Preface and acknowledgements

This thesis is the final part of my master degree in Plant Science at the Department of Plant Sciences, Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences (NMBU). The experiment was conducted in Centre for plant research in controlled environment (SKP) and Plant Ecophysiology lab, Norwegian University of Life Sciences.

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Thanks a lot.

Ås, Norway.

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Abstract

Growth chamber experiments were conducted with seedless cucumber cv. Odeon to examine the effect of night temperature on photosynthesis and growth with compensation possibilities of daytime irradiance level. Further, the effects of night temperature and the level of daytime irradiance on carbohydrate composition in different plant parts were also assessed with high performance liquid chromatography (HPLC). Daily average temperature was mainly responsible for the growth of cucumber seedling by increasing the height, leaf number, total leaf area and total dry weight. It enhances the potential of lowering night temperature in combination with high day temperature. Lowering night temperature from 22 °C to 14 °C reduced almost 34% growth as dry matter production. As long as the daily mean temperature is equal, the split night temperature had no effect on daily net photosynthesis, height; leaf area and plant dry weight. Increasing level of irradiance increased the plant's daily net photosynthesis and the magnitude of increment was significantly high at low night temperature than at high night temperature. At low night temperature (14 °C), net photosynthesis was doubled when light intensity was increased from 200 to 400 µmol m^{-2} s^{-1}. This was reflected in the total dry weight of the plants as well. The dry matter production of cucumber seedling increased almost 30% at low night temperature (14 °C) and 7% at high night temperature (22 °C) when light intensity was doubled. This indicated the potential of irradiance on compensation of losses caused by low temperature although, it will be extra energy costs related to increased light level if that has to be done by artificial lighting. Carbohydrate profiling revealed that the total carbohydrate content was significantly affected by irradiance level than temperature. Total carbohydrate concentration was increased by 8% at low night temperature (14 °C) and 20% at high light intensity (400 µmol m^{-2} s^{-1}). Among the different plant parts analyzed, the total carbohydrate content was significantly high in stem in all temperature and irradiance combinations. However, night temperature or daytime irradiance did not affect the proportion of total carbohydrate partitioned between plant parts.

Abbreviations

SKP   Centre for Plant Research in Controlled Environment

HPS   High Pressure Sodium

PPM   Parts Per Million

DMT   Daily Mean Temperature

D/N   Day/Night

h   Hour

DLC   Daily Light Cycle

PAR   Photosynthetic Active Radiation

HPLC   High Performance Liquid Chromatography

GLM   General Linear Model

SLA   Specific Leaf Area

NP   Net Photosynthesis

DR   Dark Respiration

CER   Carbon Exchange Rate

PS II   Photosystem II

Contents

1. Introduction

Cucumber (*Cucumis sativus.* L) is originated from sub-tropical regions of South Asia (Miao et al. 2007; Whitaker & Davis 1962) adapted to high temperature and high humidity for proper growth and yield (Bakker 1987; Grimstad & Frimanslund 1993). Due to harsh environmental condition (low temperature, relative humidity and low irradiance) during winter and spring period, it is difficult to produce cucumber in open field in temperate region of the world. Protected crop production is a common practice in these region that can extend the growing season as well as enhance plant production where they cannot grow optimally (Fitz-Rodriguez 2008; Papadopoulos & Hao 2000). Also, it increases the quality of fruits and vegetables that are more healthy and demanding (Pardossi et al. 2004). Although, the green house production system can boost yield and quality of crops in addition to year-round production possibilities, the system is very complex and several parameters have to be controlled to maintain better environment. Cucumber is mainly produced under protected cultivation system with semi controlled climate conditions in Northern Europe. It demands massive energy for heating greenhouse air and plant canopy during winter and early spring. To increase the temperature inside greenhouse, the heating system consume large amount of fossil fuel or other sources. This consumption of fossil fuel is not only expensive but also release significant amount of greenhouse gas (CO_2) and other air pollutants like NOx into the atmosphere (Smith et al. 2008; Von Blottnitz & Curran 2007). There is a continuous demand for high quality, energy efficient and environmental friendly cucumber production.

Temperature management is the most prioritized option for energy efficient crop production as most of the cost are related to temperature generation inside the greenhouse (Papadopoulos & Hao 2000) and it greatly affects the growth, physiology and production of greenhouse plants (Grimstad & Frimanslund 1993; Myster & Moe 1995; Went 1953). Moreover, temperature integration strategy can be used to save energy where periods of low temperature can be compensated by period of higher temperature in wide range of crops within certain limit (Rijsdijk & Vogelezang 2000). Cucumbers require high day/night temperature in addition to positive day-night temperature difference (positive DIF) for better growth and yield (Grimstad & Frimanslund 1993; Papadopoulos & Hao 2000). Within certain optimum limit, cucumber growth and yield depends on daily mean air temperature than day or night temperature (Grimstad &

Frimanslund 1993; Papadopoulos & Hao 2000). Daytime atmospheric temperature is greater than night time temperature due to solar irradiance, which means maintaining objects with low temperature during night period is efficient than daytime. Considering all these facts together, the best possible energy saving option within the low temperature strategy is low night temperature. Körner et al. (2004) and Sigrimis et al. (2000) reported that reducing night temperature by 4-5 °C can save almost 20% of the cost for energy used for heating greenhouse. While daily mean temperature and DIF has been positively correlated with cucumber growth and yield, inefficient photo assimilate partitioning has been reported as a results of low night temperature (Miao et al. 2007). Maintain proper balance between radiant and thermal energy (Liu & Heins 1997); splitting nighttime temperature have been proposed to overcome this issues (Toki et al. 1978).

Objectives of the study

Series of experiments were conducted with following objectives with the goal of energy efficient and environmental friendly cucumber production. Examine

1. The applicability of the previous finding of cucumber growth is determined by daily mean temperature not by daytime or nighttime temperature to seedless hybrid cucumber.
2. The potential of split night temperature and daytime irradiance level for their compensation potential on negative effect caused by nighttime mediated low temperature.
3. The impact of nighttime mediated low temperature and irradiance level on carbohydrate partitioning.

2. Literature review

2.1 Nomenclature

Cucumber (*Cucumis sativus.* L) is a widely cultivated plant in the gourd family, *Cucurbitaceae* and one of the most important and popular crop in commercial greenhouses (Bentivenga & Hetrick 1992; Mohammadi & Omid 2010). It is used as a vegetable crop all over the world and considered as one of the three major greenhouse vegetable crops in both Europe and North America (Zheng et al. 2010). It is also considered as one of the four major food crops of *Cucurbitaceae* family (Tatlioglu 2012), which includes watermelon, cucumber, melon and squash (Robinson & Decker-Walters 1997). It is a warm season and annual crop that grows rapidly as vine in suitable environmental condition. The main edible part of cucumber is the fruit that is used both as fresh or processed (Sarhan & Ismael 2014).

Scientific classification of cucumber

Kingdom: Plantae

    Division: Magnoliophyta

        Class: Magnoliopsida

            Order: Cucurbitales

                Family: Cucurbitaceae

                    Genus: Cucumis

                        Species: *Cucumis sativus* L.

2.2 Plant traits

2.2.1 Cucumber leaves

The leaf of cucumber plant is large (10-20 cm in the regular cucumber, 20-40 cm in the seedless cucumber). The leaves are simple, prickly, hairy and triangular that form a canopy over the fruit. Each leaf borne on long (7-20 cm) petioles. Every leaf consists of five angular lobes among which the central is the largest. The leaf surfaces are covered by many trichomes. The unbranched tendrils grow from the base of each petiole which enable the stems to climb over

other plants or objects so that the plants cannot twist themselves. The tendril tip coil around the support spirally, pulling the whole plant towards the support.

2.2.2 Cucumber shoot

Cucumber is an annual climber and the stem grows up to 2 m. It is a vine (Sarhan & Ismael 2014) that can climb up with support and run along the ground without support. The main stem of the cucumber plant begins growing erect but soon start to crawl and grows like a vine over the ground. The branching type of cucumber is sympodial (i.e., a lateral bud grows from each node and displaces the main growing point). The primary laterals, originated from the main axis can have their (secondary) laterals, and so on. All stems are roughly hairy and possess leaves singly at the nodes. The stem have an angular cross section and may turn hollow when mature.

2.2.3 Cucumber flowers

Cucumber plant consists of different kinds of flowers like staminate (male), pistillate (female) and hermaphrodite (both male and female). Normally, the flowers of cucumber are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant). The flowers are pollinated by insects and the plant is self-fertile. The flowers are yellow in color. The male flowers appear first and female flowers shortly later and the female flowers can be recognized by the swollen ovary at the base. After proper pollination, female flower develops into fruit. There are also gynoecious varieties that produce predominantly female flowers. Most of the current hybrids are gynoecious which is early and productive.

2.2.4 Cucumber fruit

Normally, cucumber is regarded as fruit. Cucumbers are the matured ovaries of a flower and fruit comes from the ovary of the flower. Botanically, the cucumbers are considered as pepoes or false berry like tomato. It is elongated and round triangular in shape. The color of the fruit is green as the immature fruits contain chlorophyll under the epidermis but upon maturity it turns yellow-white. Both the color and size of the fruit varies with different cultivar. Normally, cucumber fruits bear seeds but the greenhouse cucumbers are seedless. The seeded cucumber are short (about 15-25 cm) whereas the seedless cucumbers are long (about 25-50 cm). The outer part of

the fruit has many trichomes and it is bitter in taste. So, the skin should be peeled before eating cucumber.

2.3 Varieties of cucumber

Different kinds of cultivars are used in different region in the world depending on the growth performance, environmental requirements and market demand. Some of the important varieties according to end use are described here briefly.

2.3.1 Pickling cucumbers (*Cucumis anguria*)

The cucumber from this varieties are produced to make pickle. Although any cucumber can be pickled, these special varieties are lighter green in color, shorter, thinner-skinned which is suitable for pickled. The fruits of this cucumber grow up to about 7 cm to 10 cm long and 2.5 cm wide, have shorter growth cycle of 50-60 days and high plant populations 240,000/ha. During Pickling the cucumbers are doused in brine or vinegar with other spices although this process degrades much of the nutrients especially vitamin C.

2.3.2 Slicing cucumbers (*Cucumis sativus*)

Slicing cucumbers are used as salad item and sold fresh for immediate consumption. Compared to pickling cucumber these varieties are dark green in color, longer and thicker skin. This tougher skin provide resistant to damage during handling and shipping. Fruits of slicing varieties are preferably long, smooth, and straight. The growers can use both monoecious hybrids and gynoecious hybrids for producing slicing cucumber.

2.3.3 Burpless or greenhouse cucumber (*Cucumis sativus*)

The cucumber of this varieties are mainly produced in greenhouse. This cucumber is sweeter in taste and easy to digest. The fruits are long, narrow with rounded ends. They are gynoecious, perthenocarpic and seedless. As the greenhouse cucumber are not pollinated, the fruit development is quite uniform and smooth in appearance. The fruits can grow up to 30-35 cm long and 3.5-5 cm wide.

2.4 Nutritional benefits of cucumber

Cucumber is a vegetable fruit which is very low in calorie. It contains mostly water (95%) that plays great role in cleansing action within the body and helps to remove old waste material and chemical toxins from the body. Also, it contains different vitamins, minerals, antioxidants and phytonutrients (Sarhan & Ismael 2014) which make cucumber a very healthy vegetable. The high levels of potassium and magnesium in cucumber helps to regulate blood pressure and relaxes nerves, muscles and keeps blood circulating smoothly. Moreover, cucumber is useful for skin, bone and heart health.

Table 1: The quantitative amount of different element of 100 gm of cucumber

Nutrient	Amount
Energy	15 Kcal
Carbohydrates	3.63 g
Protein	0.65 g
Total Fat	0.11 g
Dietary Fiber	0.5 g
Vitamin A	105 IU
Vitamin C	2.8 mg
Vitamin K	16.4 µg
Sodium	2 mg
Potassium	147 mg
Calcium	16 mg
Iron	0.28 mg
Magnesium	13 mg
Phosphorus	24 mg
Zinc	0.20 mg

(Source: USDA National Nutrient data base)

2.5 Greenhouse technology for crop production

A greenhouse is a structure made of transparent materials (i.e. glass) for light penetration where plant can be grown in suitable climatic condition. Greenhouses are designed to improve crop growth and yield by providing a favorable growth environment for plant (Papadopoulos & Hao 2000). Production in greenhouse is an important technology for the region where outside climatic conditions do not support crop production due to high light intensity, low light period and very

low winter temperature. It is an established agricultural production system in mild winter climate (Castilla et al. 2008) specially northern region of the world.

According to Tognoni et al. (1997), mainly three types of greenhouse are used for crop production, as follows.

Low technology greenhouse

This greenhouse have very simple structure and mostly similar to open air cultivation. Normally, it is made of plastic covering and very often lack of heating system. The climatic condition of low technology greenhouse is poorly controlled and internal climate is dependent on external condition. The investment cost for this type of greenhouse is lower than 20-30 $/m^2 and only few plants (some vegetables and low value cut flowers) are grown in this kind of shelter (Pardossi et al. 2004).

Medium technology greenhouse

The investment cost of this kind of greenhouse ranges from 30-100 $/m^2 (Pardossi et al. 2004). This is made of metal structure and glass is used as covering material. The internal climatic condition is independent of outside and automatically controlled. Some advanced technologies such as hydroponics are used in this type of greenhouse. It is flexible, highly efficient and employed for out of season vegetables, high value cut flowers and ornamental plants (Pardossi et al. 2004).

High technology greenhouse

This type of greenhouse is highly sophisticated and mainly used for ornamentals and vegetables production in colder regions. This greenhouse technology is widely used in Northern Europe. The investment cost is quite higher and ranges from 100-200 $/m^2 (Pardossi et al. 2004). The structure is made of galvanized iron/aluminum and glass or plastic as covering material. The internal climatic condition is highly controlled including both air and root zone heating, ventilation system, humidity control, fertilization control, light conditioning and carbon dioxide enrichment which makes it unique. This greenhouse reduces the labor cost and maximize space use efficiency. This kinds of greenhouse provide high quality product to consumer.

2.6 Production of cucumber

As originated from tropical region, cucumber is regarded as a warm season (Sarhan & Ismael 2014) vegetable that can utilize high temperature, humidity, light intensity and with an continuous supply of water and nutrients for optimum production. It prefers well drained soil and does not like shade. Cucumber plants can grow fast and provide high yield in stable environmental and nutritional condition. The main stem, laterals, and tendrils also grow fast that need frequent pruning. To ensure maximum light interception, the plant should be kept vertical which allows sufficient air movement as well. In favorable environmental condition, cucumber plant can contain fruit in each leaf corner. To avoid excessive fruit load, the fruits need to be thinned as plants with excess fruit become exhausted and abort fruit. Moreover, cucumber plant is very sensitive to unfavorable condition and the productivity is affected by stress.

2.6.1 Climatic requirements for production of cucumber in greenhouse

2.6.1.1 Air and root temperature

The growth of cucumber is affected by different day and night temperature and daily mean temperature as well. Also, the optimum temperature for generative phase differ from vegetative phase (Challa 1995). The maximum growth of cucumber plant occur at 28-35 °C (Grimstad & Frimanslund 1993; Papadopoulos & Hao 2000) although it is also affected by other factors (plant age and light intensity). Fruit production requires high night temperature 19-20 °C and day temperature 20-22 °C. At the seedling stage, temperature plays important role. Seedlings require 9-16 days to emerge at 15 °C whereas, at 21 °C, it takes only 5-6 days. Overall, the cucumber plant is very sensitive to cold temperature and low temperature during growing season may cause bitter fruit. The optimum temperatures for growth of cucumbers are at night, about 18 °C, and during the day, about 28 °C accompanied by high light intensity. Cucumber was reported to grow and produce under quite different thermal regimes (Van de Vooren et al. 1978) and its photosynthesis can be balanced and maintained under wide range of climatic condition (Challa & Brouwer 1985). In addition, root temperature also affects the growth of cucumber plants and increase of soil temperature up to 30 °C increased the growth of cucumber plant significantly (Gosselin & Trudel 1985). Although, air temperature is mainly responsible for growth and

production of cucumber, a minimum root temperature of 19 °C is required, but 22-23 °C is preferable.

2.6.1.2 Light

Cucumber is a light demanding crop and can utilize high light with high temperature. Light is specially required for cucumber fruit for its size and color. The fruits of cucumber grown under low light become lighter in color during harvesting which can turn yellow easily during shelf life (Vonk Noordegraaf 1995). Moreover, Low light strongly decrease fresh and dry weight of cucumber fruit (Marcelis 1993). Also, by reducing photosynthesis, low light can reduce the carbohydrate level which affect the source-sink relation, limits the productivity of cucumber and make aborted fruit. According to Marcelis (1993), the percentage of dry-matter of both the fruits and the vegetative plant parts of cucumber increased with increasing irradiance.

2.6.1.3 Air humidity

The high relative humidity can often enhance the vegetative growth of cucumber but the symptoms of calcium deficiency are also observed under such condition (Bakker 1987; Bakker et al. 1987). Cucumber prefers a relative humidity of 75-80 % relative humidity for growth. The color of cucumber fruit reduces during storage when produced under high 24 hour average humidity condition (Bakker 1987; Fricke & Krug 1997). For optimum cucumber fruit production and quality, Bakker et al. (1987) recommended to keep the relative humidity higher at day time than that of night time because of fungal pathogen incidence that reduce the fruit quality.

2.6.1.4 Carbon dioxide

For cucumber production in greenhouse, the supplemental supply of carbon dioxide at a concentration of 800-1000 ppm has been economic with high temperature and light intensity. The yield of cucumber increase with increasing the period of carbon dioxide enrichment although it doesn't affect the fruit quality (Peet & Willits 1987). Liquid carbon dioxide is always recommended to use in greenhouse crop production as it is economically feasible and pure whereas other sources cause plant injury by gaseous pollutants such as ethylene (Mortensen 1987).

2.7 Effects of different climatic parameters on greenhouse production

Photosynthesis is a major plant functions that are the basics for plant growth and development. Both the photosynthesis and growth of plants are affected by different climatic parameters. In greenhouse, it is vital to execute all the climatic parameters effectively to achieve good yield. The temperature is the most prominent environmental parameters that affect the physical and chemical properties of plants and influence crop at both cellular and plant level (Gruda 2005). It influences vegetative growth, flower initiation, fruit growth and fruit quality (Papadopoulos & Hao 2000). The average day and night temperature for plants grown in greenhouses are generally 18-27 °C and 15-22 °C respectively (Baytorun et al. 1999). Also, the growth of greenhouse plants depend on the diurnal temperature variation as well as daily average temperature. Few studies (De Koning 1988; Rijsdijk & Vogelezang 2000) showed that plant growth in protected environment depends more on the daily mean temperature whereas other studies (Erwin & Heins 1988; Erwin & Heins 1993; Hori & Shishido 1978; Mitchell et al. 1991) showed the importance of variation in diurnal temperature on growth of the plant. Moreover, some studies (Carow & Zimmer 1977; Shanks & Osnos 1980) showed the potential of splitting night period method on floricultural crops in greenhouse. Light is another vital prerequisite for plants growth as the photosynthesis and dry matter production depend on light. Normally, the sun is used as free light energy source for greenhouses (Bot 2003; Hanan 1997) but in winter especially in northern region of the world the photoperiod becomes very low and makes the light a very limiting factor for growth. So, supplemental lighting is required to increase daily light integral (Runkle 2007) in that region. Also, supplementary lighting can increase yield during low light periods, but is generally considered commercially unprofitable (Papadopoulos & Pararajasingham 1997). However, the quality of fruits and vegetables in greenhouse, mainly depend on light quality. It improves the root growth and has great effect on earliness, quality and quantity of yield (Brazaitytė et al. 2009; Lin & Jolliffe 1996). The enrichment of carbon dioxide is an essential requirement for greenhouse crop production as carbon dioxide from outside environment is not available. Carbon dioxide enrichment can increase the dry weight, plant height, number of leaves, and lateral branching of greenhouse vegetable plants (Mortensen 1987). Extra carbon dioxide can increase the net photosynthesis of plants by decreasing oxygen inhibition of photosynthesis. The enrichment of CO_2 is effective at high irradiance levels (Mortensen & Gislerød 2012) and increase the light use efficiency of greenhouse plants (Wilson et al. 1992).

Humidity is also considered as another important climatic parameters that influence the growth, development and quality of greenhouse crops by affecting the water status of the plants (Bakker 1983; Gruda 2005). It has even been more important due to energy saving strategy for greenhouse crop production (Bakker 1987). Generally, high relative humidity favors growth although, it increases the risk of water condensing on the plants and the development of different diseases.

2.8 Photo assimilates of cucumber and its translocation

Many species (Arabidopsis, soybean, maize, sugar beet) translocate their assimilated CO_2 in the form of sucrose whereas, cucumber translocate stachyose and sucrose as well (Hu et al. 2009). The translocation of photo assimilates greatly depend on air temperature (day/night) and light intensity (Lundmark et al. 2006). Translocation of photo assimilates is strongly inhibited at low temperature (10 °C). Different studies (Miao et al. 2007; Robbins & Pharr 1987; Toki et al. 1978) reported the influence of air temperature and light intensity on photo assimilate translocation. The growth and maintenance of non-photosynthetic plant tissues (sinks) depends on the substrate (carbohydrate) that is transferred from photosynthesizing leaves (source) (Ainsworth & Bush 2011). Also, stachyose is considered to be the predominant transport sugar of the plants in this family (Hendrix 1982; Webb & Gorham 1964). The photo assimilates of plants are mainly translocated from source organs to sink organs through phloem (Ainsworth & Bush 2011). Almost 80% of the photo assimilated CO_2 in a mature leaf is transferred through phloem (Kalt-Torres et al. 1987). For plants that transport stachyose such as cucumber, use symplastic phloem loading mechanism for carbohydrate translocation. In this mechanism, sucrose moves through plasmodesmata into the companion cells and used as substrate for raffinose and stachyose synthesis (Ainsworth & Bush 2011). Hence, the cucumber plant transports mainly the stachyose from leaves but no stachyose is found in the fruits. After moving from leaf to peduncle, the stacyose is metabolized to sucrose and enters the fruit which can be further metabolized to glucose and fructose (Gross & Pharr 1982; Pharr et al. 1977). Different enzymes play role in stacyose metabolism and a mechanism proposed by Gross and Pharr (1982), describe that galactose is hydrolyzed from stachyose and can be converted to sucrose in peduncles of cucumber.
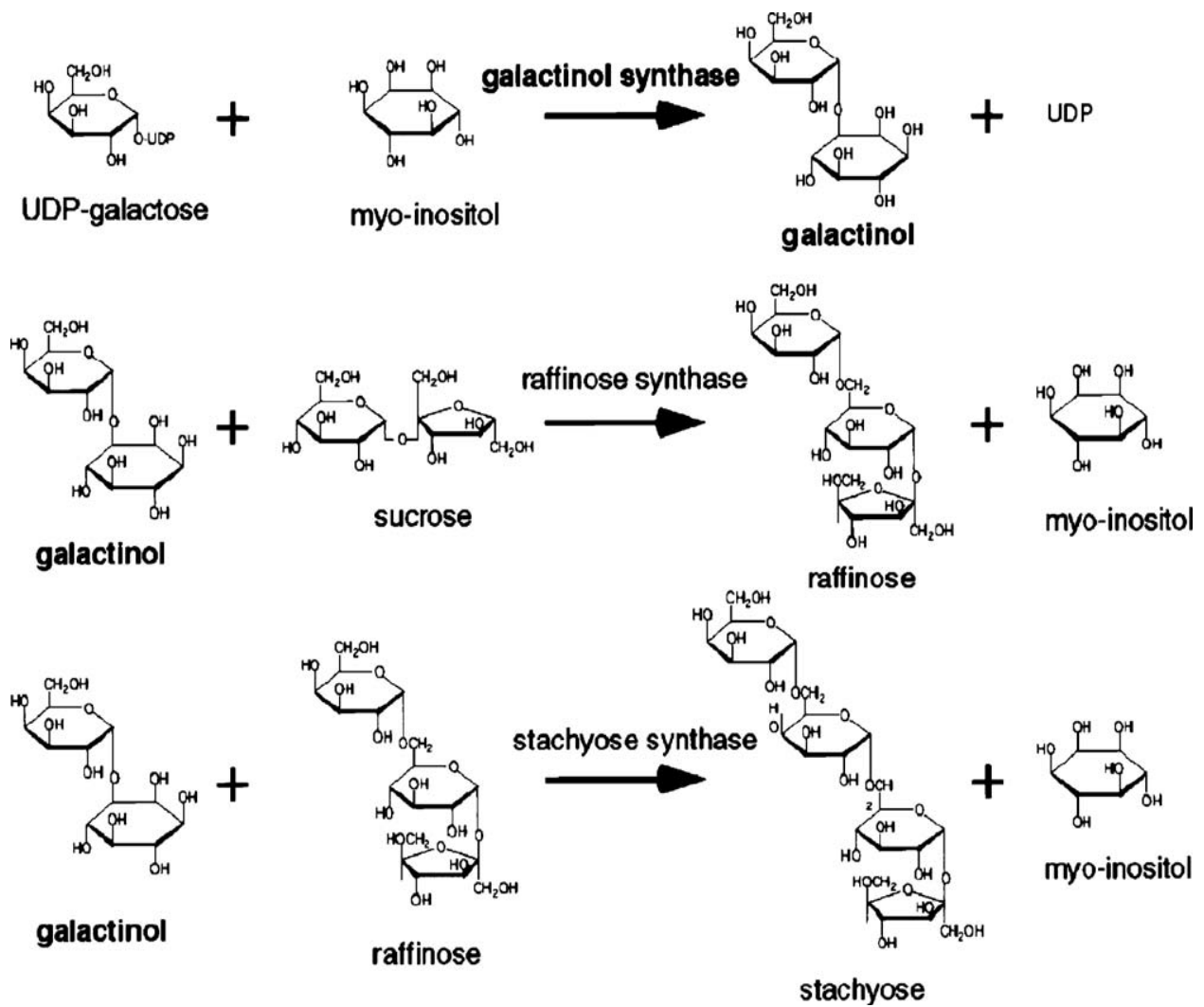
Fig 1. Stachyose biosynthetic pathway in plants (Taji et al. 2002).

3. Materials and methods

3.1 Plant material and seedling growth

Seedless cucumber cv. Odeon were propagated at Centre for plant research in controlled environment (SKP), Norwegian University of Life Sciences, Ås, Norway (59° 40' 4" N; 10° 46' 2" E) from 15th June, 2015 to 10th January, 2016. To ensure uniform seedling production, seeds of the cucumber cv. Odeon were sown in Petri dishes of 9 cm in diameter, containing water agar (10 g/l) and sealed with parafilm. Petri dishes were then incubated in greenhouse with supplemental lighting provided by high pressure sodium (HPS) (Lucalox LU400/XO/T/40, GE lighting, Budapest, Hungary) and high pressure mercury (Powerstar HQI-BT 400W/D; Osram GmbH) lamps of similar type described previously (Suthaparan et al. 2012) with 2:1 ratio. Supplemental lighting ensured daily light cycles (DLC) of 16 h with minimum photosynthetic photon flux (PPF) (400-700 nm) of 100 ± 20 µmol m^{-2} s^{-1} when outside solar irradiance fell below 200 Wm^{-2}.

Fig 2. A) Germination of cucumber seeds in Petri dishes contained water agar, B) Seedlings with cotyledon which were transferred to rock wool after germination and C) Seedlings with 1st fully developed leaf transferred to growth chambers.

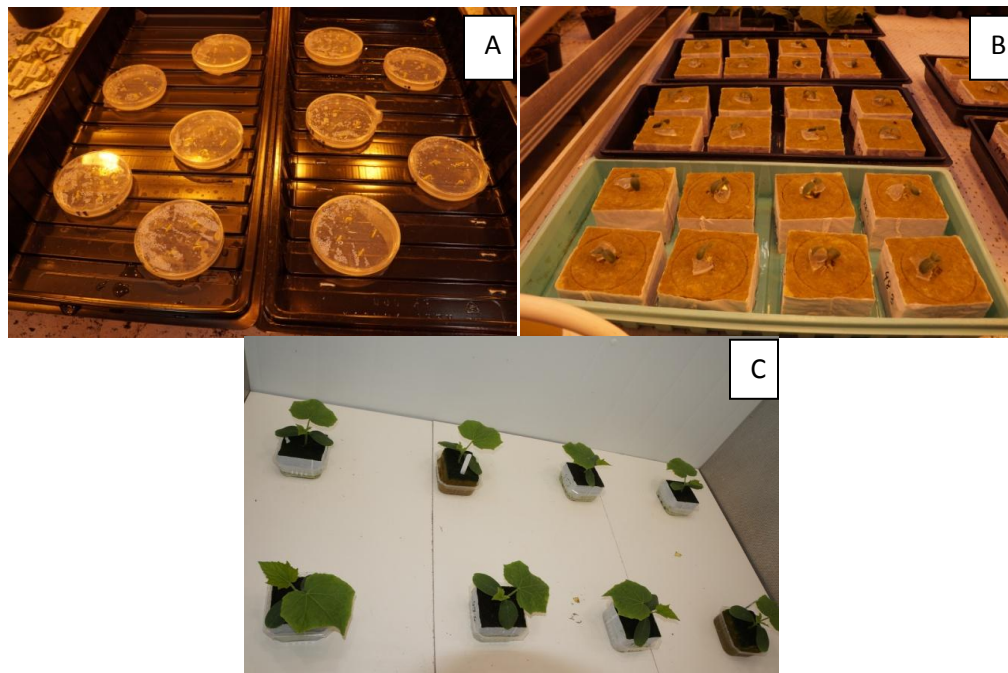Temperature and relative humidity in the greenhouse were maintained at 25 ± 5 °C and 70 ± 20 %, respectively. Three to four days after sown, germinated seedlings were transferred to rock wool block (SAINT-COBAIN CULTILENE BV, Tilburg, Netherlands) of known weight and maintained in same greenhouse condition mentioned above until they develop first true leaf. Plants were irrigated with a complete nutrient solution prepared by mixing Kristalon Indigo and YaraLiva Calcinite (1:1 [vol/vol]) (Yara International ASA; Oslo, Norway) for final electrical conductivity of 2 mS/cm. At the stage of fully developed first leaf and second leaf started to develop, the seedlings were transferred to growth chamber with different temperature and light treatments.

3.2 Growth chamber experiment

Uniformly developed seedlings were selected for the completely controlled climate chamber experiment; and accommodated with eight seedlings per chamber. Daily light cycles of 16 h was provided with high pressure mercury (Powerstar HQI-BT 400W/D; Osram GmbH) lamps. The level of CO_2 inside the climate chamber was maintained at 400 ± 30 ppm. The seedlings were irrigated with complete nutrient solution as described above for 14 days of experiment period.

3.3 Experimental design

3.3.1 Experiment 1

Experiment was conducted to examine whether the temperature mediated effect on photosynthesis and growth of cucumber is due to night temperature or daily mean temperature. Four growth chambers were used to raise seedling with four different temperature treatments along with two different daily mean temperature. The light level was kept constant for each chamber that was 150 ± 10 µmol m^{-2} s^{-1} for 16 hours. The treatments were set as i) 25/14 °C (DMT 21.33 °C), ii) 25/22 °C (DMT 24 °C), iii) 21.3/21.3 °C (DMT 21.33 °C), and iv) 24/24 °C (DMT 24 °C) D/N with values achieved as explained in Table 2. The experiment lasted for 14 days.

Table 2. Daily light cycles, day/night temperature, relative humidity and daily mean temperature measured for experiment 1. Values are mean and standard deviation of data logged 15 min interval during experiment.

Treatment	DLC	Relative humidity (%) (D/N)	Air temperature (°C) (D/N)	Daily mean temperature (DMT) (°C)
1	16 h	75/90	25.10±0.09/14.36±0.18	21.52
2	16 h	75/90	25.14±0.03/22.17±0.04	24.10
3	16 h	75/90	21.9±0.06/21.5±0.04	21.70
4	16 h	75/90	24.15±0.04/23.75±0.04	24.05

3.3.2 Experiment 2

Experiment was conducted to examine whether the early night temperature has any effect on photosynthesis and growth of cucumber. For that, the 8 h night period was split into two 4 h periods. While daytime temperature and daily mean temperature was maintained at 25 °C and 22.66 °C respectively, in all treatments, first and the second half of the nighttime temperature was set as i) 25/22/14 °C, ii) 25/14/22 °C, and iii) 25/18/18 °C for the thermo periods of 16/4/4 h with achieved values as mentioned in Table 3. The light level in all treatments was 150±10 μmol m^{-2} s^{-1}.

Table 3. Daily light cycles, day/night temperature, relative humidity and daily mean temperature measured during experiment 2. Values are mean and standard deviation of data logged 15 min interval during experiment.

Treatment	DLC	Relative humidity (%) (D/N)	Thermo periodicity	Air temperature (°C) (D/N)	Daily mean temperature (DMT) (°C)
1	16 h	75/90	16/4/4	25.68±0.68/22.21±0.09/14.7±0.12	23.27
2	16 h	75/90	16/4/4	25.65±0.24/14.81±0.95/21.32±0.76	23.12
3	16 h	75/90	16/4/4	25.30±0.0.20/18.25±0.10/18.4±0.20	23.01

3.4.3 Experiment 3

Experiment was conducted to examine the effects of daytime photosynthetic active radiation (PAR) level and its interaction with nighttime temperature on photosynthesis and growth of cucumber. For that, four growth chambers were set with the daytime (16 h) temperature of 25 °C whereas night temperature differed along with different light. In two treatments, night temperature was kept 14 °C with two different light (200 and 400 $\mu mol\ m^{-2}\ s^{-1}$) and another two treatments, night temperature was kept 22 °C with two different light (200 and 400 $\mu mol\ m^{-2}\ s^{-1}$). The daily mean temperature for first two treatments was 21.33 °C and for later treatments 24 °C with measured values as mentioned in Table 4. To increase the light intensity to 400 $\mu mol\ m^{-2}\ s^{-1}$ raised the leaf temperature to 2-3 °C, so the day temperature in that treatment was reduced to 2-3 °C.

Table 4. Daily light cycles, day/night temperature, relative humidity and daily mean temperature and irradiance measured during experiment 3. Values are mean and standard deviation of data logged 15 min interval during experiment.

Treatment	DLC	Relative humidity (%) D/N	Air Temperature (°C) D/N	Daily mean temperature (DMT) (°C)	Irradiance level ($\mu mol\ m^{-2}\ s^{-1}$)
1	16 h	75/90	24.52±0.17/14.12±0.25	21.05	200
2	16 h	75/90	24.7±0.18/14.17±0.25	21.19	400
3	16 h	75/90	25.06±0.06/22.54±0.03	24.22	200
4	16 h	75/90	25.02±0.05/22.29±0.12	24.11	400

3.4 Leaf gas exchange measurement

Leaf gas exchange measurement was carried out by using a Combined Infrared Gas Analysis System (CIRAS-1; Portable Photosynthesis System, Hitchin, Herts., UK) coupled with a 2.5 cm^2 PLC (B) broad leaf cuvette (PP system, Hitchin, UK). In each experiment, leaf gas exchange measurement was performed on 1st and 3rd fully developed leaf for 4 plants to examine the impact of acclimation in addition to treatment effect. Environmental conditions such as air temperature, CO_2 and RH were set as similar to treatment conditions in each growth chamber. Growth light used in each experiment were used as PAR source. Measurements were done 1 h

after lights on for daytime net photosynthesis, while measurements were done 1 h after lights off for dark respiration. Stabilized values within 8-10 minutes were recorded. In addition to daytime net photosynthesis and dark respiration, light responses of cucumber plants treated with different PAR level in combination with night temperatures were also measured. For that, gas exchange of third fully developed leaf was recorded with environmental conditions similar to the treatment conditions, except PAR level and its source. PAR levels of 0, 200, 400, 600, 800 and 1000 µmol $m^{-2} s^{-1}$ were provided with halogen lamp supplied by CIRAS manufacturers.

3.5 Leaf chlorophyll content measurement

At the end of each experiment, relative chlorophyll content for the top 5 unfolded leaves were measured by using hand held chlorophyll meter (SPAD-502, Minolta Camera Co., Japan). Mean value was used as representative value for plant.

3.6 Measurement of growth parameters

The plant height (cm), leaf area (cm^2), number of leaves > 4 cm in length, fresh and dry weight of stem, leaf and root were measured at the end of the experiment. Total leaf area was measured by a leaf area meter (LI3100 Licor, Lincoln, Nebraska). Samples were dried at 70°C for 10 days before the dry weight measurements.

3.7 Measurement of photo assimilate translocation

3.7.1 Extraction of carbohydrate

The amount of carbohydrates (stachyose, raffinose, glucose, sucrose and fructose) in different parts (leaf, stem, fruit) of cucumber plants were analyzed by following Pharr et al. (1985) with little modification. After finishing the 3rd growth experiment, they were repeated to collect samples for carbohydrate analysis. The samples were collected from leaf, petiole, stem and small fruit within one hour after starting night period. Around 250 mg fresh weight of each plant parts were taken. The fresh plant parts were then ground. The carbohydrates were extracted through heating the samples in 1.5 ml of 80% ethanol with two changes of ethanol at 70°C for 30 minutes for each change. The heating was carried out into an ultrasonic bath. In each change, the extracts were centrifuged at 15000 rpm/min for 3 minutes. The supernatant from each changes were added together. The ethanol was removed from the supernatant at 60 °C by using a vacuum desiccator (Eppendorf AG 22331, 8 Hamburg, Germany). It is essential to remove the ethanol

completely because it is eluted on the HPLC and it is detected by the RID detector. Therefore, it can interfere with other carbohydrate peaks. After that, added 1 ml of water with the extract and heated at 60 °C for 30 minutes. The extract was then centrifuged at 15000 rpm/min for 3 minutes and the supernatant was collected. This supernatant was then filtered through a 0.45 μm GHP membrane filter (Millipore) before chromatography.

3.7.2 Separation of carbohydrates

After extracting the samples, different carbohydrates (stachyose, raffinose, glucose, fructose and sucrose) were separated through HPLC (High Performance Liquid Chromatography). During this experiment, Agilent 1200 series of HPLC (Agilent Technologies, Waldbronn, Germany) was used to analyze cucumber samples. Carbohydrates were separated on the basis of their differential adsorption characteristics and it was analyzed by passing the solution through a column. Here, the column Agilent Hi-Plex Ca USP L19, 4.0 * 250 mm was used and separation was achieved through Refractive Index Detector. For the mobile phase, water was used as solvent and the flow rate was 0.3 ml/min and the temperature of the column was 80 °C. Ten micro liters of sample was injected into HPLC. Eluted carbohydrates were identified and quantified from retention time and area of sugar standard.
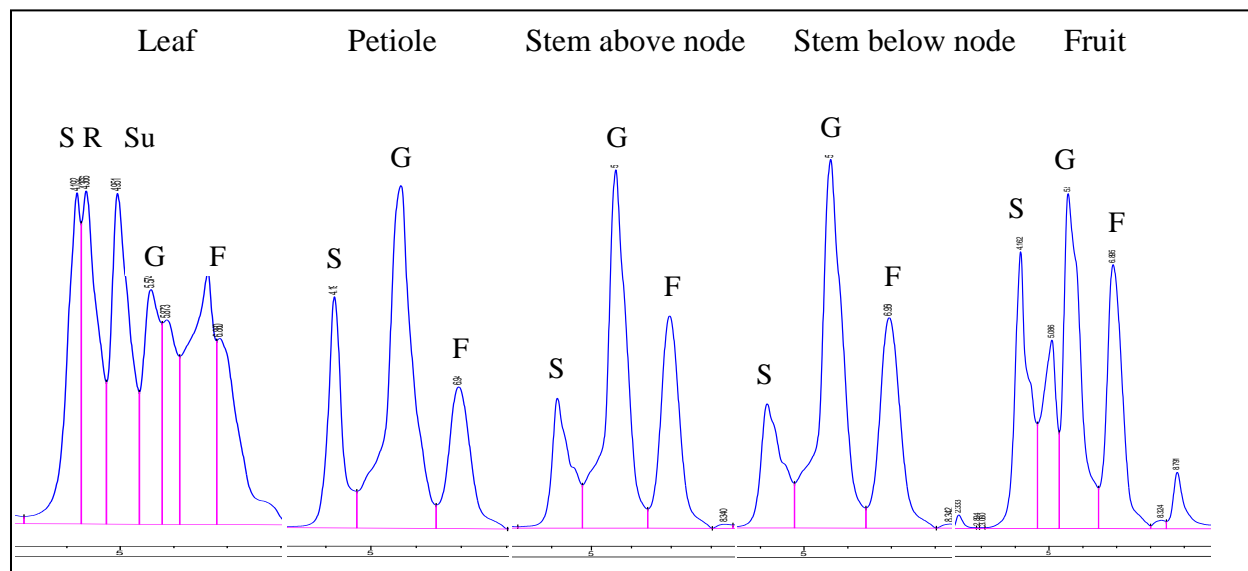

Fig 3. The HPLC chromatogram trace showing the soluble carbohydrates peak in different parts of cucumber plant. S= stachyose, R= raffinose, Su= sucrose, G = glucose, F= fructose

3.8 Statistical analysis

All the statistical analyses were run in Minitab 17 (Minitab Inc., State College, PA, USA). Analysis of variances were carried out using general linear model (GLM) to observe the effect of treatments on different parameters in each experiment. In growth analysis, temperature, light, experiment repeat, replicate and their interaction were used as factors in model. Experiment repeat and replicate were used as random factor and the parameters were plant height, leaf area, SLA, total dry weight, leaf number and chlorophyll content. In carbohydrate analysis, the model consisted temperature, light and their interaction as factors and the parameters were stachyose, raffinose, sucrose, glucose, fructose and total carbohydrate. Different levels of each significant factors were compared by Tukey's pair wise mean comparisons (P = 0.05). Means ± 1 standard error are given in text and figures.

4. Results

4.1 Experiment 1

4.1.1 Effects of low night temperature and daily mean temperature on gas exchange measurement of cucumber
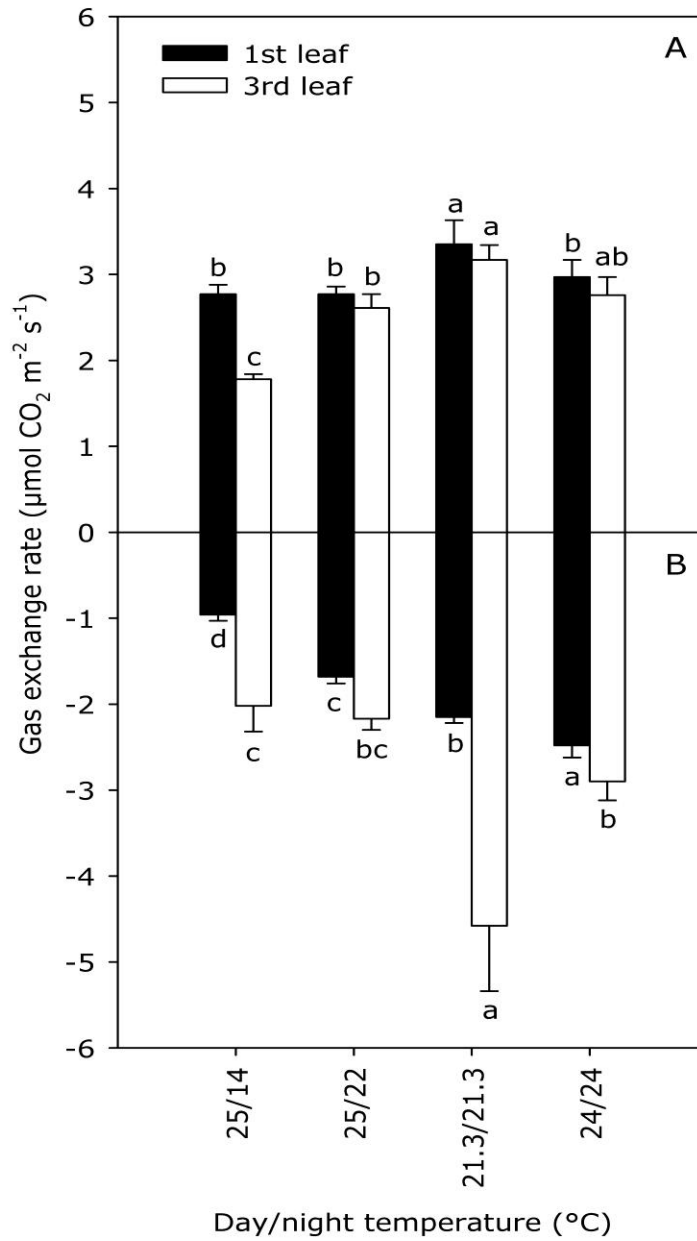
Fig 4. Effects of low night temperature and daily mean temperature on gas exchange measurement A) net photosynthesis at light period B) dark respiration at night period for both 1st and 3rd fully developed leaf of cucumber cv. Odeon grown for 14 days at four temperature treatments (25/14, 25/22, 21.3/21.3 and 24/24 °C, D/N temperature). Photoperiod 16 h and 150 µmol m^{-2} s^{-1}, CO$_2$ 400 ppm. Error bars indicate 1SE. Data are means of 8 replicate plants.

Temperature had significant effects on gas exchange measurement (P<0.001) of both 1st and 3rd fully developed leaves. For 1st leaf, the highest net photosynthesis (NP) and dark respiration (DR) were found for the plants grown at 21.3/21.3 °C (3.35 µmol CO$_2$ m^{-2} s^{-1}) and 24/24 °C (2.48 µmol CO$_2$ m^{-2} s^{-1}) (D/N) respectively. NP values were almost same for all other treatments whereas, DR decreased with decreasing night temperature (Fig 4 A-B). For 3rd leaf, both the NP and DR were highest for the plants grown at 21.3/21.3 °C (D/N) (3.17 µmol CO$_2$ m^{-2} s^{-1} and - 4.58 µmol CO$_2$ m^{-2} s^{-1} respectively) and lowest for plants grown at lowest night temperature (14 °C). Overall. Plants grown at same D/N temperature had higher photosynthesis than that of fluctuating D/N temperature (Fig 4 A-B).

4.1.2 Effects of low night temperature and daily mean temperature on growth of cucumber plants

In this experiment, Fig 5 shows highly significant response to all growth parameters (plant height: P<0.001; r^2=0.87, total leaf area: P<0.001; r^2=0.79, specific leaf area: P<0.001; r^2=0.78, leaf number: P<0.001; r^2=0.79, total dry weight: P<0.001; r^2=0.32) except leaf chlorophyll content (P=0.09; r^2=0.65). It is evident from Fig 5 that all the growth parameters showed changes with changing daily mean temperature but not with changing night temperature. Maximum height of cucumber plant (79.34 ± 1.7 cm) was found when grown at 24/24 °C D/N (DMT=24 °C) and almost same plant height (76.50 ± 1.9 cm) was observed in another treatment with same DMT (24 °) but lower night temperature (22 °C). On the other side, decreasing DMT from 24 °C to 21.3 °C reduced plant height significantly (Fig 5A) whereas at same DMT (21.3 °C), height did not decrease much although the night temperature was reduced to 14 °C from 21.3 °C (49.6 ± 1.9 cm vs. 55.4 ± 1.2 cm for 25/14 °C vs. 21.3/21.3 °C respectively). Also, number of leaves changes with changing the daily mean temperature not the diurnal variation of temperature (Fig 5D).
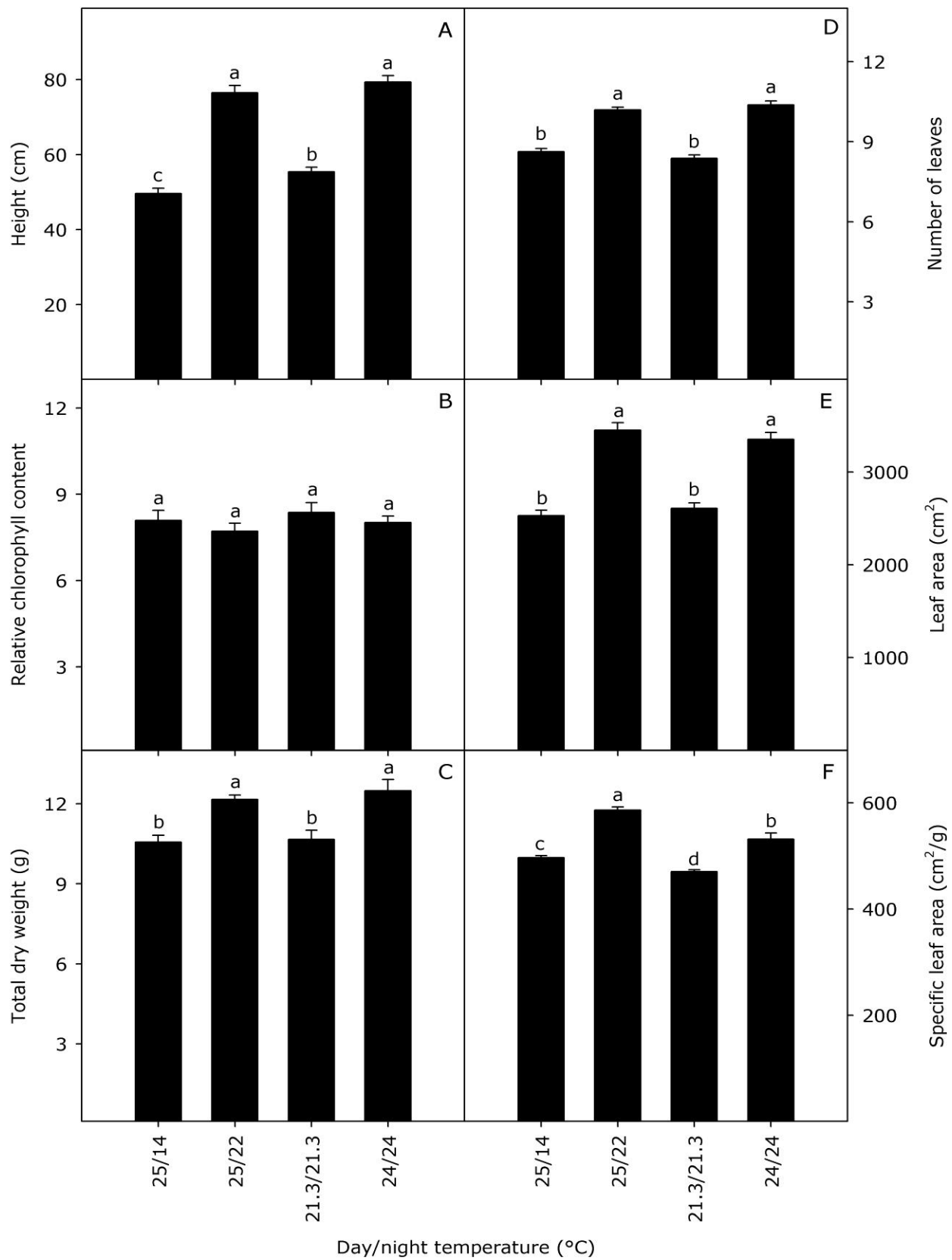
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Fig 5. Effects of low night temperature and daily mean temperature on growth parameters, plant height (cm), relative chlorophyll content, total dry weight (g), leaf number(>5), total leaf area (cm^2) and specific leaf area (cm^2/g) of cucumber cv. Odeon grown for 14 days at four temperature treatments (25/14, 25/22, 21.3/21.3 and 24/24 °C, D/N temperature). Photoperiod 16 h and 150 μmol m^{-2} s^{-1}, CO$_2$ 400 ppm. Error bars indicate 1SE. Data are means of 16 replicate plants.

Significantly, higher average leaf number (10.37 and 10.12) was observed in the plant grown at higher daily mean temperature (24°C) than that of lower daily mean temperature (8.6 and 8.3 for DMT 21.3°C). Likewise, total leaf area of cucumber plants responded to daily average temperature and not to the variation in D/N temperature. The temperature treatments (25/22, 24/24 °C) with higher daily average temperature (24 °C) had higher total leaf area (3450.6 ± 79.2 cm^2 and 3350.5 ± 75.8 cm^2 respectively) than that of the treatments with lower daily average temperature (2529.3 ± 59.1 cm^2, 2607.3 ± 59.6 cm^2; 25/14, 21.3/21.3 respectively) (Fig 5E). Moreover, the specific leaf area did not vary with daily average temperature but affected by diurnal temperature fluctuations. Highest specific leaf area of cucumber plants (586.36 ± 6.09 cm^2/g) was recorded when grown at 25/22 °C whereas lowest specific leaf area (470.5 ±3.4 cm^2/g) was found at 21.3/21.3 °C although plants at higher daily mean temperature had higher specific leaf area (Fig 5F).Similarly, total dry weight of cucumber plants was higher at high DMT than low DMT (Fig 5C). On the other hand, chlorophyll content of the leaf was almost same for all the temperature treatments (Fig 5B).

4.2 Experiment 2

4.2.1 Effects of different split night temperatures on gas exchange measurement of cucumber

Net photosynthesis (NP) of 1st leaf is slightly (P=0.04) and 3rd leaf is strongly (P<0.001) affected by different split night temperature whereas dark respiration of both leaves showed significant effect with changing split night temperature. Slightly higher net photosynthesis (3.4 μmol CO$_2$ m^{-2} s^{-1}) was found in 1st leaf when night temperature was lower in first half of night whereas 3rd leaf showed almost same net photosynthesis in both split night temperature (Fig 6A). Stable night temperature (25/18 °C) showed lowest net photosynthesis for both leaves.

Moreover, dark respiration of both leaves increased at high night period temperature and decreased at low night period temperature (Fig 6B).
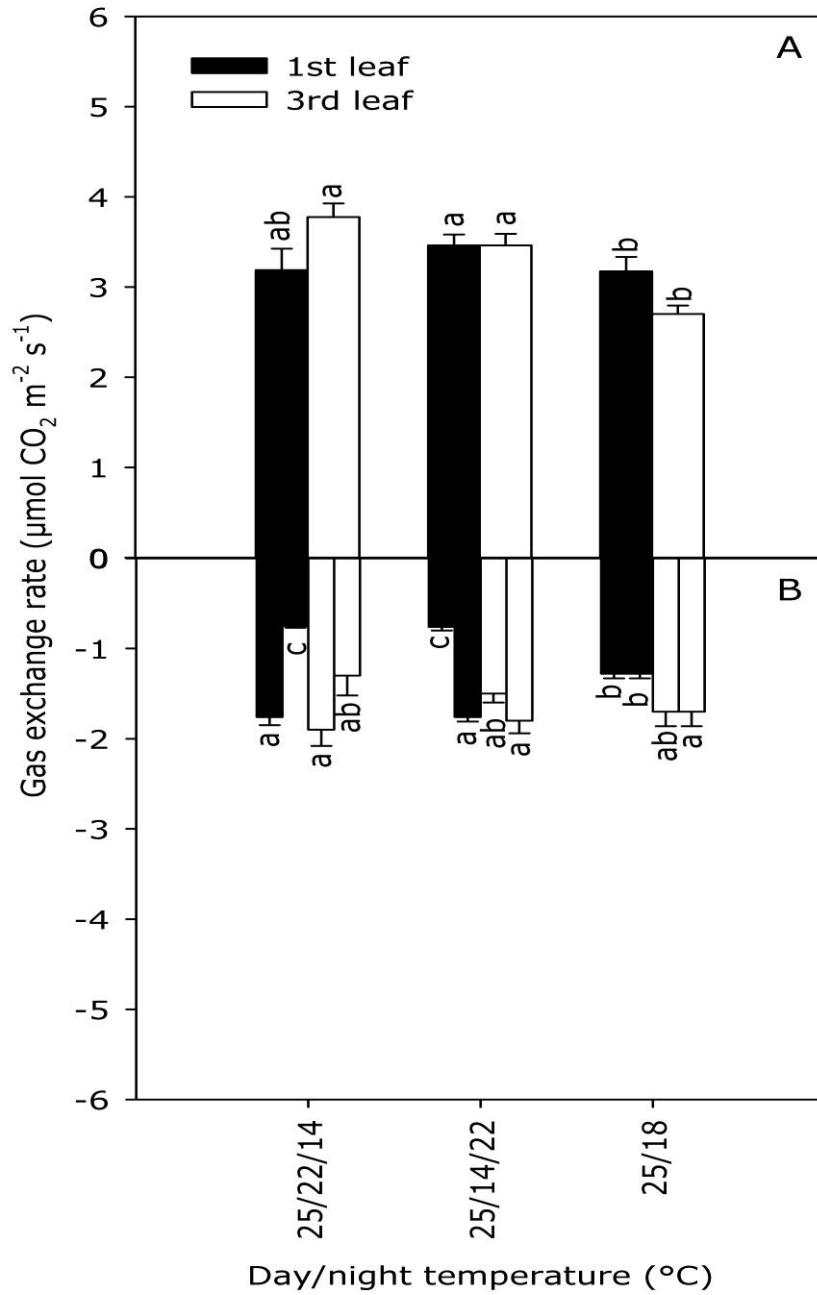
Fig 6. Effects of different split night temperatures on gas exchange measurement A) net photosynthesis at light period B) dark respiration at night period for both 1st and 3rd fully developed leaf of cucumber cv. Odeon grown for 14 days. Day temperature for 16 h, night temperature divided into 4+4 h and the treatments were (25/22/14, 25/14/22, 25/18/18 °C, D/N temperature). Photoperiod 16 h and 150 µmol m^{-2} s^{-1}, CO_2 400 ppm. Error bars indicate 1SE. Data are means of 8 replicate plants.

4.2.2 Effects of different split night temperatures on growth of cucumber plants

Partitioning the temperature at different period of night had significant impact on plant height (P<0.001; r^2=0.83), number of leaves (P<0.05; r^2=0.54) and chlorophyll content (P<0.001; r^2=0.57) whereas total leaf area (P=0.147; r^2=0.61), specific leaf area (P=0.120; r^2=0.52), and total dry weight (P=0.173; r^2=0.74) were not affected significantly by temperature treatments (Fig 7). At same daily mean temperature, significantly (P<0.001) higher plant height (73.38 ± 2.04 cm) was observed when cucumber plants were grown at lower night temperature (14 °C) at first half and higher night temperature (22 °C) at second half than other treatments (Fig 7A). Moreover, leaf numbers were found comparatively higher (9.12 ± 0.15) in plants grown at lower night temperature at first half. Total leaf area, specific leaf area and total dry weight of cucumber plants were almost same at all the experimental temperature (Fig 7 C, E, and F). Also, the chlorophyll content of the leaf was almost similar at both split night temperature (11.85 ± 0.3 and 11.78 ± 0.4 respectively). Lower chlorophyll content was found in leaf grown at stable night temperature (Fig 7B).
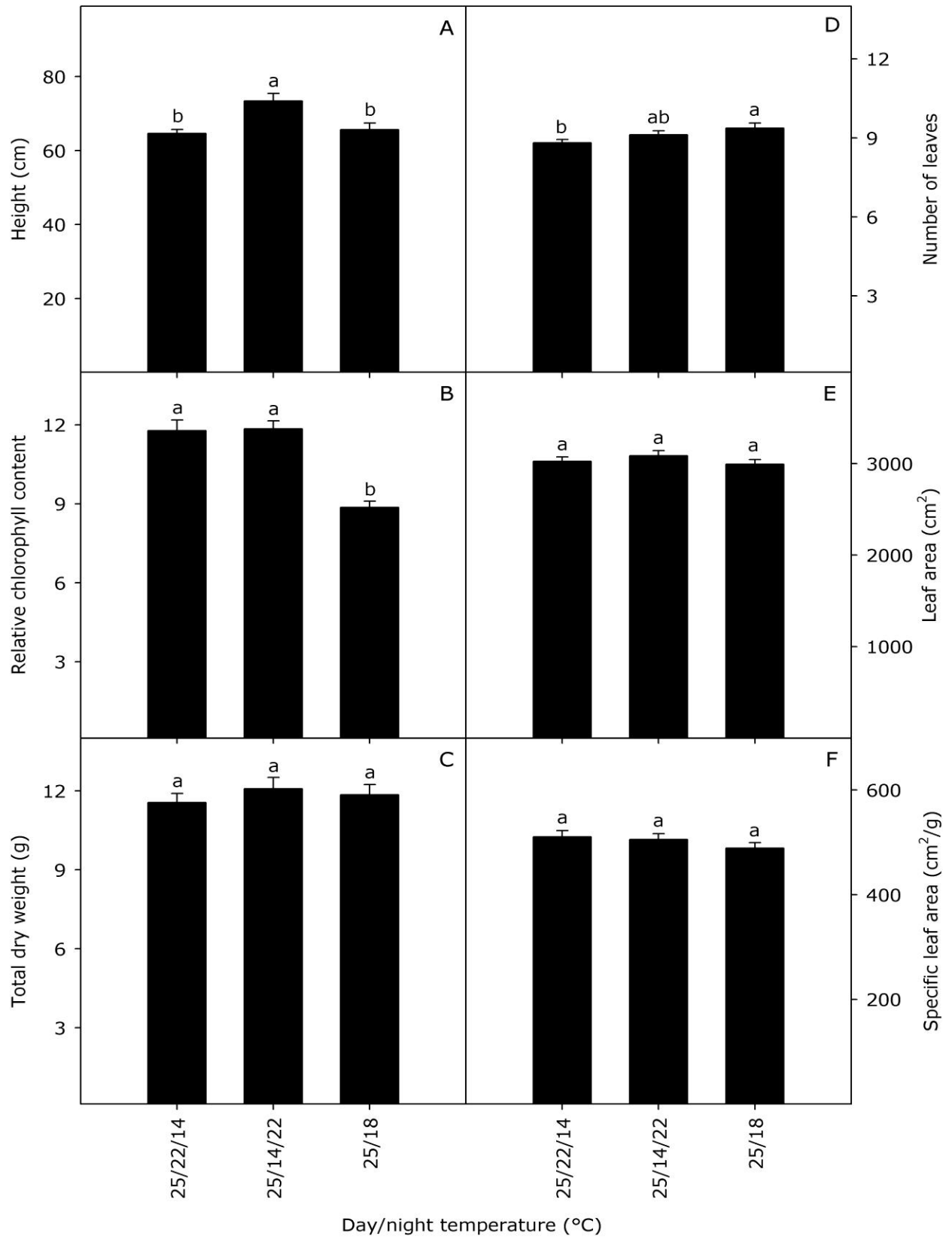
Fig 7. Effects of different split night temperatures on growth parameters plant height (cm), relative chlorophyll content, total dry weight (g), leaf number(>5), total leaf area (cm^2) and specific leaf area (cm^2/g) of cucumber cv. Odeon grown for 14 days. Day temperature for 16 h, night temperature divided into 4+4 h and the treatments were (25/22/14, 25/14/22, 25/18/18 °C, D/N temperature). Photoperiod 16 h and 150 µmol m^{-2} s^{-1}, CO_2 400 ppm. Error bars indicate 1SE. Data are means of 16 replicate plants.

4.3 Experiment 3

4.3.1 Effects of different combinations of night temperature and light intensity on gas exchange measurement of cucumber

The gas exchange measurement of first leaf was strongly (NP, P<0.001; DR, P<0.005) affected by light conditions whereas, temperature did not show any significant effect on both net photosynthesis (NP) and dark respiration (DR). Both the NP and DR increased with increasing light level for both temperature (25/14 °C and 25/22 °C) treatments and became maximum (NP=7.71 µmol CO_2 m^{-2} s^{-1}, DR= -2.4 µmol CO_2 m^{-2} s^{-1}) when cucumber plants grown at 25/14 °C and 400 µmol photon m^{-2} s^{-1} treatment. Both the NP and DR reduced to almost half when the light level was reduced to 200 µmol m^{-2} s^{-1} (Fig 8A-B). Again, in case of 3rd leaf, both the temperature and light significantly affected the gas exchange measurement of cucumber (P<0.05). Both net photosynthesis and dark respiration increased similarly with increasing light level for both temperature treatments and highest values (NP=10.03 µmol CO_2 m^{-2} s^{-1}, DR= -2.3 µmol CO_2 m^{-2} s^{-1}) were found in plants grown at 25/22 °C and 400 µmol photon m^{-2} s^{-1} treatment (Fig 8A-B).
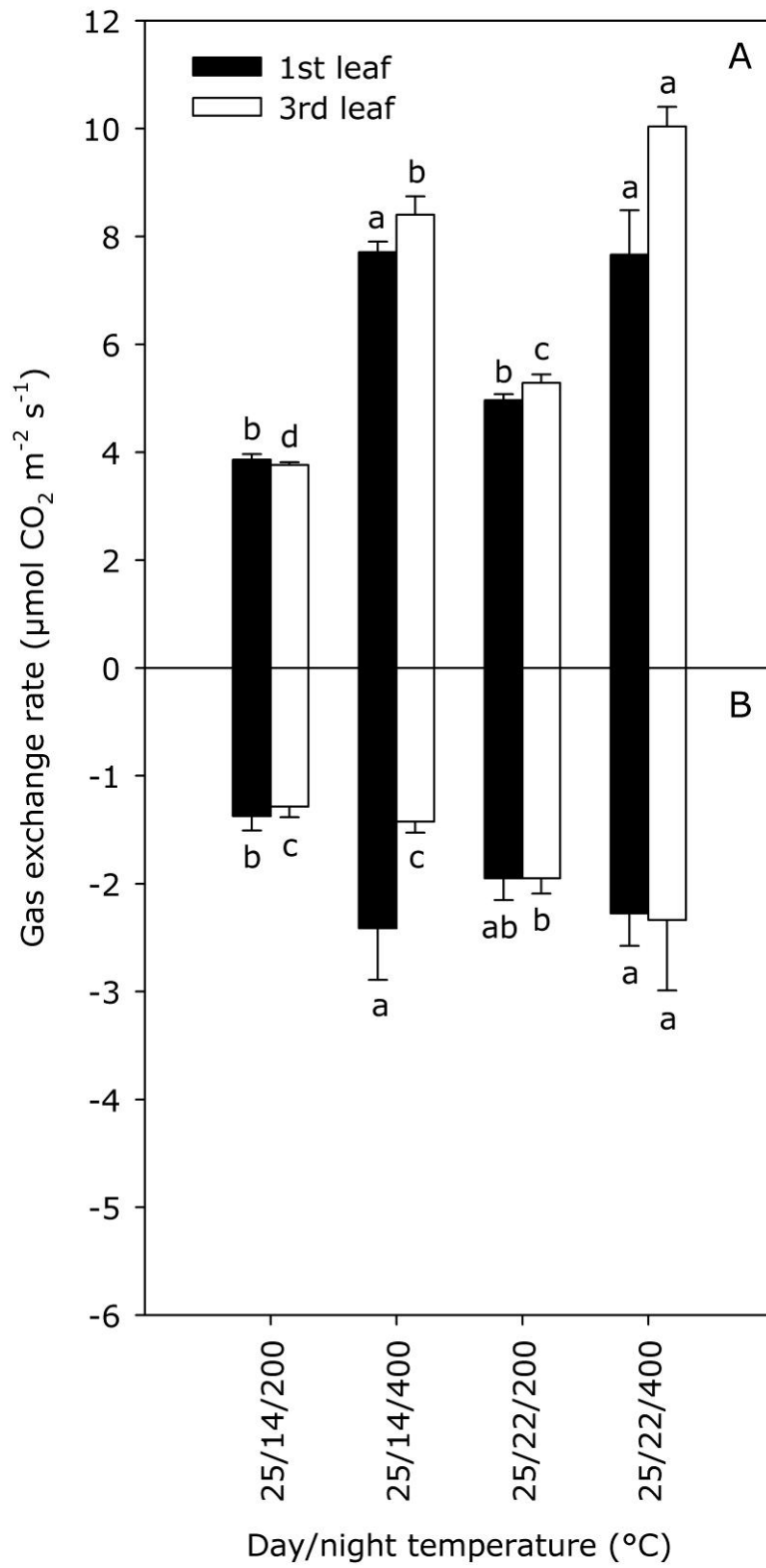
Fig 8. Effects of different combinations of night temperature and light intensity on gas exchange measurement A) net photosynthesis at light period and B) dark respiration at night period for both 1st and 3rd fully developed leaf of cucumber cv. Odeon grown for 14 days at two different night temperature (25/14 and 25/22 °C, D/N temperature) and two light intensity (200 and 400 µmol m^{-2} s^{-1}) treatments. Photoperiod is 16 h. Error bars indicate 1SE. Data are means of 8 replicate plants.

4.3.2 Effects of different combinations of night temperature and light intensity on growth of cucumber plants

In this experiment, all the growth parameters (plant height: P<0.001; r^2=0.96, total leaf area: P<0.001; r^2=0.95, specific leaf area: P<0.001; r^2=0.96, leaf number: P<0.001; r^2=0.93, total dry weight: P<0.001; r^2=0.83) and leaf chlorophyll content (P<0.001; r^2=0.62) showed response to different combinations of night temperature and light intensity (Fig 9). The effects of temperature was higher than light intensity to all parameters except specific leaf area (2-way ANOVA). The height of the cucumber plants was found highest (83.4 ± 0.77 cm) when grown at higher night temperature (25/22 °C) with lower light intensity (200 µmol m^{-2} s^{-1}) and lowest (29.8 ± 0.65 cm) at lower night temperature (25/14 °C) with higher light intensity (400 µmol m^{-2} s^{-1}). At same night temperature, plant height increased at low light intensity whereas, at same light intensity, plant height was higher at higher night temperature that gave significant (P<0.001) interaction effect of both parameters on height of the plants (Fig 9A). Moreover, average number of leaves was also highest (11.1 ± 0.08 cm) when cucumber grown at higher night temperature (25/22 °C) with lower light intensity (200 µmol m^{-2} s^{-1}). Lowering night temperature decreased leaf number and at same night temperature leaf number did not increase with increasing light (Fig 9D). Similarly, total leaf area became highest (3954 ± 36.7 cm^2) at higher night temperature (22 °C) with lower light intensity (200 µmol m^{-2} s^{-1}) and lowest (1859 ± 33.3 cm^2) at low night temperature (14 °C) and high light intensity (400 µmol m^{-2} s^{-1}). At both temperature treatments, total leaf area decreased significantly with increasing light intensity and the interaction of both temperature and light intensity had significant impact (P<0.001) on total leaf area of cucumber plant (Fig 9E). Likewise, the specific leaf area of cucumber was also found highest (473.6 ± 7.5 cm^2/g) at higher night temperature (22°C) and lower light intensity (200 µmol m^{-2} s^{-1}) which decreased with decreasing night temperature and increasing light intensity.
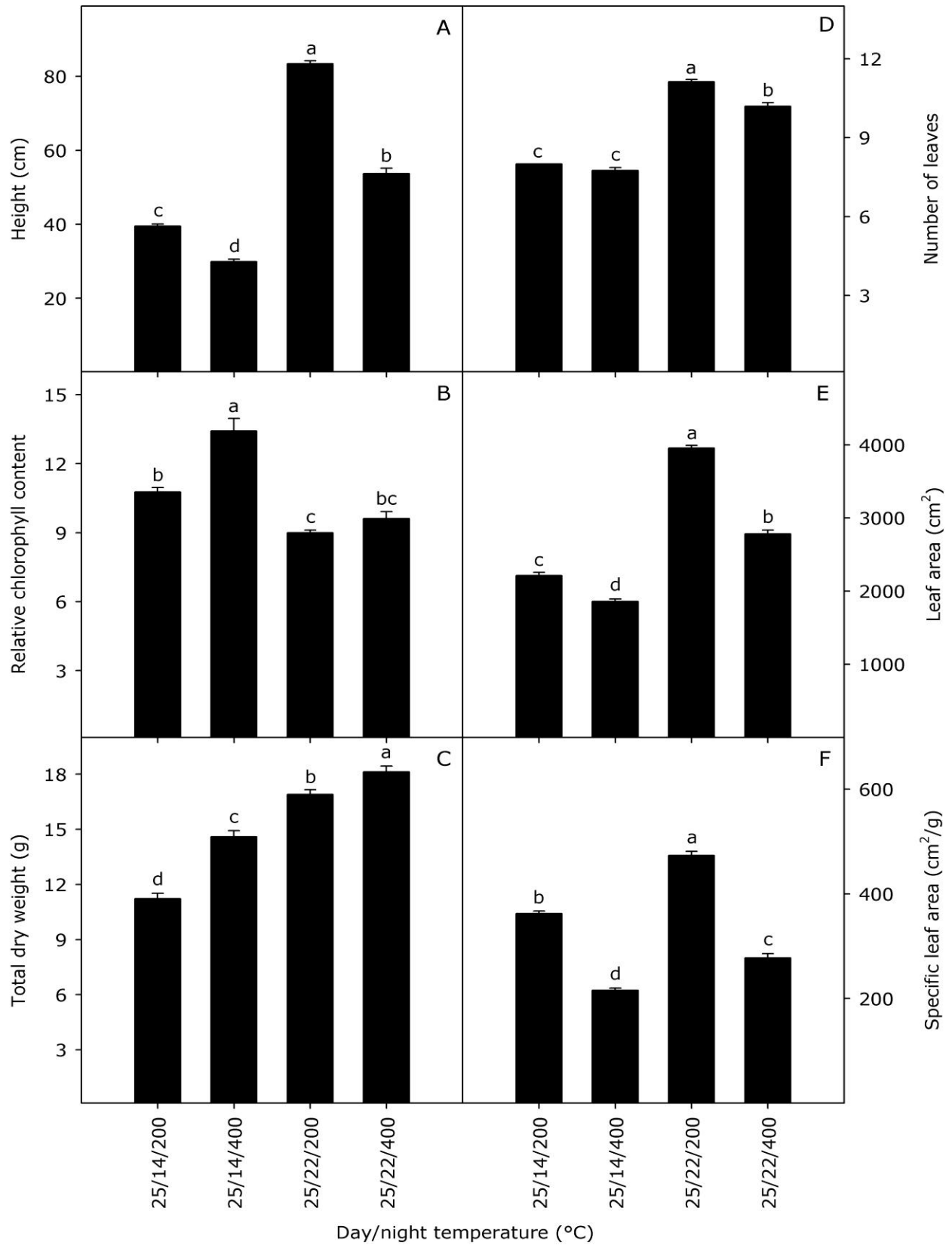
Fig 9. Effects of different combinations of night temperature and light intensity on growth parameters, plant height (cm), relative chlorophyll content, total dry weight (g), leaf number (>5), total leaf area (cm^2) and specific leaf area (cm^2/g) of cucumber cv. Odeon grown for 14 days at two different night temperature (25/14 and 25/22 °C, D/N temperature) and two light intensity (200 and 400 µmol m^{-2} s^{-1}) treatments. Photoperiod is 16 h. Error bars indicate 1SE. Data are means of 16 replicate plants.

At same night temperature, increase of light intensity significantly decreased specific leaf area for both temperature treatments (Fig 9F). Besides, total dry weight of cucumber plants was found highest (18.1 ± 0.3 g) when grown at high night temperature and high light intensity (400 µmol m^{-2} s^{-1}) whereas lowest (11.2 ± 0.2 g) at low night temperature and low light intensity (200 µmol m^{-2} s^{-1}). Interestingly, with increasing light intensity, total dry weight of cucumber plants increased for both temperature treatments although other growth parameters showed decreasing pattern (Fig 9C). Also, leaf chlorophyll content increased at high light intensity and highest chlorophyll content of cucumber leaf (13.4 ± 0.5) was measured at plants grown at low night temperature (14 °C) and high light intensity (400 µmol m^{-2} s^{-1}). For both temperature treatments (25/14 and 25/22 °C), leaf chlorophyll content increased with increasing light intensity. At same light intensity, decrease of night temperature increased the chlorophyll content of leaf (Fig 9B).

4.3.3 Light response curve at different combinations of night temperatures and light intensity

The light response curve of 3rd developed leaf at different temperature and light condition showed that the photosynthesis of cucumber leaf was not saturated with light. Plants grown at high night temperature with high irradiance level had greatest response for increasing irradiance, they started to approach for saturation and highest rate of maximum photosynthesis (23.7 µmol m^{-2} s^{-1}) was observed. On the other hand, plants grown at high night temperature with low level of irradiance and plants grown at low night temperature with high level of irradiance had similar light response and did not seems to saturate. Both treatments showed almost same maximum rate of photosynthesis (19.82 and 20.55 µmol m^{-2} s^{-1} for 14 °C/400 µmol m^{-2} s^{-1} and 22 °C/200 µmol m^{-2} s^{-1} respectively).The lowest light response was recorded in plants grown with low night temperature with low irradiance level and lowest rate of maximum photosynthesis (16.3 µmol m^{-2} s^{-1}) was observed (Fig 10).
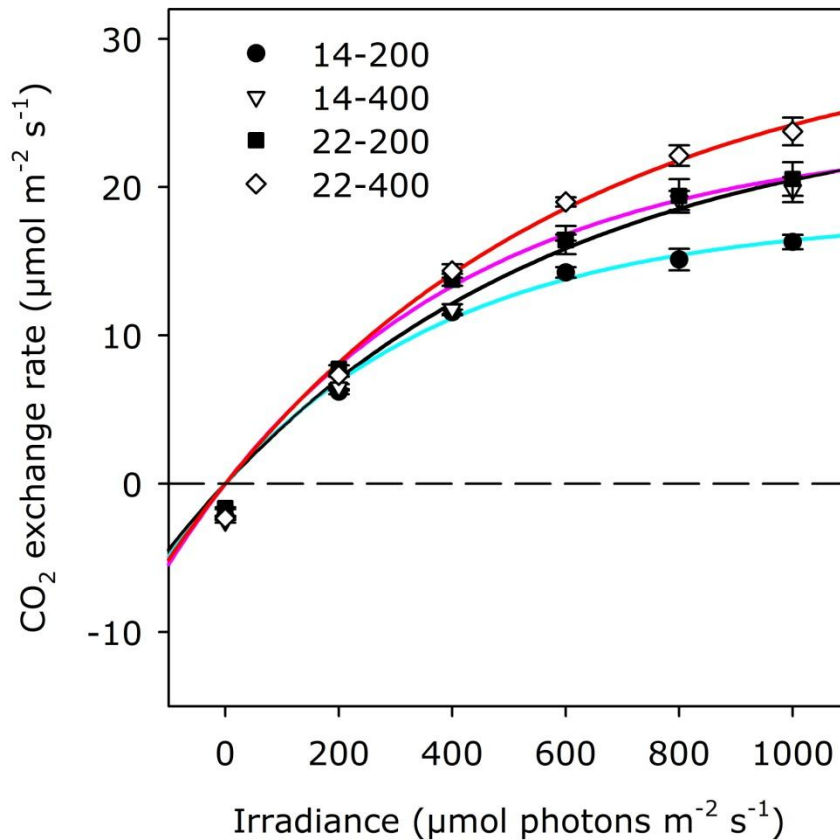
Fig 10. Photosynthetic light response curve of 3rd fully developed cucumber leaf grown at two different night temperatures (14 and 22 °C) and two different light conditions (200 and 400 μmol m^{-2} s^{-1}). Photoperiod is 16 h. Error bars indicate 1SE. Data are means of 4 replicate leaves.

4.3.4 Effects of different combinations of night temperature and light intensity on photo assimilates accumulation of cucumber plants

Among the carbohydrates stachyose, glucose and fructose were detected in all parts of cucumber whereas raffinose and sucrose were found only in leaf (Fig 12 A-E). The total carbohydrate content was strongly affected by temperature ($P<0.05$), light ($P<0.001$) and sampling position ($P<0.001$) as well. Total carbohydrate content increased 8% in plants grown at low night temperature (14 °C) than high night temperature (22 °C) whereas, the increased light from 200 to 400 μmol m^{-2} s^{-1} increased the total carbohydrate content almost 20% (n=40). No significant interaction between temperature and light was found (P=0.082). Total percentage of

carbohydrate that was accumulated at different parts in each treatment was almost similar (Fig 11). Night temperature or daytime irradiance did not affect the proportion of total carbohydrate partitioned between plant parts. Among different sampling position (Fig 12), total carbohydrate contents were higher in stem (above node, 20.6 ± 0.9 and below node 20.57 ± 0.78 mg g^{-1} FW) than leaf (12.37 ± 0.50 mg g^{-1} FW), petiole (16.89 ± 1.1 mg g^{-1} FW), and fruit (13.9 ± 0.58 mg g^{-1} FW) (n = 16).
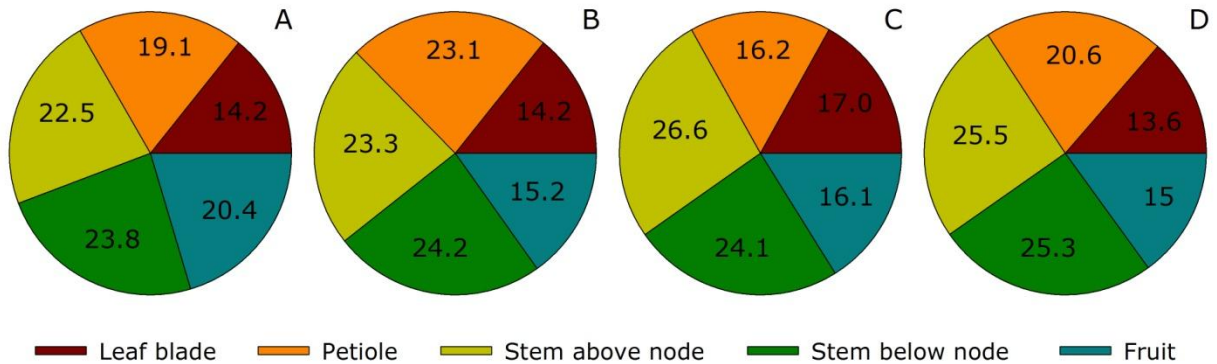
Fig 11. Effects of different night temperature and light intensity on partitioning of total percentage of carbohydrate in different parts (leaf, petiole, stem above node, stem below node and fruit) of cucumber cv. Odeon grown for 14 days at different temperature and light A (25/14/200), B (25/14/400), C (25/22/200) and D (25/22/400) D/N temperature (°C) and light (μmol m^{-2} s^{-1}).

Moreover, the concentration of stachyose varied significantly with temperature, light, and among sampling position (P<0.001, r^2=0.89) whereas, the interaction (Temperature x Light) had no significant effect (P=0.16). The cucumber plants grown at low night temperature (14 °C) had almost 35 % higher stachyose concentration than at high night temperature (22 °C) whereas, plants grown at 200 μmol m^{-2} s^{-1} had 26% more stachyose than at 400 μmol m^{-2} s^{-1} (n=40). Also, comparatively higher stachyose content was observed in petiole (4.66 mg g^{-1} FW) than stem and fruit whereas leaf contained low amount of stachyose (1.57 mg g^{-1} FW) (n=16). It showed decreasing pattern with high temperature and high light as well. Similarly, glucose concentration of cucumber plants was significantly affected by light condition and sampling position (P<0.001) but not by temperature (P=0.058). Glucose was the most quantified carbohydrates in all the plant parts. The concentration increased almost 50% in the plants when light level increased

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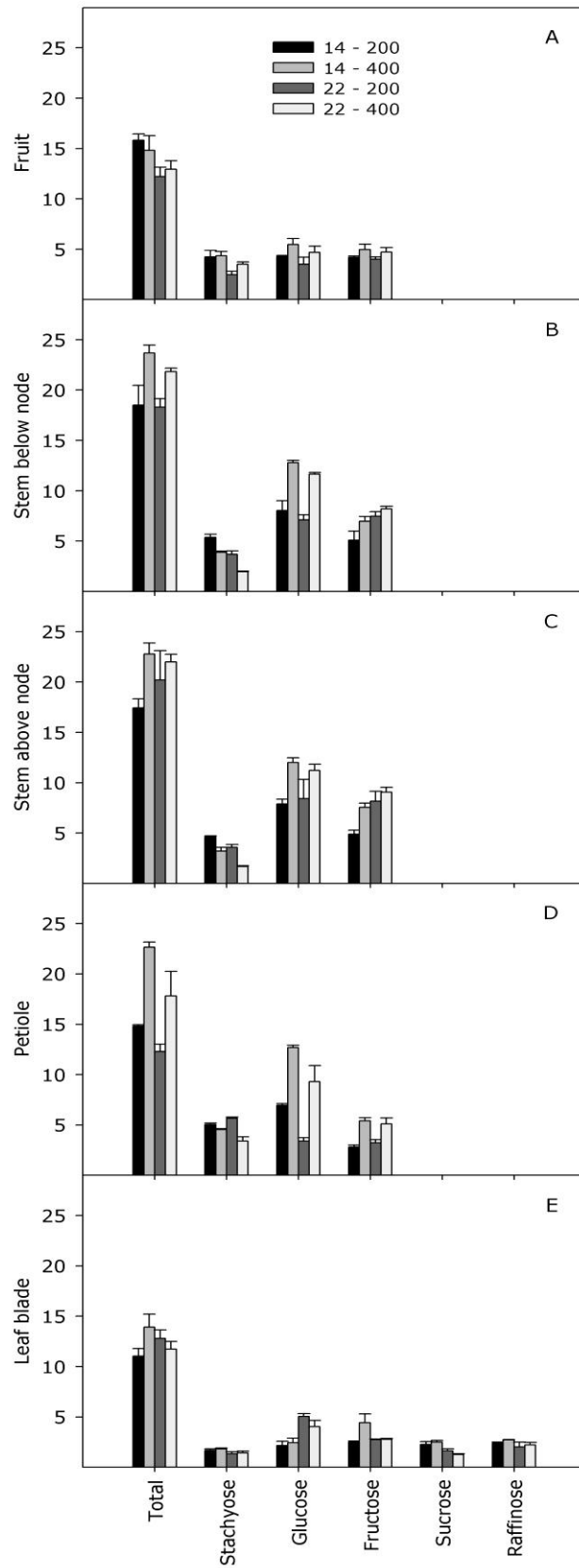
Fig 12. Effects of different night temperature and light intensity on carbohydrate concentration (stachyose, raffinose, sucrose, glucose and fructose) in A) fruit B) stem below node C) stem above node D) petiole and E) leaf blade of cucumber cv. Odeon grown for 14 days at two different night temperature (25/14 and 25/22 °C, D/N temperature) and two light intensity (200 and 400 µmol m^{-2} s^{-1}). Photoperiod is 16 h. Error bars indicate 1SE.

from 200 to 400 µmol m^{-2} s^{-1}. The stem of cucumber had the highest (9.89 mg g^{-1} FW) concentration of glucose whereas, leaf contained least amount (3.4 mg g^{-1} FW). Also, glucose content in different sampling position differed with different temperature and light gave significant (P<0.001) interaction (Temperature x sampling position and Light x sampling position) effect (Fig 12). Likewise, all the treatments (temperature: P<0.005, light: P<0.001 and sampling position: P<0.001) had significant effect on fructose concentration. The concentration of fructose was increased almost 15% at high night temperature (22 °C) and 31 % at high light intensity (400 photon m^{-2} s^{-1}). Among different parts, stem contained the highest fructose concentration (7.4 mg g^{-1} FW) and leaf contained the lowest (3.1 mg g^{-1} FW). Moreover, both the raffinose and sucrose was detected only in leaf. Sucrose concentration differed significantly with temperature (P<0.001) whereas no significant effect of treatment was found on raffinose.

5. Discussion

5.1 Effects of low night temperature and daily mean temperature on gas exchange measurement and growth of cucumber

The growth and morphology of cucumber plant is affected by not only daily average temperature, but also night- and day temperature, therefore it is vital to find out the most influential factor among them for the optimization of climatic condition (Challa & Brouwer 1985). It was evident that the daily mean temperature is the main controlling factor of growth of cucumber plant and all the growth parameters showed significant response to daily temperature integral compared to different diurnal temperature fluctuations (Fig 5). The gas exchange values were not following the same pattern as growth (Fig 4). Net carbon gain was higher at fluctuating D/N temperature because of lower respiration at low night temperature. Despite of higher leaf photosynthesis rate at high D/N temperature, overall carbon gain was lower due to high respiration at high night temperature. It means, lowering the night temperature do not have negative impacts on net carbon gain although it slightly reduces rate of photosynthesis. That may occur because low temperature reduces the enzymatic activities of PS II (Martino-Catt & Ort 1992) that can reduce photosynthesis. Also, plants grown under good light conditions at low night temperatures accumulate photosynthetic end products in the cells that may reduce photosynthesis rate (Goldschmidt & Huber 1992). However, measurements of CER after 1 h light or darkness may not give a complete picture since CER may change over time. As, the growth parameters were affected by daily average temperature, this slight decrease of photosynthesis did not have effect on total dry mass. However, plant height, leaf area, leaf number and total dry weight increased with increasing daily average temperature and decrease with decreasing daily average temperature rather fluctuation of diurnal temperature (Fig 5A-F). This confirm the previous findings of positive correlation of plant growth with daily mean temperature but not day or night temperature (Grimstad & Frimanslund 1993). It was a vital finding as the climatic optimization of greenhouse condition solely depends on this (Challa & Brouwer 1985) and it might increase the possibilities to change diurnal temperature especially night temperature without affecting growth by maintaining constant daily average temperature which is very important for saving energy. Low night temperature will (can) decrease growth, if the daily mean temperature decrease. Similar effect was found on tomatoes by Hurd and Graves

(1984) where temperature integral affected growth and yield of tomatoes but not by different diurnal temperature. Papadopoulos and Hao (2000) found that the development rate (leaf and flower number) of cucumber was affected by mean temperature, but not by the temperature difference. Same daily average temperature with lower night temperature showed reduced plant height a lesser extent suggest that night temperature has influence on cucumber height. Early studies by Went (1953) and Went (1957) suggested that stem elongation was primarily influenced by day temperature relative to night temperature. It was found that stem elongation increased largely with increasing the daily average temperature by raising day temperature only than an equal raise of day and reduction in night temperature (Grimstad & Frimanslund 1993). However, Langton and Cockshull (1997) showed that stem extension was determined by the absolute day and night temperature, not by temperature difference. Therefore, difference between diurnal temperature with same average temperature can have different impacts on plants growth and development which was shown by Friend and Helson (1976), who found higher growth at same average temperature with high day temperature than night temperature by investigating growth of seven crops. However, same daily mean temperature with much difference between D/N temperatures may have some negative impacts on growth and yield of cucumber. Marcelis and Hofman-Eijer (1993) concluded that stronger variations in D/N temperature can lead to a reinforced pushing off of the cucumber fruits and suggested to avoid this. Also, higher differences of temperature between day and night cause nutrient deficiency and yellowing of cucumber (Gruda 2005).

5.2 Effects of different split night temperatures on gas exchange measurement and growth of cucumber

The study (Khayat et al. 1985) suggested that splitting night temperature may be introduced as an economical tool to increase production of tomato plants as well as in other greenhouse crops. The photosynthesis rate was unchanged with split night temperature treatments in this study (Fig 6). Respiration increased at higher night temperature and decreased at lower night temperature. Although, positive response on growth and production of some crops was observed earlier (Carow & Zimmer 1977; Khayat et al. 1985; Zieslin et al. 1985), cucumber seedling in this experiment (Fig 7A-F) did not show any response to split night temperature treatments except plant height (P<0.001; r^2=0.83). Plants grown at lower night temperature (14 °C) at first half (4h)

had almost 15% higher plants but all other growth parameters were almost the same with split night temperature and constant night temperature as well. This suggest that to split the night period with different temperature may not be a potential for cucumber production but this requires further investigation with changing average night temperature as it is the same here in all treatments which may be responsible for the growth. Research by Gent et al. (1979) and Thorne and Jaynes (1977) implied that the first half of night temperature is physiologically more active although they got 13% less tomato production due to splitting night temperature (first 5 hours 15.5 °C and last 7 hours 7 °C) and 18 % of energy saved there. It was not evident from this study. May be the growth of cucumber seedlings does not response like tomato at different split night temperature and hence, this requires further investigation.

5.3.1 Effects of different combinations of night temperature and light intensity on gas exchange measurement and growth of cucumber

During low natural light, additional lighting increased tomato yield by 2-3 times and seedless cucumber yield by 25% (Hao & Papadopoulos 1999). At both night temperatures (14 and 22 °C), photosynthesis almost doubled with doubling the light intensity so, high light can be potential to improve growth at low night temperature. Almost, 30% higher dry matter was found when light intensity was doubled at low night temperature (14 °C) and 7 % at high night temperature (22 °C) (Fig 9). Grimstad and Frimanslund (1993) suggested that stem elongation is mostly depend on increasing the daily mean temperature by raising day temperature and be an important factor in dry matter production. So, the low night temperature can be combined with high day temperature with high solar radiation to get energy efficient cucumber production. But, high solar radiation at day time may cause reduction of CO_2 level through ventilation which can reduce the CER and growth of cucumber. A study by Mortensen et al. (2012) suggested to maintain high CO_2 concentration throughout the day in similar condition to improve the crop yield. Similarly, Hückst (2013) showed that lowering night temperature had no negative impacts on photosynthesis of tomato and suggested that night temperatures may be combined with very high day temperatures without any loss of daily photosynthesis particularly in a CO_2 enriched atmosphere. Also, high day temperatures can increase the greenhouse interior which reduce the heating costs of the following night. Higher plants and leaf area at low light intensity (Fig 9A, E) may be because of lower light saturation for the plants (Fig 10) and they required more light so

stem and leaf were expanded. Liu and Heins (1997) reported that plants grown at low light, grow and develop rapidly but become thin and weak whereas at high light grow and develop slowly but become stocky. However, total dry matter production increased with increasing light intensity and as well as temperature (Fig 9C) which is consistent with Bruggink (1992) who found that increasing light integral increased the relative growth rate of young tomato plants. Also, higher dry mass production in chrysanthemum, radish, corn, cucumber and wheat was found with increasing daily light integral (Moe et al. 2005). A study by Hao and Papadopoulos (1999) found that increased light intensity greatly affect cucumber growth, dry matter production and early yield. Higher dry mass production with high light intensity at low night temperature (29%) than high night temperature (7%) suggest that higher light may be effective for cucumber production at low night temperature and become a potential option to save energy during cucumber production. Obviously, this requires more investigation and to execute this method to observe the similar impact on morphogenesis as the effect must not be same at this vital stage like flowering and fruiting that determines the yield of cucumber (Challa & Brouwer 1985). On the other hand; growers reported that the total energy consumption increase with about 40 % with 14 °C and 400 µmol m^{-2} s^{-1}, compared to 18 °C and 200 µmol m^{-2} s^{-1} through artificial lighting for a period of 6 months cucumber production in greenhouse. So, night temperature can be reduced efficiently at spring or summer when strong natural light is available. Additionally, higher leaf chlorophyll content and very dark green cucumber leaves was observed at high light intensity which is consistent with (Hao & Papadopoulos 1999; Lin & Jolliffe 1996) and common for many plant species (Björkman 1981; McLaren & Smith 1978). As photosynthesis is proportional to chlorophyll content (Emerson 1929), it may contribute to higher production at high light intensity. Higher leaf thickness (1/SLA) at high light intensity may have contributed to high dry matter production at 400 µmol m^{-2} s^{-1} as Schuerger et al. (1997) showed that thicker leaf can intercept more light than thinner leaf. The reduction of leaf thickness under reduced light intensity was observed in wheat by Friend (1966).

5.3.2 Effects of different combinations of night temperature and light intensity on photo assimilates translocation

Stachyose, raffinose, sucrose, glucose and fructose were the primary sugars metabolized in cucumber plants. Soluble sugars are considered to serve pivotal role in stress condition especially

related to cold tolerance of many plants. Decrease of concentrations of glucose, fructose, and starch are found during alfalfa cold acclimation (Castonguay et al. 1995) whereas the concentrations of sucrose, fructose, glucose, and raffinose increase during cold acclimation in buffalograss (Ball et al. 2002). Taji et al. (2002) found that cold treated arabidopsis accumulate glucose, fructose, sucrose and raffinose. The accumulation of soluble sugars are observed in different fruits such as melon, grape and honeysuckie during cold condition (Hamman et al. 1996; Mitchell & Madore 1992). Similarly, carbohydrate metabolism plays important role on the quality of cucumber and the fruit development largely depends on photo assimilate translocation from leaves to fruits (Ho 1988). Stachyose is considered to be the main transporting sugar in cucumber which was detected in all plant parts of cucumber. Higher concentration of stachyose and raffinose at low temperature suggest that cucumber seedlings could develop cold tolerance through the accumulation of these sugars. At higher temperature and light condition, stachyose concentration depleted means during low night temperature it was stored and played protective role against cold environment. Large amount of stachyose and raffinose at low temperature was found by Meng et al. (2008). Similarly, accumulation of sugars in *Cucumis melo* was detected by Mitchell and Madore (1992) during cold stress. It was reported that these carbohydrates can act as cryoprotectants of cell structures and capable of preventing ice formation inside cells by decreasing the freezing point (Meng et al. 2008). Higher accumulation of glucose and fructose in stem at high temperature and light suggest that stachyose and raffinose was depleted into glucose and fructose from leaf with increasing temperature and light intensity. Both the temperature and light strongly influence translocation of photo assimilates (Lundmark et al. 2006; Wang et al. 2014). The mature cucumber leaf export the photo assimilate within 2 h at 20 °C and 4 h at 16 °C of air temperature (Toki et al. 1978). Moreover, significant increase in photo assimilate translocation was recorded from leaves of cucumber with alteration of light intensity from 380 μmol m^{-2} s^{-1} to 650 μmol m^{-2} s^{-1} by Robbins and Pharr (1987). Also, the main free sugars in the pedicel and fruit tissues are sucrose and hexoses (Handley et al. 1983; Pharr et al. 1977). The stachyose is synthesized in leaves which is catabolized to glucose and fructose after entering fruits (Pharr et al. 1977; Pharr et al. 1985). Higher concentration of stachyose (Fig 12A) was detected in fruit sample here, which is not common in matured cucumber fruit (De Souza et al. 2013). In this short term growth experiment, the fruit was in initial stage and may be the translocated stachyose was not converted to hexoses yet at that stage.

6. Conclusion

Cucumber plants may be grown at low daily mean temperature that can be achieved via low night temperature, if the daytime irradiance level can be increased. The price balance between heating and lighting is the primary determinant factor for energy saving. At present, the cost for providing 400 µmol m^{-2} s^{-1} with high intensity discharge lamps is much higher than cost for heating from 14 °C to 22 °C night temperature. Currently, cucumber may be produced with low night temperature during spring when the amount of solar irradiance increased significantly or in summer time where day time temperature and solar irradiances are high. Rapid advancement in modern, energy efficient photosynthetically active photon generators can have the possibilities of producing cucumber plants with low night temperature in future.

7. References

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9. Appendix

Appendix 1. Table shows the ANOVA for growth parameters (plant height, leaf area, SLA, Total dry weight, leaf number and chlorophyll content) of cucumber for 1st experiment grown for 14 days with four temperature treatments

Parameter Source	d.f	Plant height		Leaf area		SLA		Total dry weight		Number of leaves		Chlorophyll	
		F	P	F	P	F	P	F	P	F	P	F	P
Exp replication	1	25.56	0.000	12.66	0.001	5.88	0.019	1.58	0.214	5.90	0.019	119.29	0.000
Temperature (T)	3	132.59	0.000	72.30	0.000	70.46	0.000	10.65	0.000	80.06	0.000	2.29	0.090
Exp rep*T	3	2.00	0.126	3.20	0.031	8.35	0.000	0.27	0.848	1.63	0.195	1.78	0.164
Error	49												
Total	63												
r^2_{adj}		0.870		0.793		0.787		0.324		0.798		0.656	

Appendix 2. Table shows the ANOVA for growth parameters (plant height, leaf area, SLA, Total dry weight, leaf number and chlorophyll content) of cucumber for 2nd experiment grown for 14 days with three temperature treatments

Parameter Source	d.f	Plant height		Leaf area		SLA		Total dry weight		Number of leaves		Chlorophyll	
		F	P	F	P	F	P	F	P	F	P	F	P
Exp replication	1	156.23	0.000	65.38	0.000	57.24	0.000	131.92	0.000	42.52	0.000	9.44	0.004
Temperature (T)	2	37.50	0.000	2.03	0.147	2.26	0.120	1.85	0.173	5.88	0.006	30.51	0.000
Exp rep*T	2	4.62	0.017	2.45	0.101	0.20	0.822	3.31	0.048	2.02	0.147	1.23	0.306
Error	35												
Total	47												
r^2_{adj}		0.836		0.610		0.527		0.747		0.548		0.576	

Appendix 3. Table shows the ANOVA for growth parameters (plant height, leaf area, SLA, Total dry weight, leaf number and chlorophyll content) of cucumber for 3rd experiment grown for 14 days with two temperature and two light treatments

Parameter Source	$d.f$	Plant height		Leaf area		SLA		Total dry weight		Number of leaves		Chlorophyll	
		F	P	F	P	F	P	F	P	F	P	F	P
Exp replication	1	0.12	0.729	1.88	0.177	1.34	0.253	0.28	0.597	2.59	0.114	2.24	0.141
Temperature (T)	1	1178.33	0.000	915.17	0.000	318.96	0.000	245.65	0.000	821.68	0.000	72.84	0.000
Light (L)	1	396.01	0.000	299.01	0.000	1251.09	0.000	61.08	0.000	37.45	0.000	24.95	0.000
Exp rep*T	1	1.02	0.316	0.01	0.925	0.19	0.668	0.01	0.916	0.10	0.749	0.13	0.722
Exp rep*L	1	0.00	1.000	0.01	0.931	0.02	0.899	0.02	0.899	2.59	0.114	2.19	0.145
T*L	1	103.78	0.000	86.25	0.000	25.93	0.000	13.26	0.001	12.55	0.001	9.70	0.003
Error	50												
Total	63												
r^2_{adj}		0.963		0.953		0.962		0.836		0.932		0.625	

Appendix 4. Table shows the ANOVA for both photosynthesis and dark respiration of 1st developed leaf of cucumber of all three experiment

Parameter	Experiment 1				Experiment 2				Experiment 3			
Source	Net Photosynthesis		Dark respiration		Net Photosynthesis		Dark respiration		Net Photosynthesis		Dark respiration	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Exp rep	82.23	0.000	0.06	0.812	86.61	0.000	28.14	0.000	2.59	0.122	0.16	0.697
Temperature (T)	9.15	0.000	72.02	0.000	4.39	0.032	0.35	0.706	1.69	0.207	1.13	0.300
Light (L)									65.76	0.000	10.92	0.003
Exp rep*T	7.91	0.001	3.93	0.023	4.43	0.031	5.24	0.010	3.00	0.097	8.30	0.009
Exp rep*L									1.24	0.277	13.91	0.001
T*L									2.03	0.169	2.99	0.098
T*Sample time							325.41	0.000				
r^2_{adj}	**0.801**		**0.881**		**0.808**		**0.936**		**0.693**		**0.509**	

Appendix 5. Table shows the ANOVA for both photosynthesis and dark respiration of 3rd developed leaf of cucumber of all three experiment

Parameter	Experiment 1				Experiment 2				Experiment 3			
Source	Net Photosynthesis		Dark respiration		Net Photosynthesis		Dark respiration		Net Photosynthesis		Dark respiration	
	F	P	F	P	F	P	F	P	F	P	F	P
Exp replication	26.22	0.000	52.03	0.000	0.99	0.336	0.00	0.970	29.28	0.000	42.71	0.000
Temperature (T)	25.19	0.000	29.15	0.000	25.76	0.000	0.34	0.716	157.76	0.000	138.38	0.000
Light (L)									1390.10	0.000	15.38	0.001
Exp rep*T	1.56	0.229	10.30	0.000	1.36	0.288	10.74	0.000	2.68	0.116	0.00	1.000
Exp rep*L									63.89	0.000	2.23	0.149
T*L									0.20	0.659	3.49	0.075
T*Sample time							6.36	0.004				
r^2_{adj}	0.763		0.843		0.712		0.394		0.981		0.862	

Appendix 6. Table shows the ANOVA for carbohydrates (stachyose, raffinose, sucrose, glucose, fructose and total carbohydrate) of cucumber grown for 14 days with two temperature and two light treatments

Parameter Source	d.f	Stachyose		Raffinose		Sucrose		Glucose		Fructose		Total carbohydrate	
		F	P	F	P	F	P	F	P	F	P	F	P
Temperature (T)	1	72,29	0,000	2,32	0,153	27,11	0,000	3,74	0,058	9,34	0,003	5,87	0,018
Light (L)	1	44,81	0,000	0,93	0,353	0,19	0,668	83,57	0,000	41,71	0,000	31,06	0,000
Sample position (S)	4	73,27	0,000					70,86	0,000	58,76	0,000	37,13	0,000
T x L	1	2,03	0,160	0,03	0,871	2,69	0,127	0,69	0,410	6,65	0,012	3,12	0,082
T x S	4	6,30	0,000					8,07	0,000	7,73	0,000	2,35	0,063
L x S	4	15,81	0,000					12,26	0,000	1,48	0,218	4,92	0,002
Error	60												
Total	69												
r^2_{adj}		0.863		0.186		0.642		0.846		0.798		0.719	