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Quantifying the effects of daily light integral or photoperiod on maize tassel morphology across developmental stages

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Quantifying the effects of daily light integral or photoperiod on maize tassel morphology across developmental stages

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DEDICATION

This thesis is dedicated to my husband, Jarod who provided me limitless support and courage. Your encouragement to constantly push myself and grow has been more important than you could ever imagine. To my parents Mary and Mike, for whom without I would not have embarked on such a daunting task. I am humbled to have so much support and care in pursuit of this degree, and without that I would not be the scientist I am today.

TABLE OF CONTENTS

Page

LIST OF FIGURES v

LIST OF TABLES vi

ABSTRACT x

CHAPTER 1. LITERATURE REVIEW 1

CHAPTER 2. LOW DAILY LIGHT INTEGRAL INCREASES MAIZE TASSEL BARRENNESS 12

Abstract 12

Introduction 13

Materials and Methods 15

Results 19

Discussion 21

Conclusions 23

Acknowledgements 24

Literature Cited 24

Tables and Figures 28

CHAPTER 3. LONGER PHOTOPERIOD DURING VEGETATIVE OR REPRODUCTIVE GROWTH SUPPRESSES BARREN TASSEL LENGTH 38

Abstract 38

Introduction 39

Materials and Methods 41

Results 44

Discussion 45

Conclusions 46

Acknowledgements 47

Literature Cited 47

Tables and Figures 51

CHAPTER 4. GENERAL CONCLUSIONS 59

General Discussion 59

Recommendations for Future Research 60

ACKNOWLEDGEMENTS 62

LIST OF FIGURES

Chapter 2

Figure 1. Inbred A maize (*Zea mays* L.) plants throughout vegetative (V) growth stages: V3, V5, V7, V9, V11, and visible tassel (VT)..... 36

Figure 2. Tassel height, branch number, viable length and barren length for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 7 d, when 50% of plants reached V4, V5, V6, or V7, and an untreated control was also included. Data were collected 10 d after pollination (DAP). Tassels imaged above were treated during V6 and V7 compared to the untreated control..... 37

LIST OF TABLES

Chapter 2

Table 1. Photoperiod and daily light integral (DLI) for each growth environment, and set points for light intensity, day/night air temperatures, and average daily temperature (ADT) of the control chamber, the low DLI chamber, and the propagation room 28

Table 2. Vegetative and reproductive traits for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 7 d, when 50% of plants reached V4, V5, V6, or V7, and an untreated control was included. Data were collected 10 d after pollination (DAP). Each treatment consisted of 12 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test..... 29

Table 3. Tassel height, branch number, viable length and barren length for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 7 d, when 50% of plants reached V4, V5, V6, or V7, and an untreated control was also included. Data were collected 10 d after pollination (DAP). Each treatment consisted of 12 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test 30

Table 4. Vegetative and reproductive traits for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 7 d, when individual plants reached V4 - V12, and an untreated control was also included. Data were collected 10 d after pollination (DAP). Each treatment consisted of 6 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test 31

Table 5. Tassel height, branch number, viable length and barren length for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 7 d, when individual plants reached V4 - V12, and an untreated control was also included. Data collected 10 d after pollination (DAP). Each treatment consisted of 6 pseudo-

replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test 33

Table 6. Vegetative and reproductive traits for maize (*Zea mays* L.) inbred line B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 0-7 d, when individual plants reached V6, or grown continuously in high DLI (control). Data collected 25 d after pollination (DAP). Each treatment consisted of 6 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test 34

Table 7. Tassel height, branch number, viable length and barren length for maize (*Zea mays* L.) inbred line B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 0-7 d, when individual plants reached V6, or grown continuously in high DLI (control). Data collected 25 d after pollination (DAP). Each treatment consisted of 6 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test..... 35

Chapter 3

Table 1. Photoperiod and daily light integral (DLI) for each growth environment, and actual values for light intensity, day/night air temperatures, and average daily temperature (ADT) of the treatment chambers (20 and 22 h), the control chamber (16 h), and the propagation room 51

Table 2. Analyses of variance of vegetative (V) photoperiod (20 or 22 h) and reproductive (R) photoperiod (16, 20, or 22 h), and their interactions, on both vegetative and reproductive traits of maize (*Zea mays* L.) Inbred A (drought-susceptible line with a highly-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants) 52

Table 3. Analyses of variance of vegetative (V) photoperiod (20 or 22 h) and reproductive (R) photoperiod (16, 20, or 22 h), and their interactions, on both vegetative and reproductive traits of maize (*Zea mays* L.) Inbred B (drought-tolerant line with a less-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants) 53

Table 4. The effect of photoperiod (20 or 22 h) during vegetative growth on vegetative traits for both maize (*Zea mays* L.) Inbreds A (drought-susceptible line with a highly-branched tassel) and B (drought-tolerant line with a less-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants). Data are pooled across reproductive photoperiods..... 54

Table 5. The effect of photoperiod (20 or 22 h) during vegetative growth on reproductive traits for both maize (*Zea mays* L.) Inbreds A (drought-susceptible line with a highly-branched tassel) and B (drought-tolerant line with a less-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants). Data are pooled across reproductive photoperiods..... 55

Table 6. The effect of photoperiod during reproductive growth (16, 20, or 22 h) on vegetative traits for both maize (*Zea mays* L.) Inbreds A (drought-susceptible line with a highly-branched tassel) and B (drought-tolerant line with a less-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants). Data are pooled across vegetative photoperiods 56

Table 7. The effect of photoperiod during reproductive growth (16, 20, or 22 h) on the tassel height of maize (*Zea mays* L.) Inbred A (drought-susceptible line with a highly-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants). Data are pooled across vegetative photoperiods 57

Table 8. The effect of the interaction between vegetative photoperiod (V) and reproductive photoperiod (R) on barren tassel length of maize (*Zea mays* L.) Inbred A (drought-susceptible line with a highly-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants) 58

ABSTRACT

Maize (*Zea mays* L.) is an important agronomic crop throughout the world. Maize seed production and trait introgression is performed in both field and greenhouse settings (which allow for continual production seasons). The winter months within the greenhouses produce maize tassels with increased barren tassel branches (suppressed anther production) and the hypothesis is that the most limiting factor is daily light integral (DLI). Growing maize year-round in temperate regions, such as the northern part of the United States, requires the use-of greenhouse production. The low light intensities of these regions in the winter months creates a need for supplemental lighting for crops such as maize, that are high-light species. Utilizing a controlled environment, such as a greenhouse, also allows for control over the environmental factors such as temperature, light, moisture, gases and nutrients. Temperature, moisture, and nutrients can be supplied as needed, and maize does not require CO_2 regulation because it is a C4 plant. The last factor, light, is most limited on cloudy or winter days, therefore plants require supplemental lighting to provide the additional light needed to produce a high-quality maize tassel. Our objectives were to investigate how the maize plant grows and develops in response to lower DLI levels. After determining the impact that DLI has on maize quality we aimed to quantify the threshold of low DLI needed before tassel barrenness increases, and to explore opportunities to prevent the low DLI stress from occurring. This thesis describes research that was carried out to achieve these objectives and provides a discussion on the implications of these results.

CHAPTER 1. LITERATURE REVIEW

The Genus *Zea mays* L.

Maize (*Zea mays* L.), or corn, is a leading agronomic crop around the world. In the United States in 2016, 28% of the harvested crop acres were corn, second only to soybean (U.S. Department of Agriculture, 2017). More specifically, 86.7 million acres were harvested, which resulted in 51.5 billion dollars in crop values for 2016 (U.S. Department of Agriculture, 2017). This high-yielding crop produces many by-products, such as silage, forage, and livestock feed. Biofuels is another important area that maize supports, both in the form of ethanol and corn oil (used as bio-diesel). With these, among many other uses for the maize plant, high quality crops are vital for success.

Maize arose through the domestication of teosinte (*Zea mays ssp. Parviglumis*), which is indigenous to the tropical regions of Central and Latin America (Goodman, 1999; Matsuoka et al., 2002; van Heerwaarden et al., 2011). The vast genetic diversity of maize allowed for its cultivation to span the climates, from temperate areas such as southern Canada, to more tropic regions that required adaptation to longer day lengths (Goodman, 1988). The success of this plant across this wide spectrum of climates and geographic locations is attributed to its highly adaptive genetics.

Maize seed production and trait introgression is performed in both field and greenhouse settings. Although maize has been predominately grown in outdoor fields during the summer, breeding practices have increased the need for winter production. Maize production within a controlled-environment is invaluable to breeders who would be limited

to one growing season per year in the field (Ceccarelli, 2015). Since the relative maturity of maize hybrids can vary from 95 to 120 d (Dwyer et al., 1999), using greenhouses allows continual production throughout the year. Photosynthesis of maize saturates at ~2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of light, deeming this a high-light requiring plant (Fletcher et al., 2008). Because of this need for high-light, breeders rely on light-supplemented greenhouses, especially during the light limiting winter months. Like all plants, maize grows in response to its environment. Temperature, moisture, nutrients, gases, and light are all key factors that impact the growth and development of the maize plant. Excessive or limited supply from any of these influential factors can be detrimental to plant quality (Fageria et al., 2006). Regarding plants grown within a greenhouse, four of these five environmental factors can be tightly regulated. The 5th factor, light, is limited in the winter months in the northern regions of the United States (Korczynski et al., 2002).

Greenhouse Environments

Growing maize in greenhouses is accompanied by different challenges than in the field. While weather events, such as high winds, hail, or drought can damage a field, they are less problematic for the greenhouse grower (Lizumi and Ramankutty, 2015). Greenhouse managers face different obstacles, such as, manual irrigation practices and pest management. Plants can be closely monitored for quality of growth within the greenhouse more easily than in a field, and quality control measures allow for course correction if issues arise (Paparozzi, 2013). While the environmental set-points are well-known for factors such as temperature, nutrients, moisture levels, and gases, there is limited information available on the light requirements of the maize plant within a controlled environment.

Maize development

Maize development can be divided into vegetative and reproductive stages. Vegetative stages are marked by the expansion of the uppermost collared leaf. If the 3rd leaf is fully expanded the plant is at vegetative stage 3 (V3). During vegetative growth, ear traits such as potential number of rows and kernel number are determined (Stevens et al., 1986). The last vegetative stage is VT (visible tassel) and this marks the transition from vegetative to reproductive growth of the plant (Abendroth et al., 2011). During reproductive growth the male and female inflorescences are exposed, and pollination occurs. The tassel is the male inflorescence and generally begins developing between V5-V7, though has been recorded to initiate as early as V4 (Ritchie et al. 1992). The tassel branches contain anthers, which shed pollen grains on the emerged silks of the maize ear just below. This self-pollination process gives rise to the kernels that will later make up the harvestable grain, aka "yield". If the tassel is barren (lack of anthers and pollen), the subsequent pollination will also be poor, resulting in a low yield (Tollenaar and Dwyer, 1999). For a grower to invest 95-120 d into a plant that is low-yielding, is costly. By the time the grower is aware of the poor-quality tassel (at VT), it is too late. By the time the tassel is visible, damage is irreparable.

Tassel morphology

Tassel morphology is predetermined by genetics and influenced by environmental factors. The size and shape of the tassel dictates the potential for the plant to produce pollen (Vollbrecht et al., 2005). Furthermore, pollinations require a minimum quality and quantity of pollen, and if the tassel is small or barren (lacking anthers) and no pollen is produced, the

pollination will not succeed (Tollenaar and Dwyer, 1999). Tassels of maize plants grown in greenhouses during winter months are observed with anthers which are underdeveloped or not present, leading to a reduction in pollen production decreasing the yield potential of maize plant.

Light

Light is one of the most critical growth factors that influence yield and can therefore be manipulated to increase yield and quality of plants (Blanchard and Runkle, 2011; Tollenaar and Aguilera, 1992). Photosynthetically active radiation (PAR) is light that plants can utilize for photosynthesis, which occurs between 400 and 700 nanometers (Zeiger and Field, 1982). Daily light integral (DLI) is the measurement of the photosynthetic light in a day (Korczynski et al., 2002). Using DLI as a tool to manage maize growth rather than PAR allows the grower to account for variation in instantaneous PAR over a day. The outdoor DLI in Iowa can range from 10 $mol \cdot m^{-2} \cdot d^{-1}$ during winter months, to 50 $mol \cdot m^{-2} \cdot d^{-1}$ during the summer season (Korczynski et al., 2002). Additionally, the greenhouse structure can reduce the solar radiation the plants receive by 40% to 60%, due to glazing and superstructure shadowing (Eddy and Hahn, 2010). If the DLI in January is reduced up to 60% due to superstructure, the plants are only receiving a DLI of 4 $mol \cdot m^{-2} \cdot d^{-1}$ before supplemental lights.

In floriculture crops, DLI influences many growth and development traits such as, their branching, root structure, stem thickness, and time to flowering (Runkle, 2006). It is not well-documented in literature how low DLI stress impacts maize tassel quality, but tassels are observed with suppressed anther production during winter months when light is

limiting. Therefore, investigating the influence of DLI on maize tassel fertility will better prepare the greenhouse grower to produce high quality plants even in the winter months.

Increasing DLI

Electrical lighting can provide additional light to the plants. To increase the intensity of the light being provided, a grower could physically change the growing space with one of the following tactics. Increasing the number of lamps (lamp density) would increase the light intensity provided to the plants, but this option can be costly and greenhouse structure could limit the addition of additional lamps (Both, 2004). Another approach is to decrease the distance between the plant canopy and the lamp allowing for a more intense lighting. Using this tactic requires caution, so that the lamps are not placed too close to the plants, or else the associated radiant energy from the lamps can be too stressful for the plants (Crafts-Brandner and Salvucci, 2002). Growth processes, such as photosynthesis can be hindered, leading to photorespiration and the closure of stomata, if the tissue temperature is too high. Heat stress also results in desiccation of upper leaves (leaf firing) and tassel desiccation prior to pollen extrusion (tassel blast) phenotypes (Crafts-Brandner and Salvucci, 2002; Zaidi et al., 2016). A third approach to increase the light intercepted by the plants is to reduce the plant density, allowing for more light to penetrate to the lower canopy between the plants (Maddonni et al., 2001). This reduction in plant density can be costly when the decrease in plant density often translates to a reduction in number of plants grown (Both, 2004). With these different approaches to increasing light intensity, growers can determine which is the most cost effective for them.

Increasing DLI can be done by also increasing the lamp operating time. Generating a longer day length would allow for more potential accumulation of photosynthetic light by the plant (Both, 2004). The day length manipulation can alter the transition from vegetative to reproductive growth for many species, especially those who are sensitive to photoperiod (Warrington and Kanemasu, 1983). This is a consideration that must be applied when deciding if a longer day length is the best option to increase DLI.

Photoperiod

Photoperiod, or day length, can be very influential on maize growth and development. Temperate maize has a substantially reduced photoperiod sensitivity compared to that of the more tropical maize lines (Gouesnard et al., 2002; Xu et al., 2012). Tropical maize has been documented as having increased plant height, a greater total leaf number, and a delay in flowering time when grown in regions with less than 11 hours of darkness (Warrington and Kanemasu, 1983). It has been reported to use a 16 h for photoperiod throughout all growth stages of maize for seed production (Eddy and Hahn, 2010). They also documented that continuous lighting resulted in severe leaf deformity, caused by calcium deficiency symptoms. With photoperiod playing an influential role in plant quality, it is important to know the requirement of the species being grown.

Conclusions

Tassel barrenness is observed on maize plants grown within greenhouses during the light limiting (low DLI) winter months. The DLI levels during winter maize production are a

limiting factor when grown in a greenhouse. While analyzing the impact of low DLI stress on tassel growth and quality, it is important to also note the quality of the rest of the plant. The three objectives of this research were to: 1) determine if any developmental stage is most susceptible to low DLI stress; 2) to determine the low DLI threshold; and 3) study increasing DLI with longer duration of lighting. We cannot focus all efforts on the quality of the tassel and ignore the whole plant physiology. If tassel quality is improved at the cost of kernel number, the yield might not be improved. For this reason, the experiments presented in this research were aimed at documenting the vegetative and reproductive traits in response to DLI, in addition to the tassel quality.

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CHAPTER 2. LOW DAILY LIGHT INTEGRAL INCREASES MAIZE TASSEL BARRENNESS

Abstract

Maize breeding practices are increasing the use-of controlled environments for year-round production. In winter months, when ambient light is limited in the northern regions, tassel barrenness is a problem for greenhouse growers. Our first objective was to quantify the tassel morphology of two maize inbred lines in response to low daily light integral (DLI) at different growth stages. The second objective was to identify the DLI threshold (number of days with low DLI) before tassel morphology quality declined. Inbreds A and B were analyzed after being transferred from a high DLI (23.7 $mol \cdot m^{-2} \cdot d^{-1}$) to a low DLI (9.3 $mol \cdot m^{-2} \cdot d^{-1}$) for 7 d starting at vegetative growth stages (V) V4, V5, V6, V7, V8, V9, V10, V11, or V12 then placed back under a high DLI. Data were collected to characterize tassel morphology and barrenness in response to the low DLI stress. Inbred A tassels were more barren when the DLI was low during V7. Tassels of Inbred B plants responded similarly to the low DLI during V6. To identify the low DLI threshold (number of d) the previously mentioned low DLI treatment conditions were applied at V6 on Inbred B for 0, 1, 2, 3, 4, 5, 6, or 7 d. Tassel height was shorter for plants that received 5 (by 9.2 cm), 6 (by 11.8 cm), or, 7 (by 11.8 cm) d of low DLI stress during the V6 developmental stage compared to the untreated control. These results indicate that low DLI stress impacts tassel morphology of the two inbred lines tested during specific growth stages for 7 d. Inbred A was most susceptible during V7, while Inbred B was most susceptible during V6. These results

highlight the importance of providing supplemental lighting if the DLI falls below 23.7 $mol \cdot m^{-2} \cdot d^{-1}$ for more than a week during tassel development.

Introduction

Maize *(Zea mays* L.*)*, is a leading agronomic crop around the world. In the United States in 2016, 28% of the harvested field crops were corn, second only to soy (U.S. Department of Agriculture, 2017). Maize arose through the domestication of teosinte, which is indigenous to the tropical regions of Central and Latin America (Goodman, 1999; Matsuoka et al., 2002; van Heerwaarden et al., 2011). Although maize has been predominately grown in outdoor fields during the summer in temperate regions, breeding practices have increased the need for winter production as well. Relative maturity of maize hybrids can vary from 95 to 120 d (Dwyer et al., 1999); therefore, maize production within a controlled environment is invaluable to breeders who are often limited to one growing season per year in the field (Ceccarelli, 2015). Greenhouse production allows for continual growing seasons from year to year. Photosynthesis of maize saturates at 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of light, deeming this a high-light requiring plant (Fletcher et al., 2008). Using supplemental light in greenhouses make this achievable, especially during the light limiting winter months.

Maize tassel morphology is determined by genetics and influenced by environmental factors. The size and shape of the tassel dictates the pollen production potential for the plant (Vollbrecht et al., 2005). Furthermore, pollinations require a minimum quality and quantity of pollen, and if the tassel is small or barren and no pollen is produced the pollination will not succeed (Tollenaar and Dwyer, 1999). As the barren tassel length increases, the pollen potential decreases and the yield potential is diminished. Tassels of maize plants grown in

greenhouses during winter months are observed with anthers which are underdeveloped or not present, leading to lower pollen production decreasing the yield potential of maize plant.

Light is limited in the winter months in the northern regions of the United States (Korczynski et al., 2002). When other environmental stresses are reduced, it is hypothesized that light is the limiting factor in corn yield (Eddy and Hahn, 2010). Because light is one of the most critical growth factors that influence yield, it can be manipulated to increase the yield of maize plants (Blanchard and Runkle, 2011; Tollenaar and Aguilera, 1992). Daily light integral (DLI) is the quantity of photosynthetic light in a day (Korczynski et al., 2002). The greenhouse structure can reduce the solar radiation plants receive by 40% to 60% due to glazing and superstructure shadowing (Eddy and Hahn, 2010). The outdoor DLI in Iowa can range from 10 $mol \cdot m^{-2} \cdot d^{-1}$ during winter months to 50 $mol \cdot m^{-2} \cdot d^{-1}$ during the summer season (Korczynski et al., 2002).

Little work has been done to determine susceptible developmental stages to low DLI stress and the effect on tassel quality. We hypothesize that a reduction in DLI to below 10 $mol \cdot m^{-2} \cdot d^{-1}$ will suppress the anther production of the maize tassel. A shorter tassel height and decreased branch number, along with a longer barren tassel length, would result in a decline of the tassel quality. There were two objectives of this research. The first objective was to quantify the effect of low DLI on tassel morphology across vegetative growth stages, identifying any susceptible stages. The second was to identify a low DLI duration threshold before tassel morphology would be affected.

Materials and methods

Experiment 1 – Developmental stages susceptible to low DLI

Maize inbred lines were obtained from DuPont Pioneer (Inbred A and Inbred B). Both inbred lines are non-stiff stalk (NSS); however, Inbred A is a drought-susceptible line that has a relative maturity (RM) of 103 d and Inbred B is a drought-tolerant line with a shorter RM of 98 d. Furthermore, the tassel morphology of the two lines is diverse, for example, the number of tassel branches grown by Inbred B is half of the number of branches produced by Inbred A.

Maize seed was sown at a 2.5-cm depth into 32-cell flats (90.7-mL individual cell volume) containing a soilless substrate composed of (by vol.) 77% Canadian sphagnum peat, 16% perlite, and 7% vermiculite and adjusted with lime to a pH of 6.1 and irrigated with municipal water supplemented with 125 mg·L^{-1} N (Peters Excel © Cal-Mag © 15N–2.2P–12.5K Everris NA, Marysville, OH). Seeds were germinated and grown in a growth chamber (Model BDW160; Conviron©, Winnipeg, Manitoba), set points were 12-h day length, with day/night air temperature 29.1 °C /21.1 °C and a continuous 65% relative humidity (day/night vapor pressure deficit (VPD) of 1.41 kPa/0.87 kPa). The growth chamber operation and environmental set-points were programmed and maintained with the growth chamber software (CMP6050 V. 4.06; Conviron©, Winnipeg, Manitoba). The air temperatures, humidity levels, and light intensities were measured with temperature probes, humidity probes, and quantum sensors, respectively, that were built into each growth chamber and connected to data loggers. Environmental data are reported in Table 1.

After 2 weeks, seedlings were transplanted into 5.9-L pots containing a soilless substrate composed of (by vol.) 38% Canadian sphagnum peat, 51% composted bark, 8%

perlite, and 3% vermiculite and adjusted with lime to a pH of 6.0. After transplant, plants were irrigated with municipal water supplemented with 95 $mg \cdot L^{-1}$ N using the fertilizer. Two growth chambers were used; a high DLI (control) chamber and a low DLI chamber. The control chamber provided a light intensity of 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from metal halide lamps to maintain a DLI of 23.7 $mol \cdot m^{-2} \cdot d^{-1}$, while the low DLI chamber provided a light intensity of 210 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to maintain a DLI of 9.3 $mol \cdot m^{-2} \cdot d^{-1}$.

For Expt. 1, plants were sown on 13 Feb. in Johnston, IA. Plants were placed into low DLI conditions when 50% of the plants in a treatment reached V4, V5, V6, or V7 [occurring 11, 14, 18 and 24 d after sowing (DAS) respectively]. After 7 d plants were returned to the control chamber. A set of untreated plants remained in the control chamber throughout the entire experiment.

Data were collected 10 d after pollination (DAP) for vegetative traits which included final plant height (from substrate level to the flag leaf junction and the tassel tip) and final ear leaf area index (determined by ear leaf width × ear leaf length) (van Arkel, 1978). First silk emergence date and tassel first shed dates (used to calculate anthesis-silking interval or ASI), ear length, and kernel number running longitudinally and radially were used to calculate average kernel number. After gross measurements were recorded, ears and tassels were harvested for further analyses. Tassel morphology analyses was conducted by taking a digital photo of the tassel, which was held flat and parallel to the camera lens. Tassel length and size were determined using digital images and image-analyzing software (Assess 2.0 Image Analysis Software; CPL Scientific Publishing Services; Newbury, U.K.). Post-harvest tassel analyses included: tassel height (measured from lowest tassel branch node to tassel tip), tassel size (sum of all tassel branch lengths), tassel branch number, barren tassel length

(sum of all sterile branch lengths), and viable tassel length (sum of all viable branch lengths) (Table 3.).

This experiment was designed using a completely randomized design with 12 pseudo-replications (individual plants) per treatment. Analyses of variance (ANOVA) and mean separations were performed by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$ (JMP v. 12, SAS Institute, Cary, NC).

Experiment 2 – Investigation of additional developmental stages susceptible to low DLI

The same inbred lines, growing substrate, growth conditions, and fertilizer methods were utilized as previously described in Expt. 1. Plants were sown 07 July 2015 and followed the same manner as Expt. 1. The treatments were like Expt. 1, testing V4, V5, V6, V7, and an untreated control (occurring 16, 21, 27, and 29 DAS, respectively) in addition to V8, V9, V10, V11, and V12 (occurring 31, 34, 36, 38, and 41 DAS, respectively). Plants were monitored for developmental stage and placed in the low DLI treatment individually for Expt. 2, not as a group as in Expt. 1. Each treatment consisted of 6 pseudo-replications.

In addition to the data collected in Expt. 1, data on total leaf number and ear leaf number (node at which ear developed) were also collected. The statistical analyses of Expt. 2 were identical to Expt. 1.

Experiment 3 – Identifying the low DLI threshold of Inbred B

Only Inbred B was studied, while the growing medium and fertilizer methods were utilized as in Expt. 1. Plants were sown 23 June 2016 in growth chambers. Seeds were germinated and grown, until transplant on day 14, in a greenhouse propagation room. During the first two weeks of growth in a propagation room, set points were 16-h day length, with day/night air temperature of 26.7 °C /23.9 °C and 80% continuous relative humidity [day/night vapor pressure deficit (VPD) of 0.69 kPa/0.58 kPa]. The supplemental light intensity was 260 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by high-pressure sodium and metal halide lamps at a ratio of 1 to 5 respectively, resulting in a total lighting minimum of 15 $mol \cdot m^{-2} \cdot d^{-1}$ DLI (Table 1). At the time of transplant, the plants were moved to the control chamber (same as Expt. 1 and 2). All plants were grown in the high DLI chamber until reaching a specific developmental stage, and plants were moved to the low DLI chamber on an individual plant basis (same as Expt. 2) until the final treatment was carried out. The treatments began when each plant reached V6. Once the plants were moved to the low DLI chamber they remained there for 1, 2, 3, 4, 5, 6, or 7 d, after which time they were returned to the high DLI chamber. A set of untreated plants remained in the high DLI chamber.

In addition to the data collected in Expt. 2 25 DAP, data were also collected on stalk diameter. The statistical analyses of Expt. 3 were identical to Expt. 1, with 7 pseudo-replications (individual plants) per treatment.

Results

Experiment 1 – Developmental stages susceptible to low DLI

Transient low DLI stress, when applied at different developmental stages, affected the growth and development of Inbred A across vegetative, reproductive and tassel traits (Table 2 and 3). Vegetative and reproductive traits unaffected by low DLI include, plant height, ASI, ear length, and kernel number (Table 2). The tassel height was not affected by the low DLI when applied during the developmental stages V4, V5, V6, or V7 (Table 3). Compared to the untreated control, ear leaf area index was less when the low DLI stress was applied during V6 (by 141.7 cm^2) and V7 (by 62.7 cm^2) (Table 2). Fewer tassel branches developed when the low DLI stress occurred during the V4 (by 3.2 branches) and V5 (by 2.4 branches) growth stages, but not during the V6 or V7 stages in comparison to the untreated control (Table 3). Compared to the untreated control, viable tassel length was shorter under low DLI stress applied during V4 (by 27.2 cm) and V7 (by 24.8 cm). Low DLI stress at V7 led to a longer barren tassel length (by 9.2 cm) compared to the untreated control (Table 3).

The growth and development of plants from Inbred B were also affected by the low DLI treatment. Kernel number and ASI were unaffected by low DLI (Table 2). Shorter plant height (by 24.4 cm), ear height (by 12.2 cm), and ear length (by 1.7 cm) were recorded for the V7 developmental stage treatment in comparison to untreated control plants (Table 2). Tassel height was shorter than the untreated control when the treatment was applied during V6 (by 5.8 cm) and V7 (by 4.7 cm). Plants exposed to the low DLI treatment during V4 developed tassels with 3.2 fewer branches than the untreated control plants. Plants treated with low DLI during V6 and V7 resulted in suppressed viable tassel length by 46.4 cm and by 41.2 cm, respectively, and an increased barren region of 13.7 cm and of 6.7 cm,

respectively. The tassel morphology images illustrate the overall reduction in tassel quality in comparison to the untreated control (Fig. 1).

Experiment 2 – Investigation of additional developmental stages susceptible to low DLI

Additional developmental stages were found to be susceptible to low DLI for Inbred A. Compared to the untreated control, plant height was shorter when low DLI treatments were applied during V10 and V12 by 19.9 cm and by 19.5 cm, respectively. Ear leaf area index was smaller by 48.6 cm^2 for the plants treated during V6 when compared to the control (Table.4). Tassel height, tassel branch number, and viable tassel length of the treated plants were unaffected by DLI (Table 5). Tassel barrenness increased by 11 cm when plants were treated with low DLI conditions during V7 compared to the control. Vegetative and reproductive traits that were unaffected for Inbred A in response to the low DLI treatment included: ear leaf number, total leaf number, ASI, ear length, and kernel number (Table 4).

Inbred B was not found to be susceptible to low DLI treatments beyond the V4 to V8 stages. Compared to the untreated control, ear length was shorter by 1.7 cm when treated with low DLI at V7 (Table 4). Inbred B was unaffected by the low DLI stress for tassel height, tassel branch number or viable tassel length (Table 5). Barren tassel length was increased by 13.3 cm for plants treated with low DLI during V6 compared to the control (Table 5). Vegetative and reproductive traits of Inbred B plant that were unaffected by the low DLI stress applied during V4 through V12 growth stages for Inbred B compared to the untreated control included: plant height, ear leaf area index, ear leaf number, total leaf number, ASI, and kernel number (Table 4).

Experiment 3 – Identifying the low DLI threshold of Inbred B

Tassel height was shorter for plants that received 5, 6, or 7 d (by 9.2 cm, 11.8 cm, or 11.8 cm respectively) of low DLI stress during the V6 stage compared to the control (Table 7). Tassel branch number, viable tassel length, and barren tassel length were unaffected when plants were treated for 1 to 7 d of low DLI stress at V6 compared to the control (Table 7). Low DLI stress to Inbred B plants at V6 for 7 d resulted in a smaller stalk diameter by 2.6 cm compared to the control (Table 6). Vegetative traits (plant height, ear height, ear leaf area index, ear leaf number and total leaf number) were unaffected by the low DLI stress when applied during the V6 stage for 1 to 6 d when compared to the untreated control. Reproductive traits (ASI, ear length, and kernel number) were unaffected by the low DLI stress when applied during the V6 developmental stage for 1 to 7 d (Table 6).

Discussion

Plants grown under low DLI conditions produced greater barrenness likely due to diminished anther production compared to those under a higher DLI. Expt. 1 aimed to quantify the effect of low DLI on tassel morphology for developmental stages V4-V7 and, subsequently expanded in Expt. 2, V8-V12 (Expt. 2). Tassel barrenness was greatest for Inbred A when low DLIs were experienced during V7. Some of the traits that were affected under the design for Expt. 1. were unaffected in Expt. 2. The Inbred A plants were affected by low DLI across both experiments showing that ear leaf area index was smaller (V6), and barren tassel length grew (V7). Traits that were affected across both experiments for Inbred B was a shorter ear length (V7) and a longer barren tassel length (V6) (Tables 2-5). Shorter ear length under low light conditions was also documented for maize plants grown under 70% shade (30% transmittance) during vegetative growth in the field (Earley et al., 1966),

and for maize plants grown under 50% shade during reproductive growth stages (Zhou et al., 2013). Barren tassel length was longer after 7 d of low DLI stress for both Inbred A (V7) and Inbred B (V6). This tassel stress response during these vegetative growth stages is supported with literature that reports tassel morphology and development occurring between V5-V7, making the tassel highly susceptible to stress during these stages (Ritchie et al. 1992; Phillips et al., 2011). Fewer tassel branches and the decreased number of anthers on maize plants treated with 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a 12 h photoperiod (a DLI of 2.8 $mol \cdot m^{-2} \cdot d^{-1}$) at V6 and V7 has been previously documented (Bechoux et al., 2000). Our research supports these findings, with a 16 h photoperiod and light intensity of 260 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (a DLI of 14.9 $mol \cdot m^{-2} \cdot d^{-1}$), indicating that the reduction of DLI during vegetative growth stages diminishes tassel quality by increasing the barren tassel length and lowering the pollen production potential.

In addition to quantifying the impact of DLI on tassel morphology, we aimed to understand the effects of the duration of low DLI stress on tassel quality. More than 5 d of low DLI at vegetative stage 6 suppressed the tassel height of Inbred B (Table 7). Tassel height was also shorter in field maize under shaded conditions (50% transmittance) during VT and reproductive growth stages compared to non-shaded plants (Zhou et al., 2013). Tassel development at V6 (Ritchie et al. 1992; Phillips et al., 2011) increases the susceptibility of the tassel to environmental stress such as low DLI, supporting the hypothesis that high-light is an important factor for tassel quality. After 7 d of low DLI stress at V6 the stalk diameter of Inbred B was smaller compared to the control (Table 6). Previous work on the effect of low light intensity during vegetative growth of field grown maize demonstrated

that the stalk diameter above the primary ear became smaller as light is decreased (Earley et al., 1966). This positive relationship between stem diameter and DLI agrees with our results.

For both inbred lines, there were three traits that never responded to the low DLI stress across developmental stages (V4 – V12): ASI, total leaf number, and kernel number. Low light has been documented to decrease ear row number and kernel number (Zhou et al., 2013), which disagrees with our findings that the kernel number was unaffected by low DLI conditions. The discrepancy between these studies may be due to experimental design, their low light treatments were applied during VT and reproductive growth stages, which is later than any stage we tested.

Conclusions

Maize plants negatively respond to low DLI stress in several ways. As the number of days of low DLI stress increased, tassel height was suppressed for Inbred B, producing the shortest tassel under 7 d of low DLI stress (Table 7). The growth and development of Inbreds A and B under limited light conditions demonstrates that genetics also influences the responses. Both inbred lines responded with longer barren tassel lengths (Table 5). Tassel barrenness of Inbred A is most susceptible to low DLI stress during V7, while Inbred B is most susceptible during V6. This supports our hypothesis that the timing of the low DLI stress is critical to tassel quality. As barren tassel length increases the pollen potential of the plant decreases. The lack of anthers present would lead to a decrease in pollen available for pollination. The kernel yield for both farmers and breeders would suffer if relying only on the pollen from the barren tassel, such is the case with breeding programs crossing two genetic lines. This research demonstrates the importance of low DLI stress on plant quality,

and more specifically tassel morphology. With only 7 d of low DLI stress, the plant loses yield potential. In production growing settings, low DLI stress is not conveniently limited to 7 d, but rather a cyclic stress which could amplify the negative impact of the low DLI stress. To better alleviate this problem, environmental conditions must be optimized for each growing season, such as low light winter months.

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Tables and figures

Table 1. Photoperiod and daily light integral (DLI) for each growth environment, and set points for light intensity, day/night air temperatures, and average daily temperature (ADT) of the control chamber, the low DLI chamber, and the propagation room.

Treatment	Photoperiod (h)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Day temperature (°C)	Night temperature (°C)
Control chamber	12	23.7	550	29.1	21.1
Low DLI chamber	12	9.3	210	29.1	21.1
Propagation room	16	15.0	260	26.7	23.9

Table 2. Vegetative and reproductive traits for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 7 d, when 50% of plants reached V4, V5, V6, or V7, and an untreated control was included. Data were collected 10 d after pollination (DAP). Each treatment consisted of 12 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test.

	Vegetative traits			Reproductive traits		
Treatment	Plant height (cm)	Ear height (cm)	Ear leaf area index (cm^2)	ASI (d)	Ear length (cm)	Kernel (no.)
			Inbred A			
V4	217.8	-	826.8 ab[z]	-0.9	20.9	380.1
V5	208.6	-	856.5 a	-0.9	19.2	335.0
V6	200.9	-	713.2 c	-0.9	20.3	336.0
V7	194.1	-	792.2 b	-1.2	18.9	300.5
Control	210.7	-	854.9 a	-1.9	19.9	364.5
P-value	NS[y]	-	***	NS	NS	NS
			Inbred B			
V4	183.5 a	94.2 a	580.0 a	0.4	16.7 a	404.6
V5	175.4 ab	87.4 ab	526.6 ab	0.3	17.5 a	409.5
V6	176.1 ab	89.7 ab	437.2 b	0.7	16.5 ab	390.0
V7	161.7 b	80.9 b	493.1 ab	0.6	15.5 b	381.7
Control	186.1 a	93.1 a	567.3 a	0.3	17.2 a	434.6
P-value	*	*	**	NS	***	NS

[z]Means within columns that share letters are similar by Tukey's HSD test at $P \leq 0.05$.

[y]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 3. Tassel height, branch number, viable length and barren length for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 7 d, when 50% of plants reached V4, V5, V6, or V7, and an untreated control was also included. Data were collected 10 d after pollination (DAP). Each treatment consisted of 12 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test.

Treatment	Height (cm)	Branch (no.)	Viable length (cm)	Barren length
		Inbred A		
V4	30.5	8.5 b[z]	75.8 b	1.2 b
V5	31.8	9.3 b	91.0 ab	1.4 b
V6	32.7	11.8 a	103.9 a	2.1 b
V7	30.4	11.7 a	78.2 b	10.9 a
Control	31.2	11.7 a	103.0 a	1.7 b
P-value	NS[y]	***	***	***
		Inbred B		
V4	36.7 a	4.1 b	76.4 b	0.9 d
V5	34.7 a	7.0 a	81.5 b	8.3 bc
V6	30.1 b	6.1 ab	54.5 c	15.8 a
V7	31.2 b	7.8 a	59.7 c	8.8 b
Control	35.9 a	7.3 a	100.9 ab	2.1 cd
P-value	***	***	**	*

[z]Means within columns that share letters are similar by Tukey's HSD test at $P \leq 0.05$.
[y]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 4. Vegetative and reproductive traits for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 7 d, when individual plants reached V4 - V12, and an untreated control was also included. Data were collected 10 d after pollination (DAP). Each treatment consisted of 6 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test.

	Vegetative traits				Reproductive traits		
Treatment	Plant height (cm)	Ear leaf area index (cm^2)	Ear leaf (no.)	Total leaf (no.)	ASI (d)	Ear length (cm)	Kernel (no.)
				Inbred A			
V4	252.3 a[z]	850.9 ab	12.0	18.2	1.3	20.9	409.3
V5	256.6 a	867.3 a	12.2	18.5	0.8	19.9	448.0
V6	252.6 a	769.8 b	12.2	19.0	0.7	19.5	406.3
V7	245.4 a	819.0 ab	11.7	18.0	1.5	20.1	433.7
V8	245.9 a	877.1 a	12.0	18.5	1.7	18.0	421.3
V9	240.5 ab	873.9 a	12.2	18.5	1.8	20.2	390.0
V10	227.8 b	858.8 a	12.0	18.5	1.3	19.0	428.3
V11	241.3 ab	855.3 ab	11.7	18.5	1.8	20.3	432.3
V12	228.2 b	818.7 ab	11.8	18.0	1.7	19.9	401.7
Control	247.7 a	818.4 ab	12.2	18.7	1.5	18.5	432.7
P-value	***[y]	**	NS	NS	NS	NS	NS

Table 4. continued

				Inbred B			
V4	210.3	649.4 ab	10.3	15.5	-0.2	17.4 a	340.0
V5	217.3	590.0 c	10.2	15.1	-0.5	16.7 a	256.0
V6	221.1	593.0 bc	10.6	15.5	-0.5	16.5 ab	267.7
V7	214.0	649.9 ab	10.6	16.0	-0.2	14.9 b	297.3
V8	212.7	666.6 a	10.3	15.3	-0.2	15.9 ab	340.3
V9	213.3	627.8 abc	10.6	15.5	-0.2	16.5 ab	333.7
V10	204.4	664.6 a	10.0	15.0	-0.3	17.3 a	372.7
V11	200.1	663.0 a	10.0	15.1	-0.5	16.5 ab	362.7
V12	210.8	635.7 abc	10.8	15.3	-0.2	17.2 a	335.3
Control	215.1	638.7 abc	10.3	15.4	-0.2	16.6 a	325.0
P-value	NS	***	NS	NS	NS	***	NS

[z]Means within columns that share letters are similar by Tukey's HSD test at $P \leq 0.05$.

[y]NS, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

Table 5. Tassel height, branch number, viable length and barren length for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 7 d, when individual plants reached V4 - V12, and an untreated control was also included. Data were collected 10 d after pollination (DAP). Each treatment consisted of 6 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test.

Treatment	Height (cm)	Branch (no.)	Viable length (cm)	Barren length (cm)
		Inbred A		
V4	32.7	9.0	116.3	2.2 c[z]
V5	34.9	10.8	155.4	5.3 bc
V6	34.5	11.5	136.1	3.5 c
V7	35.2	12.2	138.2	17.2 a
V8	33.4	11.5	124.5	12.1 ab
V9	34.1	11.7	129.2	8.8 bc
V10	35.6	9.7	127.7	5.6 bc
V11	33.2	9.5	106.9	1.9 c
V12	33.4	12.0	141.6	4.5 bc
Control	34.1	11.7	137.1	6.2 bc
P-value	NS[y]	NS	NS	**
		Inbred B		
V4	35.6	4.5	88.9	2.5 bc
V5	32.5	3.8	76.0	3.7 bc
V6	31.4	4.5	53.6	14.3 a
V7	31.5	3.8	56.7	8.6 ab
V8	32.1	6.0	100.2	3.1 bc
V9	35.8	5.7	95.2	2.1 bc
V10	34.1	4.3	87.4	2.5 bc
V11	35.1	6.3	104.2	2.6 bc
V12	35.0	4.0	91.1	0.3 c
Control	34.2	4.2	88.8	1.0 bc
P-value	NS	NS	NS	***

[z]Means within columns that share letters are similar by Tukey's HSD test at $P \leq 0.05$.

[y]NS, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

Table 6. Vegetative and reproductive traits for maize (*Zea mays* L.) inbred line B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 0-7 d, when individual plants reached V6, or grown continuously in high DLI (control). Data collected 25 d after pollination (DAP). Each treatment consisted of 6 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test.

	Vegetative traits						Reproductive traits		
Treatment (d)	Plant height (cm)	Ear height (cm)	Ear leaf area index (cm^2)	Ear leaf (no.)	Total leaf (no.)	Stalk diameter (cm)	ASI (d)	Ear length (cm)	Kernel (no.)
					Inbred B				
0	264.1	125.0	606.8	12.5	18.8	19.2 a[z]	-2.5	16.6	196.0
1	269.9	127.4	635.6	12.4	18.8	18.2 ab	-0.4	16.1	235.4
2	255.5	120.1	621.4	12.4	19.0	17.7 ab	-0.4	16.3	213.4
3	256.5	112.6	611.7	12.2	19.1	18.6 ab	-0.2	18.2	226.5
4	259.4	122.2	598.5	12.8	18.8	17.2 ab	0.7	18.5	314.5
5	269.0	125.1	632.7	12.5	19.1	17.5 ab	-0.7	18.4	282.0
6	246.8	118.2	601.2	12.8	19.0	17.1 ab	0.1	16.9	274.8
7	247.0	115.8	592.8	12.5	19.0	16.6 b	-0.7	18.0	245.4
P-value	NS[y]	NS	NS	NS	NS	**	NS	NS	NS

[z]Means within columns that share letters are similar by Tukey's HSD test at $P \leq 0.05$.

[y]NS, ** Nonsignificant or significant at $P \leq 0.01$ respectively.

Table 7. Tassel height, branch number, viable length and barren length for maize (*Zea mays* L.) inbred line B grown under low (~9 $mol \cdot m^{-2} \cdot d^{-1}$) daily light integral (DLI) for 0-7 d, when individual plants reached V6, or grown continuously in high DLI (control). Data collected 25 d after pollination (DAP). Each treatment consisted of 6 pseudo-replications (individual plants) and mean separations were performed using Tukey's honestly significant difference (HSD) test.

Treatment (d)	Height (cm)	Branch (no.)	Viable length (cm)	Barren length (cm)
		Inbred B		
0	57.2 a[z]	9.0	82.8	6.8
1	52.3 ab	6.8	66.9	8.1
2	48.6 ab	8.1	69.5	5.6
3	53.2 ab	7.4	75.7	7.5
4	52.6 ab	9.0	76.2	5.1
5	48.0 b	7.5	75.2	3.4
6	45.4 b	8.5	71.1	3.1
7	45.4 b	9.2	77.2	3.2
P-value	***[y]	NS	NS	NS

[z]Means within columns that share letters are similar by Tukey's HSD test at $P \leq 0.05$.
[y]NS, *** Nonsignificant or significant at $P \leq 0.001$ respectively.

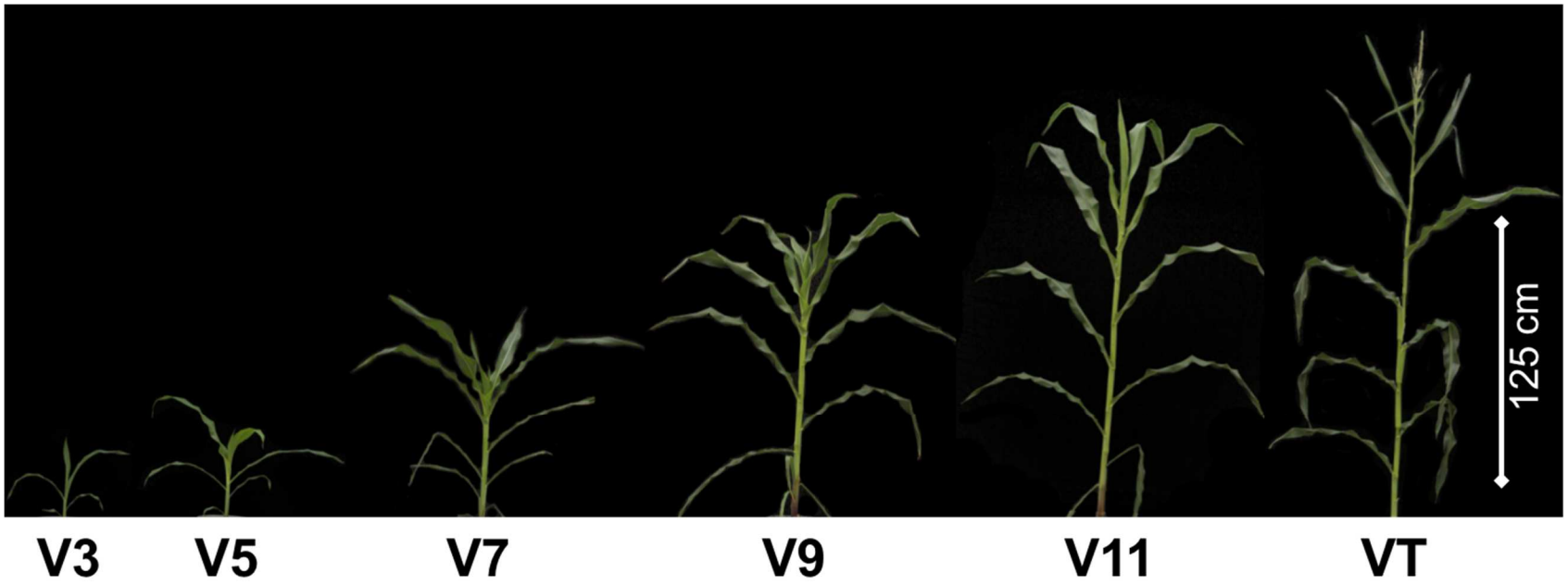

Figure 1. Inbred A maize (*Zea mays* L.) plants throughout vegetative (V) growth stages: V3, V5, V7, V9, V11, and visible tassel (VT).

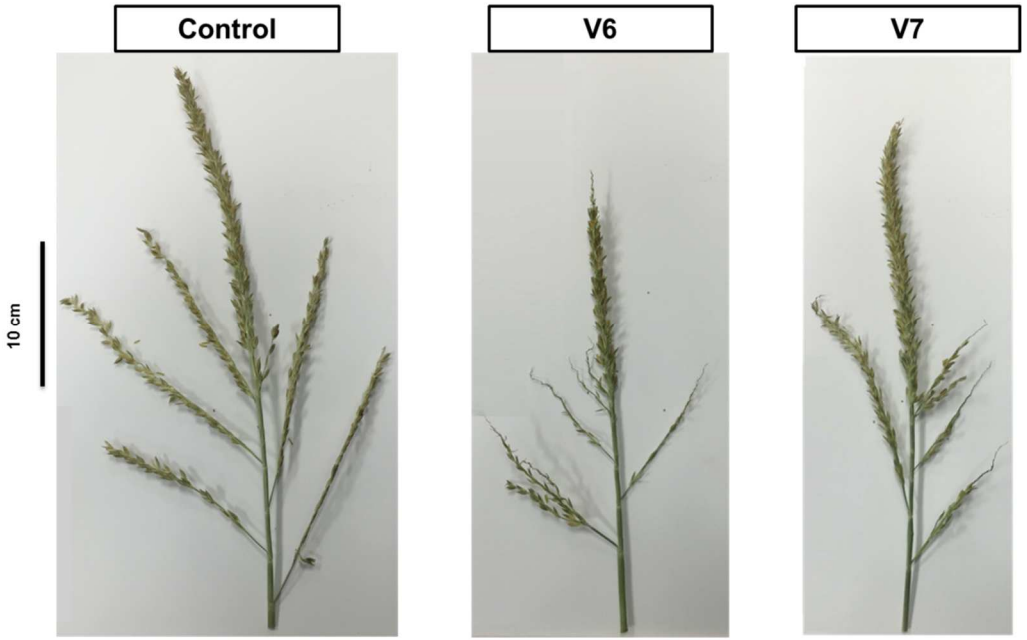

Figure 2. Tassel height, branch number, viable length and barren length for maize (*Zea mays* L.) inbred lines A and B grown under low (~9 mol·m^{-2}·d^{-1}) daily light integral (DLI) for 7 d, when 50% of plants reached V4, V5, V6, or V7, and an untreated control was also included. Data were collected 10 d after pollination (DAP). Tassels imaged above were treated during V6 and V7 compared to the untreated control.

CHAPTER 3. LONGER PHOTOPERIOD DURING VEGETATIVE OR REPRODUCIVE GROWTH SUPPRESSES BARREN TASSEL LENGTH

Abstract

The objective of this research was to quantify the growth and tassel morphology of two maize (*Zea mays* L.) inbred lines under photoperiods of different lengths during vegetative or reproductive growth. Inbreds A (drought-susceptible line with a highly-branched tassel) and B (drought-tolerant line with a less-branched tassel) were grown under photoperiods varying in length during vegetative stages (20 or 22 h) and reproductive stages (16, 20, or 22 h) in a factorial design. Vegetative photoperiod treatments began at vegetative stage 2 (V2) and continued until visible tassel (VT) stage. Plants were transferred to reproductive photoperiod treatments at VT through pollination, and final data collection took place 25 d after pollination (DAP). Both inbred lines were more susceptible to a longer photoperiod during vegetative growth than reproductive growth. Compared to the 16 h photoperiod, tassel height elongated by 4.3 cm, while viable tassel length, tassel branch number, and barren tassel length suppressed by 89.3 cm, by 4.5 branches, and by 23.6 cm respectively, with a longer photoperiod during vegetative growth for Inbred A. For Inbred B, tassel branch number was less, and barren tassel length was shorter by 3.5 branches and 19.1 cm, respectively, under a 20 or 22 h photoperiod during vegetative growth compared to 16 h photoperiod. Only the tassels of Inbred A were susceptible to a longer photoperiod during reproductive growth, with a 1.4 cm longer tassel height from 16 to 20 h, and a 4.8 cm shorter tassel height from 16 to 22 h, and a 6.2 cm shorter from 20 to 22 h. These results indicate that a longer photoperiod can alter the tassel morphology of the two inbred lines during either

vegetative or reproductive growth stages. Furthermore, this information provides a better understanding of how Inbreds A and B grow under a longer photoperiod during vegetative and reproductive growth.

Introduction

In the United States in 2016, 28% of the harvested field crop acres were corn, second only to soy (U.S. Department of Agriculture, 2017). Maize seed production and trait introgression is performed in both field and greenhouse settings. The use-of a controlled environment or greenhouse allows for regulated production of traited material and facilitates winter production. In winter production of maize in a greenhouse or controlled environment, the success of a pollination depends on the availability of pollen. Open pollinations occur in the field, while hand pollinations are required in the greenhouses to maintain trait purity standards.

Tassel morphology is predetermined genetically and influenced by environmental factors. The size and shape of the tassel dictates the pollen potential for the plant (Vollbrecht et al., 2005). Furthermore, pollinations require a minimum quality and quantity of pollen, and if the tassel is small or barren and no pollen is produced, the pollination will not succeed (Tollenaar and Dwyer, 1999). As the barren tassel lengthens, the pollen potential decreases and the yield potential diminishes. Tassels of maize plants grown in greenhouses during winter months are observed with anthers which are underdeveloped or not present, leading to a reduction in pollen production decreasing the yield potential of maize plant.

Greenhouses provide control over environmental factors such as temperature, moisture, nutrients, gases, and light. In a greenhouse, water and nutrient are prescribed and

heat is managed, while control over gasses isn't required for maize because it is a C4 plant. Being a C4 plant, the optimum photosynthesis occurs at lower CO_2 concentrations and warmer temperatures compared to C3 plants (Crafts-Brandner and Salvucci, 2002). The fifth factor, light, is at seasonally low levels in the winter months in the northern regions of the United States (Korczynski et al., 2002). Light is a limiting factor of corn yield in the winter, when all other environmental factors are supplied in abundance (Eddy and Hahn, 2010). Because maize requires high-light, it should be optimized during the winter to increase the yield potential of maize plants (Blanchard and Runkle, 2011; Tollenaar and Aguilera, 1992). Daily light integral (DLI) is the cumulative measurement of the photosynthetic light over a day (Korczynski et al., 2002). Greenhouse superstructures and glazing can reduce the solar radiation plants receive by 40% to 60% (Eddy and Hahn, 2010). The DLI requirement of corn is 20 $mol \cdot m^{-2} \cdot d^{-1}$ and Iowa outdoor DLIs range from ~10 $mol \cdot m^{-2} \cdot d^{-1}$ during winter months, to 50 $mol \cdot m^{-2} \cdot d^{-1}$ during summer (Korczynski et al., 2002). The seasonally low DLI in the winter months creates the need to use lamps for supplemental photosynthetic light.

Growers can increase their supplemental DLI by operating their lamps longer but increasing the operation time of lamps will also increase the photoperiod, or day length. Understanding how plants respond to photoperiod is essential before increasing or decreasing the day length. For example, flowering is a photoperiodic response for many plants, and growers utilize this as a tool to optimize the timing of their crops such as soybean (Purcell et al., 2014). Though photoperiod influences flowering time in soybeans, the influence of photoperiod on maize is less clear. In maize, the anthesis-silking interval (ASI) determines whether the plant can successfully self-pollinate, and longer photoperiods are documented

having increased the ASI of some crops, which decreases the pollination success (Warrington and Kanemasu, 1983).

The objective of this research was to quantify the growth and tassel morphology of two maize inbred lines grown under a longer photoperiod during either vegetative or reproductive growth. We hypothesize that extending the day length (photoperiod) will lead to diminished tassel barrenness.

Materials and methods

All experiments were conducted using two maize inbred lines obtained from DuPont Pioneer (Inbred A and Inbred B). Both inbred lines are non-stiff stalk (NSS), but Inbred A is a drought-susceptible line and Inbred B is a drought-tolerant line. Furthermore, the tassel morphology of the two lines is diverse; the number of tassel branches grown by Inbred B is half of the number of branches produced by Inbred A.

Maize seed was sown at a 2.5-cm depth into 32-cell flats (90.7-mL individual cell volume) containing a soilless substrate composed of (by vol.) 77% Canadian sphagnum peat, 16% perlite, and 7% vermiculite, adjusted with lime to a pH of 5.9 and irrigated with municipal water supplemented with 125 $mg \cdot L^{-1}$ N (Peters Excel © Cal-Mag © 15N–2.2P–12.5K Everris NA, Marysville, OH). Seeds were germinated and grown in the propagation room for the first 14 d; with a 16-h day length, with day/night air temperature and continuous humidity set points of 26.7 /23.9 °C, 80% relative humidity [day/night vapor pressure deficit (VPD) of 0.69 /0.58 kPa] and a supplemental light intensity of 260 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by high-pressure sodium and metal halide lamps at a ratio of 1 to 5 respectively, resulting in a supplemental lighting minimum of 15 $mol \cdot m^{-2} \cdot d^{-1}$ DLI (Table 1).

On 07 July 2016, two weeks after sowing, seedlings were transplanted into 5.9-L pots containing a soilless substrate composed of (by vol.) 38% Canadian sphagnum peat, 51% composted bark, 8% perlite, and 3% vermiculite, adjusted with lime to a pH of 6.1. At transplant, plants were transferred to one of three growth chambers (Model BDW160; Conviron©, Winnipeg, Manitoba), irrigated with municipal water supplemented with 95 $mg \cdot L^{-1}$ N (Peters Excel © Cal-Mag © 15N–2.2P–12.5K Everris NA). The environment across all three chambers had the same day/night air temperature set points of 29.1 /21.1 °C, and 65% relative humidity held constant (day/night VPD of 1.41 /0.87 pKa). As the photoperiod lengthened, so too did the hours of day temperature. The light intensity of all three chambers was adjusted to maintain a DLI of 23.04 $mol \cdot m^{-2} \cdot d^{-1}$, regardless of photoperiods. Photoperiod and light intensity were set in each chamber as follows: 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h day, 320 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 20-h day, or 291 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 22-h day. The growth chamber operation and environmental set-points were programmed and maintained with the growth chamber software (CMP6050 version 4.06; Conviron©, Winnipeg, Manitoba). The air temperatures, humidity levels, and light intensities were measured with temperature probes, humidity probes, and quantum sensors, respectively, that were built into each growth chamber and connected to data loggers. Plants were grown from vegetative stage 2 (V2) until visible tassel (VT), defined as the "vegetative" growth period in each of the chambers, then transferred between chambers with an equal distribution. The "reproductive" growth period began after this plant movement and continued through maturation and harvest (Abendroth et al., 2011).

Developmental stages were monitored, and V-stages were recorded weekly throughout the duration of the experiment to determine when to apply the photoperiod

treatments (data not shown). Final data collection and harvest occurred on 30 September 2016 in growth chambers. Data were collected 25 DAP for vegetative traits, which included final plant height (from substrate level to the flag leaf junction and the tassel tip), final ear leaf area index (determined by ear leaf width × ear leaf length) (van Arkel, 1978), and stalk diameter at the node subtending the primary ear. Also recorded were reproductive traits, including first silk emergence date and tassel first shed dates (used to calculate anthesis-silking interval or ASI), ear length, and kernel number running longitudinally and radially were used to calculate average kernel number. After gross measurements were recorded, ears and tassels were harvested for further analyses. Tassel morphology analyses were conducted by taking a digital photo of the tassel, which was held flat and parallel to the camera lens. Tassel length and size were determined using digital images and image-analyzing software (Assess 2.0 Image Analysis Software; CPL Scientific Publishing Services; Newbury, U.K.). Tassel analyses included: post-harvest tassel height (measured from lowest tassel branch node to tassel tip), post-harvest tassel size (sum of all tassel branch lengths), post-harvest tassel branch number, post-harvest barren tassel length (sum of all sterile branch lengths), and post-harvest tassel viable tassel length (sum of all viable branch lengths).

This experiment was designed using a completely randomized design. Each treatment consisted of 6 pseudo-replications (individual plants). Vegetative photoperiod (three levels) and reproductive photoperiod (three levels) were studied. Due to a reproductive photoperiod treatment failure, only 2 levels (20 and 22 h) were analyzed. Analyses of variance (ANOVA) and mean separations were performed by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$ (JMP v. 12, SAS Institute, Cary, NC).

Results

Inbred A

Inbred A plants exposed to a longer vegetative photoperiod produced a longer ear leaf length, a larger ear leaf area index, and a taller plant height by 4.6 cm, 56.3 cm^2, and 13.6 cm, respectively (Table 4). Ear leaf number and total leaf number lessened as the vegetative photoperiod grew longer (Table 4). Reproductive traits lengthened as the photoperiod increased from 20 to 22 h during vegetative growth, which included ear length (by 3.4 cm) and tassel height (by 4.3 cm) (Table 5), while traits such as time to silk and shed, and barren tassel length were suppressed.

Traits that were influenced by photoperiod during the reproductive growth stages, included: ear leaf width, ear leaf area index, stalk diameter, and tassel height (Table 2). As reproductive photoperiod increased from 16 to 22 h, ear leaf width, ear leaf area index, and tassel height was less, by 2.4 cm, 243.8 cm, and 6.2 cm, respectively (Tables 6 and 7). Conversely, stalk diameter enlarged by 0.9 cm as the reproductive photoperiod increased from 16 to 22 h (Table 6). As photoperiod increased from 16 to 22 h during reproductive growth, barren tassel length was shorter when grown under a 20 h day compared to a 22 h day during vegetative growth stages (Table 8).

Inbred B

As photoperiod grew longer during vegetative growth stages, total leaf number was fewer by 0.3, while ear leaf length, plant height, stalk diameter, and ear height all grew, by 8.1 cm, 29.4 cm, 1.0 cm, and 10.7 cm, respectively (Table 4). Reproductive traits that grew as photoperiod grew from 20 to 22 h during vegetative growth stages were ear length (by 7.1

cm) and kernel number (by 283.7). Tassel branch number and barren tassel length were both less than the control as the reproductive photoperiod grew, by 3.5 branches and 19.1 cm (Table 5). Stalk diameter enlarged by 2.0 cm as photoperiod grew longer from 16 to 20, and 16 to 22 h, during reproductive growth stages (Table 6).

Discussion

Inbreds A and B were more susceptible to a longer photoperiod during vegetative growth than reproductive growth (Table 2 and 3). Furthermore, none of the tassel or ear traits were affected by a longer photoperiod during reproductive growth. Tassel morphology and ear architecture are already developing by the time the plant reaches VT (Bechoux et al., 2000). The longer ear leaf length, ear length, and plant height can all be attributed to lower light intensities under the longer photoperiods during vegetative growth (Moe and Heins, 1990; Bechoux et al., 2000). The similar response of these traits to the longer photoperiods of both inbred lines suggests that the responses are conserved through the genetics of the two inbred lines.

Maize is a high-light plant, with photosynthesis saturating at ~2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fletcher et al., 2008). Tassel and ear structures are growing and developing during the vegetative growth stages, making these organs vulnerable to environmental stress (Bechoux et al., 2000). Flowering time hastened as the photoperiod grew longer during vegetative growth stages for Inbred A (Table 2 and 3). Although flowering occurred earlier, ASI was unaffected because the interval between the silk and pollen shed dates were conserved.

In maize, flowering time is reportedly hastened by both photoperiod and temperature (Bechoux et al., 2000; Craufurd and Wheeler, 2009). It is important to note that in this study

the average daily temperature (ADT) increased as photoperiod increased (Table 1). The difference between the lowest (25.8 °C) and highest (28.4 °C) average daily temperatures were less than 3 °C, yet it is still worth considering as a factor that could speed up the response of the flowering traits (Craufurd and Wheeler, 2009). With this study, however, we did not observe a negative impact on ASI due to longer photoperiods, which gives us more confidence to use day length extension to increase DLI without negatively altering the ASI.

For Inbred A plants, tassel height increased as the reproductive photoperiod increased from 16 to 20 h but was suppressed at 22 h. Leaf damage has been documented when the plants have been exposed to continuous (24 h) lighting (Eddy and Hahn, 2010). It is possible that a 22 h day length is too long for the maize plant and may explain the severe deformity of the leaves to be caused by calcium deficiency symptoms (Eddy and Hahn, 2010).

During the domestication of maize, adaptation to longer day lengths was required to move the species from tropical to temperate regions of the Americas (Goodman, 1988; Gouesnard et al., 2002; Matsuoka et al., 2002; van Heerwaarden et al., 2011; Xu et al., 2012). Tropical maize is taller with more leaves and later flowering when day lengths are >13 h compared to temperate maize (Warrington and Kanemasu, 1983). Our research found that Inbreds A and B were not as susceptible to longer photoperiods during reproductive growth. With photoperiod playing a role in plant quality to varying degrees depending on genetics, it is important to quantify the effects of photoperiod on different genetic backgrounds.

Conclusions

This study has demonstrated that Inbreds A and B are susceptible to longer photoperiods during vegetative growth stages, but less so during reproductive growth stages.

Caution must be used when increasing photoperiod above 20 h during reproductive growth stages due to the negative plant responses such as a reduction in ear leaf width and area index, along with a shorter tassel for Inbred A. Increasing lamp operation time to increase the DLI in greenhouses can be used during light-limiting seasons in northern climates, but the developmental growth stages should be taken into consideration to limit negative responses to a longer day length.

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Tables and figures

Table 1. Photoperiod and daily light integral (DLI) for each growth environment, and actual values for light intensity, day/night air temperatures, and average daily temperature (ADT) of the treatment chambers (20 and 22 h), the control chamber (16 h), and the propagation room.

Treatment	Photoperiod (h)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Day temperature (°C)	Night temperature (°C)	ADT (°C)
16 h chamber	16	23.0	400	29.1	21.1	26.4
20 h chamber	20	23.0	320	29.1	21.1	27.8
22 h chamber	22	23.0	291	29.1	21.1	28.4
Propagation room	16	15.0	260	26.7	23.9	25.8

Table 2. Analyses of variance of vegetative (V) photoperiod (20 or 22 h) and reproductive (R) photoperiod (16, 20, or 22 h), and their interactions, on both vegetative and reproductive traits of maize (*Zea mays* L.) Inbred A (drought-susceptible line with a highly-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants).

Parameter	V	R	V × R
Time to silk (d)	*[z]	NS	NS
Time to shed (d)	***	NS	NS
ASI (d)	NS	NS	NS
Ear leaf length (cm)	*	NS	NS
Ear leaf width (cm)	NS	***	NS
Ear leaf area index (cm^2)	**	***	NS
Ear leaf (no.)	***	NS	NS
Leaf (no.)	***	NS	NS
Plant height (cm)	*	NS	NS
Stalk diameter (cm)	NS	*	NS
Ear length (cm)	***	NS	NS
Ear height (cm)	NS	NS	NS
Kernel (no.)	NS	NS	NS
Tassel height (cm)	**	**	NS
Viable tassel length (cm)	***	NS	NS
Tassel branch (no.)	***	NS	NS
Barren tassel length (cm)	***	NS	**

[z]NS, *, **, *** = Nonsignificant or significant at $P \leq 0.05$, or, 0.01, or 0.001, respectively.

Table 3. Analyses of variance of vegetative (V) photoperiod (20 or 22 h) and reproductive (R) photoperiod (16, 20, or 22 h), and their interactions, on both vegetative and reproductive traits of maize (*Zea mays* L.) Inbred B (drought-tolerant line with a less-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants).

Parameter	V	R	V × R
Time to silk (d)	NS[z]	NS	NS
Time to shed (d)	NS	NS	NS
ASI (d)	NS	NS	NS
Ear leaf length (cm)	***	NS	NS
Ear leaf width (cm)	NS	NS	NS
Ear leaf area index (cm^2)	NS	NS	NS
Ear leaf (no.)	NS	NS	NS
Leaf (no.)	**	NS	NS
Plant height (cm)	***	NS	NS
Stalk diameter (cm)	*	***	NS
Ear length (cm)	***	NS	NS
Ear height (cm)	*	NS	NS
Kernel (no.)	***	NS	NS
Tassel height (cm)	NS	NS	NS
Viable tassel length (cm)	NS	NS	NS
Tassel branch (no.)	***	NS	NS
Barren tassel length (cm)	**	NS	NS

[z]NS, *, **, *** = Nonsignificant or significant at $P \leq 0.05$, or, 0.01, or 0.001, respectively.

Table 4. The effect of photoperiod (20 or 22 h) during vegetative growth on vegetative traits for both maize (*Zea mays* L.) Inbreds A (drought-susceptible line with a highly-branched tassel) and B (drought-tolerant line with a less-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants). Data are pooled across reproductive photoperiods.

Parameter	Vegetative photoperiod (h)		
	20	22	Sig.
		Inbred A	
Ear leaf length (cm)	93.8	98.4	*[z]
Ear leaf area index (cm^2)	757.9	814.2	**
Ear leaf (no.)	14.5	13.4	***
Leaf (no.)	21.4	20.6	***
Plant height (cm)	261.4	275.0	*
		Inbred B	
Ear leaf length (cm)	75.5	83.6	***
Leaf (no.)	18.9	18.6	**
Plant height (cm)	253.5	282.9	***
Stalk diameter (cm)	15.5	16.5	*
Ear height (cm)	143.2	153.9	*

[z]*, **, *** = Significant at $P \leq 0.05$, or, 0.01, or 0.001, respectively.

Table 5. The effect of photoperiod (20 or 22 h) during vegetative growth on reproductive traits for both maize (*Zea mays* L.) Inbreds A (drought-susceptible line with a highly-branched tassel) and B (drought-tolerant line with a less-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants). Data are pooled across reproductive photoperiods.

Parameter	Vegetative photoperiod (h)		
	20	22	Sig.
		Inbred A	
Time to silk (d)	78.2	75.6	*[z]
Time to shed (d)	70.4	65.6	***
Ear length (cm)	12.3	15.7	***
Tassel height (cm)	41.6	45.9	**
Viable tassel length (cm)	397.2	307.9	***
Tassel branch (no.)	19.1	14.6	***
Barren tassel length (cm)	38.5	14.9	***
		Inbred B	
Ear length (cm)	12.7	19.8	***
Kernel (no.)	64.9	348.6	***
Tassel branch (no.)	9.4	5.9	***
Barren tassel length (cm)	21.3	2.2	**

[z] *, **, *** = Significant at $P \leq 0.05$, or, 0.01, or 0.001, respectively.

Table 6. The effect of photoperiod during reproductive growth (16, 20, or 22 h) on vegetative traits for both maize (*Zea mays* L.) Inbreds A (drought-susceptible line with a highly-branched tassel) and B (drought-tolerant line with a less-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants). Data are pooled across vegetative photoperiods.

Parameter	Reproductive photoperiod (h)		
	16	20	22
		Inbred A	
Ear leaf width (cm)	9.1 a[z]	8.7 a	6.7 b
Ear leaf area index (cm^2)	881.0 a	839.9 a	637.2 b
Stalk diameter (cm)	18.8 ab	18.6 b	19.7 a
		Inbred B	
Stalk diameter (cm)	15.0 b	15.9 ab	17.0 a

[z]Means in the same row that share letters are similar by Tukey's HSD test $P \leq 0.05$.

Table 7. The effect of photoperiod during reproductive growth (16, 20, or 22 h) on the tassel height of maize (*Zea mays* L.) Inbred A (drought-susceptible line with a highly-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants). Data are pooled across vegetative photoperiods.

Parameter	Reproductive photoperiod (h)		
	16	20	22
		Inbred A	
Tassel height (cm)	44.9 ab[z]	46.3 a	40.1 b

[z]Means in the same row that share letters are similar by Tukey's HSD test $P \leq 0.05$.

Table 8. The effect of the interaction between vegetative photoperiod (V) and reproductive photoperiod (R) on barren tassel length of maize (*Zea mays* L.) Inbred A (drought-susceptible line with a highly-branched tassel). Data were collected at 25 d after pollination, with n = 6 pseudo-replications (individual plants).

	Vegetative photoperiod (h)		
	Barren tassel length (cm)		
Reproductive photoperiod (h)	20	22	Sig.
16	51.7 a[z]	11.0 a	**[y]
20	43.9 ab	16.4 a	***
22	20.1 b	17.4 a	NS

[z] Means in the same column that share letters are similar by Tukey's HSD test $P \leq$
[y] NS, **, *** = Nonsignificant or significant at $P \leq 0.01$, or 0.001, respectively.

CHAPTER 4. GENERAL CONCLUSIONS

General Discussion

The research disclosed in this thesis makes evident the influence of low daily light integral (DLI) upon maize tassel morphology. A DLI below 10 $mol \cdot m^{-2} \cdot d^{-1}$ for one week increased barren tassel length for both Inbreds A and B, leaving tassels with fewer anthers and limiting the pollen production of those plants. A DLI this low is prevalent in the winter months in Iowa and is likely one of the most limiting factors within the controlled environment of the greenhouse, with respect to maize growth and development. Timing of this low DLI stress on the plant is critical for tassel morphology. Inbred A was most affected by the low DLI during vegetative stage 7 (V7), while Inbred B was most affected during V6. Under further investigation, 1 to 6 d of low DLI was not enough to elicit the tassel barrenness response, and tassel length was not suppressed until 7 d of low DLI for Inbred B. This 7 d threshold gives the grower a tool to determine how long the low DLI stress can occur before tassel morphology begins to deteriorate.

When trying to mitigate this low DLI stress there are a few options the grower can try. Decreasing the density of the plants and or increasing the number of lamp to increase the light intensity provided will help to increase light interception and DLI, respectively. Both options are costly in terms of space when decreasing the planting density, and in terms of capital investment when installing additional lamps. Lowering the height of the lamps closer to the canopy of the plants increases the instantaneous light intensity delivered to the plants but can also increase plant temperature. An increased canopy temperature can have

detrimental effects on plant growth and development, limiting photosynthesis and desiccation the upper canopy. A third strategy would be to operate lamps providing supplemental light for a longer period of time, which would also increase the photoperiod.

The final study of this thesis aimed to quantify the impact of longer photoperiods on maize tassel morphology. Increasing the photoperiod during vegetative or reproductive growth elicited different plant responses for the two inbred lines. The tassel traits were more responsive to the changes in photoperiod during vegetative growth than during reproductive growth for both Inbreds A and B. Inbred A was responsive to an increased photoperiod during vegetative growth, with respect to tassel traits than Inbred B plants. Inbred A responded to longer photoperiods during vegetative development with a longer tassel, suppressed viable tassel length, fewer tassel branches, and a diminished barren tassel length. As photoperiod increased during vegetative growth stages, tassel branch number and tassel barrenness length were suppressed for Inbred B. Only the tassels of Inbred A were responsive to an increased photoperiod during reproductive growth, with tassel height were progressively shorter as photoperiod increased from 16 to 22 h. These results indicate that increasing photoperiod during both vegetative growth and reproductive growth stages can impact tassel morphology of both inbred lines.

Recommendations for Future Research

Optimizing the environmental conditions for any species can be a challenge. While this research characterized the effects of low DLI and photoperiod on Inbreds A and B, there are other maize lines and crops to study. One could take the methods developed in this thesis

and use them as investigative tools to characterize other maize lines to better understand which developmental stages are most susceptible to low DLI stress. Increasing photoperiod to increase the DLI and diminish tassel barrenness symptoms may not be effective for other maize lines or other crops.

Aside from applying these methods to better characterize other inbred lines or other crops, another important aspect to investigate is the pollen quality of these plants under low DLI stress and to determine if increasing photoperiod would increase the viable pollen present. Increasing pollen quantity is important, but of more importance is increasing the viability of that pollen. If an increase in photoperiod does not increase the pollen productivity, then the objective of retaining viable tassels is not achieved. For, without viable pollen, the tassel cannot truly be considered viable itself.

While this research studied the effect of 16, 20, and 22 h photoperiod on these two inbred lines, other photoperiods should be studied. A combination of managing light intensity and photoperiod to maintain a target DLI would be an approach, and the hours of lamp operation would change each day depending on the light intensity of that day. On cloudy days a photoperiod of 20 or 22 h may be needed, but on a sunnier day, a 16-h photoperiod may be all that is needed to achieve the DLI target. Further investigation into the increase of photoperiod as tool to manipulate DLI is needed and would be beneficial to add flexibility to the growth and production of crops throughout the seasons.

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