

Photoperiodic influence of light-emitting diode (LED) on vegetative parameters of *Spinacia oleracea* L. (Spinach)

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Abstract. This research aims to determine sustainable strategies to optimize crop growth and yield, by testing the possibilities of using light-emitting diode (LED) technique to influence the vegetative parameters of spinach. A speed-breeding chamber was constructed using LEDs as a light source under varying photoperiods (19, 17, 15, and 13 hours). The control was established to be the normal light duration of 11 hours during the study period. Spinach vegetative parameters involving morphological parameters such as stem length, root length, and leaf area as well as physiological parameters such as plant weight and percentage necrosis and chlorosis were investigated for 30 days after transplanting. The results showed a significant ($p < 0.05$) increase in morphological parameters of spinach with increasing photoperiod. The spinach plant under the long photoperiod was observed to show the highest morphological and physiological properties. About a 30% increase in root length was observed in the speed-breeding chamber with the longest photoperiod duration compared to the control conditions. Significantly improved spinach plant weight was observed for 19-hour photoperiod compared to the shorter exposure ($p < 0.05$). A lower percentage of necrosis and chlorosis was observed in spinach with longer LED exposure. This research indicated that LED-induced speed breeding is very effective in improving the vegetative properties of spinach. It can be argued that

a 19-hour LED-induced photoperiod is the optimum photo duration required by spinach to improve vegetative growth. Future research should be conducted to investigate the influence of similar LED-induced photoperiods on other species of vegetables.

Keywords: breeding chambers, light-emitting diode, photoduration, photoperiodic influence.

Introduction

Over the years, the global population has increased and it is expected to continue to do so in the next years. Mathematically, it has been estimated that the world population will expand by 75 % by 2050 (Ray *et al.*, 2013). This poses a serious concern on food security and availability for the growing population. For this purpose, several strategies have been considered on how to improve food productivity to meet up with the ever-increasing food demand (Musa and Ikhajiagbe, 2021; Moses *et al.*, 2023). Previously, local farmers relied on traditional methods of breeding, which can no longer meet the food requirements of the ever-increasing population. Consequently, farmers are under constant pressure to optimize crop production and improve yield in less time, with higher nutritional value (Enerijiofi *et al.*, 2024). It has been documented that temperature, light duration and humidity are important abiotic conditions that determine plant growth and development (Rouphael *et al.*, 2012; Rahman *et al.*, 2019). Research has further proved that plant yield and cultivation time can be easily influenced by the normal light conditions using speed breeding techniques. Since plants has several photoreceptors that can be used in signaling and regulation of photomorphogenesis in them (Galvão and Fankhauser, 2015; Musa and Ikhajiagbe, 2024a).

Speed breeding technique involves several strategies where abiotic features like temperature and light are manipulated in order to speed up vegetation, flowering and seed development (Hussain *et al.*, 2018). Speed breeding is a very important strategy that was initiated by the US National Aeronautics and Space Administration (NASA). It has helped farmers in fast cultivation of crops (NASA, 2016). African researchers are using speed breeding to generate new kinds of crops more quickly. The speed breeding technique involved artificially allowing plants to have a continuous light for 20–22 hours. Since plants can undergo photosynthesis for long period in order to bring about increased yield. In a normal condition where a plant takes 6 months to grow, speed breeding may reduce this long period to about 50% per year.

Previous researchers have employed the speed breeding technique to influence the photoperiod and temperature of various important crops to optimize its yield (Rouphael *et al.*, 2012; Rahman *et al.*, 2019). However, researchers are skeptical regarding photoperiod manipulation due to the various sources and forms of artificial lights. Among many artificial light sources, light-emitting diode (LED) has been proven to have the highest colour rendering index (CRI), indicating its high photoelectrons, which may be the best in plant growth optimization (Khan *et al.*, 2017). Light sources with high CRI can easily adjust the light quality, light intensity, and photoperiod of plants (Chiuruywi *et al.*, 2018). However, previous studies used the blue and red illumination in improving the growth of basil microgreens (Collard *et al.*, 2017). Even though, these lights have been proven to have less CRI than the white LED light (Rahman *et al.*, 2019). According to Monostori *et al.* (2018), speed breeding research, especially on light manipulation can best be studied using light-specific plants. For this purpose, the current research focuses on spinach plant as a test plant. The study was aimed at using speed breeding technique specifically the white LED in optimizing vegetative properties of *Spinacia oleracea* to meet up with the global rising demand for food, as the global population increases.

Materials and methods

Sample collection

A composite soil sample was collected from the Admiralty University of Nigeria farm (6.16471 °N, 6.57544 °E), located in the University campus at Ibusa-Ogwashi-Uku expressway, Delta State. About 4 kg of soil was measured and distributed into 15 polyether bags; some of the soil was also used for the nursery bed.

Collection of *Spinacia oleracea* seed

Seeds of *S. oleracea* (No. 21-38135/979100042077) were obtained from Songhai Delta at Amukpe in Sapele, Delta State, on 10th of July 2022.

Preparation of the nursery bed

About 150 seeds were dispersed in the prepared nursery bed and allowed to germinate for 4 days. After 4 days, seedlings of similar height were picked and transplanted.

Seedling transplantation

About 10 seedlings of *S. oleracea* were transferred into each of the 15 experimental polythene bags. It was ensured that all seedlings transplanted were ranging between 1.9 cm and 2.0 cm in height and were all transplanted at same time into the 15 experimental polythene bags following the Randomized Block Design (RBD) in three replicates as described by Musa *et al.*, 2017.

Construction of the speed breeding chamber

Transparent rubbers of 50 X 50 cm were used as the breeding chamber. Five speed breeding chambers were constructed using the transparent plastic rubber. White LED bulbs were attached at the extreme end of each chamber (Fig. 1). Each speed-breeding chamber was made to have a unique photoperiod duration as described in Tab. 1. The experiment was conducted in Asaba during its wet season (0.04 inches precipitation) according to Wolter *et al.*, (2019) with an average of 11 hours sunlight duration. The speed-breeding chamber also served as a screen house that shielded the plants from pests, but not from rainfall. The experimental polyether bags were then situated in the speed-breeding chamber with three experimental polythene bags per speed breeding chamber as replicates. The soils in all the polyether bags were stirred once every week to enhance aeration and no chemicals was used during the study. The experimental pots were weeded every two days following Musa and Ikhajiagbe (2021). The experiments lasted for 30days (August 12th – October 10th), the spinach was harvested on the 30th day.

Table 1. LED-induced photoperiod duration.

Speed breeding chamber code	Light duration
A	19 hours
B	17 hours
C	15 hours
D	13 hours
E (Control)	11 hours



Figure 1. Constructed speed breeding chamber.

Morphological parameters

Morphological parameters that are analytical of vegetative growth and yield of *S. oleracea* were examined. The morphological parameters were divided into two. The above ground parameters include the number of leaves, stem length, leaf area and leaf length, while the below ground parameters were root length and secondary root number. The leaf number were calculated by counting at every six (6) days intervals from the transplanting date to 30th day. The stem length was measured in (cm) at six (6) days intervals from day 1 after transplanting to day 30. Leaf area (cm²) was calculated using an android application (Leaf-IT) using the method of Julian *et al.*, (2017) at six (6) days interval from transplanting day to day 30. Leaf length was calculated in (cm) at interval of six (6) days. The number of leaves were calculated by counting at an interval six (6) days from the day of transplanting to the 30th day. The length of fresh root was measured in (cm) at 20th and 30th day after transplanting. Also, the number of secondary roots were accurately detected and calculated at 20th and 30th day after transplanting.

Physiological parameters

To analyze the effect of LED on the vegetative parameters of *S. oleracea*, physiological parameters were investigated. Leaf tip chlorosis and necrosis was measured at 10, 20 and 30 days after transplanting as follows:

$$\text{Leaf tip necrosis/chlorosis (\%)} = \frac{\text{Number of spinach leaf with signs of necrosis or chlorosis}}{\text{Number of spinach leaf}} \times 100$$

Weight of fresh leaf (g) was measured using an analytical weighing balance at day 10, 20 and 30 after transplanting. The chlorophyll *a* and *b* levels at 10 and 30 days after transplanting were calculated as seen in Arnon *et al.*, (1949); Maxwell and Johnson (2000).

Statistical analysis:

The recorded data were presented as means and standard errors of three replicates and subjected to statistical analysis. A One-Way Analysis of Variance (ANOVA) at a significance level of *p*=0.05 was used throughout the study to determine if there were significant differences in the values recorded values.

Results

Effect of photoperiod on spinach morphological parameters

The influence of LED-induced photoperiod on spinach plant was evaluated using morphological parameters. The morphological parameters that were assayed in this research were divided into; the above ground parameters and below ground parameters.

The above ground parameters

The number of spinach leaves was observed to be highest (5.9) at 30 DAT in the speed-breeding chamber A, while the lowest leaf number (3.7) at 30 DAT was observed in the control (E). At 6 DAT, three (3) leaves were observed in the speed breeding chamber A, B and C, while in the speed breeding chamber D and the control (E), only two (2) leaves were observed. Furthermore, at 30 DAT, there was a significant increase ($p < 0.05$) in leaf number was observed in the speed breeding chamber A compared to the other speed breeding chambers (B, C, D and control) (Tab. 2).

Table 2. Response of spinach photoperiod on leaf number.

Sample	Leaf number				
	6 DAT	12 DAT	18 DAT	24 DAT	30 DAT
A	3.0 \pm 0.23 ^a	3.0 \pm 0.12 ^a	4.9 \pm 0.11 ^a	5.2 \pm 0.01 ^a	5.9 \pm 0.22 ^a
B	3.0 \pm 0.13 ^a	3.0 \pm 0.34 ^a	4.0 \pm 0.25 ^b	4.0 \pm 0.11 ^b	4.0 \pm 0.22 ^b
C	3.0 \pm 0.54 ^a	3.0 \pm 0.32 ^a	3.6 \pm 0.43 ^c	3.7 \pm 0.21 ^c	4.0 \pm 0.12 ^b
D	2.3 \pm 0.55 ^b	2.3 \pm 0.22 ^b	3.6 \pm 0.11 ^c	4.3 \pm 0.11 ^d	4.3 \pm 0.12 ^c
E	2.0 \pm 0.01 ^b	2.0 \pm 0.32 ^c	3.0 \pm 0.76 ^d	3.6 \pm 0.21 ^e	3.7 \pm 0.32 ^d

Results with same alphabetic superscripts on same column did not show significant difference from each other ($p > 0.05$). Results were recorded in mean and standard error of three replicates. DAT = Days after transplant. A = 19 hours photoperiod, B = 17 hours photoperiod, C = 15 hours photoperiod, D = 13 hours photoperiod and E = 11 hours photoperiod (control).

The stem length (cm) is one of the important parameters in growth studies. The spinach under speed breeding chamber A was observed to show the highest stem length at all the assayed days. Also, at 30 DAT, it was observed that the spinach under the control (E) showed the least (2.4 cm) stem length. Furthermore, significant difference ($p < 0.05$) was observed in the stem length between all the speed breeding chambers (A, B, C and) and the control (E). In addition, significant increase ($p < 0.05$) in stem length were observed as the days progress, except for the control (E), where no significant ($p > 0.05$) increase in stem length was observed from 18 DAT to 30 DAT. Furthermore, leaf length observed, range from 2.3 – 5.3 cm throughout the 30-day study. At 30 DAT, speed breeding chamber A was observed to show the highest (5.3 cm) leaf length, while the control (E) showed the lowest (2.5 cm) leaf length (Tab. 3). Since leaf area is a major indicator of plants ability to utilize light, the spinach under the speed breeding chamber A had the highest (7.7 cm^2) leaf area at 30 DAT, compared to other speed breeding chambers (B, C and D). The spinach under the

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control condition (E) was observed to show the lowest (2.2 cm^2). There was a significant difference ($p < 0.05$) in leaf area of spinach under all the assayed speed breeding conditions at 30 DAT. Delayed increases in leaf area was observed in the spinach under the control condition (E) and (D) (Tab. 4).

Table 3. Response of spinach photoperiod on stem length and leaf length.

Sample	Stem length (cm)					Leaf length (cm)				
	6	12	18	24	30	6	12	18	24	30
	DAT									
A	2.6 \pm 0.3 ^a	3.2 \pm 0.2 ^a	3.5 \pm 0.2 ^a	3.7 \pm 0.3 ^a	4.4 \pm 0.1 ^a	2.1 \pm 0.2 ^a	2.9 \pm 0.2 ^a	4.6 \pm 0.1 ^a	4.8 \pm 0.2 ^a	5.3 \pm 0.1 ^a
B	3.0 \pm 0.2 ^b	3.0 \pm 0.4 ^b	3.1 \pm 0.2 ^b	3.2 \pm 0.3 ^b	3.5 \pm 0.1 ^b	2.0 \pm 0.3 ^a	3.3 \pm 0.3 ^b	3.8 \pm 0.2 ^b	4.1 \pm 0.2 ^b	4.5 \pm 0.3 ^b
C	2.0 \pm 0.5 ^c	2.3 \pm 0.3 ^c	2.5 \pm 0.3 ^c	2.6 \pm 0.2 ^c	2.9 \pm 0.1 ^c	2.0 \pm 0.4 ^a	2.0 \pm 0.5 ^c	2.3 \pm 0.1 ^c	2.5 \pm 0.1 ^c	2.8 \pm 0.2 ^c
D	1.7 \pm 0.3 ^c	1.7 \pm 0.2 ^d	2.0 \pm 0.4 ^d	2.2 \pm 0.1 ^d	2.5 \pm 0.1 ^d	1.6 \pm 0.3 ^b	2.5 \pm 0.4 ^d	2.6 \pm 0.1 ^d	2.7 \pm 0.3 ^c	2.7 \pm 0.2 ^c
E	2.0 \pm 0.3 ^c	2.0 \pm 0.4 ^e	2.3 \pm 0.4 ^e	2.4 \pm 0.6 ^e	2.4 \pm 0.1 ^e	2.0 \pm 0.3 ^a	2.5 \pm 0.2 ^d	2.5 \pm 0.1 ^c	2.5 \pm 0.3 ^c	2.5 \pm 0.3 ^c

Results with same alphabetic superscripts on same column did not show significant difference from each other ($p > 0.05$). Results were recorded in mean and standard error of three replicates. DAT = Days after transplant. A = 19 hours photoperiod, B = 17 hours photoperiod, C = 15 hours photoperiod, D = 13 hours photoperiod and E = 11 hours photoperiod (control).

Table 4. Response of spinach photoperiod on leaf area.

Sample	Leaf area (cm^2)				
	6 DAT	12 DAT	18 DAT	24 DAT	30 DAT
A	5.0 \pm 0.2 ^a	5.6 \pm 0.5 ^a	6.3 \pm 0.1 ^a	7.1 \pm 0.2 ^a	7.7 \pm 0.3 ^a
B	3.3 \pm 0.4 ^b	3.6 \pm 0.1 ^b	3.8 \pm 0.1 ^b	4.1 \pm 0.1 ^b	4.4 \pm 0.1 ^b
C	3.5 \pm 0.2 ^c	3.6 \pm 0.4 ^b	3.6 \pm 0.2 ^c	3.8 \pm 0.1 ^c	3.9 \pm 0.2 ^c
D	2.3 \pm 0.1 ^d	2.4 \pm 0.2 ^c	2.4 \pm 0.1 ^d	2.4 \pm 0.1 ^d	2.7 \pm 0.1 ^d
E	2.0 \pm 0.5 ^e	2.1 \pm 0.1 ^d	2.1 \pm 0.1 ^e	2.2 \pm 0.1 ^e	2.2 \pm 0.1 ^e

Results with same alphabetic superscripts on same column did not show significant difference from each other ($p > 0.05$). Results were recorded in mean and standard error of three replicates. DAT = Days after transplant. A = 19 hours photoperiod, B = 17 hours photoperiod, C = 15 hours photoperiod, D = 13 hours photoperiod and E = 11 hours photoperiod (control).

The below ground parameters

The result of the below ground parameters of spinach plant showed that spinach root length increased significantly ($p > 0.05$) with continuous photoperiod. Spinach plant from the speed-breeding chamber A was observed to have the longest (16.0 cm) root length at 30 DAT, while the shortest root length (6.4 cm) was observed in the spinach plant from the control condition (E). There was statistically significant difference ($p < 0.05$) in the root length of the spinach plant under all the photoperiod condition at all the assayed days (Tab. 5). At 20 DAT, it was observed that spinach root length in the speed breeding chamber C and D showed no significant difference ($p > 0.05$) (Fig. 2).

Table 5. Response of spinach photoperiod on root length and number of secondary roots.

Sample	Root length (cm)		Number of secondary roots	
	20 DAT	30 DAT	20 DAT	30 DAT
A	11.0 \pm 0.1 ^a	16.0 \pm 0.2 ^a	30 \pm 0.4 ^a	38 \pm 0.1 ^a
B	8.0 \pm 0.2 ^b	11.0 \pm 0.2 ^b	21 \pm 0.2 ^b	26 \pm 0.1 ^b
C	3.8 \pm 0.2 ^c	7.0 \pm 0.1 ^c	15 \pm 0.1 ^c	19 \pm 0.1 ^c
D	3.7 \pm 0.2 ^c	6.8 \pm 0.3 ^d	12 \pm 0.2 ^d	16 \pm 0.1 ^d
E	3.4 \pm 0.2 ^d	6.4 \pm 0.2 ^e	10 \pm 0.1 ^d	13 \pm 0.1 ^e

Results with same alphabetic superscripts on same column did not show significant difference from each other ($p > 0.05$). Results were recorded in mean and standard error of three replicates. DAT = Days after transplant. A = 19 hours photoperiod, B = 17 hours photoperiod, C = 15 hours photoperiod, D = 13 hours photoperiod and E = 11 hours photoperiod (control).

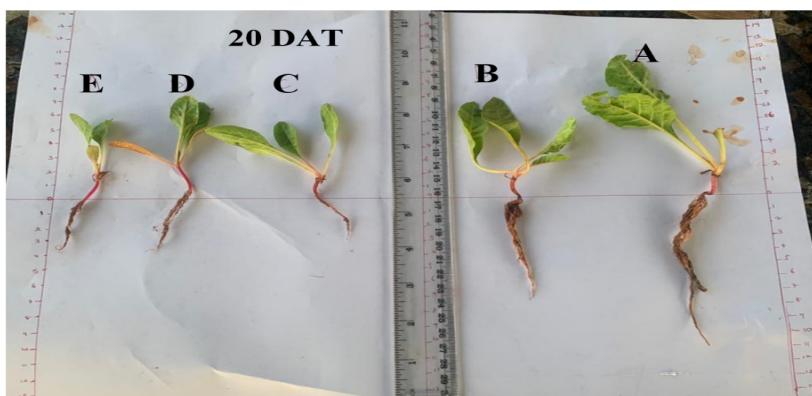


Figure 2. Root length parameters. DAT=Days after transplant. A= 19 hours photoperiod, B= 17 hours photoperiod, C= 15 hours photoperiod, D= 13 hours photoperiod and E= 11 hours photoperiod (control).

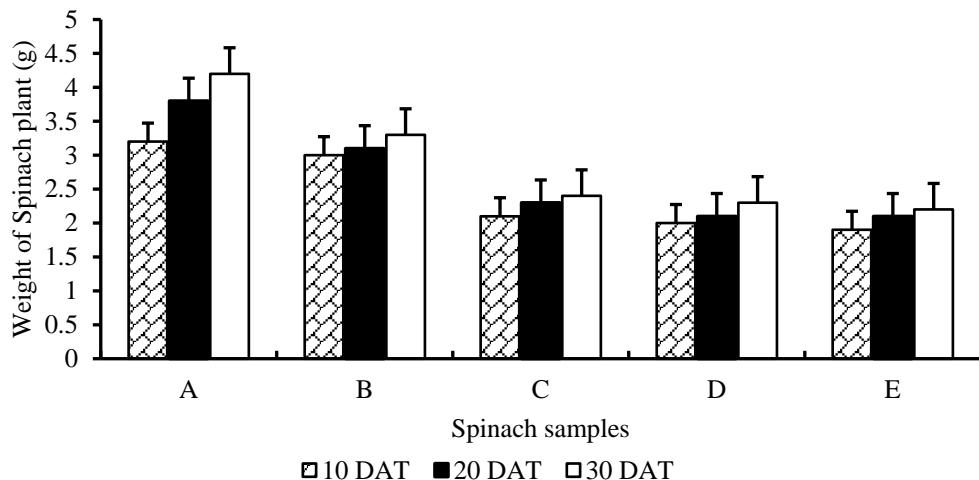
Effect of photoperiod on Spinach physiological parameters

Greater percentage (35%) of the spinach leaves under the control (E) condition showed visible signs of leaf tip necrosis and chlorosis, while lower (15 %) of the spinach leaves from the speed breeding chamber C showed visible signs of leaf tip necrosis and chlorosis at 30 DAT. No visible signs of chlorosis and necrosis were observed in the spinach leaf under the speed-breeding chamber A and B throughout the 30 DAT period of study. However, at 10 DAT, lower leaf necrosis and chlorosis were observed on the spinach plant under the speed breeding chamber D and the control (Tab. 6). Furthermore, it was observed that the spinach plants under the speed breeding chamber A had the highest weight (4.2 g) at 30 DAT which showed a significant difference ($p < 0.05$) when compared to the weight of other spinach plant from B, C and D speed breeding chambers. The lowest spinach weight (2.2 g) was observed in the control (E). However, there was no significant difference ($p > 0.05$) between the weight of spinach plant under C and D speed breeding chamber, as well as the control (E) (Fig. 3). Chlorophyll-a and Chlorophyll-b content of spinach leaves at day 10 and 30 after transplanting also showed a significant increase between spinach leaves in speed breeding chamber A and others (B, C, D and Control). The control speed breeding chamber showed the least chlorophyll-a and chlorophyll-b content (Tab. 7).

Table 6. Response of spinach photoperiod on leaf tip necrosis and chlorosis.

Sample	LTNC (%)		
	10 DAT	20 DAT	30 DAT
A	No sign	No sign	No sign
B	No sign	No sign	No sign
C	No sign	10	15
D	20	25	25
E	30	30	35

Results with similar alphabetic superscripts on same column did not differ from each other ($p > 0.05$). Results were in mean and standard error of three replicates. DAT = Days after transplant. A = 19 hours photoperiod, B = 17 hours photoperiod, C = 15 hours photoperiod, D = 13 hours photoperiod and E = 11 hours photoperiod (control). LTNC = Leaf tip necrosis and chlorosis.

**Figure 3.** Response of spinach photoperiod on plant weight.**Table 7.** Response of spinach photoperiod on chlorophyll-a and b.

Sample	Chlorophyll-a (mg/cm ²) FW on		Chlorophyll-b (mg/cm ²) FW on	
	10 DAT	30 DAT	10 DAT	30 DAT
A	6.4	8.9	2.9	3.6
B	6.1	8.1	2.6	3.2
C	6.1	8.0	2.6	3.0
D	4.8	5.2	3.1	3.6
E	4.1	5.0	2.8	3.3

DAT = Days after transplanting, FW =fresh weight, A = 19 hours photoperiod, B = 17 hours photoperiod, C = 15 hours photoperiod, D = 13 hours photoperiod and E = 11 hours photoperiod (control).

Discussion

LED-induced speed breeding technique has been widely used in improving algal breed in recent times. This research aimed at using the LED-induced speed breeding technique under different photoperiod to improve vegetative parameters of spinach plant. Since spinach plant is known to be a long day plant, allowing spinach to achieve its required photoperiod may be essential in improving the growth of spinach plant. Plant morphology and physiology can easily be influenced

by environmental photoperiods, majorly due to some plant photoreceptors that are sensitive to LED (Wolter *et al.*, 2019). From the current study, the increased number of spinach leaf observed in the speed-breeding chamber A (19 hours LED-induced photoperiod) may be linked to the effectiveness of the LED in improving vegetative parameters of spinach. This suggests that the LED level in the present study must have signal plant photoreceptors, leading to increase in growth parameters of the test plant. Similar results were found in Piovene *et al.*, (2015) experiment, where strawberry plant blooming was influenced with blue ratio lightening than the normal blooming period. In addition, blooming of Azalea was also improved using white ratio lightening source (Wolter *et al.*, 2019; Musa and Ikhajiagbe, 2024b). This research is in agreement with a study by Wolter *et al.*, (2019) where he discovered that extra plant leaf was observed in *Amaranthus hybridus* after 40 days red light induced photoperiod.

At elevated LED intensity, the increased stem length, leaf length and leaf area that was witnessed in the current study may be due to the increased plant photosynthetic activities. In this study, it can be concluded that due to the high LED light intensity, plants were able to optimize nutrient assimilation, bringing about increased growth of vegetative parameters. According to Piovene *et al.*, (2015), about 40% increase in plant biomass and 60% increase in plant yield properties were recorded using LED in comparison with organic fertilizers (Collard *et al.*, 2017; Musa and Ikhajiagbe, 2023). However, the reduced spinach vegetative properties observed in the control can be linked to the reduce photoperiod witnessed in the control set up.

Root morphology is a good indicator of a plant's health since strong roots may help a plant grow correctly by supplying it with enough water and nutrients. In our experiment, the improved root parameters observed at increasing photoperiod may be due to the improved vegetative properties, leading to more nutrient intake and water usage. Research by Hogewoning *et al.*, (2010) explained that light photoperiod can induce the activation of phloem and xylem receptors, leading to more nutrient and water suction for plants during translocation, bring about root elongation as well as increased number of secondary roots. This result is in agreement with the work of Dong *et al.*, (2014) who observed increased root parameters. Furthermore, improved light intensity to a minimum level have been documented to bring about rapid cell growth and development (Musa and Ikhajiagbe, 2021).

Physiological parameters are significant in understanding the response of vegetables to photoperiod. For this purpose, physiological parameters such as leaf tip necrosis and chlorosis were studied. The higher signs of necrosis and chlorosis observed with reduced photoperiod indicated that the plant leaf is not receiving required duration of photoperiod, which may be the reason why

vegetative decline in spinach has been directly proportional to reduced photoperiod. According to Musa and Ikhajiagbe (2021), photosynthetic parameters are known to improve root properties of vegetables. Furthermore, the high photosynthetic pigments observed from the result of chlorophyll-a and chlorophyll-b in the 19 hours LED induced treatment (A) compared to the control indicated the positive effect of the increased LED photoperiod on plant photosynthesis. Since good light source can improve plant photosynthesis and improve exchange of stomatal gas (Collard *et al.*, 2017), this may be reason that makes the spinach in the speed breeding chambers with high LED photoperiod had higher plant morphological, as well as physiological parameters.

Conclusion

The outcomes demonstrated that white LED light could be utilized in altering the growth, yield and metabolism for spinach plant. The 19 hours LED-induced photoperiod proved to be the most effective and optimum light level for spinach plant. For the optimal light combinations to be achieved, it is important that the white LED lightening source be used. Based on the findings, it appears that spinach may develop to its peak quality in 6 weeks under white LED induced photoperiod with continuous 19 hours duration per day.

Conflict of interest. None exist among the authors

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