag contained one seedling only.

Measurement of seedlings growth and developmental characteristics

Seeds were considered to have germinated once the radicle had protruded at least 2 mm from the testa. The days of required to seed germinate, and its germination rate were recorded. The vine length, number of leaf and leaf area were measured weekly. The root development was measured after seedling already uprooted from the polybags. The length of root was measured by using ruler. The whole seedling was taken out from the poly bag. Fresh weight of the seedlings was taken by weighing the plants by using weighing balance. Then, the plants were dried in oven at 60°C for 24 hours and its dry weight was taken.

Determination of chlorophyll and pigments content

Chlorophyll content was measured by using SPAD-502 meter (Minolta Japan) according to the method described by Saifuddin et al. (2009). This SPAD meter was lightweight, hand-held device that widely used for accurate, fast and non-destructive measurement of leaf chlorophyll content by means of absorbance or transmittance measurements. The measurement was taken by meter which was simply clamped over leafy tissue. The meter showed the indexed chlorophyll content reading. Carotenoid content was measured by using spectrophotometer. This measurement was taken to measure the photosynthetic pigments of leaf. The leaves samples were collected for each treated seedling. Then, the samples were cleaned and air dried. The veins of the leaf were removed, and the leaf was cut into small pieces. Samples were weighed about 1 g by using electronic balance for each treatment. The weighed samples were crushed by using mortar and pestle and were homogenized in 10 ml of 80% acetone for each 0.25 g samples. Then, the homogenate was filtered through mounted in glass funnel. The filtrate was poured into 3 mL cuvette and its absorbance was measured in wavelengths of 663 nm, 645 nm and 480 nm wavelengths for measurement of chlorophyll a, chlorophyll b and carotenoid, respectively. The readings were taken by spectrophotometer devices. The concentrations of chlorophyll and carotenoids were calculated by using the formula of Arnon (1949).

The formula to calculate the pigments concentration is as shown below:

Chlorophyll a (mg/L) = 12.7 × A663 – 2.69 × A645

Chlorophyll b (mg/L) = 22.9 × A645 – 4.68 × A663

Carotenoid (µg/g) =

= [A480 + (0.114 × A663) – (0.638 × A645)] / 112.5

Stomatal conductance, chlorophyll fluorescence and photosynthetic yield and leaf Total Soluble Solid (TSS)

A Portable Leaf Porometer, Model SC-1 was used to measure the stomatal conductance (CID Bio-science, USA). Measurement was taken for three replicates at different spot of a single leaf. The average of all three replicates was calculated and the results were recorded. Chlorophyll fluorescence was measured by using JUNIOR-PAM Chlorophyll Fluorometer. The JUNIOR-PAM was controlled by PC through USB interface. The fluorometer requires no external power supply as it is powered by the PC via the USB line. WinControl-3 software was installed, and PC was connected to JUNIOR-PAM through USB. The magnet was clamped to the leaf, and then the chlorophyll fluorescence readings were measured and saved in the PC. Initial fluorescence (F0), maximal fluorescence (Fm) and photosynthetic yield (Fv/Fm) were obtained. Then, variable fluorescence (Fv) was calculated. The TSS of leaf was measured by using handheld refractometer. The leaf sample was crushed by using the pestle and mortar and the juice was extract. Then, 1 to 2 drops of the leaf extract were placed onto the sensor of hand refractometer. The TSS readings were recorded as described by Khandaker et al. (2012).

Statistical Analysis

The experiment was arranged under a Completely Randomized Design (CRD) with five (5) replications. The data were analyzed by using SPSS statistical software. The one-way ANOVA was applied to evaluate significant differences in the parameter studied for the different treatments. Differences at $p < 0.05$ were considered as significant.

Results and Discussion

Germination and physiological characteristics

In this current study, the planted seeds of watermelon were observed every day to obtain its required period to germinate. Table 1 below showed the data on the days of required to seed to germination. The results showed that the seeds incubated with 30°C and 35°C required less days to germinate than the other treatments and control (25°C). Seeds treated under low temperature treatment including the control and 20°C germinate later than seeds incubated with 30°C and 35°C. It was recorded that seed incubated with higher temperature require less days to germinate. Thomas (1981) stated that the survival and performance of seeds after sowing is affected by physical, mechanical, chemical and biotic factors. Temperature, light, drought, flooding and gaseous environments are physical factors which influence seedling emergence. Low temperature after the sowing of many warm-season vegetables can lead to asynchronous seedling emergence (Thompson, 1974). Similar positive effects on seed germination of cucumber are also reported by Ellyzatul et al. (2018). May be the high temperature reduced the concentration of ABA in embryo which stimulate the germination of seeds after sowing or planting.

Germination rate of watermelon seed were observed in the first week after sowed the seeds in germination tray. Germination rate of seed were calculated and represented in Table 1 in percentage value. The data on Table 1 shows the

Table 1. Effect of temperature on days require to seed germinate, vine length, number of leaf and leaf area of watermelon seedlings

Treatment, °C	Days to germinate	Germination Rate, %	Vine length, (cm)	Number of leaf	Leaf area, (cm^2)	Root length, (cm)
Control (25)	3^b	73^b	8.3^a	4^b	8.70^b	11.0^a
20	3^b	87^b	7.0^b	3^b	7.90^c	8.00^b
30	2^a	87^b	7.5^b	4^b	9.70^a	9.80^b
35	2^a	100^a	9.1^a	4^b	10.9^a	13.3^a
40	3^b	100^a	7.2^b	4^b	9.80^a	10.5^a

Different letter in same column differ the significant different in 5% level germination rate of seed treated with different temperature was affected significantly. Based on Table 1below, it can be observed that the germination rate is higher when treated with temperature 35°C and 40°C. The lowest germination rate was recorded in control and 20°C treatment. Our results are supported by the findings of Thompson (1974), who stated that low temperature after the sowing of many warm-season vegetables can lead to asynchronous seedling emergence and reduced the rate of germination. Therefore, temperature is one of the physical factors which influence seedling emergence (Thomas, 1981). Dimalla et al. (1997) also reported that incubation of pecan embryos at 30°C resulted in an increase in the level of endogenous cytokinins and gibberellins and also gave optimum germination.

The vine length was measured in centimeter (cm) unit and the data were presented in Table 1. Seed treated with 35°C gave the highest vine length growth of watermelon at 3rd week of observation. In this study, vine length of watermelon significantly affected ($p < 0.05$) by the temperature treatments. Our result showed that the vine of watermelon grows better at 35°C. According to Nerson (2007), the optimum temperature produced the most rapid plant growth and development. The lowest temperature 20°C including control (25°C) give the least vine growth through out the observation periods (Figure 1). This indicates that the temperature has significant effect on plant growth and development of watermelon. May be the increased temperature increase the cell division.

Number of leaf of watermelon seedlings was recorded during the experimental period. The results showed that the temperature treatments did not produce any siginifcant effect on the production of leaves. Most of the watermelon seedlings produced same number of leaf except lowest temperature at 3rd week of observation. All the treatments except 20°C produced the same number of leaf which were

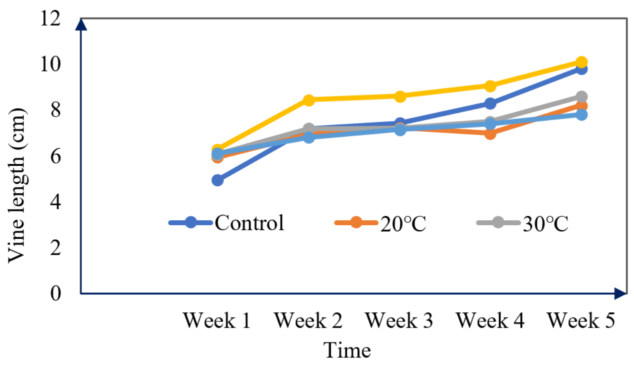

Fig. 1. The effects of different temperature on the vine length growth of watermelon seedlings per week

4 leaves. Seedling under 20°C produced less number of leaf which were 3 leaves. This result suggested that treatment with optimum temperature could increase number of leaf of germinated seedlings. Lower temperature may be inhibit the cell division, cell expansion and morphogenesis of plant. Terry (1968) reported that temperature had significant effect on assimilate distribution in sugar beet and the above and below 24°C the rates of cell division and cell expansion decreased in sugar beet (Figure 2).

Table 1 shows that leaf area of watermelon seedlings was significantly affected by temperature treatments. It showed that the seedlings treated with 35°C gave the largest leaf area, while seedlings treated under 20°C produced the least leaf area. These indicated that the leaf area of watermelon seedlings was affected by temperature and the leaf grows better under high temperature. Baker and Nie (1994) stated that the plants grow slowly and develop leaves with a reduced area when exposed to low temperature during growth. Incubated seed before germination with 30 to 35°C may induce the production of cytokinin and gibberellin hormones which stimulated the cell division and cell of seedlings.

The root length of watermelon seedlings were recorded in week 3 in order to observe the root development under different temperature regimes. From Table 1, it can be seen that root length of watermelon seedling was the highest under 35°C in week 3, while seedling under 20°C showed the least root length in week 3. It was noticed that root under 20°C showed high increased on root length while under 35°C showed least increased in root length from week 3 to week 5. It was also observed that after transplanting root length of seedlings increased with the advancement of temperature. Sattelmacher et al. (1990) also reported that elongation of soybean and potato roots affected positively with the temperature. On the other hand, Ralmi et al (2016) reported that the plant resistance decrease at high temperature above 28°C. May be the optimum temperature allevated the level and activity of cytokin hormne which help to increase the root length. Our results are supported by the findings of Terry (1968), who stated that root length of sugar beet seedlings at 15°C was smaller than at 25°C even through there was a larger concentration of soluble carbohydrates in the leaves at lower temperature.

Chlorophyll content (SPAD value)

The chlorophyll content is an important experimental parameter in agronomy and plant biology research (Lamb et al., 2012). The results showed that the chlorophyll content in leaves of watermelon seedlings was affected significantly with different temperature treatment (Figure 3).

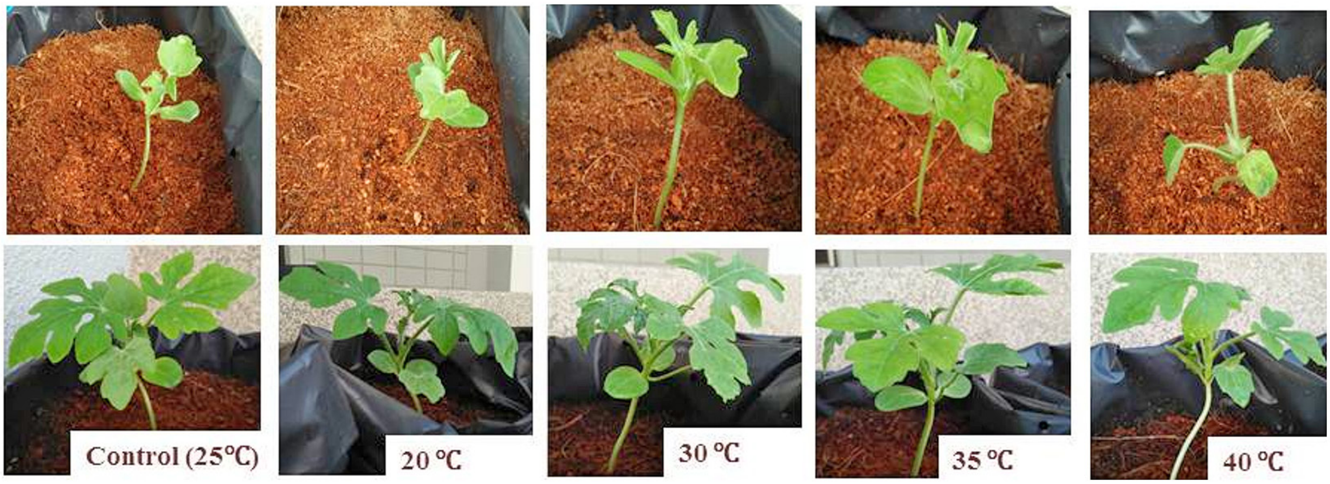

Fig. 2. The growth of watermelon seedlings with different temperatures at Week 3 and Week 4

The seeds incubated with 35°C produced the seedlings with higher leaf chlorophyll content compared to other treatments and control, while the control have the least chlorophyll content (Figure 3). This showed that the leaf chlorophyll content increases when the seeds incubated with 30°C and 35°C. MacWilliam and Naylor (1967) has been suggested that chlorosis develops under conditions of low temperature because chlorophyll is photo-oxidized prior to its integration in pigment–protein complexes of the thylakoid membranes where the pigments are less prone to photo-oxidative damage.

Pigments content

Plant exposed to high photosynthetic photon flux (PPF) present Chl *a/b* ratios around 3.2-4.0 and plants growing in environments with reduced PPF have a ratio around 2.5–2.9 (Lichtenthaler & Wellburn, 1983). Figure 4 below showed the effects of temperature on leaf chlorophyll *a*, chlorophyll *b* and total chlorophyll content of watermelon seedlings.

Seed incubated at 20°C showed higher chlorophyll *a* content with 27.93 mg/L followed by control treatment with 25.58 mg/L, 40°C with 25.40 mg/L and 35°C with 25.37 mg/L, respectively. The lowest reading of chlorophyll *a* comes from treatment under 30°C with only 20.96 mg/L. This indicated that accumulation of leaf chlorophyll *a* content is higher within the range of 20 to 25°C. While for chlorophyll *b*, the highest reading was showed under treatment of 20°C with 45.22 mg/L

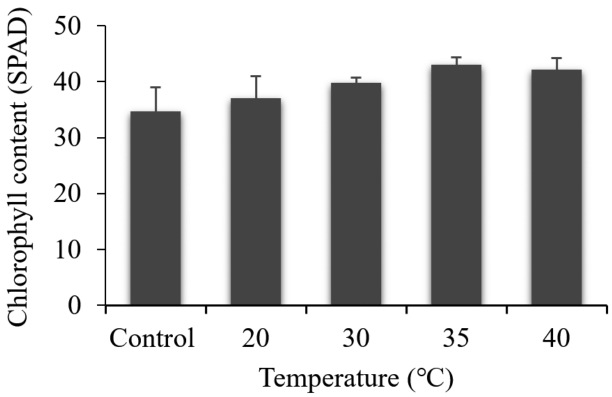

Fig. 3. Leaf chlorophyll content of watermelon seedlings under different temperature. Bars indicate ± S. E.

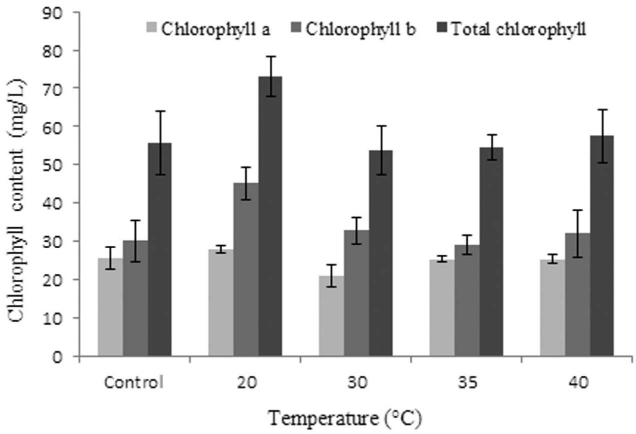

Fig. 4. Effects of temperature on leaf chlorophyll *a*, chlorophyll *b* and total chlorophyll content of watermelon seedlings. Bars indicate ± S. E.

followed by 30°C with 32.92 mg/L, then treatment of 40°C with 32.06 mg/L and 35°C with 29.25, respectively. Control treatment showed the lowest chlorophyll *b* with 30.16 mg/L. The mean for both chlorophyll *a* and chlorophyll *b* were not significantly difference at $p < 0.05$ level. The results also showed that the total leaf chlorophyll content was also higher in 20°C treated seedlings (Figure 4). Xu et al. (2002) reported that temperature have significant effect on accumulation of leaf chlorophyll content. They also stated that reducing soil temperature from 35 to 20°C while maintaining air temperature at 35°C increased leaf chlorophyll content, photosynthetic rate, non-structural carbohydrate content, and growth rates of shoots and roots of *A. stolonifera*.

Chlorophyll fluorescence

Measurement of the chlorophyll fluorescence is a quick, precise and non-destructive technique, widely used in investigating damage or repair caused in the photosynthesis plant system by various types of stresses (Govindjee, 1995). Chlorophyll fluorescence gives information about the state of photosystem II (Khandaker et al., 2011). Jahan et al (2014) reported that that the light-harvesting complexes in PSII might modulate GSH-regulated plant growth and development. Chlorophyll fluorescence values of treated and untreated seedlings were taken at the 3rd week of observation. The effects of temperature on chlorophyll fluorescence of watermelon seedlings were insignificant (Table 2).

As can be seen from Table 2, lower fluorescence (Fo), maximum fluorescence (Fm) and variable fluorescence (Fv) of treated and untreated watermelon seedlings were not affected significantly. The highest Fo was recorded under 30°C while 40°C showed the least Fo value. It was noticed that seeds incubated with 20°C showed the highest Fm and Fv values. The difference between Fo and Fm is the variable fluorescence, Fv. It has been shown theoretically and empirically that Fv/Fm gives a robust indicator of the maximum quantum yield of PSII chemistry (Genty et al., 1992). The Fv/Fm ratio is positively correlated to PSII quantum yield and an indirect measurement of plant physiologic status (Maxwell & Johnson, 2001).

Photosynthetic yield (Fv/Fm) was recorded at 3rd week after transplant, results obtain indicated that no significant difference was observed amongst the treatments and control. However, the highest photosynthetic yield recorded under 20°C and the lowest photosynthetic yield showed by seedling treated with 30°C (Table 2). Temperature effects on photosynthesis may occur through an increased oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Ribeiro et al., 2004). In addition to the inhibition of Rubisco activity, high temperature may also limit photosynthesis by stomatal closure under photorespiration condition (Ribeiro et al., 2004). Fv/Fm is important in determine the plant stress. For unstressed leaves, the value of Fv/Fm is highly consistent, with values of ~0.83, and correlates to the maximum quantum yield of photosynthesis (Demmig & Björkman, 1987). The existence of any type of 'stress' that results in inactivation damage of PSII or often referred to as photo inhibition or the induction of sustained quenching results in a lowering of Fv/Fm (Long et al., 1994). The results from the study showed that the Fv/Fm of watermelon seedlings were below 0.83 which means that the plants were under stress. These may be due to lower chlorophyll accumulation in the leaves of small seedlings or moderate high temperature stress. Guchou et al. (2007) reported that the moderate high temperature stress led to a decrease in Fv/Fm, namely the primary photochemical quantum efficiency and causes a partial inhibition of PSII.

Stomatal conductance

Stomata occupy a central position in the pathway for the transport of water vapour, CO_2, and O_2. The regulation of stomatal conductance (gs) is the main mechanism by which plants control gas exchange and leaf temperature (Salleo et al., 2000). Based on the obtained data, the stomatal conductance of watermelon seedlings was affected significantly with the temperature treatments. The highest reading was showed from treatment under 35°C followed by the 40°C, 25°C and 30°C. While the lowest reading was showed under 20°C. It can be noticed that the stomatal conductance was increased with temperature. Similarly, Urban et al. (2017)

Table 2. Effects of different temperature on chlorophyll fluorescence and stomatal conductance of watermelon leaves

Treatment, °C	Lower fluorescence (Fo)	Maximum fluorescence (Fm)	Variable fluorescence (Fv)	Photosyn. Yield (Fv/Fm)	Stomatal conduct. (mmol m^{-2} s^{-1})	Carotene content (µg/g)
Control (25)	15.66^a	51.66^a	36.00a	0.69^a	309^a	0.014^b
20	13.66^a	56.00^a	42.33^a	0.75^a	236^b	0.016^a
30	17.66^a	53.33^a	35.66^a	0.65^a	243^b	0.013^b
35	16.33^a	51.00^a	34.66^a	0.67^a	334^a	0.017^a
40	13.10^a	53.00^a	40.33^a	0.73^a	313^a	0.012^b

Different letter at same column differ significant difference at 5% level of significant stated that stomatal conductance increased with rising temperature despite the decrease in leaf water potential, increase in transpiration, increase in intercellular CO_2 concentration and was decoupled from photosynthesis.

Carotene content and TSS content in leaves

Carotenoids play an important role in the light harvesting complex and in the photoprotection of the photosystems. Several studies have shown that these compounds are very important in protecting the photosynthesis apparatus against photo damage, by inter-conversions among the xanthophyll molecules (Young et al., 1997). Table 2 above shows that 35°C treatment have the highest value of carotenoid which is 0.017 µg/g, followed by 20°C treatment with 0.016 µg/g, the control treatment with 0.014 µg/g and 30°C treatment with 0.013 µg/g, respectively. The lowest reading of carotenoid was showed at 40°C treatment with 0.012 µg/g. Xu et al. (2002) also reported that moderate temperature 30 to 35°C increased the accumulation pigments contents in the leaves. It was recorded that seeds incubation with different temperature did not produce any significant effect on leaf TSS content of watermelon seedlings (Figure 5).

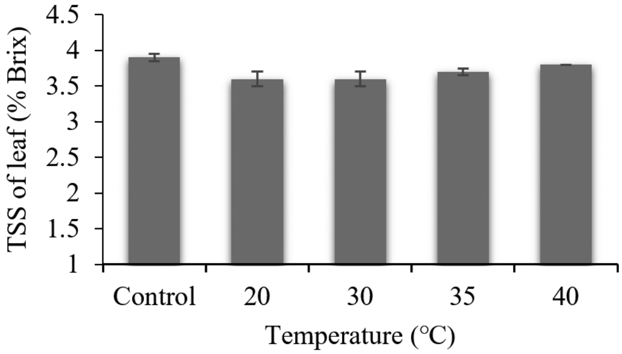

Fig. 5. Effects of different temperature treatment on leaf total soluble solids content of watermelon seedlings. Bars indicate ± S.E.

However, higher TSS value in leaf was recorded under 25°C which is 3.9% Brix followed by 40 and 35°C with a value of 3.8 and 3.7% Brix. Treatment under 20°C showed the lowest TSS value 3.6% Brix. This findings are supported by the results of Xu et al. (2002),who stated that moderate temperature increased the accumulation of assimilates and non structure carbohydrate content in the aerial parts of the plants.

Biomass of plant

The fresh and dry weights of watermelon seedlings treated with different temperature were present in Figure 6.

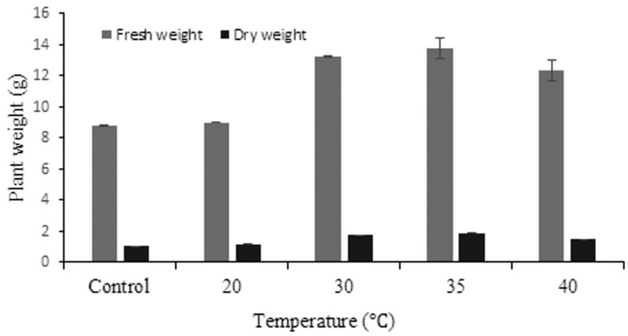

Fig. 6. Temperature effects on fresh and dry weight of watermelon seedlings. Bars indicate ± S.E.

There was highly significant difference of fresh and dry weight of treated and untreated watermelon seedlings (Figure 6). Results showed that seeds treated with 35°C yielded higher fresh weight and dry weight than the other treatments as well as control, while control showed the lowest fresh weight and dry weight of seedlings. Da Silva et al. (2012) reported that dry matter production in plants increased when the temperature reached higher values. They also stated that temperature may have affected plant physiology during the process of absorption and trans location of nutrients. Dry matter is what remains after all of the water is evaporated out and it is an indicator of the amount of nutrients that are available to the plant. Dry matter contents are important traits in plant ecology because they are associated with many critical aspects of plant growth and survival (Shipley & Vu, 2002). It has been also reported that dry matter content in plant parts depend on the variety or cultivar of the plants (Moneruzzaman et al., 2011). Therefore, the plant with higher dry weight could give a better growth and development.

Conclusion

As a conclusion, the plant physiological activities and growth of watermelon seedlings can be improved by the incubation of seeds with temperature during germination. The results showed that 35°C temperature reduce the days require to seed germination, increase seed germination, leaf number, leaf area, root length, vine length, chlorophyll content and chlorophyll fluorescence of watermelon seedlings. In addition, chlorophyll *a* and b, total chlorophyll, carotene content, stomatal conductance and TSS content also affected positively with the temperature treatment. Seed germination, root growth, vine length, leaf growth, and fresh and dry biomass accumulation of watermelon seedlings were increased significantly with 35°C treatment. Finally, it can be concluded that the temperature 35°C was the best treatment for stimulation of seed germination, root development and seedlings growth of watermelon.

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