

Original Research

Differential Effect of Day and Night Temperature Regimes on the Growth and Biochemical Attributes of Violet Rape (*Brassica campestris* ssp. *chinensis* L.)

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Abstract

The present study investigated the influences of different day and night temperature regimes on the growth of violet rape (*Brassica rapa* var. *chinensis*) and pinpointed the optimal temperature combination for both yield and quality of the plant. For this purpose, the plants were grown under 6 temperature combination conditions: W1 (30/20°C, 12-h light/12-h dark), W2 (25/15°C, 12-h light/12-h dark), W3 (20/10°C, 12-h light/12-h dark), W4 (30/15°C, 12-h light/12-h dark), W5 (25/10°C, 12-h light/12-h dark), and W6 (30/10°C, 12-h light/12-h dark), and in addition we measured various morphological, yield, and biochemical traits. Results demonstrated that temperature regimes considerably affected the growth parameters of the violet rape compared with control. The W3 treatment with an average temperature of 20/10°C displayed the highest plant growth traits, including plant height (12.74±0.24 cm), leaf length (8.20±0.08 cm), leaf width (5.44±0.16 cm), and leaf area (33.46±0.83 cm²). Moreover, a highly significant correlation ($p<0.05$) was found between temperature and soluble protein, chlorophyll, anthocyanin, nitrate nitrogen contents, and root activity; whereas the vitamin C and soluble sugar contents remained significantly unaffected except in the W1 treatment, under different temperature combinations between day and night. This study concluded that high temperature exerts an adverse effect on plant growth, and therefore a low day and night temperature combination is suggested in order to achieve high yield and quality for irrigated violet rape.

Keywords: temperature regimes, violet rape, morphological traits, anthocyanin, root activity

Introduction

In recent decades, considerable research has focused on the influence of climate change on plant growth and final crop yield [1-2]. The efficiency of plant growth and development depends on the temperature surrounding the plant, and every species possess a defined temperature for its optimal growth. Reports have shown that over the last 100 years, the average temperature has been raised by $0.74\pm 0.18^{\circ}\text{C}$ [3-4], and heat waves are projected to become more penetrating, frequent, and last longer than currently [5]. Though extreme heat events may have the most dramatic impact on plant output, nevertheless there has been very little work carried out to document these effects. China also suffers considerably from climate change [6]. From 1960 to 2009, high-temperature stress caused by global warming has been extensively reported in the major planting areas in China [7-10].

Chinese cabbage (*Brassica campestris* ssp. *chinensis* L.), also known as violet rape (a member of the cruciferous family), is a highly consumable leafy vegetable in China and Southeast Asia [11]. As compared to other family members, it doesn't have a well-developed root system, producing sessile leaf with a short stem. Due to high water and fertile soil requirements, this plant cannot tolerate water for longer periods [12-13]. Previous studies have shown that temperature for violet rape seed germination ranges $4\text{-}40^{\circ}\text{C}$, but the most suited temperature lies between $20\text{ and }25^{\circ}\text{C}$, in which it grows optimally. Generally, it prefers the cold climate in spite of potential resistant capability to the high temperature of summer as compared to other family members [14]. At high temperature (above 25°C), its growth becomes weaker together with poor quality and bitter taste. Violet rape is a cool-loving plant and maintains normal growth and physiology when exposed to low temperature [15].

A large number of recent studies have shown that standardization of varying temperatures and/or radiations can improve crop yield (up to 15%), which also differs for various species [1]. However, the limitation of most previous reports lies in that only one-stage temperature influences on the growth of plants was examined, while no studies have been conducted investigating the comprehensive effects of day and night temperature set points on the growth of plants – particularly on violet rape. Therefore, the objective of our current study was to explore the influence of different temperature regimes between day and night on agronomic traits, growth, and quality of violet rape.

Materials and Methods

Experimental Site

The experiments were conducted in climate-regulated chambers under the phytotron at the Department of Agriculture and Biology, Shanghai Jiao Tong University, China ($121^{\circ}38'E$, $31^{\circ}12'N$) from December 2015 to March 2016.

Agronomic Practice and Sowing

Violet rape (*Brassica campestris* ssp. *chinensis* L.) seeds were kindly provided by Shouguang Renhe Seed Industry Co., Ltd. China. For sowing purposes, uniform and healthy seeds were chosen by hand-picking, while seeds with any visible defect, insect damage, and/or malformation were discarded. Prior to sowing, the seeds were soaked in water and grown into a standard 200-hole tray with one seed in one hole under natural environmental conditions without any fertilizer supplementation during the seedling stage. When the seedlings developed up to 3 apparent leaves, they were transplanted into a standard 32-hole tray having a loading capacity of about 4 l of substrate. Each hole of the tray was 50 cm (length) and 26 cm (width), accommodating a capacity of about 0.125-l substrate. The seedlings were firmly transplanted into the tray containing osmocote (4 g/L) as basal fertilizer and water (3 liters), and soaked it for 30 min until substrate absorbed sufficient water. The tray was then shifted to an artificial climate chamber containing 85.0% relative humidity and fluorescent light (Philips) intensity ranging from 4,500 lx to 4,600 lx. The temperature combination conditions provided are given in Table 1. The plants were irrigated after every 5 days and finally harvested in March 2016.

Morphological Indicators

After harvesting, fresh plant samples were transported to the laboratory and morphological parameters such as leaf area, leaf numbers, and fresh and dry weights of roots and shoots were measured. The dry weights of roots and shoots were analyzed by placing the samples in an incubator (Himac CF15R) at 105°C for 24 h. Leaf area per plant was calculated by the formula:

$$K = \frac{A}{L \times W} \quad (1)$$

Table 1. Experimental design showing different temperature treatments for growth characteristics of violet rape.

Treatment	W1	W2	W3	W4	W5	W6	CK
Day temperature ($^{\circ}\text{C}$)	30 ± 1.0	25 ± 1.0	20 ± 1.0	30 ± 1.0	25 ± 1.0	30 ± 1.0	20 ± 1.0
Night temperature ($^{\circ}\text{C}$)	20 ± 1.0	15 ± 1.0	10 ± 1.0	15 ± 1.0	10 ± 1.0	10 ± 1.0	20 ± 1.0

...where A indicates the area, K is the coefficient constant whose value is 0.7501, and L and W represent the length and width of leaf per plant, measured by means of a manual ruler.

Biochemical Parameters

Vitamin C Content

Ascorbic (AA) acid content was determined as reported previously [16]. In an Erlenmeyer flask (250-mL) containing 2.0 g of fresh ground leaf powder, 1.0 mL of $ZnSO_4$ (30%) and potassium ferrocyanide (15%) were thoroughly mixed and then 1.0% oxalic acid was added to the final volume of 100 mL. The resulting homogenate was filtered and 2.0 mL of 2, 6-dichlorophenolindophenol and 5.0 mL of xylene solutions were added to the 4.0 mL of the filtrate, vortex mixed, and optical density was recorded at 500 nm.

Anthocyanin Measurement

For anthocyanin content measurement, 1.0 mL of acidic methanol [HCl, 1.0% (w/v)] was added to 300 mg of freshly ground plant material and samples were incubated at room temperature for 18 h under mild agitation. The plant material was centrifuged (1.0 min, 14,000 rpm) and 400 mL of clear supernatant was mixed with 600 mL of acidic methanol. Absorption of each sample was determined at 530 and 657 nm in a double-beam UV-vis spectrophotometer (HITACHI U-2900 spectrophotometer) [6].

Soluble Sugar and Protein Analysis

For soluble sugar analysis, fresh leaf sample (0.2 g) was taken in a test tube with 5.0 mL of distilled water and placed in a boiling water bath (100°C) for 30 min. The sample mixture was filtered into a volumetric flask and 5.0 mL Anthrone solution was added to 1.0 mL of filtrate followed by incubation in a water bath for 10 min. After 10 min, the solution was taken out, cooled to room

temperature, and absorbance was measured at 620 nm [17]. A rapid and versatile method of Bradford using Bovine serum albumin (BSA) as standard was used for the quantification of total protein contents [18].

Nitrogen Nitrate and Chlorophyll Content

Nitrogen nitrate contents of dried plant sample were analyzed following the method of [19]. Briefly, in a beaker, 0.5 g root tips, 5.0 mL 2, 3, 5-triphenyltetrazolium chloride (TTC) solution (0.4%), and 5.0 mL of phosphate buffer were added and the beaker was incubated at 37°C for 3 h. At designated incubation time, 2.0 mL of H_2SO_4 (1.0 M) was added to terminate the reaction and mixture absorbance was monitored at 485 nm. Leaf chlorophyll content was analyzed in a manner reported previously [20].

Statistical Analysis

All the results including growth, physiological index, or biochemical parameters were reported as means along with standard deviations from 3 independent measurements. Differences between treatments were calculated by one-way ANOVA and Duncan's multiple range tests at 5.0% level of probability.

Results

Morphological Characteristics of Violet Rape at Day and Night Temperature Fluctuations

The morphological attributes during violet rape (*Brassica campestris* ssp. *chinensis* L.) development process such as leaf number, plant height, leaf length, leaf width, and leaf area were determined at different day and night temperature set points, and responses thus obtained are summarized in Table 2. The appearance of violet rape plants and their leaves cultivated under different growth conditions in temperature-controlled chambers are shown in Fig. 1. Results revealed that no significant difference ($p > 0.05$) was observed at varying temperature regimes

Table 2. Influence of differential day and night temperature set points on the morphological indexes of violet rape.

Treatment	Leaf number	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm ²)
W1	12.60±0.75a	8.74±0.58c	5.28±0.47de	4.08±0.29d	16.16±2.82c
W2	10.60±0.40ab	9.14±0.21c	6.00±0.16cd	5.06±0.16ab	22.77±0.87b
W3	12.40±0.60a	12.74±0.24a	8.20±0.08a	5.44±0.16a	33.46±0.83a
W4	11.75±0.43a	10.72±0.45b	6.37±0.20bc	4.75±0.14bc	22.71±4.69b
W5	12.20±0.66a	11.26±0.32ab	7.18±0.31b	5.38±0.18a	28.97±2.11a
W6	9.60±0.40b	6.70±0.58d	4.26±0.29f	2.82±0.22e	9.01±1.18d
CK	11.60±0.81a	7.38±0.46d	4.94±0.39ef	4.30±0.21cd	15.93±2.03c

Bold values indicate the highest response of any respective parameter; mean values indicated by dissimilar lowercase letters are significantly different from each other ($p < 0.05$); values are means ± SDs (n = 15)

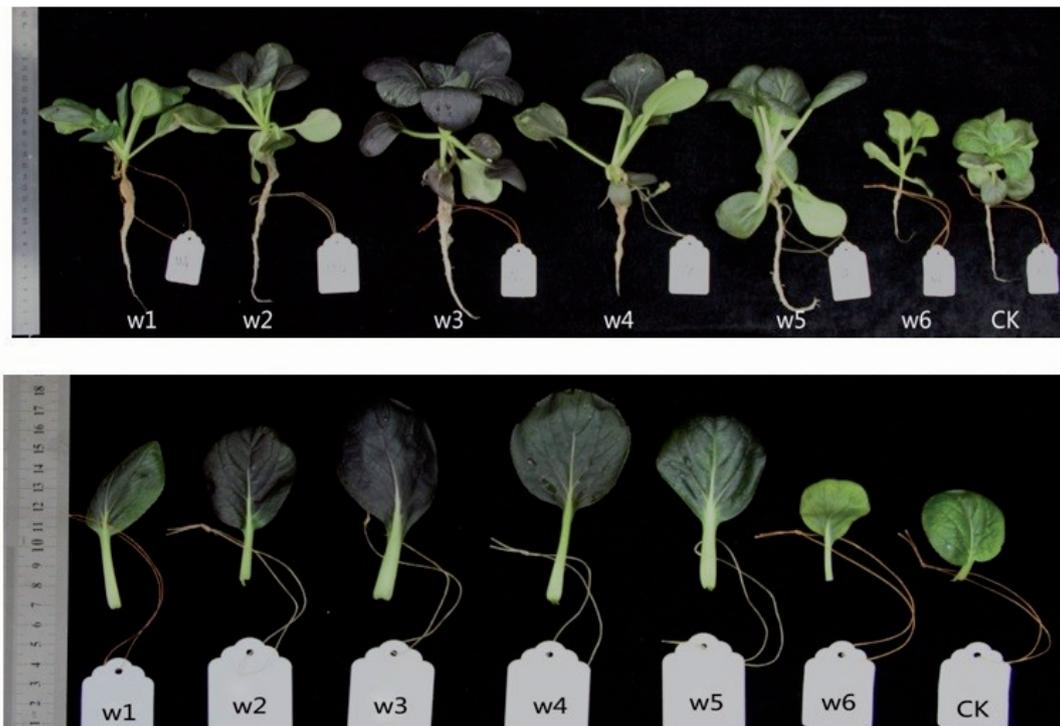


Fig. 1. The appearance of violet rape plant cultivated under different growth conditions in temperature-controlled chambers: Control (CK) 20°C, 12-h light/12-h dark cycle; W1 30/20°C, 12-h light/12-h dark cycle; W2 25/15°C, 12-h light/12-h dark cycle; W3 20/10°C, 12-h light/12-h dark cycle; W4 30/15°C, 12-h light/12-h dark cycle; W5 25/10°C, 12-h light/12-h dark cycle; and W6 30/10°C, 12-h light/12-h dark cycle (upper panel, overall views of plants at different temperature treatments; lower panel, view of plant leaves at different treatments).

for leaf numbers, and all the temperature set points to give rise to more than 10 leaves except W6 treatment (30/10°C, 12-h light/12-h dark). Noticeably, the highest leaf numbers (up to 12.60) were recorded in W1 (30/20°C, 12-h light/12-h dark) followed by W3 (20/10°C, 12-h light/12-h dark cycle), and W5 (25/10°C, 12-h light/12-h dark). Nevertheless, these treatments were statistically not significant with respect to control (CK, 20°C, 12-h light/12-h dark). The plant's height showed a fluctuating trend at different temperature combinations. The highest plant height was noted in W3 (12.74 cm), followed

by W5 (11.26 cm), whereas it was smallest in the W6 (6.70 cm) combination. Moreover, a significantly positive correlation ($p < 0.05$) was found at various temperature treatments for leaf length, leaf width, and leaf area and, as a consequence, the W3 temperature regime exhibited superior results for all these agronomic traits.

As shown in Table 3, among all the treatments, W3 portrays a better performance for plant fresh weight, shoot fresh and dry, as well as root fresh and dry weights. The recorded improvements were 68.09%, 66.10%, 80.71%, 61.22%, and 85.71% for plant fresh weight, shoot

Table 3: Influence of differential day and night temperature set points on the growth parameters of violet rape.

Treatment	Plant fresh weight (g)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Root-shoot ratio
W1	5.15±0.73c	4.37±0.62c	0.79±0.11c	0.36±0.05b	0.05±0.01a	0.18±0.01ab
W2	5.80±0.40c	4.78±0.38c	1.01±0.1ab	0.32±0.03b	0.05±0.01a	0.21±0.03a
W3	10.25±0.69a	8.85±0.55a	1.40±0.30a	0.49±0.04a	0.07±0.02a	0.16±0.03ab
W4	5.63±0.51c	5.08±0.43c	0.56±0.11bc	0.33±0.03b	0.03±0.00ab	0.11±0.02b
W5	8.19±0.45b	7.17±0.48b	1.01±0.11ab	0.42±0.02ab	0.05±0.01a	0.14±0.02ab
W6	1.58±0.14e	1.41±0.15e	0.17±0.05c	0.09±0.01d	0.01±0.00b	0.13±0.05ab
CK	3.27±0.29d	3.00±0.26d	0.27±0.09c	0.19±0.00c	0.01±0.00b	0.09±0.03b

Bold values indicate the highest response of any respective parameter; mean values indicated by dissimilar lowercase letters are significantly different from each other ($p < 0.05$); values are means ± SDs (n = 15)

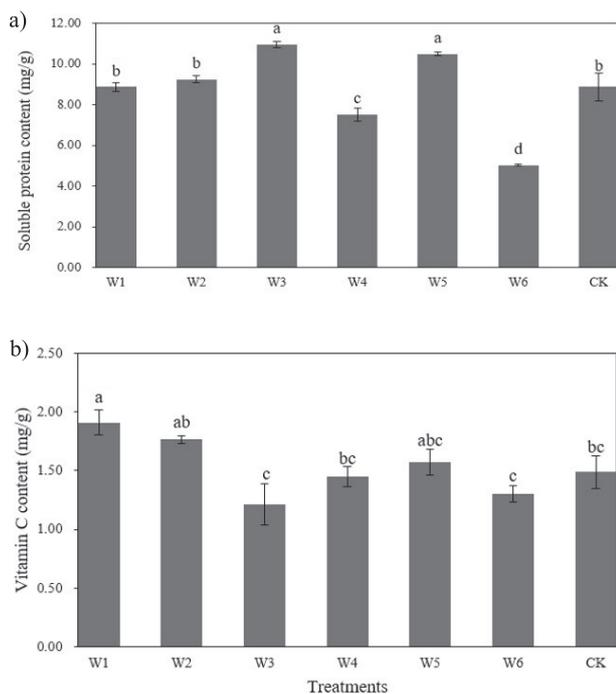


Fig. 2. a) Soluble protein content of violet rape cultivated under different day and night temperature set points and b) vitamin C content of violet rape cultivated under different day and night temperature combinations; mean values indicated by dissimilar lowercase letters represent significant differences from each other ($p < 0.05$).

fresh weight, root fresh weight, shoot dry weight and root dry weight, respectively, as compared to control (CK). Minimal plant fresh weight was observed in W6 treatment (only 1.58 g), and even less than half of the CK. On the other hand, the root-shoot ratio was found to be 57.14% higher in W2 contrasted with control.

Biochemical Parameters of Violet Rape at Day and Night Temperature Regimes

The effects of different day and night temperature amalgamations were also investigated on different biochemical parameters of violet rape such as

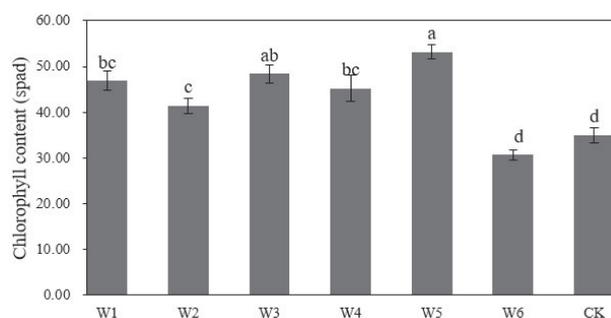


Fig. 3. Chlorophyll content of violet rape cultivated under different day and night temperature set points; mean values indicated by dissimilar lowercase letters are significantly different from each other ($p < 0.05$).

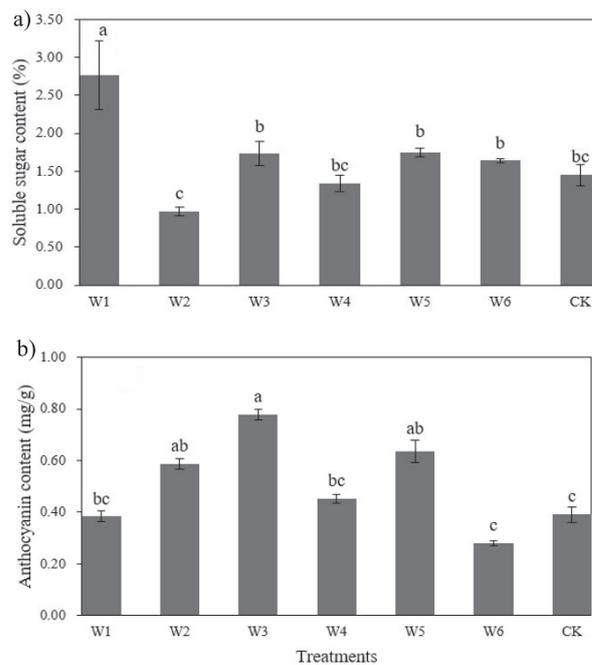


Fig. 4. a) Soluble sugar content of violet rape cultivated under different day and night temperature combinations and b) the anthocyanin content of violet rape cultivated under different day and night temperature combinations; mean values indicated by dissimilar lowercase letters are significantly different from each other ($p < 0.05$).

soluble protein, vitamin C, chlorophyll, soluble sugar, anthocyanin, nitrate nitrogen contents, and root activity. Notably, soluble protein contents were found to be significantly different ($p < 0.05$) at all the temperature treatments (Fig. 2a). In contrast to CK, the day and night temperature combination of W3 and W5 led to 23.96% and 18.38% higher protein contents, respectively. Whereas W6 and W4 treatments decreased the protein content by 43.40% and 15.45%, respectively, as compared to CK. As shown in Fig. 2b, the vitamin C content remained significantly unchanged under different temperature combinations between day and night. Maximum vitamin C recorded in W1 (1.91 mg/g) was 28.19% greater than CK, while other groups did not show any noticeable change in vitamin C compared to CK. In all six temperature regimes, chlorophyll contents were found to be highest in W5 (53.18 spads), followed by W3 (48.38 spads; Fig. 3). The percentage enhancements were 34.11% and 27.58%, respectively, compared to CK. The chlorophyll in W6 was 12.56% lower than CK, but this decline was not significant. Similarly, no significant difference between different treatments was observed for soluble sugar content except the W1 treatment. The soluble sugar content in the W1 and W2 treatment was 91.03% higher and 33.10% lower than that of control (Fig. 4a). Violet rape temperature treatments exhibited a significant influence on the content of anthocyanin (Fig. 4b). In W3, best anthocyanin content attained (0.78 mg/g) was 50% superior to control, while in case of W1

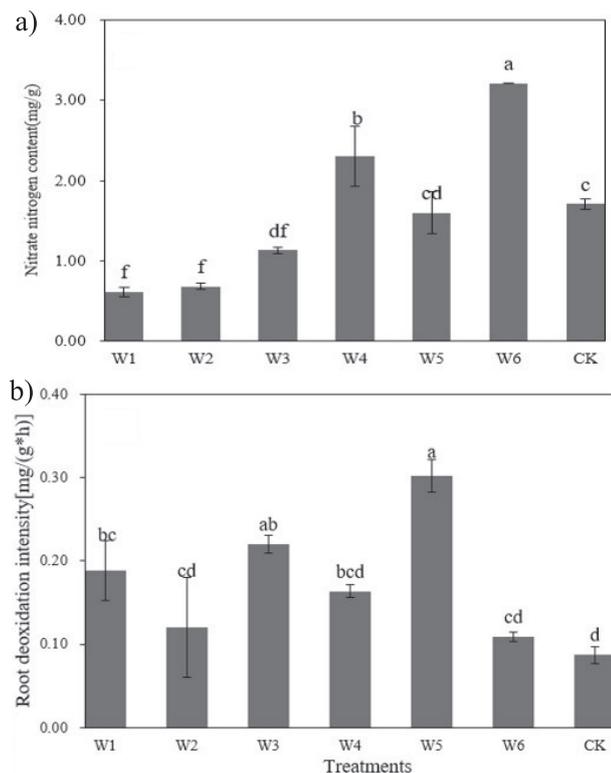


Fig. 5. a) The nitrate nitrogen content of violet rape cultivated under different day and night temperature set points and b) root de-oxidation intensity of violet rape cultivated under different day and night temperature combinations; mean values indicated by dissimilar lowercase letters are significantly different from each other ($p < 0.05$).

and W6 treatments, anthocyanin content was 2.56% and 39.28% less than CK. Fig. 5a) portrays nitrate-nitrogen contents on different day and night temperatures to show a positive correlation. The order for nitrate-nitrogen contents was $W1 < W2 < W3 < W5 < W4 < W6$. The nitrate contents in W1, W2, and W3 were 64.33%, 60.23%, and 33.92% lower than that of CK, while it was the highest in W6, about 87.72% higher than CK. The obvious differences in root activity intensity were observed at varying temperature combinations (Fig. 5b). W5 possessed the strongest root activity – 0.30 mg/(g*h), which was 70% greater in comparison to CK. The root activities in W5, W3, and W1 were 3.3, 2.4, and 2.1 times higher, respectively, with respect to control.

Discussion

Temperature is the main ecological factor that considerably influences growth development of agricultural plants [21-22]. Sometimes the developmental process of violet rape is not essentially the same in different planting dates in spite of the same cumulative climate data of those sowing dates. Therefore, the standardized amalgamation of day and night temperature set points is of great value for efficient growth of any

plant. The knowledge of optimized day and night temperature regimes would undoubtedly contribute to a suitable sowing date schedule so as to assure each stage in its optimal temperature range. Low-temperature level during plant growth stage drastically decelerates leaf enlargement, leaf numbers, and total uptake at maturity [23-24]. On the other hand, high-temperature stress during or after flowering is quite unfavorable for plant growth, and several authors have reported a marked reduction in plant biomass, which eventually resulted in poor quality and production of food crops [25-26]. In common bean (*Phaseolus vulgaris*), morpho-physiological attributes such as phenology, plant-water relations, shoot and root growth, and elongations are seriously hindered due to elevated heat stress [27]. In some other plant species, high-temperature causes noteworthy elongated stems and leaves, while total biomass was reduced [28-29]. The green leaf area and productive tillers were drastically diminished under high temperature (30/25°C, day/night) [30]. Various plants show programmed cell death in specific cells or tissues following exposure to extreme heat stress due to aggregation and/or denaturation of proteins, while a moderately higher temperature for longer duration causes shedding of leaves or even death of the whole plant [31-32].

Deng and co-workers (2015) investigated the average maximum and minimum daily temperatures in the rice phenological stages, ranging from 22.4 to 29.1°C, 26.2 to 31.8°C, and 21.6 to 30.6°C in vegetative, reproductive, and grain-filling stages, respectively [1]. The optimum temperature in vegetative and grain-filling stages was recorded to be 28°C and 22°C, respectively. They recommended a range of 26 to 28°C to be the optimal average daily temperature in vegetative stage to get the best crop yield. In another study, Sanchez et al. (2014) evaluated the one-stage temperature influences on the growth of plants and found 28.4°C to be optimal for tillering [33-34]. It has been reported that a combined temperature optimization strategy is useful in practical applications, while the one-stage study is more suitable for basic/elementary research [1].

The cooperating influences of environmental temperatures on growth and physiological attributes of *Pisum sativum* were investigated by Martel and Qaderi (2016), and found that higher temperatures and supplemental UV-B radiation significantly reduced the plant growth rate by decreasing stem height, diameter, and leaf area [35]. The findings were in agreement with earlier reports on crops, including canola [36] and peas [37] as well as some higher plants [38].

According to the best of our knowledge, no other such climatic study has been reported with violet rape evaluating biochemical parameters in response to different temperature combinations. For turnip roots (*B. rapa L.*), Shattuck and coworkers, (1991b) observed that low growth temperature for short periods does not affect ascorbic acid content [39]. The vitamin C content seems to be the highest at low growth temperatures in broccoli [40]. As compared to 7-12°C, the elevated

temperatures (15-20°C) led to 38% reduction in the content of ascorbic acid in greenhouse-grown broccoli. In a controlled climate study (21/15°C and 15/9°C (day/night)), Steindal and coworkers (2013) recorded a 16% reduction in vitamin C content at the higher temperatures for 12 h and 24 h day length. Conversely, Molmann et al. (2015) found no significant differences in vitamin C content between broccoli grown at 12°C and 18°C [41]. The utmost levels of total sugar content were found for treatments 21/15/9°C and 15/9/6°C, both with a significant difference to the lower level at 21°C [42]. Shattuck et al. (1991a) reported that a low growth temperature (0-12°C) for 11 days before harvesting considerably increased the total sugar content [39-43]. Comparable results have also been reported by Davik (1992) and Shattuck et al. (1991a).

Our study in controlled climate chambers clearly showed the biochemical responses to various designated treatments and contributed an insight of possible temperature effects on morphological and biochemical properties in violet rape. As for consumer's preference, the findings revealed that high cultivation temperatures do not benefit eating quality. Nevertheless, the results should be strengthened by field studies, where several other environmental factors including light conditions, day-length, light intensity, and spectral composition might influence both morphological and biochemical properties.

Conclusions

It is of great concern to select an appropriate day and night temperature regime so as to accommodate optimal local climatic conditions according to the requirements of a plant to achieve the utmost growth and yield. The results revealed that W3 combination with an average temperature of 20/10°C and 12-h light/12-h dark led to highest plant growth and morphological attributes. Though the biochemical characteristics of violet rape showed great variations in different temperature set points, lower temperatures were found to be better. These findings would definitely provide guidance to farmers for the cultivation of other crops (such as wheat, maize, and rapeseed, etc.) aimed at improving yield and quality.

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