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Photoperiod effect on vegetative growth and bulbing initiation of four garlic genotypes

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Abstract. *Kendarini N, Aisyah SI, Maharijaya A, Sobir. 2022. Photoperiod effect on vegetative growth and bulbing initiation of four garlic genotypes. Biodiversitas 23: 4716-4723.* The main factors influencing garlic growth and development were genetics, photoperiod, and temperature. Low temperature and short photoperiod were required for initial growth, followed by a long photoperiod and high temperature for bulbing and bolting. The study was conducted to evaluate the response of garlic genotypes to photoperiod on the vegetative growth and bulbing initiation, and physiological traits (C/N ratio, GA, and total chlorophyll content). Four garlic genotypes used in this experiment consisted of two Chinese garlic genotypes (hardneck and softneck) and two local garlic cultivars (Sangga Sembalun and Tawangmangu Baru). The garlic plants were subjected to the treatment of short photoperiod (control) and long photoperiod (night break and extension methods). The results showed that the Chinese hardneck and softneck had plant vegetative growth with a higher vigor than Sangga Sembalun and Tawangmangu Baru in all photoperiod treatments. Sangga Sembalun and Tawangmangu Baru had bulbing index below 0.3 in all photoperiods. The Chinese hardneck and softneck genotypes had bulbing index greater than 0.5 in all photoperiod treatments, except the hardneck in the night break treatment. The bulbing initiation in Chinese garlic genotypes was delayed in long photoperiod treatments. High temperature during the experiment affected bulbing initiation in long photoperiod treatment. The garlic genotypes had different responses to photoperiod treatments in C/N ratio, GA, and total chlorophyll content. The vegetative growth increased under extension treatment, and night break accelerated bulb formation. Long photoperiod increased C content and C/N ratio in the leaves while it decreased N content and photosynthetic pigment content. The late maturity time (Chinese hardneck and Chinese softneck) depended on long photoperiods for the bulbing formation. In contrast, local garlic cultivars with the early and mid-season types (Sangga Sembalun and Tawangmangu Baru) were insensitive to photoperiod.

Keywords: *Allium sativum*, bulbing index, day length, maturity time

INTRODUCTION

Garlic (*Allium sativum* L.) is the second most important crop of the Allium group. Fresh garlic is commonly used for cooking, and the dehydrated product is used as condiments and in the food industry. Garlic is also used in traditional and modern medicine for its antibacterial and antioxidant activities, antifungal, antiviral, anti-protozoal, and anti-cancer properties (Hernawan and Setyawan 2003; Bayan et al. 2014). Garlic also contains protein, minerals, and vitamins A, B6, B1, and C (Sethi et al. 2014).

Cloves of garlic are mainly clonally propagated by seed cloves because flowers are usually sterile, leading to a narrow genetic variability (Shemesh-Mayer et al. 2013). Commercial cultivars are classified into hardneck and softneck. Hardneck garlic or bolting types (*A. sativum* ssp ophioscorodon) produce scape or flower stalk within pseudostem, which causes it to be rigid. Softneck garlic or non-bolting types (*A. sativum* ssp sativum) do not produce scape (Etoh and Simon 2002). Hardneck has large cloves, easy peel, shorter shelf-life, and is adapted to a colder climate. Softneck prefers a warmer climate and stores longer. Garlic cultivars were divided into three categories based on their maturity time, i.e., early type with bulb production up to 120 days, mid-season type with 120-180 days duration, and late-type with more than 200 days duration (Cunha et al. 2014).

Plants should achieve adequate growth before bulbing begins so that the foliage can produce large bulbs and high yields. In garlic, plant growth and development were mainly affected by genetics, photoperiod, and temperature (Rabinowitch and Goldstein 2020). The vegetative stage required a low initial temperature and short photoperiod, then long photoperiod and high temperature for flower stalk elongation and bulb and clove formation (Atif et al. 2019). According to Zakari et al. (2017), vegetative growth (plant height and number of leaves) was positively correlated with garlic bulb yield. Photoperiod regulates plant growth and development through photosynthetic function. Photosynthetic pigments allow plants to absorb energy from light, so foliar chlorophyll content is a crucial factor affecting the performance of plant photosynthesis (Taiz and Zeiger 2006). The chlorophyll content is considered an indicator of plants' photosynthetic capacity (Ling et al. 2011), which is affected by photoperiod treatment (Dong et al. 2016). The effect of photoperiod on vegetative growth varies depending on the species or varieties. Long photoperiod increased leaf number, plant height, and photosynthetic pigment content (Son et al. 2020), plant height, bulb diameter, and pseudostem diameter, shortened growth period (Wu et al. 2016; Atif et al. 2019), leaf area, relative growth plants, dry weight (Shibaeva and Markovskaya 2013). Short photoperiod increased chlorophyll content, net photosynthetic rate, and accelerated flowering and maturity stage (Dong et al. 2016).

The bulbs of local cultivars are small; according to Brewster (2008), garlic produces poor bulb quality in the warm temperature and short photoperiod in the tropics. The genetic material used in this study is a genotype based on the life cycle: early, middle, and late modifications of the reference, according to Cunha et al. (2014). We elucidated the effects of different photoperiods on plant growth, bulbing initiation, the C/N ratio, and gibberellic acid endogen content in four garlic genotypes with different genetic backgrounds and characteristics. The study results were expected to provide information on response patterns from the Chinese and the local Indonesian garlic in different photoperiod treatments. This information can be useful when introducing garlic cultivars for breeding and cultivation practice improvement in garlic that is suitable for the characteristics of the local climate in Indonesia.

MATERIALS AND METHODS

Plant materials and experimental design

The experiment was performed under a screen house at the Punten Village of Batu City, East Java Province, Indonesia (7°50'15.0"S 112°31'34.6"E) at an altitude of 1100 m above sea level. The average minimum and maximum temperatures were 17.11°C and 30.56°C, respectively, and RH ranged from 60 to 80%. The genetic materials used were four garlic genotypes obtained from different locations (Table 1). Genotypes of garlic consist of two China garlic: hardneck-type (China hardneck) and softneck-type (China softneck), and two local cultivars: Sangga Sembalun and Tawangmangu Baru. The genotypes were selected upon their differences in genetic makeup and day length grouping traits, thus categorized as early, mid, and late-season garlic (Table 1). The experiment was arranged in a nested randomized complete block design with two factors: photoperiod treatment as the main plot and garlic genotypes as the subplot. The photoperiod treatment consisted of short photoperiod (SP) as a control treatment, and long photoperiod (LP) treatments used night break and extension methods.

Light treatment and arrangement

The plants were grown under natural light from the day of planting until 36 days after planting (DAP); afterward, the photoperiod treatment was given until harvesting time. The plants were treated with different photoperiods consisting of short photoperiod (<12 h) and long photoperiod (>14 h). In short photoperiod (SP), the plant was exposed to natural light as a control treatment (C), and the long photoperiod (LP) consisted of natural daylight followed by 4 hours of supplemental light. Night break and extension methods were used as long photoperiod treatments. The night break treatment was done by turning the lights from 22:00 to 02:00 and from 17:00 to 21:00 for the extension. LED grow-light 28 watts (2100 lux) was used as a supplemental lighting source. The LED lights were arranged in a row of 45 cm apart from each other and mounted 30 cm above the plants, and it was raised as the plant's height was getting higher (Figure 1B). Black plastic mulch was used as shutters separating photoperiod treatment and rolled up during daylight.

Soil preparation and planting

The soil preparation consisted of plowing, harrowing, and raised bed formation. The basal fertilizer used was goat manure provided at a dose of 20 t ha^{-1}. The soil acidity was corrected using 2 t ha^{-1} of $Ca(OH)_2$, and it was broadcasted and mixed thoroughly on the plot three weeks before planting. The standard agronomical practice was applied during the experiment. Healthy and dormant-free cloves were selected as seeds. Clove size and weight were uniforms within genotypes. Pre-planting seed cloves were treated using a *Trichoderma*-based product as a biological control to suppress the growth of plant pathogenic microorganisms and regulate the rate of plant growth. Cloves were single-planted into a space of 10 cm x 10 cm at a depth of 3-4 cm. Rice straw mulch was applied to the plots to maintain soil humidity and prevent weeds.

Table 1. Genotype, origin, genotype source location, bulb weight, clove number per bulb, clove weight and diameter, maturity time, and maturity time category

Genotype	Chinese hardneck	Chinese softneck	Sangga Sembalun	Tawangmangu Baru
Origin	Chinese	Chinese	Indonesian local cultivar	Indonesian local cultivar
Genotype source location	Local market in Batu, West Java	Local market in Batu, West Java	Local grower in Sembalun, local West Nusa Tenggara	grower in Tawangmangu, Solo Mid-Java
Bulb weight (g)	36.51±5.90	42.84±7.61	10.66±3.30	17.59±3.79
Clove number per bulb	7.15±0.66	12.77±0.86	8.89±1.40	10.20±1.44
Clove weight (g)	5.88±0.89	3.38±0.58	1.21±0.20	1.56 ±0.22
Clove diameter (mm)	25.07±2.38	16.11±1.48	11.40±1.20	12.16±1.21
Maturity time (DAP)	>200	>200	<120	120-180
Category of maturity time	Late	Late	Early	Middle

Note: *Category of the maturity time modification based on Cunha et al. (2014)

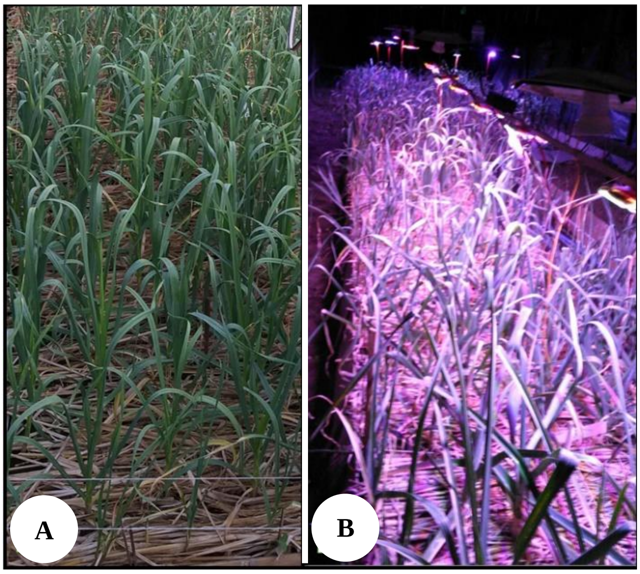

Figure 1. Garlic plants in the control treatments bedding plot (A) and lighting arrangement in the bedding plot in the long photoperiod treatments (B)

Plant growth measurement

The observation was done 12 weeks after planting (WAP) on 15 plant samples selected randomly for vegetative growth in morphological character viz., plant height, leaf number, and pseudostem diameter. The plant heightmeasurement was started from the basal part of the leaf sheath (pseudostem) to the tip of the longest leaf. Pseudostem (false stem) diameter was recorded at the leaf sheath's largest diameter above the bulb's top (± 1 cm from the above-ground). Initiation of the bulbing or bulbing index was observed by bulb diameter and pseudostem diameter. The bulbing index was expressed as the ratio of pseudostem diameter to bulb diameter (Mann 1952).

Chlorophyll total content, C and N contents determination, and gibberellic acid content

The leaf samples for chlorophyll, C/N ratio, and gibberellic acid content were collected at 12 WAP. Chlorophyll content was measured using a modification of the method described by Sims and Gamon (2002). 20 mg of fresh leaves were extracted in 2 mL of acetone tris (85:15 v/v) and then centrifuged at 8000 rpm for 5 min. The supernatant was taken at about 1 mL and mixed with 3 mL of acetone. The extract's absorbance was measured at 663 and 645 nm using the spectrophotometer UV-Shimadzu. Calculations of chlorophyll a and b contents were based on the following equations: chlorophyll a: [12.7 x A663 – 2.69 x A647]; chlorophyll b: [22.9 A647 – 4.68 A663]. Leaf total N content was analyzed using the Kjeldahl method, and total C-organic was determined using the Walkley and Black method. Gibberellic acid content was analyzed using a method by Barendse (1987).

Data analysis

Data analysis was done using analysis of variance (ANOVA) in a nested design, and $p<0.05$ was considered significant. The mean separation test was done by the Least Significant Difference (LSD)'s test at $p<0.05$. Microsoft Excel 2010 and SAS software (SAS Institute ver. 9.4) were used in the data analysis.

RESULTS AND DISCUSSION

Analysis of variance results showed that genotype significantly affected all the observed characters (Table 2). It implied that there was variability among the tested garlic genotypes. The photoperiod significantly affected all characters except pseudostem diameter. It means the observed characters differed at the different photoperiods except for the pseudostem diameter. Interactions of genotype and photoperiod significantly affected all observed traits. That implied genotypes had different responses in different photoperiods.

The interaction of genotype and photoperiod on plant height and leaves number

This research showed that interaction between genotype and photoperiod affected garlic plants' plant height and leaf numbers. The Chinese hardneck genotype had the highest plant height in each photoperiod treatment (control, night rest, and extension), and the other genotypes were not significantly different (Table 3). Differences in plant height are caused by differences in the genetic makeup of each genotype. The Chinese hardneck genotype had clove seed with a larger diameter and heavier bulb weight than the other genotypes (Table 1).

Table 2. Analysis of variance result on the effect of genotype, photoperiod, and interaction of genotypes and photoperiod

Characters	Genotype	Photoperiod	Genotype x photoperiod	CV (%)
Plant height	**	*	*	10.28
Leaves number	**	*	*	7.67
Diameter of pseudostem	**	tn	*	15.81
Bulbing index	**	*	**	10.58
C/N ratio	**	**	**	4.07
Chlorophyll total	*	**	**	2.85
Gibberellic acid	**	**	**	4.55

Note: CV: coefficient of variation; **: highly significant at 1% level of significance; *: significant at 5% level of significance; ns: nonsignificant at 5% level of significance

Larger seed cloves usually produce plants with vigor performance, while smaller ones produce opposite plants (Gautam et al. 2014). The Chinese hardneck agreed with the statement regarding morphological performance and plant growth than the local cultivars (Figure 2A). Chinese hardneck genotypes had the highest plant height in this experiment and previous studies, among other genotypes (data not presented). The research results by Ishthifaiyyah and Sobir (2018) showed that the local garlic genotypes had shorter plant heights and less vigor than garlic plants from the introduced genotypes.

The Chinese softneck genotype had the highest number of leaves in the control treatment, but in a night break and extension, treatments were not significantly different from Sangga Sembalun and Tawangmangu Baru (Table 3). The Chinese softneck genotype had shorter leaf internodes, so compared to other genotypes with the same plant height; the Chinese softneck will have more leaves. According to Rabinowitch and Goldstein (2020), the number of leaves varied depending on the genotype. Similar to the plant height, several studies also found that the size of clove and bulbs affected plant growth, including leaf number (Gautam et al. 2014; Ibrahim et al. 2020; Desta et al. 2021). The Chinese softneck genotype was the second larger clove size (Table 1). Larger cloves had a higher plant height and number of leaves per plant; it might be because cloves and bulbs were more abundant in nutrients in the early growth stage, which allowed for vigorous growth and resulted in more plant height and leaf number and better development when compared to smaller bulbs (Desta et al. 2021). However, this pattern is not always followed by all genotypes. Chinese hardneck and softneck genotypes have large cloves, but the Chinese hardneck genotype was the highest in plant height, while the Chinese softneck was the highest in leaf number. These differences indicate that the two genotypes are genetically different. Hardneck garlic is *A. sativum* ssp ophioscorodon, and softneck type is *A. sativum* ssp sativum (Etoh and Simon 2002). The results also showed that the number of leaves of all genotypes in each treatment had more than seven leaves per plant (Table 3). The number of leaves of all genotypes is sufficient to form bulbs. According to Rabinowitch and Goldstein (2020), the minimum number of leaves is 6-30 before transitioning from the vegetative to the reproductive state. The short photoperiod gave better plant growth than the long photoperiod treatment for all the garlic genotypes. This was related to garlic plants' life cycle, which tends to be shorter at long photoperiod treatments (data not presented). Plant growth under short photoperiods was not as fast as those grown under long photoperiods. A longer growth process had been shown in plants under shorter photoperiods, whereas when the photoperiod was extended, the duration of the plant stage was shortened significantly in several plants (Yunze and Shuangsheng 2014; Wu et al. 2016). additional irradiation and high temperatures during plant growth become abiotic stresses for certain plants.

Table 3. The interaction of genotype and photoperiod on plant height and leaves number

Genotype	Plant height (cm)			Leaves number		
	Control	Night break	Extension	Control	Night break	Extension
Chinese hardneck	74.61a	65.72a	67.11a	7.94b	7.39b	7.11b
Chinese softneck	54.11b	52.94b	53.06b	9.50a	9.22a	8.72a
Sangga Sembalun	55.17b	53.61b	50.83b	8.06b	8.06ab	7.83ab
Tawangmangu Baru	58.50b	55.22b	51.89b	8.11b	8.94a	7.72ab

Note: Numbers followed by the same lowercase letter notation in the same column are not significantly different on the Least Significant Different (LSD) test α=5%

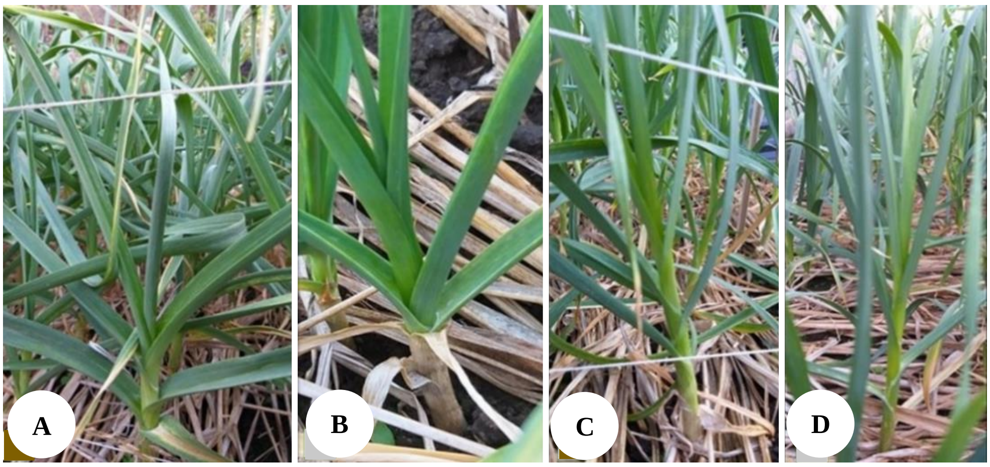

Figure 2. Morphological performance of Chinese hardneck (A), Chinese softneck (B), Sangga Sembalun (C), and Tawangmangu Baru (D)

Table 4. The interaction of genotype and photoperiod on pseudostem diameter and bulbing index

Genotype	Pseudostem diameter (mm)			Bulbing index		
	Control	Night break	Extension	Control	Night break	Extension
Chinese hardneck	6.72a	6.98a	5.39a	0.67a	0.41b	0.65a
Chinese softneck	5.89ab	5.54b	4.68a	0.57b	0.53a	0.55b
Sangga Sembalun	4.99b	4.67b	4.48a	0.24c	0.24c	0.29c
Tawangmangu Baru	5.26b	5.32b	4.66a	0.29c	0.27c	0.31c

Note: Numbers followed by the same lowercase letter notation in the same column are not significantly different on the Least Significant Different (LSD) test α=5%

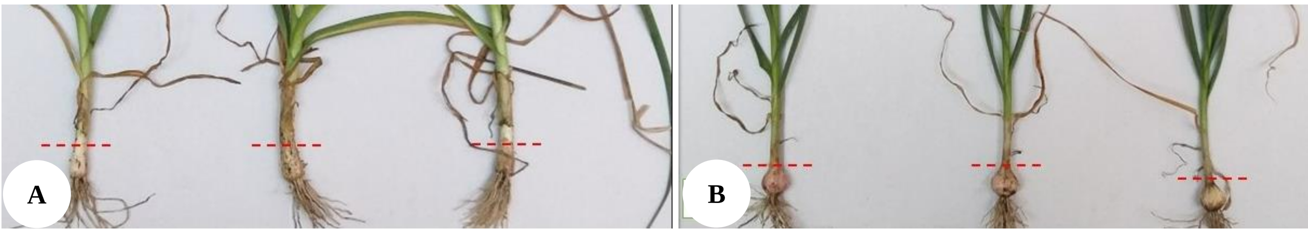

Figure 3. The difference in the bulbing index appearance between Chinese hardneck (A) and Sangga Sembalun (B) (indicated by the red dotted line). Treatment from left to right: control-extension-night break

The interaction of genotype and photoperiod on pseudostem diameter and bulbing index

The interaction effect of genotype and photoperiod significantly affected pseudostem diameter. In the control treatment, the Chinese hardneck genotype had the highest pseudostem diameter but non significantly different from the Chinese softneck genotype (Table 4). However, in the control treatment, the Chinese softneck genotype was not significantly different from local garlic genotypes in pseudostem diameter. Pseudostem diameter was not different among garlic genotypes in the extension treatment. While in the night break treatment, the Chinese hardneck was the highest pseudostem diameter genotype (Table 4, Figure 3A). Chinese hardneck was the tallest garlic plant among other genotypes. It was not surprising that the Chinese hardneck had large pseudostem diameters since the genotype had the highest plant height. The pseudostem is a cylindrical false stem arranged from every leaf base, enveloping the sheath of the newer developing younger leaf (Rabinowitch and Goldstein 2020) composed of circular leaves (a false stem made of the rolled base leaves). Plants in active vegetative growth usually have a larger pseudostem, indicating that several leaf sheaths are still intact within the pseudostem. A slight decrease in pseudostem diameter suggested the slowed plant growth. The absence of an increase in pseudostem diameter indicates that active vegetative growth has stopped. The reduction in the size of the pseudostem diameter indicates that the pseudostem will become hollow unless the flower stalk (scape) emerges and grows in bolting-type cultivars (Rabinowitch and Goldstein 2020).

The bulbing index was significantly affected by the interaction of genotype and photoperiod. Bulb initiation or bulbing index is defined as the ratio between the pseudostem and equatorial diameter of the bulb. Plants begin to form bulbs if the bulbing index value is below 0.50 (Mann 1952). Local garlic cultivars (Sangga Sembalun and Tawangmangu Baru) had the lowest bulbing index than Chinese garlic (hardneck and softneck) in each photoperiod treatment (Table 4). Local garlic cultivars (Sangga Sembalun and Tawangmangu Baru) had bulb index values ranging from 0.24 to 0.26, which indicated those genotypes were at the mature bulb stage. Garlic plants are determined in the mature bulb stage if their bulbing index is from 0.20 to 0.30 (Mann 1952). In this experiment, the bulbing index of local cultivars was lowest both in the short and long photoperiod. Those implied that genotypes might be less sensitive to photoperiods. Based on the maturity time, Sangga Sembalun and Tawangmangu Baru are garlic genotypes in the early and mid-season categories, respectively (Table 1). This condition followed the statement that garlic genotypes of the early and mid-season maturity group have a very low dependence on long photoperiods to form bulbs (Cunha et al. 2014). Local garlic genotypes have been adapted to the local tropical climate, with short photoperiods (natural daylight less than 12 hours) and warm to high temperatures. So, they can form the bulbs in that agro-climate.

China hardneck and softneck did not form bulbs in the short photoperiod in the previous study (data not presented), and garlic did not form bulbs either in the control treatment in this experiment. Chinese hardneck and softneck had longer than 200 DAP, so they belong to the late season genotype (Table 1). Garlic genotype categorized as a late-season type was photoperiod and temperature dependent and was the slowest to start bulbs (Cunha et al. 2014). Bulbs only form under long photoperiod conditions (Atif et al. 2019). Hardneck and softneck garlic showed a bulbing index greater than 0.50 with long photoperiod (night break and extension). Therefore, it was indicated that the bulb initiation had not started in those genotypes, except the Chinese hardneck had a bulbing index of 0.41 during the night break (Table 4). This condition differed from the statements, which stated that a longer photoperiod and higher temperature promoted earlier in bulbing formation (Khokhar 2017, Atif et al. 2020). The high temperature during the experiment affected the initiation of bulbs in Chinese garlic. Bulbing in Chinese garlic required extended time under high temperatures and long photoperiod conditions. However, they could still form bulbs under that condition (data not shown). Pseudostem diameter tended to be low in the extension treatment.

Meanwhile, the night break gave a low bulbing index in all garlic genotypes. Night interruption or night break is also an effective approach to induce flowering in *Hypericum perforatum* (Chen et al. 2010). The length of the uninterrupted dark period is one of the most critical factors that control the flowering responses of plants to a succession of light-dark cycles. It is assumed that, during the long dark period, the photoreceptor-activated flowering signals are too low to promote flowering in long-day plants (Chen et al. 2010).

The interaction of genotype and photoperiod on C/N Ratio, chlorophyll total content, and gibberellic acid content

The results show that China softneck, China hardneck, and Sangga Sembalun had the highest CN ratio in the night break, extension, and control treatments, respectively (Table 5). Sangga Sembalun, Chinese softneck, and Chinese hardneck tended to have high C content in the control, night break, and extension treatment, respectively (Figure 4A). Chinese softneck had a low N content in the control and night break treatments but had high N content in the extension treatment (Figure 4B). The C/N ratio was used as an indicator of plant growth and development. A low CN ratio indicates a plant is in the vegetative phase, while a high CN ratio is associated with a change in the plant from the vegetative stage to the reproductive phase (flowering) (Corbesier et al. 2002). According to Michael et al. (2015), garlic plants form bulbs when the plants have passed the juvenile phase. Garlic plants will begin to form bulbs indicated by a bulbing index value of less than 0.5 (Mann 1952). Genotypes Local garlic formed bulbs in all photoperiod treatments (Short and long photoperiod). Sangga Sembalun showed this condition in the control treatment, which had the highest CN ratio and low bulbing index. However, a different pattern was shown in Chinese garlic, which had a high C/N ratio but a high bulb index of more than 0.5. The high bulbing index indicated that bulb initiation had not yet formed. Different genotypes have different physiological states, so it causes differences in growth and development. Sangga Sembalun has a short maturity time (<120 DAP) compared to Chinese garlic, which has a long maturity time (200 DAP). It caused a different length of vegetative phase before entering the juvenile and mature state. If the genotypes were grown in the same environment or same treatment, but their response is different, it means the difference was caused by genetics. The C/N ratio tended to be high in the long photoperiod, but Sangga Sembalun had a different pattern. A high C/N ratio indicated sucrose (carbohydrate) accumulated in the leaf before flowering. Atif et al. (2021) stated that longer photoperiods and higher temperatures significantly increased the content of sugars in garlic bulbs.

Changes from vegetative meristems to reproductive meristems also affect the concentration of endogenous gibberellic acid (ga) because ga is one of the factors that play a role in the flowering pathway in plants (Poethig 2013). Bulb initiation will occur after the plant has passed the juvenile phase (rao 2016). The results showed that the chinese hardneck in the short photoperiod treatment (control) had the highest concentration of ga. Chinese softneck and hardneck genotypes contained the highest GA in the night break treatment. Chinese hardneck was the genotype with the highest GA concentration but was not significantly different from Sangga Sembalun in the extension treatment (Table 5). According to Atif et al. (2020), GA will increase along with the length of the photoperiod. As with the C/N ratio, the genotypes used in this experiment had different genetic makeup and maturity time. Therefore, the stage of each genotype was also different, which might affect the endogenous GA concentration. Sohn et al. (2011) stated that the GA content would increase before bulb differentiation occurs, and after that, it begins to decrease until harvest. Although, China softneck tended to elongate the internodes on long photoperiod treatments. Elongation of leaf internode is the influence of GA and is one of the signs of the plant entering the generative stage. But the Chinese softneck genotype had the highest GA in the night break treatment and had longer internodes compared to the control treatment, but the bulbing index of the Chinese softneck was greater than 0.5. It meant the bulb had not formed yet. The information is to be investigated further, focusing on the Chinese softneck in the following research.

Table 5. The interaction of genotype and photoperiod on C/N ratio, total chlorophyll content, and gibberellic acid content

Genotype	C/N ratio			Gibberellic acid (mg g^{-1})			Chlorophyll total (mg g^{-1} FW)		
	Control	Night break	Extension	Control	Night break	Extension	Control	Night break	Extension
Chinese hardneck	11.95d	12.28d	14.10a	47.44a	44.92a	48.11a	1.81d	1.99c	2.10d
Chinese softneck	12.76c	23.71a	13.25b	41.55b	46.71a	36.19c	4.22a	3.28b	3.62a
Sangga Sembalun	16.96a	14.63c	12.92bc	37.66c	36.99b	45.72a	3.91b	3.46a	2.81c
Tawangmangu Baru	15.20b	15.35b	12.31c	41.98b	31.65c	40.72b	3.81c	3.48a	3.50b

Note: Numbers followed by the same lowercase letter notation in the same column are not significantly different on the Least Significant Different (LSD) test α=5%

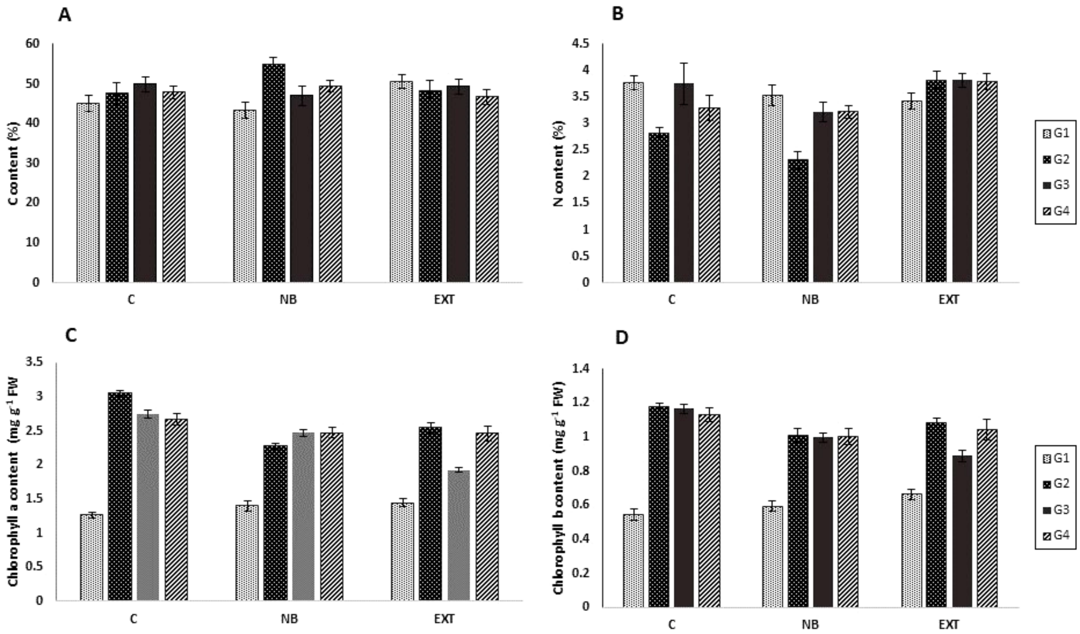

Figure 4. Content of Carbon (C) (A), Nitrogen (N) (B), chlorophyll a (C), and chlorophyll b (D). G1=Chinese hardneck, G2=Chinese softneck, G3=Sangga Sembalun, G4=Tawangmangu Baru, C=Control, NB=Night break, EXT=Extension

Photoperiod experiments showed the Chinese softnecks had the highest total chlorophyll content in the control and extension treatments. Meanwhile, the highest total chlorophyll content genotype in the night break treatment was Tawangmangu Baru and Sangga Sembalun. China hardneck genotype had the lowest total chlorophyll content in all photoperiod treatments (Table 5). The Chinese softneck genotype had chlorophyll a and b contents higher under short photoperiod than under long photoperiod (Figure 4C and 4D). Chinese hardneck had the lowest chlorophyll a and b in all photoperiod treatments. Different genotypes have differed in genetic makeup, leading to varied chlorophyll pigment content in their growth and development (Putri et al. 2021). The difference in total chlorophyll content can be related to whether the plant growth is in an active vegetative state, juvenile, or entering the adult phase in each genotype. Differences in maturity time also affected the ongoing phase of the garlic genotype. The total chlorophyll content increased from the beginning of development to flowering and decreased by the end of the growing season, while the maximum content of chlorophyll a was in the flowering phase, and it would be decreased in the fruiting phase (Abdurazakova et al. 2020). As in the C/N ratio and GA content, the total chlorophyll content will depend on the state the genotype goes through at that time. According to El-Magd et al. (2013), the increase in total chlorophyll occurred in the active vegetative phase to a maximum extent and then began to decrease along with leaf senescence. Each genotype had a different pattern in each photoperiod treatment in this experiment. But we did analyze total chlorophyll content, C/N ratio, and GA content just one time at 12 WAP in all treatment combinations. It caused a lack of information on the changing chlorophyll total content pattern in leaves related to the phase in garlic plants. But from this experiment, information can be explored further for the following research to reveal the physiological and metabolic changes due to different photoperiod focusing in two genotypes with the distinguished response in this experiment. The total chlorophyll content tended to be higher under short photoperiod than that under long photoperiod. This was probably related to the maturity level of the leaves. The local garlic genotypes (Sangga Sembalun and Tawangmangu Baru) were entering the maturity stage, indicated by a low bulbing index value. Total chlorophyll content has been used as an indicator of leaf ontogeny with three developmental stages: young or developing stage, mature stage, and senescent stage. The first sign of autumn senescence leaves chlorophyll degradation (Keskitalo et al. 2005). THE genotypes that received the long photoperiod treatment had a faster life cycle than the control. Garlic genotypes under long photoperiods were harvested between 8.67 and 47 days earlier than the control treatment (data not presented). However, some researchers revealed that chlorophyll content is also related to the ability of plants to cope with abiotic stress. Chlorophyll is synthesized in the daytime in angiosperms because protochlorophyllide oxidoreductase is active only in the light, but chlorophyll also decreases during the day rather than the night (Mattila et al. 2018). Short photoperiod treatment exhibited higher chlorophyll contents and photosynthetic rate in adzuki bean (Dong et al. 2016), and chlorophyll content was negatively correlated with the extended photoperiod (Haque et al. 2015). According to Adams and Langton (2005), increased leaf expansion in lp was frequently accompanied by a reduction in net assimilation rate, and it causes a reduction in total chlorophyll per unit area of the leaf.

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