Physiological characterization of winter canola under heat and drought stress during flowering and pod-filling stages

by

Meghnath Pokharel

B.S., Tribhuvan University, 2013
M.S., University of Arkansas at Pine Bluff, 2016

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Canola (*Brassica napus* L.), also known as oilseed rape or double low rapeseed, is an economically valuable oilseed and an emerging bio-energy crop. Global climate models predict a significant increase in both day time and night time temperatures and erratic rainfall patterns at the global and regional scales, which can induce both yield and quality losses in winter canola. Three studies were conducted with an overall goal to quantify the impact of abiotic stress exposure during flowering and pod-filling stages in winter (biennial) canola. In the first study, the impact of high night temperature (HNT) exposure during flowering and pod-filling stages on the time-of-day of flowering, physiological traits, yield, oil content and seed fatty-acid composition were quantified. Two independent HNT stress experiments involving ten (experiment 1) and six (experiment 2) canola cultivars were conducted using walk-in climate-controlled environment chambers following a split-plot design. The results from both experiments demonstrated that peak flower opening shifted towards earlier hours in the morning. The photochemical efficiency of Photosystem (PSII) was significantly decreased and thylakoid membrane damage was significantly increased in the leaves of susceptible cultivars. Quantitative impact of heat stress was confirmed with increased sensitivity to HNT exposure from gametogenesis until maturity resulting in a significantly higher yield loss compared to stress exposure from post-flowering until maturity. HNT significantly decreased oil concentration, but increased protein concentration and saturated fatty acid levels in seeds of the susceptible cultivars. However, HNT had no impact on the unsaturated fatty acids in both hybrids and open-pollinated cultivars. Our findings conclude that canola hybrids are better suited to regions experiencing heat stress compared to open-pollinated cultivars. The second study was conducted to quantify the effect of HNT, high day time (HDT) and a combination of high day and night temperature (HNDT) stress on the reproductive processes

during flowering, affecting yield, oil content and seed fatty-acid composition in winter canola through two independent experiments using walk-in climate-controlled environment chambers. Based on the results, HDT had the most significant impact on seed-set during flowering. HDT stress significantly shifted flowering towards early morning hours, induced floral sterility, flower abortion and complete loss of yield with two weeks of stress imposition during flowering. However, total dry matter accumulation, total number of pods, pods and seed weight per plant were significantly increased or unchanged which demonstrated significant plasticity in canola to overcome short episodes of HDT damage. Long duration heat stress under field conditions recorded significant decreases in pod number, grain yield and oil concentration. The impact of drought stress on the effective quantum yield of photosystem II and yield components was assessed during reproductive stages using field based rain-out shelters. Drought stress had a significant negative impact on quantum yield of photosystem II, biomass and the yield components in winter canola. Collectively, the findings from these studies indicate the possibility of using canola hybrids in regions that are currently faced with warmer climate and support breeding efforts towards developing winter canola with enhancing resilience to abiotic stresses under future warming scenarios.

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S.V. Krishna Jagadish

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Dedication

I wholeheartedly dedicate my dissertation work to my beloved wife, Prabha Sapkota Pokharel and my respected parents, Danada Pani Pokharel and Mishrimaya Pokharel for their unconditional love, blessings, sacrifice and support.

Chapter 1- Introduction and literature review

1.1 Canola

Canola (*Brassica napus* L.), also known as oilseed rape or double-low rapeseed, belongs to the *Brassicaceae* plant family as does mustard, broccoli, brussel sprouts and cauliflower. It is a three to five feet tall plant that produces small yellow flowers, which later develops pods and seeds. Canola is an edible form of rapeseed that has been bred with < 2% concentration of erucic acid in the oil and <30mmol g^{-1} of glucosinolates in the oil free meal (Morrision et al., 2016). High concentration of erucic acid in the oil increases coronary heart disease and the blood cholesterol in human, while higher amount of glucosinolates in the oil free meal reduces feed efficiency and weight gain in animals (Kumar et al., 2010). 'Tower' was the first canola variety released in 1974 with both low erucic acid and low glucosinolate concentrations (Morrision et al., 1993).

There are two types of canola cultivars based on time of cultivation - winter (biennial) and spring (annual) canola. In the southern Great Plains of the United States, sowing of winter canola mostly starts in September or October and the plant undergoes vernalization throughout winter. Canola blooms in April and is harvested in May of the subsequent year. Winter canola is well-suited to the environmental conditions of the southern Great Plains and generally produces 20 to 30% higher yield than spring canola (Boyles et al., 2012).

1.2 Importance of canola

Canola is currently one of the most productive and important oilseed crops grown worldwide (Aksouh-Harradj et al., 2006). It contains high oil concentration ranging between 40 and 50% of dry weight of seeds (Reyes, 2007) and is the third most abundant vegetable oil crop in the world after palm kernel (*Elaeis guineensis*) and soybean (*Glycine max*) (USDA, 2015). Canola oil, with

its high unsaturated/saturated fatty acid ratio, provides significant health benefits which include lowering total cholesterol levels, reduce cancer cell growth and increase insulin sensitivity (Lin et al., 2010) and hence possesses an increased demand among diet-conscious consumers (Grombacher & Nelson, 1992). In addition, the meal, a by-product of oil extraction, is used for animal feed because of its high protein content, ranking it second in global production after soybean meal (Elferjani & Soolanayakanahally, 2018). Canola oil also has a high nutritive value due to the presence of numerous aliphatic acids and vitamins (Tian et al., 2017). It is one of the cultivated medicinal food plants in the Middle Asia, North Africa and Western Europe (Saeidnia & Reza, 2012).

Globally, canola cultivation is expanding due to its importance in food and bio-diesel industries (FAO, 2006). By 2014, production increased to 68.9 million metric tons, and the harvested area expanded to 33.7 million hectares (FAO, 2017), compared to 35 million metric tons in 2000. Winter canola is a potential food and bio-energy crop for the United States as significant land area was occupied during recent years. USDA-NASS reports indicate 32,000, 56,000, and 28,000 hectares were occupied by canola in Oklahoma in 2015, 2016, and 2017, respectively. Similarly, Kansas planted 21,080, 10,000, and 20,000 hectares of winter canola during the same time period. Canola is considered an alternative crop to cereals, predominantly in wheat-based monoculture cropping systems under semiarid conditions (Zentner et al., 2002). Burton et al. (2008) indicated that canola can be used in crop rotations to break the disease and pest cycles and improve weed management in wheat-based cropping systems.

1.3 Abiotic stresses in winter canola production

Global mean air temperature has increased by 0.5°C and is further expected to increase by 1.5–4.5°C by 2100 (IPCC, 2014). Climate models also predict that daily minimum temperature is

increasing more rapidly compared to daily maximum temperature (Vose et al., 2005; Sillmann et al., 2013). This implies that both the day and night air temperatures are increasing and are predicted to continue increasing. Further, unpredictable and erratic rainfall pattern are forecast to become more frequent. More precipitation in wet regions and less precipitation in dry regions are predicted, which will widen the precipitation gap between wet and dry regions as well as between wet and dry seasons, leading to increased drought and flooding events (IPCC, 2013). Both heat and drought stresses are considered to be the most damaging abiotic stresses for crop production. The co-occurrence of heat and drought stress is expected to become more frequent under anticipated future warmer environments. A recent study spanning 1964 and 2007 reported that drought and heat events resulted in losses of 1.82 and 1.19 billion metric tons, respectively, in cereal production (Lesk et al., 2016). Both the frequency and the intensity of drought episodes and heat waves have been increasing, and the consequences for crop yields are more disastrous than those from other climatic extremes, such as flooding, frost, or hail (Pachauri et al., 2014; Zscheischler et al., 2014).

With the increasing interest in canola and the expanding demand for its products, cultivation has expanded to warmer regions, exposing the crop to heat and drought stresses and frequent yield losses. A long term study on weather and canola yield in Saskatchewan, Canada from 1967 to 2001 by Kutcher et al. (2010) found that for every 1°C increase in mean temperature, canola yields declined between 18.4 $kg\,ha^{-1}$ and 75 $kg\,ha^{-1}$ per day when maximum temperature exceeded 30°C during the growing season. This demonstrates that canola yield is challenged by current warming temperatures, which would become even more damaging considering the predicted increase in temperature as a result of climate change. Hence, it is increasingly important to enhance abiotic stress tolerance in field crops including canola to combat the impending threat posed by climate change on global food security.

1.4 Sensitive stages in canola to heat stress

The increasing threat of climate change is already having a substantial impact on agricultural production worldwide as heat waves cause significantly yield losses, with greater risks predicted for future global food security (Ray et al., 2019). Of the major forms of abiotic stresses that plants are exposed to in nature, heat stress has an independent mode of action on the morphology, physiology and the metabolism of plants including canola. Morrison (1993) reported complete floral sterility in Argentine canola (cvs. Delta and Westar) exposed to 27/17°C day/night temperatures through a controlled environment cabinet study. Another study by Morrision & Stewart (2002) concluded that the threshold temperature for *Brassica* species during flowering to be 29.5ºC, beyond which seed yield losses were recorded. Other studies have imposed more severe heat stress ranging between 30 and 32ºC (Polowick & Sawhney, 1988) and 35/15ºC (Angadi et al., 2000; Gan et al., 2004). Complete pod sterility and pod abortion was recorded in these studies due to failure of fertilization leading to parthenocarpy (development of pods without fertilization of ovules). Being a cool season winter crop, both spring and winter canola production are extremely sensitive to increasing temperature particularly during reproductive (Singh et al., 2008; Angadi et al., 2000) and pod-filling stages (Weymann et al., 2015; Gan et al., 2004; Young et al., 2004).

1.4.1 Impact of heat stress during flowering

Achieving high aboveground biomass is essential for canola to construct a favorable canopy and to produce sufficient number of pods and seeds for obtaining high yield (Zhang & Flottmann, 2016). Angadi et al. (2000) observed variable responses in dry matter production between two growth stages at 35/15°C day/night, with early flowering being more sensitive (21% decrease) compared with early pod filling (8% decrease in biomass). Heat stress imposed during flowering

in canola negatively impacted photosynthetic capacity and grain yield (Elferjani & Soolanayakanahally, 2018). High temperature stress during reproductive stages has been reported to reduce seed yield in canola (*B. napus*) cultivars in different studies (Morrison, 1993; Angadi et al., 2000; Gan et al., 2004). Within the reproductive stage, Angadi et al. (2000) reported flowering to be the most sensitive stage to heat stress (35/15°C day/ night) from a controlled environment chamber study on three *Brassica* species – *B. napus, B. rapa,* and *B. juncea*. In the same study, heat stress was imposed separately for 7d at early-flowering (stage 6.1) and at early-pod (stage 7.1) stages. Angadi et al., (2000) documented that with 35/15°C day/ night imposed at early flowering, yield per plant was reduced by 52%, while the same stress at the early-pod stage reduced yield per plant by 18%. This differential impact occurred because the flowers that opened during the stress imposition were unable to produce viable pods, and floral buds were also aborted. Heat stress during early-pod formation (i.e., after passing the critical flowering stage before heat stress was imposed) were able to form pods. Supporting the above findings, Tayo & Morgan, (1975) documented that 75% of flowers that opened within 14 days from the start of flowering under optimum conditions were able to convert into fertile and filled pods in *B. napus*. Therefore, the first 14 days of flowering in canola determines the final seed yield and thus heat stress exposure during this period is expected to have the greatest impact on seed yield.

Young et al. (2004) examined pollen viability, germination and pollen tube growth under heat stress (35°C) during early flowering in canola cultivars. They observed that pollen taken from plants exposed to 4 d of heat stress had lower *in vitro* pollen germination rates (17.5%) than pollen from control-grown plants (59.2%). The lower germination percentage for pollen from heat stress treated plants occurred regardless of whether *in vitro* germination was carried out at 23°C or 35°C. Similarly, heat stress during *in vitro* germination also had a detrimental effect on pollen tube

growth. Young et al. (2004) concluded that more than 50% reduction in pod set and yield was due to reduced pollen viability or pollen tube growth which impaired micropyle penetration, fertilization and post fertilization events. In another study, Singh et al. (2008) evaluated *in vitro* pollen germination and pollen tube length responses in 12 canola cultivars to a range of temperatures (from 5 to 35^{o}C) and concluded that the temperatures below or above the optimum temperatures (23.6°C) caused a significant linear reduction in the pollen germination and the pollen tube growth in all 12 canola cultivars.

Polowick & Sawhney, (1988) captured the impact of heat stress (32/26°C; day/night) on the fertility and seed set in canola. They observed that flowers on plants raised under heat stress were stunted and a majority of them (> 90%) failed to open but had the stigma protruding beyond the closed sepals. The fewer flowers which opened were abnormally shaped and contained shriveled petals and shrunken stamens. They also observed that the length of stamens (male reproductive organs) on normal plants exceeds that of the gynoecium (female reproductive organ) at flowering. However, the stamens in heat stressed plants were barely half as long as the gynoecium. Hence, the authors concluded heat stress causes differential developmental rate in the reproductive organs, leading to female hyperplasia in the flowers of *B. napus.*

Total seed yield in canola is determined by the number of pods, seeds per pod and seed weight (Angadi et al., 2000; Gan et al., 2004). Angadi et al. (2000) observed that short episodes of heat stress during flowering increased the total number of pods but reduced the number of fertile pods. Similarly, Gan et al. (2004) also recorded a significant reduction in the pod numbers, seeds per pod and seed weight when heat stress (35/18^{o}C day/night) was imposed during early flowering and pod-filling stages. The flower number and pod/flower ratio are also considered important factors that determine canola seed yield (Angadi et al., 2000; Morrison & Stewart, 2002; Gan et

al., 2004). Severe stress of 35/15 °C during flowering, progressively reduced the number of flowers that opened during the stress period. Only 27% flowers were opened between the 4th and 6th day of the stress period than during the first two days of heat stress in canola (Angadi et al., 2000). Under field conditions, high temperature stress (> 35°C) during flowering can prematurely end flowering, limiting seed set in canola (Faraji et al., 2009). On the other hand, in contrast, Young et al. (2004) did not observe similar changes in the number of flowers that opened under heat stress (35°C) exposure for 4 h each day lasting one or two weeks after the initiation of flowering.

1.4.2 Impact of heat stress during pod-filling

Pod filling is another sensitive stage in canola to heat stress. Gan et al. (2004) revealed that the average seed yield per plant was reduced by 58% with heat stress (35/18°C day/night) imposed for 10 days during early flowering, and by 77% yield reduction coinciding with pod development, compared to the control. They concluded that heat stress during pod development was more sensitive than early flowering. The rationale behind this finding was that the plants stressed at an earlier stage exhibited greater recovery after release of heat stress while the level of recovery was not the same with stress exposed during pod development, resulting in significantly lower seed yield. In addition, the seeds formed at the later developmental stage would not have enough time to develop/recover fully due to reduced grain-filling duration. Similarly, McGregor (1981), indicated the possibility of considerable recovery if damage occurs during early flowering by producing more branches or more flowers on new secondary inflorescences or more/heavier seeds per pod after the release of stress. Similar levels of plasticity were observed by Young et al. (2004), wherein significantly higher number of inflorescences resulted in an increase in pod and seed production in heat stress treated plants after the release of the stress treatment.

1.4.3 Impact of heat stress on seed composition and quality

Canola seed composition is influenced by environment and mainly by temperature during seed development (Canvin, 1965; Si et al., 2003). Previous studies have suggested that the period between two and five weeks after flowering is the most active stage of synthesis and storage of seed components (Fowler & Downey, 1970; Deng & Scarth, 1998) and is, therefore, considered to be the most sensitive time for changes in seed composition and quality (Appelqvist, 1973). Furthermore, oil concentration in canola (*B. napus*) has been reported to be determined during the seed-filling period and variation in oil concentration is closely related to prevailing temperature during that period. For instance, Faraji (2012) documented that there was a negative linear relationship between air temperature during seed-filling period and oil concentration in both open pollinated and hybrid cultivars of canola. He further stated that high temperatures increased the rate of plant development thereby shortening the seed-filling period and reducing the oil concentration potential in all the investigated canola cultivars.

A warm climate during the pod-filling stage resulted in seed oil content reduction in oilseed rape (Zhu et al., 2012; Singer et al., 2016). Seed oil and seed protein content are negatively correlated in *Brassica* species (Grami et al., 1977; Jensen et al., 1996). Canola oil concentration was reduced but protein concentration was increased even after heat treatments of four days of 38°C for four hours from first flowering lasting 29 days (Aksouh-Harradj et al., 2000; Aksouh-Harradj et al., 2006). Seed oil stems mostly from photosynthetic carbon assimilation of leaves and green pod walls, which are the major sources of photosynthates (Aschan & Pfanz, 2003; Bennett et al., 2011; Hua et al., 2012) and later carbohydrates converted into triacylglycerol through a metabolic pathway (Baud & Lepiniec, 2010). Abiotic stressors during flowering and pod filling would affect pod development and subsequently reduce the available photo-assimilates for

triacylglycerol biosynthesis and oil accumulation in the seeds. In addition, oxygen availability in the pod is also cited as a limiting factor in seed development (Porterfield et al., 2000). Vigeolas et al. (2003) reported that low oxygen concentration in *B. napus* seeds caused reductions in the adenosine triphosphate (ATP) level and the triacylglycerol content.

Fatty acid composition is the balance between saturated, monounsaturated and polyunsaturated fatty acids and is considered to be an important aspect of canola seed quality. A longer period of moderate temperature (28/20°C for 9 days) from 20 to 29 days after flowering (DAF) and a short period of high temperature (38/23°C for 5 days) from 25 to 29 DAF significantly altered the fatty acid profile by increasing the levels of saturated fatty acids [palmitic (16:0) and stearic (18:0) acids] and oleic acid (18:1) and decreasing the levels of linoleic and linolenic acids in different canola cultivars (Deng & Scarth, 1998; Aksouh-Harradj et al., 2006). Similarly, in an earlier study, reduction in linoleic and linolenic acids and an increase in oleic acid content was observed under constant post-flowering temperatures above 27°C in zero-erucic acid winter oilseeds (Canvin, 1965). Similarly, Prichard et al. (2000) showed an increase in saturated fatty acids and a decrease in linolenic acid under high temperature regimes (32/24°C and 37/25°C for three days). However, the effect on oleic and linoleic acid content was inconsistent and reversible depending on the timing of the stress exposure. Furthermore, oleic acid content was increased with the short episode of extreme heat stress (37/25°C for three days) from 10 DAF but decreased when imposed from 20 DAF (Prichard et al., 2000). Similarly, linoleic acid was increased when the temperatures of 32/24°C and 37/25°C were imposed from 20 DAF but decreased when the same temperatures stress was from 30 DAF in seeds obtained from the whole plant (Prichard et al., 2000). In summary, the accumulation of saturated fatty acids (stearic and palmitic acids) under heat stress during seed maturation has been reported by a number of previous studies (Calvin,

1965; Deng & Scarth, 1998; Aksouh-Harradj et al., 2006), including other oilseed crops (Green, 1986). Inconsistent impacts in canola, particularly for oleic acid and linoleic acid, under heat stress needs further investigation.

1.5 Impacts of high night temperature on canola cultivars

Climate models predict that daily minimum temperature is increasing more than twice that of daily maximum temperature (IPCC, 2014). Greater increase in night temperature compared to day temperature is resulting in narrower diurnal (difference between the maximum and minimum temperature during a day) temperature amplitude (Impa et al., 2019). Crop growth and development has shown to have differential effects of maximum day and minimum night-time temperatures using modelling approach (Lobell & Ortiz-Monasterio, 2007). Past studies have reported that high night temperature (HNT) is more detrimental to grain growth than high daytime temperatures (Morita et al., 2002 [rice]), which is shown to result in 10% reduction in grain yield with every °C increase (Peng et al., 2004 [rice]). In cotton, higher than optimum night temperature during flowering resulted in significant reductions in the number of flower buds per plant (Loka et al., 2016), number of seeds per locule and the number of seeds per boll (Echer et al., 2014). In wheat, early reproductive organ development in later tillers coinciding with HNT exposure resulted in a lower number of productive spikes due to the sensitivity of pollen development, leading to reduced grain number per plant under growth chamber (Impa et al., 2019) and field conditions (Garcia et al., 2015). Pollen viability and pollen germination were negatively affected by HNT leading to lower seed-set percentage and ultimately reduced grain yield in rice (Mohammed & Tarpley, 2009). Previous studies have reported an increase in thylakoid membrane damage and a decrease in photochemical efficiency and chlorophyll content in wheat both at

24/20°C and 24/23°C (Prasad et al., 2008), grain sorghum at 32/28°C (Prasad & Djanaguiraman, 2011) and soybean at 30/29°C (Djanaguiraman, 2013) grown under high nighttime temperature.

Most studies have investigated the impacts of high day temperatures on canola. Only few reports have addressed the impact of HNT on canola and these have been to investigate impact on fatty acid composition in the developing seeds (Zou et al., 2018; Baux et al., 2013). A strong negative correlation was reported between night temperature and oleic acid content in sunflower (Izquierdo et al., 2002; Izquierdo & Aguirrezábal, 2008) and soybean (Zuil et al., 2012). Using 10 years of field data and following a modelling approach, Baux et al. (2013) observed an increase in oleic acid (C18:1) in conventional oilseed rape (OSR) and high-oleic low-linolenic (HOLL) rapeseed varieties associated with increasing night temperature during the post flowering period, which was compensated by a decrease in linoleic acid (C18:2) and linolenic acids (C18:3). It was concluded that linolenic acid (C18:3) was highly sensitive to increasing night temperature during post flowering (Guthier et al., 2017). On the other hand, Zou et al. (2018) documented a significant decrease in the relative proportions of C18:0, C18:1, C20:1 but an increase in the proportion of C18:2 and C18:3 in seeds in both low and high oil content cultivars under HNT (19°C) compared to low night (9°C) temperature. Reports on the impacts of HNT on *B. napus* cultivars are highly contradictory. Therefore, it is important to fill this knowledge gap and to better understand the effects of high night temperatures on oil content and quality.

1.6 Sensitive stages in canola to drought stress

Increasing water scarcity, caused by global climate change and increasing competition for available water resources, is a major constraint for crop production and global food security (Rosegrant et al., 2009). Globally, more than 1.2 billion hectares of land under rainfed agriculture are at risk of water-deficit (or drought) stress (Kijni, 2006; Passioura, 2007). Singh et al. (2002)

documented that in India, long periods of drought stress resulted in 60 to 100% yield losses in different crop species including canola. Drought stress is becoming the most important factor limiting crop production in agricultural systems in semiarid regions (Molasadeghi et al., 2011). Drought stress could result in greater yield reduction compared to any other abiotic stress and is considered the most damaging abiotic stress on crops (Boyer, 1982; Araus et al., 2002; Farooq et al., 2009). The effect of drought stress is a function of genotype, intensity and duration of stress, other weather variables (prevailing air temperature and relative humidity), and the crop growth and developmental stages (Robertson et al., 2004). Canola is not well adapted to drought prone conditions (Wright et al., 1998). Water-deficit stress has deleterious effects during vegetative and reproductive growth stages in canola cultivars (Gan et al., 2004; Ghobadi et al., 2006; Rad & Abbasian, 2011).

1.6.1 Impact of drought during flowering

The crop stage of occurrence and duration of drought stress were considered more important than the intensity of stress (Korte et al., 1983). In support, Haq et al. (2014) reported reductions in seed yield, wherein they concluded the reproductive stage to be more sensitive to drought stress than seedling and vegetative stages in canola cultivars. Within the reproductive stage, flowering is highly sensitive because of losses to pollen viability, reductions in pollen tube growth and poor fertilization under water-limited conditions, which leads to lower seed yield in canola (Faraji et al., 2009). In previous observations, greater rapeseed yield reduction was obtained when water stress occurred at flowering and then at pod-development (Masaud et al., 2007). Furthermore, seed yield was limited by a relatively short period of soil moisture shortage (50 to 75% available water depletion) during the flowering stage, wherein the number of pods per plant and the number of seeds per plant were significantly reduced (Ghobadi et al., 2006).

Applying drought stress (50% field capacity) at the flowering stage caused a significant reduction in the number of pods per plant, number of seeds per pod, 1000-seed weight, seed yield, seed oil content, and oil yield of rapeseed cultivars (Rahnema et al., 2006; Nasri et al., 2008). The number of pods per plant is the most important component of the seed yield in rapeseed (Angadi et al., 2000). Daneshmand et al. (2008) observed a significant decrease in the number of pods per plant (up to 59%) and number of seeds per pod with water-deficit imposed during the flowering stage, and the decrease was attributed to insufficient fertility and flower abscission (Rad and Zandi, 2012; Sinaki et al., 2009). Canola showed severe reductions in pod dry matter and pod numbers as a result of flower and pod drop, with this problem being more prevalent under severe drought stress (Wright et al., 1995). Similar results have been reported for chickpea (Ghassemi-Golazani et al., 2008) and soybean (Demirates et al., 2010). Din et al. (2011) revealed that both the number of seeds per pod and seed size were significantly affected by drought stress imposed during early stages of flowering and pod-filling in canola cultivars (Johnston et al., 2002). A decrease in soil moisture below field capacity resulted in a reduced number of branches per plant in previous experiments (Sadaqat et al., 2003; Naeemi et al., 2007). Since canola is an indeterminate plant, vegetative growth continues even after the reproductive stage is initiated and drought stress at flowering decreases plant height by significantly affecting vegetative growth and assimilation, thus resulting in reduced number of branches per plant (Tahir et al., 2007).

1.6.2 Impact of drought stress during pod-filling

The pod-filling stage in canola is also sensitive to water-deficit conditions. Abiotic stress such as drought at the later stages of reproductive growth (pod filling) can result in source limitation for seed yield by inducing increased leaf shedding and hastening maturity (Gan et al., 2004). In a recent study, Zirgoli & Kahrizi (2015) showed that soil moisture below field capacity at seed filling

decreased the seed-filling rate and duration, leading to reduced seed weight compared to normal conditions in 10 rapeseeds (*Brassica napus* L.) varieties. Krogman & Hobbs (1975) indicated that both leaves and pods (when still green) are important for photosynthesis and seed yield and the water stress during the seed-filling period did not affect sink capacity (seeds per plant) but decreased source capacity leading to the reduction of seed yield via reduction in seed weight. Similarly, the lowest 1000-seed weight was obtained when soil moisture stress fell below the field capacity at the seed-filling stage (Ahmadhi & Bahrani, 2009). Plants maintained at 40% available soil water depletion from late flowering (> 80%) to maturity showed significant decrease in number of pods per plant, 1000-seed weight, final seed yield and seed oil percentage in oilseed rape cultivars (Pasban, 2009). An experiment conducted in winter canola cultivars by Darjani et al. (2013) concluded that interrupting irrigation at pod-development stage and beyond significantly reduced the number of flowering branches per plant, pod number per plant, number of seeds per pod and ultimately grain yield. In summary, drought stress imposed at a later stage (pod filling) reduced sink size (Mendham & Salisbury, 1995) and shortened the duration of seed filling and decreased the opportunity of the crop to recover (Gan et al., 2004).

1.6.3 Impacts of drought stress on various physiological characteristics of canola

Drought stress negatively affects many physiological plant processes, including photosynthesis, transpiration, stomatal conductance, chlorophyll content and metabolite accumulation which negatively impacts plant productivity (Reddy et al., 2004). Photosynthesis is the key process which contributes towards the final yield of the crop. Water deficiency during late vegetative and early reproductive growth stages reduced the photosynthetic rate in leaves of canola cultivars (Gammelvind et al., 1996). Higher production of reactive oxygen species (ROS) led to increased electron leakage in photosynthetic and respiratory organelles under drought stress (Moghadam et

al., 2009). Similarly, reduction in molecular oxygen and generation of ROS have also been reported to disrupt metabolism by oxidizing photosynthetic pigments, membrane lipids and proteins (Yordanov et al., 2000; DaCosta & Huang, 2007). Drought imposed from late flowering to maturity in different rapeseed cultivars decreased their leaf water potential, stomatal conductance, and leaf relative water content (RWC) (Pasban, 2009). The leaf RWC indicates the leaf water status and is considered to be an important marker of the level of drought stress (Sánchez-Blanco et al., 2002). All these changes disturb the normal process of photosynthesis, leading to lower production of photosynthates and ultimately poor yield in rapeseed (Mondal & Khajuria, 2000).

Chlorophyll concentration can be used as an indicator for source capacity to synthesize photosynthates (Zhang et al., 2007). In canola cultivars, Sharma et al. (1993) and Din et al. (2011) observed a decrease in chlorophyll (*a* & *b)* contents by 38% and 45%, respectively under drought stress at flowering as compared to the sufficiently watered plants. In addition, a decrease in chlorophyll content was also reported in canola plants (Sakova et al., 1995; Gibon et al., 2000) and in the leaves of mustard genotypes under drought stress (Singh et al., 2003). Drought not only causes dramatic loss of pigments but also leads to disorganization of thylakoid membranes (Ladjal et al., 2000). Further, the decrease in chlorophyll content under water-deficit stress was mainly due to a decrease in activity of chlorophyllase and an increase in ROS accumulation that damaged the chloroplasts (Gill & Tuteja, 2010).

1.6.4 Impacts of drought stress on oil and seed fatty acids composition

Oil content **-** Faraji et al., (2009) has reported that drought stress during the flowering and grain-filling period caused a negative impact on seed formation and oil content in canola cultivars. In an experiment on summer grown rapeseed, Wright et al. (1995) observed that early drought resulted

in low oil content in seeds as compared to the control treatment. In another study, reduction of 36.9% to 31.4% in oil content of rapeseed was recorded when drought stress was applied at post flowering and seed-development stages, respectively (Mailer & Cornish, 1987). Ghobadi et al. (2006) investigated the impact of short (flowering) and long (pod-filling) term water-deficit stress (75% of available water depletion) and concluded that seed oil content was significantly decreased under both drought regimes while protein content was only increased under long term drought stress. However, oil content did not decline significantly under mild drought stress, while decreased considerably when the stress level was increased (Jensen et al., 1996). In contrast to the above findings, Zarei et al. (2010) in a field study found no differences in canola oil content (an average of 37.3%) with different irrigation intervals of 7, 10, 14 days, consuming 6750, 5250 and 4500 m^3/ha/season, respectively. Similarly, Elferjani & Soolanayakanahally, (2018) also reported no significant effect on oil content when drought stress was maintained at 30% field capacity during the flowering and pod-filling stages in canola cultivars.

Fatty acid composition - Water-deficit stress imposed during vegetative growth and flowering has shown to reduce oleic acid in *B. napus* seeds (Bouchereau et al., 1996; Champolivier & Merrien, 1996). Aslam et al. (2009) showed that drought stress was associated with lower monounsaturated fatty acid and higher polyunsaturated fatty acids content in canola seed oil. This was due to a reduction in the desaturation of stearic to oleic acid as a result of premature seed maturity as observed by Rakow & McGregor (1975). In a recent study, Elferjani & Soolanayakanahally (2018) found that drought stress at 30% field capacity increased the relative content of unsaturated fatty acids but decreased saturated fatty acids. In contrast, Moghadam et al. (2011) recorded an increase in the percentage of stearic acid (a fraction of saturated fatty acids) and glucosinolates (Ullah et al., 2012) but had lower percentage (16%) of polyunsaturated fatty acids, such as linoleic acid, in

all canola genotypes due to a shorter growing season under drought stress conditions. In cotton leaves, Anh et al. (1985) reported that water deficiency decreased the degree of fatty acid unsaturation which was attributed to the inhibition in the biosynthesis of polyunsaturated fatty acids and suppression in the activities of desaturases.

1.7 Management strategies to minimize heat and drought stress damage in canola

- The selection of tolerant canola cultivars would be one of the best strategies to maintain yield and quality in warming environments. Seed fatty acid composition, number of pods aborted per plant and oil content were not significantly affected by high temperature stress in some of the cultivars (Aksouh-Harradj et al., 2001). Thermo-tolerance variation between *Brassica* species has been reported, with *B. rapa* found to be the most sensitive followed by *B. napus* and *B. juncea* (Angadi et al., 2000). Koscielny et al. (2018) reported genetic variation among *B. napus* genotypes to heat tolerance. If genetic variation within the primary gene pool exists and exploited, this would expedite the process of improving thermo-tolerance within *B. napus*.
- Early flowering and shorter maturation periods may enhance heat and drought avoidance in canola by completing flowering and seed development before onset of terminal heat and drought stress (Din et al., 2011).
- Canola hybrids have shown comparatively more abiotic stress tolerance due to high seed yield under normal as well as hot and dry conditions (Gehringer et al., 2007). Karamanos et al. (2005) observed that canola hybrids yielded more than open-pollinated cultivars by 17 to 33%. When hybrids were subjected to heat stress, the oil content declined less than the inbreds, while protein did not increase at the same rate as the inbreds. These results depict the ability of hybrids to minimize the negative effect of heat stress on the seed yield and quality in canola (Koscielny et al., 2018).

- Foliar boron application in spring canola has shown to mitigate heat stress effects during flowering and pod-development stages specifically by reducing heat-induced pod abortion and increasing yield in warm growing seasons (Ramsahoi et al., 2013).

Canola is an economically valuable oilseed and an emerging bio-energy crop. With the increasing demand for its products, canola cultivation has expanded to much warmer and dry regions, increasing canola's exposure to heat and drought environments. Canola is a cool season spring or winter crop and poorly adapted to heat and drought conditions (Wright et al., 1998). Hence, canola production is extremely sensitive to both high day and high night temperature, and drought stress particularly during reproductive stages (Singh et al., 2008; Angadi et al., 2000; Faraji et al., 2009) and pod filling (Weymann et al., 2015; Gan et al., 2004; Young et al., 2004; Zirgoli & Kahrizi, 2015). This dissertation research comprises three studies which focus on exploring heat and drought impacts on physiological processes, yield and seed quality in winter canola. In the first study, we quantified the impact of high night temperature exposure during flowering and pod-filling stages, the time-of-day of flowering, physiological traits, yield, oil content and seed fatty-acid composition. The second study was conducted to quantify the effect of short episodes of high day time, night time and combined high day and night temperature on the reproductive processes affecting yield and oil composition in winter canola through a controlled environment chamber experiment. In the second part of this study, we quantified the impact of long duration high day time temperature on the same physiological and agronomic parameters using unique field-based heat tents. In the third study, we assessed the impact of drought stress on the effective quantum yield of PSII and yield components in winter canola during reproductive stages using field based rain-out shelters.

1.8 References

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Chapter 2 - High night-time temperature during flowering and pod filling affects flower opening, yield and seed fatty acid composition in winter canola

Abstract

Winter canola (*Brassica napus* L.), also known as winter oilseed rape and double-low rapeseed, is highly sensitive to increasing temperatures during the reproductive stages. Although the impact of high day-time temperature stress on yield and quality has been documented in canola, similar information under high night-time temperature (HNT) stress is limited. Using six hybrids and four open-pollinated cultivars, we observed a marked shift in peak flowering towards earlier, cooler hours of the morning under HNT. The photochemical efficiency of photosystem II was decreased (3%), with an increase in thylakoid membrane damage (13%) in the leaves of susceptible cultivars under HNT stress. Similarly, the susceptible cultivars recorded reduction in biomass (34%), pod number (22%), pod weight (37%) and total seed weight (40%) per plant while the same set of agronomic traits were not affected among the tolerant cultivars. Quantitative impact of heat stress was confirmed with increased sensitivity to HNT exposure from gametogenesis until maturity resulting in a greater yield loss compared to stress exposure from post-flowering until maturity. HNT decreased oil concentration, but increased protein concentration and saturated fatty acid levels in seeds of the susceptible cultivars. However, HNT had no impact on the unsaturated fatty acids in both hybrids and the open-pollinated cultivars. Breeding targets based on fatty acid composition for enhancing canola seed quality may not be easily amenable due to the inconsistency documented with the compositional changes under heat stress. In summary, our findings conclude that canola hybrids are better suited to regions experiencing heat stress compared with open-pollinated cultivars, indicating the possibility of a complete shift to hybrid canola cultivation under predicted warmer climates in the future.

2.1 Introduction

Canola (*Brassica napus* L.), also known as oilseed rape or double-low rapeseed, is an economically valuable oilseed crop associated with high quality oil (Downey, 1990; Zhang et al., 2003). Cultivation of canola is currently expanding due to its importance in oilseed and bio-diesel industries (FAO, 2006). Canola cultivars have been developed as both spring (annual) and winter (biennial) types based on the planting season and vernalization requirement (Wang et al., 2011). As a cool season crop, both spring and winter canola are extremely sensitive to increasing temperature particularly during the reproductive stage including gametogenesis, pollination, fertilization and early embryogenesis (Singh et al., 2008; Angadi et al., 2000) and pod-filling stage (Weymann et al., 2015; Young et al., 2004). Over the past century, global mean air temperature has increased by 0.5°C and is predicted to further increase by 0.3 to 4.8°C by 2100 (IPCC, 2014). Follow up analysis on the overall mean temperature increase has revealed minimum night temperature to have increased more than twice that of the maximum day temperature (Easterling et al., 1997; Vose et al., 2005) and this trend is predicted to continue into the future (IPCC, 2014). There are two routes through which high night-time temperature (HNT) affects crops. First, through a direct reduction in grain-filling duration and loss in overall yield and harvest index (Bahuguna et al., 2017; Impa et al., 2019). Second, narrowing diurnal temperature amplitude leads to a profound negative impact on crop productivity through enhanced night respiration (Sunoj et al., 2016; Impa et al., 2019).

The seed yield of canola is primarily determined by the number of pods, seeds per pod and seed weight (McGregor, 1981). Photo-assimilate supply during fertilization determines seeds per pod, whereas seed weight depends on the continued supply of photosynthates after fertilization until maturity. In addition, other studies have indicated that the number of flowers that translate

into pods is a key determinant of seed yield. Therefore, flower numbers and pod/flower ratio are also important factors that determine canola seed yield (Angadi et al., 2000; Morrison & Stewart, 2002; Gan et al., 2004). High temperature damages photosynthetic membranes, leading to chlorophyll loss (Ristic et al., 2007), decrease in efficiency of photosystem II (PSII) (Pradhan et al., 2012) and photosynthesis (Al-Khatib & Paulsen, 1999; Prasad et al., 2008). Reproductive organs of canola plants exposed to 32^{o}C are affected negatively, resulting in reduced male and female reproductive organ viability (Polowick & Sawhney, 1988). For reasons highlighted above, temperatures above optimum often decrease seed set, grain number, grain-filling duration, grain-filling rate and individual grain weight (Al-Khatib & Paulsen, 1984; Wheeler et al., 1996; Ferris et al., 1998) and ultimately result in reduced grain yield and harvest index across many crops including canola (Gibson & Paulsen, 1999; Prasad et al., 2008). However, current information relates mostly to high day-time temperature (HDT), with limited reports on the response of canola exposed to HNT.

Canola seed composition is significantly influenced by environmental conditions, particularly by temperature changes during seed development (Aksouh-Harradj et al., 2006; Brunel-Muguet et al., 2015; Elferjani & Soolanayakanahally, 2018). The active phase of synthesis and storage of seed components in canola occurs between two and five weeks after the start of flowering (Fowler & Downey, 1969; Rakow & McGregor 1975), and this phase is considered to be vulnerable to high temperature conditions. Previous studies demonstrate that HDT stress during pod filling results in reduced oil concentration and increased protein concentration in oilseed crops (Aksouh-Harradj et al., 2006; Zhu et al., 2012; Singer et al., 2016 [canola]), (Dombos & Mullen, 1992; Gibson & Mullen, 1996 [soybean]). Similarly, a negative-correlation between monounsaturated and polyunsaturated fatty acids has been observed with HDT stress exposure in

a number of oilseed crops, including canola (Deng & Scarth, 1998; Aksouh-Harradj et al., 2006; Baux et al., 2013, Elferjani & Soolanayakanahally, 2018), sunflower (Nagao & Yamazaki, 1984; Flagella et al., 2002) and soybean (Dornbos & Mullen, 1992, Gibson & Mullen, 1996). In summary, day-time heat stress is shown to alter the oil and protein composition and reduce beneficial mono- and polyunsaturated fatty acids, while the impact of HNT on these fatty acids is not fully known in canola or other *Brassica* species.

Crop growth and development can be differentially sensitive to higher day and night-time temperatures (Lobell & Ortiz-Monasterio, 2007). In rice, HNT is shown to be more detrimental to grain growth than HDT (Morita et al., 2002). In field grown rice, every °C increase in night temperature resulted in 6 to 10% reduction in grain yield (Peng et al., 2004; Lyman et al., 2013). In cotton, higher than optimum night temperature during flowering resulted in a significant reduction in the number of flower buds per plant (Loka & Oosterhuis, 2016), number of seeds per locule and the number of seeds per boll (Echer et al., 2014). Currently, the impact of HNT on the yield, yield components and quality of canola is not well known, with previous studies related to heat stress focusing on HDT. Hence, to fill this knowledge gap, climate controlled walk-in chamber studies were conducted to investigate the impact of HNT stress on (i) the time-of-day of flower opening dynamics, physiological responses during flowering and pod-filling stages affecting yield and its components; (ii) pod set and pod weight, with stress exposure encompassing different developmental stages during gametogenesis, flowering and pod filling; and (iii) changes in oil, protein concentration and seed fatty acid composition in canola.

2.2 Material and Methods

2.2.1 Crop husbandry

Five canola hybrids; 46W94, Edimax CL, Hekip, Mercedes and Popular and five open-pollinated canola cultivars; DKW44-10, DKW46-15, HyCLASS225W, Riley and Wichita were chosen for Experiment 1. Findings from Experiment 1 were independently validated using a set of contrasting cultivars by selecting four tolerant hybrids (46W94, Edimax CL, Mercedes, Popular) and two susceptible open-pollinated cultivars (DKW44-10, DKW46-15) for the second experiment. The seeds of all cultivars were treated with Helix XTra and sown at a depth of about 2 cm in 4-cm deep trays. The growth medium consisted of a skid loader scoop of soil (135.9 kg), 79.28 kg of Sun Gro Metro Mix 360 (Sun Gro Horticulture, Agawam, Massachusetts), perlite (8,618.3 g), and fertilizers: 113.4 g of Osmocote (13-13-13), 113.4 g of Osmocote (14-14-14), 113.4 g of gypsum, 113.4 g of ammonium phosphate (18-46-0), and 113.4 g of elemental sulfur and micronutrients. This mix was used to fill 150 pots, each with a 3.78 L capacity.

2.2.2 Walk-in growth chambers and heat treatments

The HNT experiments were conducted using walk-in climate-controlled environment chambers in the Department of Agronomy, Kansas State University, Manhattan, KS, USA. The experimental design was a randomized complete block with a split-plot treatment structure, with temperature regime as the main plot and cultivar as the subplot. Each cultivar had six replicate plants in each treatment, in both the experiments.

The seedlings were established in a greenhouse maintained at 23/15°C (day-time maximum/night-time minimum). Two-week-old seedlings were vernalized in a plant growth chamber (Percival Mfg. Co., Model 1-37X, Perry, Iowa) for 2 months at 4°C with a photoperiod

of 8h. Following vernalization, seedlings were transplanted into 3.78 L pots and maintained in large climate-controlled environment chambers (249 cm width, 137 cm length, and 180 cm high; Conviron, Winnipeg, MB, Canada) under day/night temperatures of 23/15 °C (control) with a photoperiod of 16/8 h light/dark. Six plants for each cultivar were exposed to HNT of 23/20 °C, lasting 10 hours per day i.e., from 8:00 PM to 6:00 AM, starting on the 7th day after first sign of flowering and continued until physiological maturity. The plants were watered regularly based on visual soil appearance to avoid any water-limited condition and the pots were rearranged within the chamber once every 12 days to minimize positional effects. The photosynthetic photon flux density at the leaf level was ≥800 µmol m^{-2} s^{-1}. The relative humidity (RH) in all growth chambers was set to 70% during the day and night. Air temperature and relative humidity were continuously monitored at 15-min intervals in all growth chambers throughout the experiments using HOBO data loggers (Onset Computer Corp., Bourne, MA, USA).

2.2.3 Observations

2.2.3.1 Time-of-day of flower opening

The time-of-day of flower opening was recorded at hourly intervals for three consecutive flowering days following Ishimaru et al. (2010), starting from the first day of flowering under the control treatment and from the day of HNT imposition under the stress treatment. Three plants were chosen to record the cumulative number of open flowers at hourly intervals starting from 6:00 AM to 2:00 PM. The total number of flowers that opened at hourly interval was recorded cumulatively without physically touching the flowers, as human touch is shown to alter the flowering pattern (Chiluwal, et al., 2019; Kobayasi et al., 2010). Hourly counts were summed to get a single value over three

flowering days for each cultivar and treatment. Flower opening time was recorded in both the experiments.

2.2.3.2 Chlorophyll a fluorescence and chlorophyll index

Chlorophyll fluorescence is used to evaluate the status of the photosynthetic machinery under various stresses (Chen et al., 2010). Chlorophyll a fluorescence was measured using a modulated fluorometer (OS30p, OptiSciences, Hudson, NH, USA) on the second leaf from the top of the main stem, following 30-min dark adaptation. The observation was recorded in six replicate plants per cultivar from both control and HNT treatments. The photochemical efficiency of PSII represents the ratio of variable fluorescence (Fv) i.e., the difference between maximum fluorescence (Fm) and minimum fluorescence (Fo), over maximum fluorescence (Fm); and the thylakoid membrane damage was determined by the ratio of minimum fluorescence (Fo) to maximum fluorescence (Fm) (Maxwell and Johnson, 2000).

Chlorophyll index was measured using a self-calibrating chlorophyll meter (Soil Plant Analyzer Development [SPAD], Model 502, Spectrum Technologies, Plainfield, IL, USA). Measurements were taken in the same six replicate plants on three different places on the second leaf and averaged to get a single value for a plant. All traits were measured between 10:00 AM and 1:00 PM on the 7^{th} and 14^{th} day after HNT treatment was initiated. Control measurements were taken simultaneously at both time-points.

2.2.3.3 Quantitative impact of heat stress

To capture the impact of stress exposure on pod weight and seed weight per pod over different developmental stages, flowers were marked with three different colors of acrylic paint: black (on the stem [Figure 2.1a]) and blue and red (on the flower pedicle [Figure 2.1b,c]). This process

follows an approach well established in rice (Jagadish et al., 2007, 2008, 2010) and wheat (Aiqing et al., 2018). The first set of flowers were marked with black on the stem starting on the 3rd day following flower initiation and a second black mark was placed on the upper portion of the stem following the 6th day. After completing the black markings, plants were moved to the HNT chambers on the 7th day (Figure 2.1a). A similar set of plants were marked and maintained under control conditions. These black marks captured the flowers that opened and completed pollination and fertilization under control conditions. Similarly, flowers opening on the 7th and 14th day after stress initiation were marked with blue and red paint, respectively, on the flower pedicles (Figure 2.1b,c). Flowers receiving the blue mark were considered to have stress imposed after gametogenesis, i.e., including flowering and pod-filling stages, while those receiving the red mark were considered to have stress imposed starting from gametogenesis until maturity.

Addressing the quantitative impact of heat stress on an indeterminate crop like canola is challenging, as the number of flowers and pods considered for each developmental stage will be significantly different due to the rate of growth within and across branches. Hence, we followed a normalization procedure to make unbiased comparisons of the quantitative impact of heat stress.

$$\text{Single pod weight (g)} = \frac{\text{Total weight of marked pods (g)}}{\text{Total number of marked pods}}$$

$$\text{Seed weight per pod (g)} = \frac{\text{Total seed weight from marked pods (g)}}{\text{Total number of marked pods}}$$

2.2.3.4 Yield and yield components

At physiological maturity, plants were hand harvested by cutting at the stem base. Number of pods per plant was recorded. Marked pods under control and HNT treatments were collected separately. Vegetative parts (leaves and stems), marked pods, and all remaining pods were dried at 60, 40, and 40°C, respectively, for one week. Dry matter accumulation was determined as the weight of

stems plus the leaves that were retained on the plant. Pods were threshed manually after drying to separate seeds. Pod number, pod weight, and seed weight of marked pods were recorded between control and HNT. Similarly, pod number, pod weight, and seed weight per plant were recorded.

2.2.3.5. Oil, protein concentration, and fatty acid composition

Oil and protein concentration, and fatty acid composition were measured from 8 and 2 g seed samples, respectively, at the University of Idaho Brassica Breeding and Research Program's Oilseed Quality Laboratory (http://www.cals.uidaho.edu/brassica/) (Stamm et al., 2015). In Experiment 1, the focus was on quantifying the impact of HNT on oil concentration and the fatty acid composition. The oil concentration in Experiment 1 was determined by Nuclear Magnetic Resonance (Hammond, 1991; Howard and Daun, 1991). However, in Experiment 2, the aim was to capture the impact of HNT on both protein and oil. To measure both protein and oil concentration, near infrared spectrophotometry (NIR) (FOSS Analytical XDS Rapid Content Analyzer, FOSS North America, Eden Prairie, MN) was used. Due to a highly consistent response of majority of the cultivars in both Experiments 1 and 2, the percentage of saturated and unsaturated fatty acids were determined from a select set of contrasting cultivars (Mercedes, Edimax CL, DKW44-10 and DKW46-15) by using gas chromatography (Hammond, 1991).

2.2.4. Heat susceptibility index

Heat susceptibility index (HSI) values for important traits, such as grain yield have been widely used for selecting tolerant genotypes in spring canola (Koscielny et al., 2018), wheat (Bhardwaj et al., 2017) and barley (Parashar et al., 2019). Cultivars with higher HSI values greater than 1 for many of the important traits were considered to be susceptible to heat stress and those with lower HSI values lower than 1 were considered as tolerant (Fisher and Maurer, 1978). In the present study, HSI was calculated as a measure of HNT tolerance for dry matter accumulation,

yield and yield components and oil concentration with high night temperature stress and control by using the formula as suggested by Fisher and Maurer (1978).

HSI = [l-YD/YP]/D

Where,

YD = mean of the genotypes in HNT stress.

YP = mean of the genotypes under control.

D = 1- [mean YD of all genotypes/mean YP of all genotypes].

2.2.5. Data analysis

Both the experiments were laid out in a randomized complete block design with a split-plot treatment structure. Temperature was the main plot and cultivar was the subplot. Analysis of variance (ANOVA) was performed using the Proc GLM procedure in SAS 9.4 (SAS Institute, 2013). Means were separated using least significant difference (LSD) at probability level of 0.05 (p = 0.05). Graphs were created using SigmaPlot 12.5 (Systat Software, 2013).

2.3 Results

2.3.1 Heat susceptibility index

In Experiment 1, the winter canola cultivars, 46W94, Edimax CL, Mercedes, and Popular recorded low HSI ranging between -1.96 and 0.96 for a number of key traits including dry matter accumulation, total pod number, pod weight, seed weight and oil concentration. However, DKW44-10, DKW46-15, HyCLASS225W and Hekip had high HSI ranging between 0.61 and 4.91 for the same set of traits in response to HNT. Cultivars Wichita and Riley did not show a consistent trend and the HSI values were minimum for some of the traits and maximum for others (Table 2.1). Similarly, in Experiment 2, Mercedes, Edimax CL, Popular and 46W94 had the lowest

range in HSI (-2.10 to 0.63) for the same set of traits with DKW44-10, DKW46-15 recording the highest range in HSI (from 0.73 to 6.42) in response to HNT (Table 2.1). Based on the average HSI values across traits and experiments, Mercedes, Edimax CL, Popular and 46W94 were classified as tolerant, DKW44-10, DKW46-15, HyCLASS225W and Hekip as susceptible and Riley and Wichita as moderately tolerant winter canola cultivars to HNT stress. The same classification has been used throughout this dissertation to compare responses of cultivars exposed to HNT and control conditions.

2.3.2 Flowering patterns

No flower opening was observed before 6 a.m. or after dark (Figure 2.2). The majority of flowers opened during the morning from 6:00 a.m. to 12:00 p.m. under control conditions, with the largest proportion of flowers opening by 7 a.m. This trend was more conspicuous with a significant shift in peak flower opening towards earlier morning hours (6:00 a.m. to 7:00 a.m.) when exposed to HNT stress in both the experiments (Figure 2.2).

2.3.3 Photochemical efficiency of PSII, thylakoid membrane damage, and chlorophyll index

Photochemical efficiency of PSII (Fv/Fm) and thylakoid membrane damage (Fo/Fm) were affected by HNT stress, with a significant interaction between treatment and cultivars at 7 and 14 days of stress exposure in both experiments (Table 2.2 and Table 2.3). In Experiment 1, after 7 days of HNT exposure, the four susceptible cultivars (DKW46-15, DKW44-10, HyCLASS225W, and Hekip) had an average reduction in Fv/Fm of 3% compared to <1% in the six tolerant and moderately tolerant cultivars (Riley, Wichita, 46W94, Edimax CL, Mercedes, and Popular) (Table 2.2). Likewise, a significant increase in thylakoid membrane damage was recorded in the susceptible cultivars (12%) compared to the moderately tolerant and tolerant cultivars (1%). Similar responses were obtained in Experiment 2, with the two susceptible cultivars (DKW46-15

and DKW44-10) averaging a 3% reduction and 16% increase in Fv/Fm and Fo/Fm, respectively (Table 2.2). However, the four tolerant cultivars (46W94, Edimax CL, Mercedes, and Popular) were not significantly affected with <1% reduction in Fv/Fm and 2% increase in Fo/Fm. After 14 days of HNT stress, a similar effect on Fv/Fm and Fo/Fm was documented in the tolerant and moderately tolerant and the susceptible cultivars in Experiment 1, and the tolerant and susceptible cultivars in Experiment 2 (Table 2.3). Across both experiments, the susceptible cultivars averaged 3% lower Fv/Fm and 13% higher thylakoid membrane damage (Table 2.2). Chlorophyll index was not significantly affected by HNT stress across both experiments and exposure times. However, a significant cultivar by treatment interaction was recorded after 7 days of HNT stress in Experiment 2, wherein the susceptible cultivars had on average 7% lower chlorophyll index compared to control while the tolerant cultivars were unaffected under HNT (Table 2.2).

2.3.4 Yield and yield components

2.3.4.1 Dry matter accumulation

Dry matter accumulation differed significantly between temperature, cultivar and temperature by cultivar interaction (Table 2.4). In Experiment 1, we observed variable responses among the six tolerant and moderately tolerant cultivars. Cultivars Popular, Wichita, and Riley on average had 10% less dry matter accumulation under HNT stress than the control, while 46W94, Edimax CL, and Mercedes showed significantly greater dry matter under HNT stress (Table 2.4). In contrast, a significant decrease (23%) in average dry matter accumulation was recorded for the four susceptible cultivars. In Experiment 2, Mercedes and 46W94 exhibited an 8% average reduction in dry matter accumulation under HNT stress while Popular and Edimax CL did not show a reduction (Table 2.4). On the other hand, dry matter accumulation was significantly lower (45%) in the two susceptible cultivars under HNT stress than control. Across both experiments, HNT

stress decreased dry matter accumulation by 34% in susceptible cultivars (Table 2.4). The tolerant cultivars showed minimal to no reduction in dry matter accumulation.

2.3.4.2 Pod number per plant

Cultivar and the temperature by cultivar interaction differed significantly with pod number per plant for both Experiment 1 and Experiment 2. However, the temperature effect was significant only in Experiment 1 (Table 2.4). In Experiment 1, Mercedes, Edimax CL and Wichita on average recorded 5% lower total pod number per plant than the control under HNT stress. HNT stress significantly decreased pod number per plant (22%), averaged across all susceptible cultivars (Table 2.4). In Experiment 2, pod number per plant of the tolerant cultivars was 3% lower than the control, while the susceptible cultivars recorded a similar decline in pod number as in Experiment 1 (22%), compared to the control.

2.3.4.3 Pod weight per plant

The pod weight per plant followed a similar response as pod number, with the interaction between the treatment and cultivars and their independent impact changing significantly (Table 2.5). In Experiment 1, the susceptible cultivars had 34% lower total pod weight per plant than the control, while the tolerant cultivars recorded 12% lower pod weight compared to the control (Table 2.5). A similar response was obtained in Experiment 2. On average, HNT stress decreased total pod weight per plant significantly in the susceptible cultivars (40%) but not the tolerant cultivars (5%).

2.3.4.4 Seed weight per plant

In both the experiments, seed weight per plant was significantly affected by temperature, cultivar and their interaction (Table 2.5). In Experiment 1, tolerant and moderately tolerant cultivars recorded an 8 and 20% reduction in total seed weight under HNT stress, respectively. Total seed

weight in the susceptible cultivars was 36% lower than the control, with cultivar HyCLASS225W showing the highest reduction of 47% (Table 2.5). In Experiment 2, DKW46-15 and DKW44-10 recorded a 45 and 41% reduction in total seed weight, respectively (Table 2.5). Among the tolerant cultivars, Popular and 46W94 showed a 5% reduction in total seed weight while Mercedes and Edimax CL yielded a 4% increase. Averaged across experiments, total seed weight per plant was reduced by 40 and 6% in the susceptible and tolerant cultivars under HNT stress, respectively (Table 2.5).

2.3.5 Quantitative impact of heat stress

2.3.5.1 Single pod weight

In Experiment 1, night temperature had significant effect on single pod weight of the blue and red marked pods but not on the pods marked in black (Table 2.6). In Experiment 2, single pod weight for the differentially marked pods was significantly affected by temperature and cultivar, and the interaction was significant only for pods marked in red (Table 2.6).

In Experiment 1, tolerant and moderately tolerant cultivars averaged 14, 22, and 22% reduction in single pod weight of black, blue, and red marked pods, respectively under HNT stress compared to the control. The susceptible cultivars averaged 18, 44, and 52% reduction in single pod weight of black, blue, and red marked pods, respectively when exposed to HNT stress (Table 2.6). Similarly, in Experiment 2, tolerant cultivars averaged 15, 5, and 0% reduction and the susceptible cultivars averaged 45, 37, and 28% reduction in single pod weight for the black, blue, and red marked pods, respectively, under HNT stress. Across experiments, HNT stress decreased single pod weight of susceptible cultivars by 32, 41 and 40% with black, blue, and red color marked pods, respectively (Table 2.6).

Reduction in total pod weight per plant as a result of HNT stress was strongly and positively correlated with reductions in single pod weight in blue (R^2= 0.59) and red (R^2= 0.44) marked pods but not with black marked pods (R^2= 0.05) in Experiment 1. In Experiment 2, the relationship was stronger between total pod weight reductions per plant and single pod weight reductions, for the black (R^2= 0.80), blue (R^2= 0.62) and red (R^2= 0.84) marked pods (Figure 2.4).

2.3.5.2 Seed weight per pod

Similar to single pod weight in Experiment 1, seed weight per pod of the blue and red marked pods was significantly affected by temperature (Table 2.7). While in Experiment 2, seed weight per pod of all three groups of marked pods differed significantly with temperature and cultivar but the interaction was significant only for pods with red markings (Table 2.7).

In Experiment 1, the tolerant and moderately tolerant cultivars averaged 18, 20 and 17% lower seed weight per pod than the control with black, blue, and red marked pods, respectively. The susceptible cultivars averaged 19, 39, and 49% lower seed weight per pod than the control for black, blue, and red marked pods, respectively (Table 2.7). Similarly, in Experiment 2, the tolerant cultivars averaged, 21, 6 and 5% lower seed weight per pod and the susceptible cultivars averaged 47, 36, and 33% lower seed weight per pod than the control for the black, blue, and red marked pods, respectively. Across experiments, the highest reduction in seed weight per pod under HNT stress was observed for the blue and red marked pods. HNT stress significantly decreased seed weight per pod of susceptible cultivars by 33, 38 and 41% with the black, blue and red marked pods, respectively (Table 2.7).

The regression analysis showed that in both experiments there was strong positive relationships between total seed weight per plant and seed weight per pod reductions under HNT

stress for all marked pods (R^2= 0.50 to R^2= 0.87), except for black marked pods in Experiment 1 (Fig. 2.5).

2.3.6 Oil and protein concentration

Significant temperature, cultivar, and temperature by cultivar interaction effects were recorded for total oil concentration in both experiments and protein concentration in Experiment 2 (Tables 2.8 and Table 2.9). In Experiment 1, under HNT stress, the tolerant and moderately tolerant cultivars had 3% reduction in oil concentration compared to 12% reduction in the susceptible cultivars (Table 2.8). Among the tolerant cultivars, Mercedes had the lowest reduction (2%) while the oil concentration of 46W94 was increased by 10%. The largest decline in oil concentration (14%) was documented for HyCLASS225W and Hekip. In Experiment 2, the susceptible cultivars yielded 13% lower oil concentration compared to 2% for the tolerant cultivars (Table 2.9). The greatest reduction in oil concentration was recorded in DKW44-10 (17%) compared to the least reduction of 1% in Popular and 46W94. Across experiments, total oil concentration was reduced by 13% and 3% in the susceptible and tolerant set of cultivars, respectively.

HNT stress significantly increased protein concentration of the two susceptible cultivars, DKW46-15 and DKW44-10, by 20 and 13%, respectively (Table 2.9). On average, a significant increase of 16% protein concentration was recorded in the susceptible cultivars compared to a 2% reduction in the tolerant cultivars.

2.3.7 Fatty acids profile

2.3.7.1 Saturated fatty acids

HNT stress from flowering to physiological maturity significantly increased saturated fatty acids (Table 2.10). In Experiment 1, on average the susceptible cultivars recorded 8 and 14% increase

in palmitic acid and stearic acid compared to 2 and 12% increase in the tolerant cultivars, respectively (Table 2.10). Similar responses were obtained in Experiment 2, with the susceptible and tolerant cultivars, on average recording 8 and 2% increase in palmitic acid, and 17 and 14% increase in stearic acid, respectively. Across experiments, the highest percentage increase in palmitic acid (12%) was observed in DKW46-15. On the other hand, Edimax CL recorded the highest percentage increase in stearic acid (20%) followed by DKW44-10 (17%) and DKW46-15 (16%) (Table 2.10).

2.3.7.2 Unsaturated fatty acids

Among the four unsaturated fatty acids that we measured in our experiments, oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) were not significantly affected by temperature. Only, gadoleic acid (20:1) was significantly affected by temperature and cultivar in both experiments, and the temperature by cultivar interaction in Experiment 1 (Table 2.11).

In Experiment 1, the gadoleic acid concentration of susceptible and tolerant cultivars was reduced by 15 and 3%, respectively. Similar responses were documented in Experiment 2, with the susceptible and tolerant cultivars recording 12 and 3% reduction in gadoleic acid, respectively (Table 2.11). Across experiments, the highest and least percentage decrease in gadoleic acid were observed in DKW44-10 (18%) and Mercedes (2%), respectively.

2.4 Discussion

HNT advances time-of-day of flowering towards morning hours

Flowering is known to be the most sensitive stage to heat stress, and prevailing air temperature during flowering (flower opening) has been closely linked to reproductive success or failure (Yoshida et al., 1981; Jagadish et al., 2007; Bheemanahalli et al., 2017). To date, this is the first attempt to record time-of-day of flowering under HNT stress exposure in canola *Brassica* species.

Coast et al. (2015) reported significant variation in flowering characteristics including duration of a spikelet remaining open during flowering, and start and peak flowering among rice cultivars exposed to night-time temperatures of 24, 30, and 35^{o}C. They observed advancement in the time of start and peak flowering by a few hours in some rice cultivars when exposed to HNT. However, in winter canola, we only observed a noticeable shift in peak flowering under HNT but not in time of start of flowering. In rice, earlier time-of-day of flowering is shown to help escape key physiological processes such as pollen germination from late-morning and early-afternoon heat stress (Ishimaru et al., 2010; Hirabayashi et al., 2014), reducing spikelet sterility under stressful conditions in the field (Bheemanahalli et al., 2017). Similarly, wheat exposed to HDT recorded peak flowering either during early hours of the morning or late in the evening when temperatures were cooler (Aiqing et al., 2018). Although earlier times of peak flowering minimizing yield losses under warmer day temperatures (Ishimaru et al., 2010; Kobayasi et al., 2010) or shifts in peak flowering (this study) are documented, the mechanisms behind this phenomenon are not known and need to be investigated further. The flexibility in flower opening time to earlier, cooler hours of the morning as observed in our study could be an important adaptive trait for sustaining productivity of winter canola under future warmer (day and night) environments.

HNT reduces PSII efficiency and increases membrane damage, affecting yield

The properties of the photosynthetic system and thylakoid membrane activities including key enzymes depends on the thermal stability of membranes and Reactive Oxygen Species (ROS) accumulation (Bjorkman et al., 1980). Previous studies involving different crops have reported a significant reduction in membrane thermo-stability when grown under high night temperatures (Mohammad & Tarpley, 2009; Prasad et al., 2008; Prasad & Djanaguiraman, 2011; Djanaguiraman et al., 2013, Loka & Oosterhuis, 2016). The above resulted in damaged PSII

reaction center and electron flow (Djanaguiraman et al., 2010; 2013), and increased proton leakage under night-time heat stress (Schrader et al., 2004; Djanaguiraman et al., 2013; Loka & Oosterhuis, 2010, 2016). In our study, similar physiological changes would have led to reduced photochemical efficiency of PSII and increased thylakoid membrane damage during flowering even with moderate night-time temperature of 20^{o}C lasting one or two weeks. This response is in agreement with previous HNT studies in wheat (Narayanan et al., 2015; Prasad et al., 2008) and soybean (Djanaguiraman et al., 2013). Furthermore, lower PSII activities is shown to reduce photosynthetic rate (Tang et al., 2018), thus providing fewer assimilates to the reproductive processes, leading to reduced seed-set. The availability of sugars during flowering is a key factor determining grain number (Demotes-Mainard & Jeuffroy, 2004) because inadequate availability of assimilates may cause floret death or impaired fertilization leading to lower seed set (Kirby, 1988). Increased thylakoid membrane damage causes electrolyte leakage, leading to reduced cellular homeostasis and photosynthetic rate (Djanaguiraman et al., 2013, Al-Khatib and Paulsen, 1990).

Extended duration of HNT exposure has a quantitative impact on canola yield

The potential and final pod numbers are related to cumulative dry matter production of canola until the beginning of flowering and the end of flowering, respectively (Habekotte, 1993; Faraji, 2012). Hence, a decrease in dry matter accumulation due to long duration stress could be one of the reasons behind the reduced pod numbers in our study. In wheat, reproductive organ development in later tillers, when coincided with HNT exposure, resulted in less grain number per plant (Garcia et al., 2015; Impa et al., 2019). Similarly, in our study, a majority of the flowers underwent bud maturation and flowering under stress conditions (see Figure 2.1), indicating reproductive organ development to be equally sensitive to HNT in cereals and oilseed crops. Final seed weight is determined by both rate and duration of grain growth. In wheat, seed weight was highly sensitive

to increasing night-time temperature and decreased above 17°C due to reduced grain-filling duration (Prasad et al., 2008). Other studies in rice have observed HNT to be more detrimental to grain weight than HDT (Morita et al., 2002, 2005).

Exposure to HNT encompassing a combination of sensitive reproductive stages such as gametogenesis + flowering and pod filling (red color marked pods) resulted in significantly greater reduction in pod and seed weight compared to just grain filling (pods with black mark), supporting the hypothesis of a quantitative impact of heat stress in canola, similar to rice (Jagadish et al., 2007; Rang et al., 2011). In addition, HNT leading to narrow diurnal temperature amplitude, i.e. between day and night temperatures, has been shown to have stronger negative impact in corn than under large diurnal amplitude (Sunoj et al., 2016). Similarly, we had a lower diurnal temperature amplitude (8^{o}C difference with control compared to 3^{o}C under HNT), which could be another reason for a larger decline in yield among susceptible, open-pollinated cultivars compared to hybrids. Interestingly, canola hybrids demonstrated significantly higher tolerance to HNT and produced more yield compared to the open-pollinated cultivars. Increased stress tolerance in hybrid cultivars, as compared with open-pollinated cultivars, has been well documented in corn (Troyer & Wellin, 2009). Based on these findings, for regions currently experiencing warmer temperatures during the reproductive period, shifting from open-pollinated cultivars to hybrids is strongly recommended, with a possible complete shift to hybrid canola cultivation under predicted warmer climates in the future.

HNT decreases oil concentration but increases protein concentration

The oil content and composition in canola seeds are affected by environmental factors (Jensen et al., 1996; Si et al., 2003). To our knowledge, this is the first study to record the impact of HNT stress on oil and protein concentration in winter canola. We found that the susceptible cultivars

showed significant decreases in oil concentration and increases in protein concentration with stress exposure from early flowering to physiological maturity. Previously, warm temperatures during the pod-filling stage have been reported to reduce seed oil concentration in oilseed rape (Zhu et al., 2012; Singer et al., 2016). Seed oil stems mostly from photosynthetic carbon assimilation of leaves and green pod walls, which are significant sources of photosynthates (Aschan and Pfanz, 2003; Bennett et al., 2011; Hua et al., 2012). Later, the carbohydrates are converted into triacylglycerol (Baud & Lepiniec, 2010). Abiotic stressors during and after flowering would affect pod development and subsequently reduce the available photo-assimilates for triacylglycerol biosynthesis and subsequently oil accumulation in the seeds. Seed triacylglycerol and seed protein content are negatively correlated in *Brassica* species (Grami et al., 1977; Jensen et al., 1996). Thus, stressors decreasing the oil content in seeds would concurrently increase the protein fraction (Rossato et al., 2001; Rathke et al., 2006). A similar response is recorded in wheat, wherein HNT reduced grain starch concentration resulted in a significant increase in protein and lipid accumulation (Impa et al., 2019).

HNT alters saturated fatty acids but not unsaturated fatty acids

In canola, Deng & Scarth (1998) and Aksouh-Harradj et al. (2006) recorded an increased level of monounsaturated fatty acids (C18:1) and decreased polyunsaturated (Gibson & Mullen, 1996) fatty acids (C18:2, C18:3) under high day-time temperature conditions. However, Elferjani & Soolanayakanahally (2018) found an opposite response. Using 10 years of field data and following a modelling approach, Baux et al. (2013) observed an increase in oleic acid (C18:1) in conventional oilseed rape and high-oleic low-linolenic (HOLL) varieties associated with minimum temperature, coincided by a decrease in linoleic acid (C18:2) and linolenic acids (C18:3) concentration. In contrast, Zhou et al., (2018) reported that HNT significantly decreased the total fatty acids and

relative proportions of C18:0, C18:1, C20:1, in seeds of both low and high oil concentration cultivars but increased the proportions of C18:2 and C18:3 in both cultivars under HNT (19 °C) compared to low night (9 °C) temperatures. However, findings from our study revealed increased levels of saturated (C16:0 and C18:0) fatty acids without significant changes in unsaturated fatty acids (C18:1, C18:2, C18:3) except for C20:1 (Table 2.10 and Table 2.11). Taken together, the response of canola cultivars to either HDT or HNT in terms of the fatty acid composition is highly dynamic and variable based on the genotype x environment interaction. Considering the conflicting results with altered composition or consistency of saturated and unsaturated fatty acids under heat stress conditions, setting breeding targets to improve quality based on fatty acid composition will be challenging.

2.5 Conclusions

Our findings revealed a quantitative impact of heat stress, with stress exposure from gametogenesis until maturity having a significantly greater impact than flowering or post-flowering until maturity. The ability of canola to shift the peak flower opening time to earlier, cooler hours of the morning could be an important adaptive trait for sustaining productivity of canola under future warmer (day and night) environments. Canola hybrids recorded significantly higher tolerance to HNT by maintaining or in some cases producing higher yield and less alteration in oil concentration than open-pollinated cultivars. HNT also increased saturated fatty acids and protein in the open-pollinated canola cultivars. Taken together, our findings reveal significantly higher tolerance to HNT in canola hybrids and point towards a possible complete shift to hybrid canola cultivation under predicted warmer climates in the future.

2.6 References

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Table 2.1 Heat susceptibility index for yield and its attributes and oil concentration under HNT stress compared to control in Experiment 1 and 2. The tolerant entries are highlighted in bold, while the moderately tolerant/sensitive and the sensitive group of entries are presented in normal and italics, respectively.

Cultivars	Dry matter accumulation	Pod number plant^{-1}	Pod weight plant^{-1}	Seed weight plant^{-1}	Oil concentration
Experiment 1					
Mercedes	-1.08	0.59	0.45	0.47	0.47
Edimax CL	-1.35	0.28	0.76	0.66	0.71
Popular	0.96	-1.16	0.38	0.46	0.76
46W94	-0.95	-1.21	-0.23	-0.05	-1.96
Wichita	2.79	1.29	1.53	1.07	0.55
Riley	0.71	-1.38	0.73	0.98	0.78
DKW46-15	2.61	2.54	1.45	1.25	1.59
DKW44-10	2.05	2.80	1.12	1.32	1.80
HyCLASS225W	3.05	4.91	2.02	2.19	2.42
Hekip	0.61	1.11	1.83	1.83	2.47
Experiment 2					
Mercedes	0.39	0.61	0.42	-0.54	0.63
Edimax CL	-0.49	-0.44	-0.19	-0.15	0.48
Popular	-0.45	-2.10	0.28	0.40	0.23
46W94	0.47	0.57	0.36	0.49	0.01
DKW46-15	2.19	6.42	4.09	3.81	1.63
DKW44-10	2.48	0.73	2.35	3.46	3.65

Table 2.2 Photochemical efficiency of PSII, thylakoid membrane damage and chlorophyll index on the 7th day under control and HNT treatments. Tolerant and moderately tolerant cultivars are in normal font and the four and two susceptible cultivars from Experiment 1 and 2, respectively, are in italics. Values presented are mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; NS = Non-significant based on ANOVA, T = Treatment, C = Cultivar

Cultivars	Photochemical efficiency of PSII		Thylakoid membrane damage		Chlorophyll index	
Experiment 1	Control	HNT	Control	HNT	Control	HNT
Mercedes	0.838 ± 0.0041	0.833 ± 0.0006	0.163 ± 0.0039	0.167 ± 0.0006	60.75 ± 1.04	58.58 ± 1.06
Edimax CL	0.831 ± 0.0079	0.834 ± 0.0032	0.161 ± 0.0085	0.165 ± 0.0032	55.15 ± 0.95	54.58 ± 1.71
Popular	0.829 ± 0.0022	0.835 ± 0.0069	0.171 ± 0.0022	0.165 ± 0.0068	60.02 ± 2.46	63.03 ± 0.95
46W94	0.835 ± 0.0021	0.838 ± 0.0041	0.164 ± 0.0023	0.161 ± 0.0042	60.20 ± 1.82	63.78 ± 1.46
Wichita	0.819 ± 0.0046	0.828 ± 0.0000	0.179 ± 0.0038	0.173 ± 0.0011	64.20 ± 2.39	67.05 ± 0.50
Riley	0.830 ± 0.0085	0.830 ± 0.0028	0.169 ± 0.0086	0.169 ± 0.0028	64.55 ± 2.75	62.63 ± 2.31
DKW46-15	0.840 ± 0.0075	0.815 ± 0.0002	0.159 ± 0.0073	0.177 ± 0.0045	59.55 ± 2.95	63.85 ± 3.55
DKW44-10	0.831 ± 0.0005	0.816 ± 0.0015	0.168 ± 0.0006	0.184 ± 0.0012	60.65 ± 1.45	62.85 ± 3.95
HyCLASS225W	0.833 ± 0.0050	0.816 ± 0.0005	0.166 ± 0.0050	0.183 ± 0.0004	62.16 ± 1.39	63.70 ± 4.10
Hekip	0.844 ± 0.0005	0.823 ± 0.0024	0.155 ± 0.0004	0.176 ± 0.0024	57.75 ± 2.25	59.30 ± 3.61
T	*		*		NS	
C	*		*		**	
T x C	**		*		NS	
Experiment 2						
Mercedes	0.832 ± 0.0050	0.837 ± 0.0031	0.168 ± 0.0032	0.163 ± 0.0031	57.32 ± 0.54	54.68 ± 0.12
Edimax CL	0.829 ± 0.0022	0.834 ± 0.0009	0.168 ± 0.0012	0.165 ± 0.0009	53.53 ± 1.96	54.38 ± 0.18
Popular	0.831 ± 0.0019	0.829 ± 0.0022	0.169 ± 0.0020	0.170 ± 0.0022	56.13 ± 1.22	58.77 ± 0.83
46W94	0.837 ± 0.0045	0.830 ± 0.0015	0.162 ± 0.0045	0.170 ± 0.0016	58.98 ± 1.14	58.40 ± 1.75
DKW46-15	0.837 ± 0.0024	0.818 ± 0.0042	0.161 ± 0.0036	0.183 ± 0.0013	62.55 ± 3.20	60.45 ± 2.2
DKW44-10	0.839 ± 0.0040	0.821 ± 0.0049	0.160 ± 0.0037	0.187 ± 0.0040	74.22 ± 0.91	66.95 ± 1.86
T	**		***		NS	
C	NS		*		***	
T x C	***		***		*	

Table 2.3 Photochemical efficiency of PSII, thylakoid membrane damage and chlorophyll index on the 14th day under control and HNT treatments. Tolerant and moderately tolerant cultivars are in normal font and the four and two susceptible cultivars in Experiment 1 and 2, respectively, are in italics. Values presented are mean ± SE. *, **, *** significance at 5 %, 1 %, 0.1 %; NS = Non-significant based on ANOVA, T = Treatment, C = Cultivar

Cultivars	Photochemical efficiency of PSII		Thylakoid membrane damage		Chlorophyll index	
Experiment 1	Control	HNT	Control	HNT	Control	HNT
Mercedes	0.826 ± 0.0041	0.827 ± 0.0027	0.173 ± 0.0047	0.172 ± 0.0027	58.10 ± 0.48	56.03 ± 0.60
Edimax CL	0.832 ± 0.0020	0.831 ± 0.0020	0.165 ± 0.0032	0.170 ± 0.0018	54.13 ± 0.45	55.80 ± 1.57
Popular	0.826 ± 0.0020	0.829 ± 0.0070	0.174 ± 0.0019	0.171 ± 0.0069	57.52 ± 2.37	61.60 ± 1.56
46W94	0.828 ± 0.0010	0.827 ± 0.0025	0.170 ± 0.0011	0.171 ± 0.0026	59.62 ± 0.87	61.00 ± 1.25
Wichita	0.829 ± 0.0021	0.827 ± 0.0045	0.170 ± 0.0022	0.171 ± 0.0043	62.40 ± 1.02	63.00 ± 2.20
Riley	0.832 ± 0.0025	0.818 ± 0.0045	0.167 ± 0.0028	0.181 ± 0.0047	59.50 ± 1.00	59.03 ± 0.35
DKW46-15	0.832 ± 0.0038	0.813 ± 0.0010	0.170 ± 0.0014	0.186 ± 0.0009	60.00 ± 2.70	58.00 ± 4.00
DKW44-10	0.833 ± 0.0015	0.818 ± 0.0010	0.165 ± 0.0014	0.181 ± 0.0009	63.70 ± 2.80	59.65 ± 0.45
HyCLASS225W	0.830 ± 0.0037	0.809 ± 0.0050	0.169 ± 0.0035	0.190 ± 0.0050	60.90 ± 2.91	63.25 ± 0.75
Hekip	0.832 ± 0.0006	0.818 ± 0.0015	0.167 ± 0.0007	0.181 ± 0.0015	60.46 ± 0.52	54.70 ± 2.10
T	***		***		NS	
G	NS		NS		***	
T x G	**		*		NS	
Experiment 2						
Mercedes	0.825 ± 0.0034	0.821 ± 0.0021	0.174 ± 0.0034	0.179 ± 0.0021	53.38 ± 0.77	52.06 ± 1.08
Edimax CL	0.836 ± 0.0034	0.824 ± 0.0032	0.170 ± 0.0052	0.175 ± 0.0032	47.50 ± 0.78	50.85 ± 1.70
Popular	0.829 ± 0.0025	0.830 ± 0.0027	0.171 ± 0.0025	0.170 ± 0.0026	52.42 ± 0.96	57.25 ± 1.19
46W94	0.825 ± 0.0027	0.822 ± 0.0028	0.175 ± 0.0026	0.178 ± 0.0029	57.08 ± 1.35	55.22 ± 1.46
DKW46-15	0.842 ± 0.0023	0.826 ± 0.0010	0.158 ± 0.0023	0.174 ± 0.0009	57.08 ± 0.91	56.80 ± 0.90
DKW44-10	0.833 ± 0.0015	0.816 ± 0.0005	0.166 ± 0.0014	0.184 ± 0.0115	70.93 ± 1.55	63.13 ± 2.65
T	***		***		NS	
G	**		*		***	
T x G	*		*		**	

Table 2.4 Dry matter accumulation (g) and pod number per plant under control and HNT treatments. Tolerant and moderately tolerant cultivars are in normal font and the four and two susceptible cultivars from Experiment 1 and 2, respectively, are in italics. Variation in traits is given as mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; NS = Non-significant based on ANOVA, T = Treatment, C = Cultivar

Cultivars	Dry matter accumulation (g)		Pod number plant^{-1}	
Experiment 1	Control	HNT	Control	HNT
Mercedes	28.07 ± 2.78	30.63 ± 1.51	513.50 ± 27.33	490.00 ± 11.37
Edimax CL	30.14 ± 0.95	33.68 ± 0.33	485.00 ± 14.19	474.50 ± 16.07
Popular	30.87 ± 1.86	28.31 ± 1.54	462.00 ± 18.77	503.25 ± 26.68
46W94	31.97 ± 2.28	34.60 ± 1.49	484.00 ± 16.94	529.33 ± 28.38
Wichita	32.02 ± 1.96	24.28 ± 1.38	436.33 ± 10.99	393.00 ± 20.51
Riley	27.99 ± 1.15	26.28 ± 1.71	415.33 ± 23.07	459.50 ± 36.68
DKW46-15	30.47 ± 1.03	23.59 ± 0.76	495.33 ± 11.39	398.33 ± 58.71
DKW44-10	31.91 ± 1.30	26.24 ± 0.74	381.33 ± 4.09	299.00 ± 27.03
HyCLASS225W	35.23 ± 0.63	25.93 ± 1.41	509.50 ± 16.00	316.50 ± 18.50
Hekip	27.75 ± 1.35	26.29 ± 2.78	454.50 ± 48.51	415.50 ± 1.50
T	**		*	
G	**		***	
T x G	**		**	
Experiment 2				
Mercedes	26.01 ± 1.14	24.08 ± 2.09	559.40 ± 17.61	538.50 ± 25.82
Edimax CL	24.79 ± 1.57	27.13 ± 1.29	494.00 ± 7.66	507.40 ± 14.99
Popular	25.30 ± 2.36	27.48 ± 2.28	508.00 ± 39.76	573.20 ± 16.04
46W94	35.83 ± 1.88	32.60 ± 4.82	615.20 ± 21.62	593.67 ± 47.79
DKW46-15	27.58 ± 3.69	16.03 ± 2.07	529.50 ± 26.18	322.33 ± 60.95
DKW44-10	51.32 ± 4.86	27.05 ± 1.42	369.25 ± 30.71	352.71 ± 24.82
T	**		NS	
G	***		***	
T x G	***		**	

Table 2.5 Pod weight and seed weight per plant (g) under control and HNT treatments. Tolerant and moderately tolerant cultivars are in normal font and the four and two susceptible cultivars from Experiment 1 and 2, respectively, are in italics. Variation in traits is given as mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; NS = Non-significant based on ANOVA, T = Treatment, C = Cultivar

Cultivars	Pod weight plant^{-1} (g)		Seed weight plant^{-1} (g)	
Experiment 1	Control	HNT	Control	HNT
Mercedes	55.75 ± 3.39	50.46 ± 1.23	31.15 ± 1.58	28.05 ± 1.01
Edimax CL	59.55 ± 4.22	49.95 ± 4.19	33.22 ± 1.72	28.53 ± 3.00
Popular	55.92 ± 5.24	51.46 ± 1.95	31.61 ± 3.29	28.50 ± 1.02
46W94	53.13 ± 2.15	55.68 ± 3.30	28.77 ± 1.15	29.06 ± 1.72
Wichita	44.75 ± 2.52	30.28 ± 2.66	18.97 ± 1.36	14.67 ± 1.03
Riley	47.30 ± 4.15	40.01 ± 3.50	24.15 ± 1.67	19.11 ± 1.01
DKW46-15	45.71 ± 2.01	31.74 ± 3.67	22.99 ± 1.22	16.91 ± 1.88
DKW44-10	38.20 ± 0.99	29.13 ± 0.62	19.18 ± 1.26	13.80 ± 1.08
HyCLASS225W	61.91 ± 2.01	35.44 ± 5.09	31.76 ± 1.60	16.97 ± 1.34
Hekip	54.26 ± 6.02	33.24 ± 6.75	29.02 ± 2.88	17.72 ± 4.92
T	***		***	
G	***		***	
T x G	*		*	
Experiment 2				
Mercedes	58.96 ± 3.13	55.93 ± 2.55	30.71 ± 3.17	32.66 ± 1.46
Edimax CL	58.28 ± 2.99	59.62 ± 3.43	31.84 ± 2.22	32.42 ± 2.40
Popular	53.95 ± 2.00	52.06 ± 2.88	30.61 ± 0.87	29.19 ± 2.44
46W94	66.72 ± 3.22	63.77 ± 5.07	36.18 ± 2.48	34.11 ± 1.86
DKW46-15	45.20 ± 3.03	22.37 ± 3.52	26.63 ± 2.87	14.69 ± 3.47
DKW44-10	33.35 ± 0.83	23.67 ± 1.66	18.78 ± 0.78	11.14 ± 1.18
T	***		**	
G	***		***	
T x G	**		*	

Table 2.6 Single pod weight (g) from three different groups of marked pods exposed to control and HNT conditions during different developmental stages. The tolerant cultivars are presented in normal font while the susceptible cultivars are in italics. Variation in traits is given as mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; NS = Non-significant based on ANOVA, T = Treatment, C = Cultivar

Cultivars	Black		Blue		Red	
Experiment 1	Control	HNT	Control	HNT	Control	HNT
Mercedes	0.135 ± 0.012	0.118 ± 0.014	0.135 ± 0.003	0.130 ± 0.013	0.103 ± 0.000	0.097 ± 0.015
Edimax CL	0.158 ± 0.043	0.112 ± 0.021	0.124 ± 0.013	0.096 ± 0.016	0.124 ± 0.046	0.074 ± 0.012
Popular	0.109 ± 0.023	0.075 ± 0.008	0.146 ± 0.010	0.107 ± 0.010	0.116 ± 0.005	0.110 ± 0.012
46W94	0.134 ± 0.018	0.136 ± 0.009	0.121 ± 0.022	0.105 ± 0.016	0.119 ± 0.013	0.094 ± 0.007
Wichita	0.110 ± 0.022	0.081 ± 0.031	0.168 ± 0.035	0.100 ± 0.012	0.146 ± 0.022	0.108 ± 0.052
Riley	0.093 ± 0.025	0.103 ± 0.002	0.157 ± 0.023	0.117 ± 0.009	0.138 ± 0.046	0.092 ± 0.012
DKW46-15	0.124 ± 0.003	0.095 ± 0.012	0.132 ± 0.040	0.077 ± 0.011	0.121 ± 0.044	0.061 ± 0.008
DKW44-10	0.099 ± 0.028	0.073 ± 0.019	0.158 ± 0.032	0.077 ± 0.001	0.178 ± 0.037	0.078 ± 0.018
Hekip	0.122 ± 0.009	0.116 ± 0.000	0.143 ± 0.012	0.086 ± 0.031	0.120 ± 0.001	0.059 ± 0.007
T	NS		***		***	
C	NS		NS		NS	
T x C	NS		NS		NS	
Experiment 2						
Mercedes	0.138 ± 0.011	0.098 ± 0.013	0.147 ± 0.016	0.139 ± 0.009	0.122 ± 0.002	0.120 ± 0.006
Edimax CL	0.153 ± 0.003	0.153 ± 0.010	0.139 ± 0.006	0.141 ± 0.009	0.116 ± 0.005	0.117 ± 0.008
Popular	0.113 ± 0.011	0.092 ± 0.004	0.103 ± 0.011	0.094 ± 0.009	0.101 ± 0.008	0.109 ± 0.006
46W94	0.124 ± 0.016	0.110 ± 0.006	0.123 ± 0.008	0.115 ± 0.008	0.122 ± 0.005	0.120 ± 0.003
DKW46-15	0.124 ± 0.012	0.063 ± 0.007	0.122 ± 0.012	0.087 ± 0.003	0.105 ± 0.008	0.075 ± 0.011
DKW44-10	0.122 ± 0.016	0.073 ± 0.017	0.129 ± 0.021	0.070 ± 0.014	0.136 ± 0.008	0.098 ± 0.004
T	**		*		**	
C	**		**		***	
T x C	NS		NS		**	

HYCLASS225W had very few flowers (between 1 and 5) that opened on days 7 (blue) and 14 (red) after stress was initiated and hence excluded from the table.

Table 2.7 Seed weight per pod (g) from three different groups of marked pods exposed to control and HNT conditions during different developmental stages. Tolerant and moderately tolerant cultivars are in normal font and the four and two susceptible cultivars from Experiment 1 and 2, respectively, are in italics. Variation in traits is given as mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; NS = Non-significant based on ANOVA, T = Treatment, C = Cultivar

Cultivars	Black		Blue		Red	
Experiment 1	Control	HNT	Control	HNT	Control	HNT
Mercedes	0.075 ± 0.006	0.066 ± 0.010	0.083 ± 0.003	0.078 ± 0.009	0.064 ± 0.000	0.056 ± 0.010
Edimax CL	0.092 ± 0.025	0.059 ± 0.014	0.076 ± 0.008	0.051 ± 0.010	0.071 ± 0.027	0.039 ± 0.009
Popular	0.059 ± 0.014	0.040 ± 0.004	0.085 ± 0.005	0.063 ± 0.006	0.067 ± 0.003	0.069 ± 0.006
46W94	0.075 ± 0.012	0.071 ± 0.010	0.071 ± 0.012	0.058 ± 0.009	0.063 ± 0.006	0.050 ± 0.006
Wichita	0.054 ± 0.014	0.041 ± 0.018	0.082 ± 0.016	0.053 ± 0.004	0.07 0± 0.006	0.051 ± 0.019
Riley	0.044 ± 0.014	0.051 ± 0.001	0.087 ± 0.012	0.061 ± 0.003	0.077 ± 0.028	0.044 ± 0.006
DKW46-15	0.067 ± 0.003	0.055 ± 0.005	0.070 ± 0.016	0.051 ± 0.010	0.059 ± 0.016	0.034 ± 0.004
DKW44-10	0.052 ± 0.016	0.041 ± 0.011	0.090 ± 0.020	0.047 ± 0.000	0.096 ± 0.021	0.044 ± 0.012
Hekip	0.067 ± 0.007	0.069 ± 0.001	0.084 ± 0.008	0.049 ± 0.021	0.066 ± 0.001	0.033 ± 0.005
T	NS		***		***	
C	NS		NS		NS	
T x C	NS		NS		NS	
Experiment 2						
Mercedes	0.071 ± 0.005	0.054 ± 0.007	0.078 ± 0.009	0.075 ± 0.005	0.068 ± 0.002	0.066 ± 0.002
Edimax CL	0.083 ± 0.003	0.080 ± 0.005	0.080 ± 0.002	0.078 ± 0.003	0.065 ± 0.002	0.063 ± 0.005
Popular	0.064 ± 0.007	0.046 ± 0.004	0.062 ± 0.006	0.052 ± 0.005	0.066 ± 0.004	0.061 ± 0.004
46W94	0.077 ± 0.013	0.053 ± 0.005	0.060 ± 0.009	0.062 ± 0.004	0.069 ± 0.004	0.064 ± 0.000
DKW46-15	0.065 ± 0.008	0.031 ± 0.001	0.067 ± 0.006	0.047 ± 0.002	0.057 ± 0.003	0.039 ± 0.001
DKW44-10	0.054 ± 0.012	0.032 ± 0.007	0.062 ± 0.011	0.037 ± 0.006	0.074 ± 0.004	0.050 ± 0.001
T	**		*		***	
C	**		**		**	
T x C	NS		NS		*	

HYCLASS225W had very few flowers (between 1 and 5) that opened on days 7 (blue) and 14 (red) after stress was initiated and hence excluded from the table.

Table 2.8 Oil concentration per plant (%) under control and HNT treatments from Experiment 1. Tolerant and moderately tolerant cultivars are in normal font and the four susceptible cultivars are in italics. Values presented are mean ± SE. *, *** significant at 5%, 0.1T = Treatment, C = Cultivar

Cultivars	Oil concentration (%)	
Experiment 1	Control	HNT
Mercedes	43.83 ± 0.58	42.73 ± 0.84
Edimax CL	42.23 ± 0.96	40.60 ± 1.50
Popular	42.19 ± 0.58	40.46 ± 0.79
46W94	36.70 ± 2.22	40.59 ± 0.46
Wichita	38.16 ± 0.88	37.03 ± 0.80
Riley	40.46 ± 0.66	38.75 ± 0.42
DKW46-15	42.18 ± 1.03	38.56 ± 0.51
DKW44-10	35.22 ± 0.82	31.79 ± 0.17
HyCLASS225W	42.66 ± 0.72	37.07 ± 0.05
Hekip	40.48 ± 0.68	35.08 ± 0.85
T	***	
C	***	
T x C	*	

Table 2.9 Oil concentration (%) and protein concentration (%) under control and HNT treatments from Experiment 2. Tolerant and moderately tolerant cultivars are in normal font and the two susceptible cultivars are in italics. Variation in oil and protein concentration is given as mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; NS = Non-significant based on ANOVA, T - Treatment, C – Cultivar

Cultivars	Oil concentration (%)		Protein concentration (%)	
Experiment 2	Control	HNT	Control	HNT
Mercedes	45.71 ± 0.94	44.38 ± 0.85	19.02 ± 0.41	19.55 ± 0.50
Edimax CL	42.82 ± 1.04	41.87 ± 0.91	20.13 ± 0.57	19.33 ± 0.61
Popular	43.84 ± 0.52	43.39 ± 1.16	18.97 ± 0.62	18.45 ± 0.82
46W94	44.92 ± 0.47	44.89 ± 0.25	18.46 ± 0.53	17.95 ± 0.18
DKW46-15	41.15 ± 0.33	38.07 ± 0.32	20.51 ± 0.80	24.45 ± 0.60
DKW44-10	33.53 ± 0.53	27.90 ± 0.64	23.79 ± 0.39	26.68 ± 0.49
T	***		*	
C	***		***	
T x C	***		**	

Table 2.10 Saturated seed fatty acid composition (%) under control and HNT treatments. Tolerant cultivars are in normal font while the susceptible cultivars are in italics. Variation in fatty acid concentration is given as mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; NS = Non-significant based on ANOVA, T - Treatment; C – Cultivar

Cultivars	Palmitic acid (16:0) (%)		Stearic acid (18:0) (%)	
Experiment 1	Control	HNT	Control	HNT
Mercedes	4.13 ± 0.03	4.30 ± 0.06	1.16 ± 0.07	1.26 ± 0.03
Edimax CL	4.46 ± 0.12	4.46 ± 0.15	1.00 ± 0.00	1.17 ± 0.09
DKW46-15	4.20 ± 0.26	4.60 ± 0.10	1.43 ± 0.03	1.63 ± 0.03
DKW44-10	3.85 ± 0.05	4.05 ± 0.05	1.05 ± 0.03	1.20 ± 0.00
T	*		***	
C	*		***	
T x C	NS		NS	
Experiment 2				
Mercedes	4.27 ± 0.07	4.40 ± 0.06	1.13 ± 0.03	1.20 ± 0.06
Edimax CL	4.50 ± 0.10	4.47 ± 0.07	1.00 ± 0.00	1.23 ± 0.03
DKW46-15	4.30 ± 0.10	4.80 ± 0.10	1.43 ± 0.03	1.67 ± 0.07
DKW44-10	3.93 ± 0.03	4.10 ± 0.00	1.37 ± 0.03	1.60 ± 0.06
T	**		***	
C	***		***	
T x C	*		NS	

Table 2.11 Unsaturated seed fatty acid composition (%) under control and HNT treatments. Tolerant cultivars are in normal font while the susceptible cultivars are in italics. Variation in fatty acid concentration is given as mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; NS = Non-significant based on ANOVA, T - Treatment; C - Cultivar

Cultivars	Oleic acid (18:1) (%)		Linoleic acid (18:2) (%)		Linolenic acid (18:3) (%)		Gadoleic acid (20:1) (%)	
Experiment 1	Control	HNT	Control	HNT	Control	HNT	Control	HNT
Mercedes	64.80 ± 0.45	65.03 ± 0.39	18.23 ± 0.20	18.87 ± 0.27	9.17 ± 0.34	8.20 ± 0.21	1.17 ± 0.03	1.13 ± 0.03
Edimax CL	62.00 ± 0.72	63.83 ± 1.28	19.87 ± 0.48	18.43 ± 0.71	9.43 ± 0.12	8.87 ± 0.75	1.30 ± 0.06	1.27 ± 0.09
DKW46-15	65.06 ± 0.84	64.03 ± 0.93	18.50 ± 0.70	18.80 ± 0.35	7.67 ± 0.19	8.20 ± 0.51	1.23 ± 0.03	1.10 ± 0.00
DKW44-10	63.75 ± 0.61	62.50 ± 0.2	19.90 ± 0.39	20.85 ± 0.25	10.52 ± 2.26	8.55 ± 0.35	1.65 ± 0.06	1.35 ± 0.05
T	NS		NS		NS		**	
C	*		**		NS		***	
T x C	NS		*		NS		*	
Experiment 2								
Mercedes	64.73 ± 0.58	64.83 ± 0.35	19.17 ± 0.32	18.77 ± 0.38	8.37 ± 0.22	8.23 ± 0.03	1.17 ± 0.03	1.17 ± 0.03
Edimax CL	62.53 ± 0.97	63.43 ± 0.32	20.13 ± 0.56	19.03 ± 0.26	8.93 ± 0.38	8.60 ± 0.06	1.30 ± 0.06	1.23 ± 0.03
DKW46-15	63.37 ± 1.56	64.07 ± 2.04	19.70 ± 1.14	18.57 ± 0.87	8.07 ± 0.27	8.00 ±0.90	1.30 ± 0.06	1.13 ± 0.07
DKW44-10	62.73 ± 0.74	64.17 ± 0.43	19.80 ± 0.56	19.37 ± 0.27	8.03 ± 0.32	7.90 ± 0.10	1.73 ± 0.03	1.57 ± 0.03
T	NS		NS		NS		*	
C	NS		NS		NS		***	
T x C	NS		NS		NS		NS	

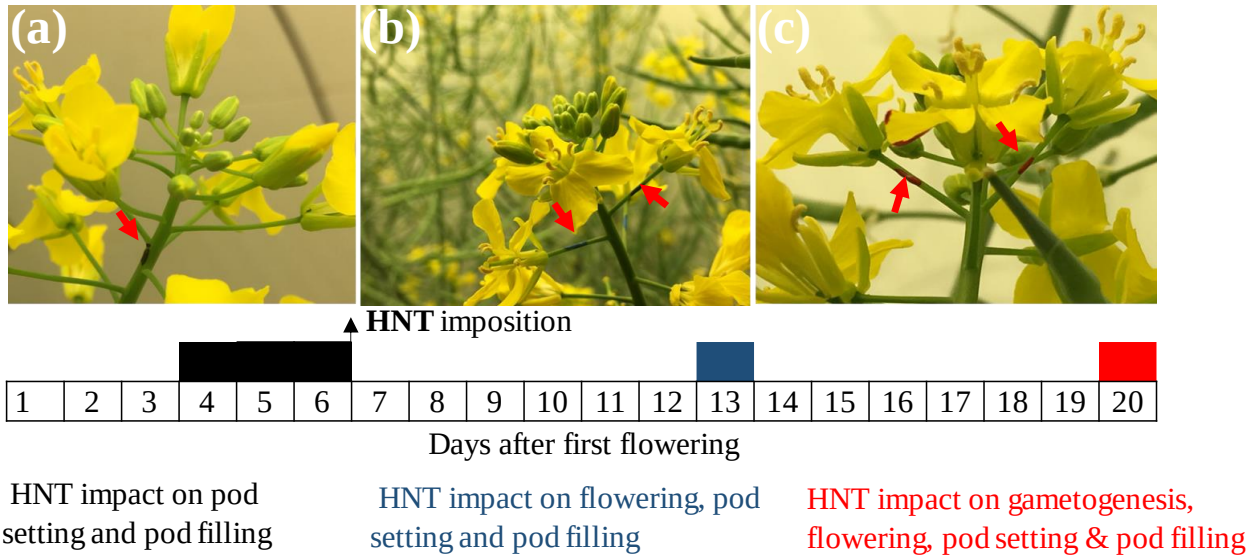

Figure 2.1 Schematic illustration of HNT stress exposure including different developmental stages- Gametogenesis, flowering, pod setting, pod-filling stages. Red arrows point to black (a), blue (b) and red markings (c). "1" on the number scale indicates the start of flowering.

Figure 2.2 Canola at the flowering stage - Picture was taken at 5:30 a.m. Red arrows indicate mature but unopened flower buds before the lights were turned on. Flowers that are open were from previous days flowering.

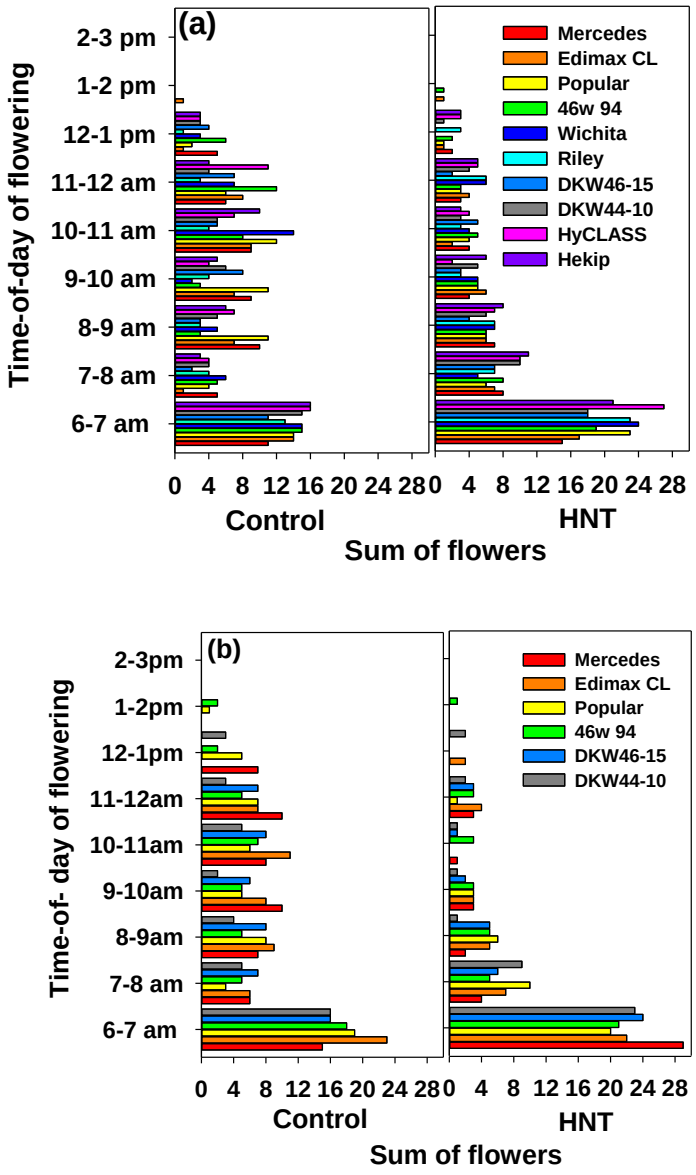

Figure 2.3 Time-of-day of flower opening in different canola cultivars under Control and HNT treatments from Experiment 1 (a) and Experiment 2 (b). Data presented in both experiments is the sum of flowers collected at hourly interval from three replicate plants for each cultivar, for three consecutive flowering days, starting from the first day of flowering and day of HNT imposition under control and HNT treatments, respectively.

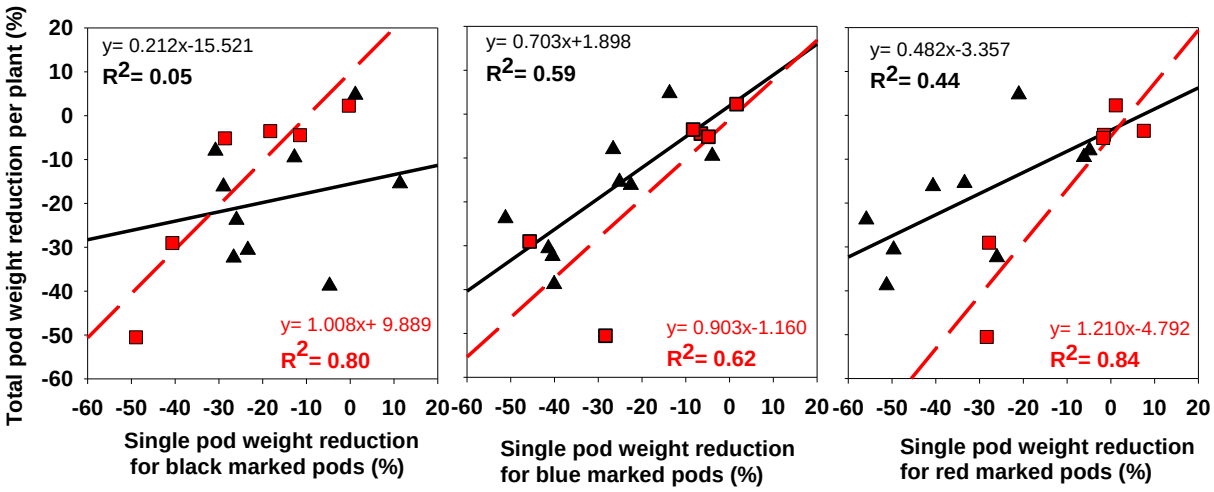

Figure 2.4 Relationship between total pod weight reduction per plant (%) and single pod weight reduction (%) for black, blue and red marked pods under HNT stress from Experiment 1 (solid black line with triangles) and Experiment 2 (dashed red line with squares).

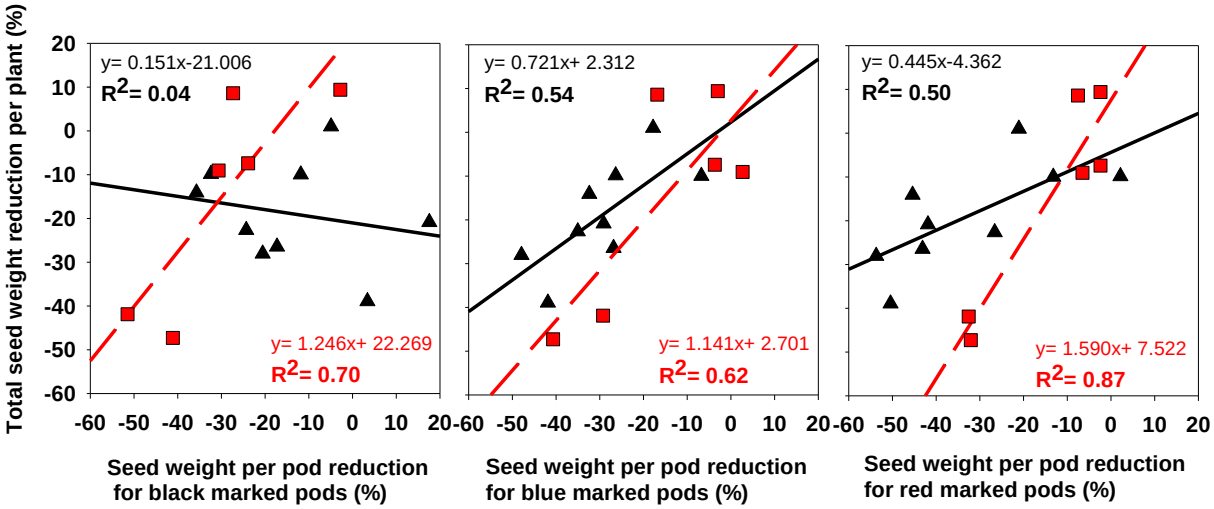

Figure 2.5 Relationship between total seed weight reduction per plant (%) and per pod seed weight reduction (%) for black, blue and red markings under HNT stress from Experiment 1 (solid black line with triangles) and Experiment 2 (dashed red line with squares).

Chapter 3 - Heat stress affects flowering pattern, pod set and seed quality in in chamber and field grown winter canola

Abstract

The impact of heat stress coinciding with reproductive stages in winter canola was studied using walk-in controlled environment chambers and field-based tents. Six different cultivars (46W94, Edimax CL, Mercedes, Popular, DKW44-10 and DKW46-15) were used in both the experiments following a split-plot design. Under controlled chambers, all six cultivars were exposed to HDT (day/night; 34/15°C), HNT (23/20°C), HDNT (34/20°C) and control (23/15°C) conditions for 14 days coinciding with flowering. Under field conditions, custom built "heat tents" were used to impose heat stress starting seven days after 50% of flowering till maturity. The results demonstrated that HNT, HDT and HDNT stress significantly shifted flower opening time towards early morning hours, while HDT and HDNT induced significant floral sterility and complete yield loss with two weeks of stress exposure. However, biomass, seed weight and oil concentration at maturity were either significantly increased or unchanged which demonstrated significant plasticity in canola to overcome damage caused by short episode of HDT and HDNT during flowering. The long duration heat stress under field conditions recorded significant decrease in yield parameters and oil concentration in the canola cultivars. Incorporating greater post-stress plasticity will help develop canola as an ideal alternative crop under future climates predicted to enhance frequency of short heat stress episodes during critical reproductive stages.

3.1 Introduction

Canola (*Brassica napus* L.), also known as oilseed rape or double-low rapeseed, is currently one of the most productive and important oilseed crops grown worldwide (Zhang & Flottmann, 2016). The increasing threat of climate change is already having a substantial impact on agricultural production worldwide, as heat waves can cause significant yield losses (Gornall et al., 2010). Over the past century, global mean air temperature has increased by 0.5°C which is predicted to further increase by 1.5–4.5°C by 2100 (IPCC, 2014). Further, the daily minimum temperature is reported to be rapidly increasing at twice the rate of the daily maximum temperature (Sillmann et al., 2013; Screen, 2014; Sadok & Jagadish, 2020). Taken together, being a cool season winter crop, canola production is extremely sensitive to variations in both day and night temperature particularly during reproductive (Singh et al., 2008; Angadi et al., 2000) and pod-filling stages (Weymann et al., 2015; Young et al., 2004). Thus, it is important to quantify the independent effect of high day temperature (HDT), high night temperature (HNT) and combined high day and night temperature (HDNT) in order to ameliorate the impact of heat stresses, particularly in winter crops including winter canola. Support for pursuing such investigation in field crops is obtained from mapping exercises, wherein a differential occurrence of HDT, HNT or their combination was observed across different rice growing regions of the world (Laborte et al., 2012).

Within the reproductive stage, flowering has been identified to be the most sensitive stage to heat stress due to its negative impact on floral fertility (Angadi et al., 2000). Complete sterility in Summer rape (cultivars Delta and Westar) was observed on exposure to 27/17°C day/night temperature from late bud development through early seed formation (Morrison, 1993). Significant seed yield losses were documented beyond a critical threshold temperature of 29.5°C (Tmax) during flowering in *Brassica* species (Morrison & Stewart, 2002). Imposing severe heat

stress during flowering [32/26°C day/ night (Polowick & Sawhney, 1988) and 35/15°C (Angadi et al., 2000; Gan et al., 2004)] resulted in complete sterility or abortion of pods. Heat stress during flowering in canola can prematurely end flowering, resulting in lower seed set (Faraji et al., 2009). Pollen viability and pollen tube growth were adversely affected with 35^{o}C exposure during flowering resulting in 50% reduction in pod set and yield in canola, without a reduction in number of flowers (Young et al., 2004). In addition, heat stress (35/18°C) coinciding with pod development was shown to be highly sensitive as seed-filling duration was significantly shortened, leading to reduced seed weight (Gan et al., 2004). The potential to recover from heat stress damage induced during flowering by producing additional new branches to accommodate more flowers and pods after the stress subsided has been observed by McGregor (1981). Although similar findings on damage recovery or plasticity were noticed by Young et al. (2004), Angadi et al. (2000) and Gan et al. (2004), the degree of recovery, related to number of inflorescences, pods and seed yield gained after stress removal, was not quantified.

Higher night temperatures can induce differential sensitivity to crop growth and development compared to high day temperatures (Lobell & Ortiz-Monasterio, 2007). HNT exposure had severe impact on rice grain growth compared to HDT (Morita et al., 2002). In field grown rice, 6 to 10% reduction in grain yield was associated with every 1°C increase in season-long night temperature (Lyman et al., 2013; Peng et al., 2004). Similarly, HNT stress during flowering in cotton recorded significant reductions in the number of flowers per plant (Loka & Oosterhuis, 2016) and the number of seeds per boll (Echer et al., 2014). A previous study documented a quantitative impact of HNT (23/20°C) from gametogenesis until maturity resulting in a significantly higher yield loss in winter canola. HNT also resulted in a significant shift in flowering time, decreased photochemical efficiency of PSII, pod numbers, grain yield and oil

concentration but increased protein concentration (Pokharel et al., 2020). However, the impact of high night combined with high day temperature stress during flowering has not been investigated in canola.

Between two and five weeks after flowering is considered to be the most active stage of synthesis and storage of seed components (Fowler & Downey, 1970; Deng & Scarth, 1998). Reduced oil concentration but increased protein concentration in seeds has been reported even from short episodes of heat stress (four days of 38°C for four hours each day) after 29 days from first flowering in canola (Aksouh-Harradj et al., 2006; Zhu et al., 2012; Zhang et al., 2014). This was attributed to reduced photosynthetic carbon assimilation in leaves and green pod walls (Hua et al., 2012). Except for a recent report (Pokharel et al., 2020), there is limited information on HNT impact on seed oil and protein and no reports on a simultaneous testing of HDT, HNT and their combination on seed oil and protein composition.

Most of the previous heat stress studies on rapeseed or canola have been limited to controlled environment chambers conditions due to the lack of a field-based phenotyping facility (Faraji et al., 2012; Singh et al., 2008; Gan et al., 2004; Angadi et al., 2000). Findings from these studies could vary considerably compared to field conditions due to the inherent limitations that are associated with controlled environment chamber studies such as limited light and reduced wind speed (Bahuguna et al., 2017). There have been efforts to quantify the impact of heat stress under field conditions in rice (Shi et al., 2013; Bahuguna et al., 2017) and wheat (Hein et al., 2019; Bergkamp et al., 2018) using field-based heat tents. These facilities have allowed testing of crops for heat stress response under realistic field conditions. Similar efforts to phenotype and compare the physiological and agronomic responses of winter canola exposed to heat stress under field conditions have not been attempted. In addition, the integration of field-based studies with

controlled environment studies has been proposed to be complementary and has been suggested as a better phenotyping approach under chilling stress in sorghum (Chiluwal et al., 2018).

Considering the identified knowledge gaps, a controlled environment walk-in chamber experiment was conducted to quantify the impact of high day, high night temperature stress and their combination on (i) time-of-day of flower opening dynamics and physiological responses during flowering affecting yield and its components (ii) pod set, and pod and seed weight within stress exposure during flowering and (iii) changes in oil and protein concentration in winter canola. Based on results from the controlled environment study, a field based heat-tent study was conducted to investigate the impact of HDT stress on physiological responses during flowering and pod-filling stages affecting yield and its components, changes in oil and protein concentration and fatty acid composition in winter canola.

3.2 Material and Methods

3.2.1 Crop husbandry

Four canola hybrids; 46W94, Edimax CL, Mercedes and Popular and two open-pollinated canola cultivars; DKW44-10, DKW46-15 were chosen for the controlled environment walk-in chambers study. The growth medium was similar to Pokharel et al. (2020) which consisted of a skid loader scoop of soil (135.92 kg), 79.28 kg of Sun Gro Metro Mix 360 (Sun Gro Horticulture, Agawam, Massachusetts), perlite (8618.26 g), and fertilizers: 113.40 g of Osmocote (13-13-13), 113.40 g of Osmocote (14-14-14), 113.40 g of gypsum, 113.40 g of ammonium phosphate (18-46-0), and 113.40 g of elemental sulfur and micronutrients. This mix was used to fill 150 pots, each with a 3.78 L capacity.

3.2.2 Walk-in growth chambers and heat treatments

HDT, HNT and HDNT stress experiment was conducted using the walk-in controlled environment chamber facility in the Department of Agronomy, Kansas State University, Manhattan, KS, USA. The experiment was laid out in a randomized complete block with a split-plot design wherein temperature regimens were the main plot and the different cultivars were the subplot.

The seeds of all six canola cultivars were sown and maintained at 23/15°C (day/night) in the greenhouse. Two-week-old seedlings were subjected to vernalization for eight weeks at 4°C, in a plant growth chamber (Percival Mfg. Co., Model 1-37X, Perry, Iowa). The vernalized seedlings were transplanted into 3.78 L pots and maintained under control temperatures of 23/15°C (day/night) and a photoperiod of 16/8 h light/dark until beginning of heat stress exposure using controlled environment walk-in chambers (249 cm wide, 137 cm deep, and 180 cm high; Conviron, Winnipeg, MB, Canada). Canola cultivars were exposed to four different temperature regimens, with six replicate plants for each cultivar and each of the four treatments listed below and graphically presented in Figure 3.1:

1. High day temperature (HDT): 34/15°C (day maximum/night minimum), lasting 7 hours per day, starting from 9:00 AM to 4:00 PM, starting on the 4th day after first sign of flowering and continued for two weeks.
2. High night temperature (HNT): 23/20°C, lasting 10 hours per day, starting from 8:00 PM to 6:00 AM, starting on the 4th day after first sign of flowering and continued for two weeks.
3. High day and night temperature (HDNT): 34/20°C, with 34°C lasting 7 hours per day, i.e., from 9:00 AM to 4:00 PM and 20°C lasting 10 hours per night, starting from 8:00 PM to 6:00 AM, starting on the 4th day after first sign of flowering and continued for two weeks.

4. Control temperature: 23/15°C with the photoperiod of 16/8 h light/dark kept consistent across all four treatments.

The plants from all temperature regimens were watered regularly based on visual soil appearance to avoid any water-limited condition. The photosynthetic photon flux density at the leaf level was ≥800 μmol m^{-2} s^{-1}. The relative humidity (RH) in all growth chambers was set to 70% during the day and night. Air temperature and RH were continuously monitored at 15-min intervals in all growth chambers throughout the experiment using HOBO data loggers (Onset Computer Corp., Bourne, MA, USA).

3.2.3 Plot establishment and experimental design under field experiment

The field experiment was conducted in the 2018-2019 growing season using field-based "heat tents" at Agronomy North Farm (39 11'N, 96 35'W), Department of Agronomy, Kansas State University, Manhattan, KS, USA. The experiment included four canola hybrids; 46W94, Edimax CL, Mercedes and Popular and two open-pollinated canola cultivars; DKW44-10 and DKW46-15. The seedlings of the 46W94 hybrid were significantly damaged due to winter frost and failed to recover, hence no data was collected from this hybrid.

The field experiment was laid out in a randomized complete block design following a split-plot arrangement with 4 replications for each cultivar and treatment. The temperature regimens (Control and HDT) were the main plot and the cultivars were the subplot. Plot preparation prior to planting included multiple tillage passes of a disc, cultivator, and harrow in the summer of 2018 to prepare the seedbed for planting. Ammonium polyphosphate (10-34-0) was applied at a rate of 15 liters/hectare and ammonium thiosulfate (12-0-0-26) was applied at a rate of 18 liters/hectare at planting. Weed control in the canola plots was managed using a pre-emergent herbicide, trifluralin, at the rate of 0.4 liter/hectare, along with hand weeding as needed to minimize weed

pressure throughout the growing season. The recommended rate of 13 kg/hectare of nitrogen was top dressed in the form of urea (46-0-0) at the end of March 2019, using a variable rate drop spreader (Gandy Company, Owatonna, MN).

The seeds were sown on 13th Sept. 2018 using a seed planter. Ten blocks (5 m wide and 6 m long) were planted in the field. Four uniform blocks out of ten were selected for HDT stress treatment using field-based heat tents and an equal number of blocks were selected for control (ambient) temperature treatment. Each block had six sub plots (1.5 x 3 m) with each subplot planted with one of the six cultivars. Two grams of seeds were sown in each subplot, which contained 6 rows with 0.25 m spacing between rows. All plots were randomly distributed within each block and blocks were randomly selected for imposing HDT treatment using heat tents.

3.2.4 Heat Stress imposition using heat tents

HDT stress was imposed using field-based heat tents, which were manually placed over the plots approximately 7 days after 50% of flowering (Figure 3.2). Flowering in the tested canola cultivars was initiated between 216 and 218 days after planting (DAP) and reached physiological maturity between 260 and 263 DAP, indicating uniform phenology across the tested cultivars. Each heat tent (5.4 m wide, 7.2 m long, and 3.0 m high at the apex) consisted of a galvanized steel framework and a moveable overhead flap (0.6 m) on the top that opened and closed depending upon the level of heat inside the tent. The steel framework was covered by clear polyethylene film that transmitted 92% of the solar radiation (6 mil Sun Master® Pull and Cut Greenhouse Film), which was source for generating latent heat inside the tents. The temperature inside the tents was regulated by a thermostat set at 34°C. When the temperature inside the tent was above the target temperature (34°C), the thermostat triggered the actuators to open the overhead flap, allowing ambient air circulation to avoid excessive heating. On the other hand, when the temperature was below 34°C,

the overhead flap was programed to automatically close to retain heat inside the tent. Along with the overhead flap, each tent had a 15 cm clearance at the bottom on all four sides to allow better air circulation and temperature regulation within the heat tent (for additional details, see Bergkamp et al., 2018). Air temperature and RH were continuously monitored at 15-min intervals in all four heat tents and outside controls throughout the experiments using HOBO data loggers (Onset Computer Corp., Bourne, MA, USA). The control plots were maintained under open field conditions. Daily average maximum and minimum temperatures both within and outside the heat tents during the entire stress period are presented in Figure 3.3.

3.2.5 Observations

3.2.5.1 Time-of-day of flower opening

The time-of-day of flower opening was recorded at hourly intervals for three consecutive flowering days following Chiluwal et al. (2019) and Pokharel et al. (2020). The flowering pattern was recorded starting from the first day of flowering under the control treatment and from the day of heat stress exposure under HNT, HDT and HDNT treatments. Three plants were chosen to record the cumulative number of open flowers at hourly interval starting from 6:00 AM to 2:00 PM. The total number of flowers that opened at hourly interval was recorded cumulatively to avoid any manual stimuli, which is known to affect the flowering pattern (Chiluwal et al., 2019; Kobayasi et al., 2010). Newly opened flowers recorded over three flowering days from three plants at hourly intervals were summed to get a single value for each cultivar and treatment. Flower opening time was recorded only in controlled environment chambers experiment.

3.2.5.2 Effective quantum yield and chlorophyll index

For both chamber and field experiments, effective Quantum Yield (QY) of photosystem II in the light adapted state was measured using FluorPen (Photon System Instruments, Ltd., Brno, Czech

Republic) and chlorophyll index was measured using a self-calibrating chlorophyll meter (Soil Plant Analyzer Development [SPAD], Model 502, Spectrum Technologies, Plainfield, IL, USA).

In the growth chamber study, both quantum yield and chlorophyll index were recorded after two, four, seven and 14 days after each of the four treatments were initiated. Both measurements were taken at three different places on the second and third leaf from the top of the main stem and averaged to get a single value for a plant. These measurements were taken on all six replicate plants for each cultivar between 10:00 AM and 1:00 PM, across all four treatments.

In the field experiment, both quantum yield and chlorophyll index were recorded two and four weeks after HDT imposition. Three representative plants per cultivar were selected from each subplot per tent to measure both the traits. Both measurements were taken at three different places on the fourth leaf from the top of the main stem and averaged to get a single value for a plant. Simultaneously, QY and chlorophyll index measurements were also recorded at the same time points on control plants.

3.2.5.3 Marking of flowering branches

To capture the direct impact of different heat stress regimens on pod number, pod weight and seed weight, flowering branches were marked with two different colors of acrylic paint: black (Figure 3.4A) and red (Figure 3.4B). Since the flowering period lasts for more than two weeks in indeterminate canola plants, the marking approach was essential to consider only flowers that were exposed to heat stress for subsequent analysis following Pokharel et al. (2020). Three days after the start of flowering, all flowering stems were marked with black paint above the last opened flower (Figure 3.4A), while the stems were marked with red paint after two weeks of stress imposition (Figure 3.4B) in all four treatments including the control. Flowers that opened during the two weeks of stress period and positioned within these markings were considered for analyzing

the heat stress impact during flowering. After the completion of red marking, after 14 days of stress exposure, plants were moved back to control conditions (23/15°C). No markings were made with the field experiment due to the long-duration stress, which encompassed flowering and lasted until maturity.

3.2.5.4 Yield and yield components

At physiological maturity, plants were hand harvested by cutting the stem at the base. Number of pods per plant was recorded at the time of harvest. Marked pods, exposed to two weeks of stress period under control, HNT, HDT and HDNT treatments were collected and dried separately. Vegetative parts (leaves still attached to the plant), marked pods, and all remaining pods were dried at 60, 40, and 40°C, respectively, for one week following Pokharel et al. (2020). Aboveground biomass was determined as the weight of retained leaves, stem and pod weight per plant, including marked pods. After drying the pods, the pod weight from the marked portions and other remaining pods per plant were recorded, separately, for all four treatments. Harvested pods from the growth chamber study were threshed manually after drying to separate seeds while the pods harvested from field experiment were threshed using an ALMACO belt thresher (ALMACO, Nevada, IA). Finally, seed weight of marked pods and remaining pods per plant were recorded under control, HNT, HDT and HDNT treatments.

To record the yield and yield related parameters from the field experiment under both control and HDT treatments, 12 representative plants were hand harvested out of four sub-plots from each of the cultivar and treatments. Twelve plants were then randomly divided into 3 groups, each having 4 biological plants. Later, aboveground biomass, pod numbers, pod and seed weight from four biological plants were averaged to get a single value for respective traits forming a replication.

3.2.5.5 Oil and protein composition

Oil and protein concentration per plant were measured from 8 g seed samples using near infrared spectrophotometry (NIR) (FOSS Analytical XDS Rapid Content Analyzer, FOSS North America, Eden Prairie, MN) from both experiments. The percentages of different fatty acids were determined only from field experiment with 2 g seed samples using gas chromatography (Hammond, 1991) at the University of Idaho Brassica Breeding and Research Program's Oilseed Quality Laboratory (http://www.cals.uidaho.edu/brassica/).

3.2.6 Data analysis

All the statistical analysis was carried out using SAS 9.4 (SAS Institute, 2013). Analysis of variance (ANOVA) for split-plot design was performed using the generalized linear model (GLM) procedure. Means between treatments were compared using least significant difference (LSD) at a $p < 0.05$ significance level. Temperature regimens, genotypes and measured traits were considered as fixed effects and the replications were considered as random effects.

3.3 Results

3.3.1 Results from controlled chamber study

3.3.1.1 Flowering patterns

No flower opening was observed before 6:00 AM in all of the canola cultivars under four treatments, similar to Pokharel et al (2020). The majority of flowers opened during the morning from 6:00 AM to 12:00 PM., distributed with similar proportion under control conditions. However, the trend was more conspicuous with a significant shift in peak flower opening towards earlier morning hours (6:00 AM to 7:00 AM). The pattern was the same across all the investigated canola cultivars exposed to high temperature stress (HNT, HDT and HDNT) in the controlled environment chamber experiment (Figure 3.5).

3.3.1.2 Effective quantum yield and chlorophyll index

Effective quantum yield (QY) differed significantly among temperatures, cultivar and their interaction after two, seven and 14 days of heat stress imposition and only temperature had a significant impact after 4 days of heat stress exposure (Table 3.1 and Table 3.2). Mercedes and Edimax CL had a significant reduction of 3% and 4% in QY only after 7 days of HDNT stress imposition (Table 3.1). Significant reductions of 5, 3 and 5% in QY were observed for Popular after two, four and 14 days with HDT (Table 3.1 and Table 3.2). DKW46-15 had significant reduction in QY with 3, 6, 6, and 5% under HDT after two, four, seven and 14 days after heat stress, respectively, and a reduction of 3, 4 and 4% under HDNT after four, seven and 14 days after heat stress imposition, respectively, compared to control. Similar to DKW46-15, QY in DKW44-10 was significantly reduced by 3, 4, 5 and 5% on two, four, seven and 14 days after HDT, respectively, and 3, 4 and 6% under HDNT on four, seven and 14 days after heat stress imposition, respectively (Table 3.1 and Table 3.2).

Chlorophyll index was significantly affected by temperature and cultivar after two, four, seven and 14 days of heat stress imposition but temperature by cultivar interaction was only significant after seven and 14 days of heat stress exposure (Table 3.3 and Table 3.4). Among the treatments, significant reduction was observed only in HDNT stress exposure. Across the cultivars, DKW44-10 recorded a significant reduction of 14, 8, 8 and 6% in chlorophyll index after two, four, seven and 14 days from HDNT stress imposition, respectively, while DKW46-15 had significant reduction of 8% only after 7 days of HDNT exposure (Table 3.3 and Table 3.4).

3.3.1.3 Impacts of heat stress on floral morphology

The morphology of the flowers was not affected under both control and HNT treatments. The size of flowers was consistent and all four petals were normally shaped. The length of stamens (male

reproductive organ) exceeded the gynoecium (female reproductive organ) in all the flowers under control (Figures 3.6A and 3.6E) and HNT (Figures 3B and 3F). On the other hand, we observed significant changes in the flower morphology on plants exposed to seven days of HDT and HDNT stress. The flowers produced by the canola cultivars under HDT and HDNT were stunted and a majority of them (> 90%) failed to open but had the stigma protruding beyond the closed sepals (Figures 3.6C and 3.6D). The few flowers that opened were abnormally shaped with shriveled petals and shrunken stamens (Figures 3.6G and 3.6H).

3.3.1.4 Quantitative heat stress impact and whole plant response on yield components

3.3.1.4.1 Aboveground biomass

Significant differences for temperature, cultivar, and the temperature by cultivar interaction were observed in aboveground biomass (Table 3.5). There was no significant difference between control and HNT. Aboveground biomass was significantly increased in all cultivars with two weeks of HDT and HDNT compared to control and HNT exposure during flowering (Table 3.5). Averaged across the cultivars, two weeks of HDT and HDNT exposure increased aboveground biomass by 42 and 28% respectively, compared to control (Table 3.5).

3.3.1.4.2 Pod number

Pod numbers collected from flowers exposed to two weeks of heat stress during flowering were significantly affected by temperature, cultivar and the temperature by cultivar interaction (Table 3.6). Pod numbers were significantly decreased by HDT and HDNT treatments in all the cultivars, compared to the control, while there was no significant reduction in pod number between HNT and control treatments. Averaged across cultivars, with two weeks of stress, pod numbers were reduced by 93% in HDT and 98% in HDNT treatments compared to the control (Table 6).

At the whole plant level, pod number per plant at maturity was significantly affected by temperature and cultivar and their interaction (Table 3.6). After two weeks of heat stress, no significant reduction in total pod number was observed among cultivars with HDT exposure except for Mercedes, which recorded a 36% increase in total pod number (Table 3.6). Similarly, HDNT exposure recorded no significant difference in 46W94, Edimax CL and Popular but induced even higher pod number in Mercedes (32%) and DKW46-15 (26%) compared to control. Across HDT and HDNT treatments, Mercedes showed the highest plasticity in overcoming the negative impact of short episodes of heat stress during flowering (Table 3.6).

3.3.1.4.3 Pod weight

Temperature, cultivar and their interaction differed significantly for pod weight with 14 days of heat stress imposition (Table 3.7). All the canola cultivars recorded significant reductions in pod weight with 14 days of HDT and HDNT treatments while there was no significant reduction between HNT and control treatments (Table 3.7). Averaged across cultivars, HDT and HDNT resulted in a 96% and 99% reduction in pod weight with 14 days of heat stress imposition compared to control.

Although total pod weight per plant at maturity differed significantly between temperature, cultivar, and the temperature by cultivar interaction, the response was opposite compared to the weight of pods obtained from 14 days stress period (Table 3.7). The total pod weight per plant was not significantly affected by two weeks of high temperature treatments in all the cultivars. Mercedes, DKW44-10 and 46W94 recorded 47, 45 and 28% increases in total pod weight after release of 14 days of HDT exposure, respectively (Table 3.7). Significant increases in pod weight per plant were observed with Mercedes (38%) and DKW46-15 (32%) after releasing plants from HDNT exposure (Table 3.7).

3.3.1.4.4 Seed weight

Similar to pod number and pod weight with 14 days of heat stress exposure during flowering, seed weight was significantly affected by temperature, cultivar and their interaction (Table 3.8). A significant reduction in seed weight was documented with 14 days of HDT and HDNT exposure in all the cultivars, compared to control and HNT treatments. Averaged across cultivars, seed weight was reduced by 98% in HDT and 99% in HDNT treatments compared to the control (Table 3.8).

Similar to pod number and pod weight at the whole plant level, seed weight per plant at maturity was significantly affected by temperature, cultivar and by the temperature and cultivar interaction. (Table 3.8). Short episodes of HDT increased the total seed weight by 53, 46 and 30% in DKW44-10, Mercedes and 46W94, respectively. Similarly, after HDNT was released, Mercedes, DKW44-10 and DKW46-15 recorded 45, 34 and 27% increases in seed weight per plant, respectively, compared to the control (Table 3.8).

3.3.1.5 Oil and protein concentration

Significant temperature, cultivar, and temperature by cultivar interaction effects were recorded for total oil and protein concentrations in this controlled environment experiment (Table 3.9). After two weeks of heat stress imposition, HDT exposed plants recorded significant increases in oil concentration by 17, 16, 13, 11 and 9% in Mercedes, 46W94, DKW44-10, Popular and DKW46-15, respectively, compared to the control. Similar to HDT, oil concentration was significantly increased by 15, 13, 11 and 7% in DKW44-10, Mercedes, DKW46-15 and 46W94 cultivars, respectively, with HDNT exposure compared to the control (Table 3.9). The cultivar Edimax CL did not show any significant difference in oil concentration among the treatments. In contrast to the oil concentration, all the canola cultivars recorded significant reductions in protein with both

HDT and HDNT treatments. The highest reduction in protein concentration was observed in Mercedes in both HDT (23%) and HDNT (20%) treatments while the lowest reduction was recorded in Edimax CL (9%) in HDT and Popular (8%) in the HDNT treatment (Table 3.9).

3.3.2 Results from field study

3.3.2.1 Effective quantum yield and chlorophyll index

Quantum yield of PSII was not significantly affected by temperature, cultivar and the temperature by cultivar interaction after two and four weeks of HDT stress exposure, except for cultivar at four weeks after stress (Table 3.10). Similarly, no significant difference among temperatures was observed in chlorophyll index after two and four weeks of heat stress exposure but was significantly affected by cultivar after four weeks, with a temperature by cultivar interaction only after two weeks of stress exposure (Table 3.10).

3.3.2.2 Yield and yield components

3.3.2.2.1 Aboveground biomass

Aboveground biomass differed significantly by temperature and cultivars but not with temperature by cultivar interaction under field conditions (Table 3.11). No significant difference was observed in Popular, Mercedes and Edimax CL cultivars but aboveground biomass per plant was significantly reduced in DKW44-10 (25%) and DKW46-15 (15%) with HDT treatments versus ambient conditions (Table 3.11).

3.3.2.2.2 Total pod number per plant

Significant temperature and cultivar main effects were observed for pod number per plant in the field experiment (Table 3.12). Mercedes, Edimax CL and Popular showed no significant reduction in pod number between treatments while, DKW44-10 and DKW46-15 recorded significant

reductions of 27 and 40%, respectively, compared to the control. Across the cultivars, Popular (14%) and DKW44-10 (40%) recorded the lowest and highest reduction in total pod number per plant under long duration HDT stress exposure (Table 3.12).

3.3.2.2.3 Total pod weight per plant

Pod weight per plant differed significantly between temperature, cultivar, and the temperature by cultivar interaction (Table 3.12). HDT resulted in a significant reduction of total pod weight in DKW46-15 (28%) and DKW44-10 (34%) cultivars. However, Popular, Mercedes and Edimax CL recorded no significant difference in pod weight between HDT and ambient conditions (Table 3.12).

3.3.2.2.4 Total seed weight per plant

Similar to pod weight per plant, seed weight per plant was significantly affected by temperature, cultivar and the temperature by cultivar interaction in the field experiment (Table 3.12). Cultivars DKW46-15 and DKW44-10 recorded a 40% reduction while Edimax CL recorded a 20% lower total seed weight with HDT stress compared to ambient conditions. Among the cultivars, Popular had the lowest reduction (9%) with HDT exposure (Table 3.12).

3.3.2.3 Oil and protein concentration

Oil and protein concentrations were significantly affected by temperature, cultivar, and temperature by cultivar interaction, while the interaction effect was not significant for protein concentration (Table 3.13). HDT stress resulted in significant reduction of 13, 10 and 8% in oil concentration for DKW46-15, DKW44-10 and Edimax CL, respectively, compared to the ambient conditions. Popular and Mercedes did not show a significant reduction in oil concentration under HDT. However, protein concentration was significantly increased in all winter canola cultivars under HDT (Table 3.13). Averaged across the cultivars, HDT resulted in 15% increase in protein

concentration, with highest increase of 16% recorded in both DKW44-10 and Edimax CL, compared to the control (Table 3.13).

3.3.2.4 Fatty acid profile

3.3.2.4.1 Saturated fatty acids

Significant temperature, cultivar, and temperature by cultivar interaction effects were recorded for both the saturated fatty acids with HDT stress under field conditions (Table 3.14). No significant different was observed for Mercedes, Edimax CL and DKW44-10 with both saturated fatty acids between HDT and control. Across the cultivars, DKW46-15 recorded a significant increase in stearic acid (24%) while Popular recorded a significant decrease in palmitic acid (8%) under HDT exposure, compared to ambient conditions (Table 3.14).

3.3.2.4.2 Unsaturated fatty acids

Among the unsaturated fatty acids, oleic acid and linoleic acids were only significantly affected by cultivar but not by temperature and temperature by cultivar interaction. Linolenic and Erucic acid differed significantly between temperature and cultivar but not by their interaction. Significant temperature, cultivar, and temperature by cultivar interaction was recorded only in gadoleic acid (Table 3.15). All the cultivars recorded no significant different in oleic acid, linoleic and linolenic acids between HDT and control except DKW46-15 wherein HDT stress resulted in a significant reduction with linolenic acid by 13% (Table 3.15). Among the cultivars, DKW46-15 and DKW44-10 recorded a significant increase of 34% and 19% in gadoleic acid with long duration HDT stress, respectively. Similarly, erucic acid concentration was also significantly increased in DKW46-15 (63%) and DKW44-10 (69%) with HDT stress, compared to ambient field conditions (Table 3.15).

3.4 Discussion

HNT, HDT and HDNT stresses advances time-of-day of flowering towards morning hours

Flowering is identified as the most sensitive stage to heat stress in canola and the flowers that open during higher temperatures can become sterile or develop into significantly smaller pods (Angadi et al., 2000). We observed a significant shift in peak flowering towards early morning with HNT, HDT and HDNT, but not a shift in time-of-start of flowering. In rice, early time-of-day of flowering advances peak flowering by 1.5 to 2 h, helping the sensitive flowering processes escape from late-morning and early-afternoon heat stress (Hirabayashi et al., 2015; Jagadish et al., 2015). This heat escape strategy, defined as early morning flowering, has shown to be effective in mitigating heat stress induced spikelet sterility in rice under controlled environment conditions (Hirabayashi et al., 2015) and field conditions (Bheemanahalli et al., 2017). Similarly, peak flowering in wheat was observed to be either earlier in the morning or later in the evening when the temperature was cooler, with this pattern becoming more prominent under HDT (Aiqing et al., 2018). Coast et al. (2015) recorded variation in flowering characteristics including the duration a spikelet remained open during flowering and start and peak flowering among rice cultivars exposed to night temperatures of 24, 30, and 35ºC. Although differences in time-of-day of flowering have been observed, mechanisms behind this phenomenon are not known in crops (Jagadish, 2020). As observed in canola, earlier opening of flowers could help escape heat stress sensitive physiological processes including anther dehiscence, allowing pollination and fertilization to occur under cooler morning hours. This trait has the potential to maintain productivity of winter canola under predicted warmer (day and night) environments.

HDT and HDNT negatively affects reproductive organs, pod number and yield

In our study, pod number, pod weight and seed weight were reduced by up to 98% with two weeks of HDT and HDNT exposure during flowering. Similar results of 81 to 96% yield reduction on the main stem was recorded by imposing 35/15°C day/night temperature during early flowering (Gan

et al., 2004; Angadi et al., 2000). This was primarily because the flowers that opened during the stress (35/15°C) could not produce any fertile pods and negatively affected developing floral buds and pods. Canola plants subjected to high temperatures during flowering often display increased abortion of flowers, pods and even leaves, due to an increased concentration of abscisic acid (ABA) (Nilsen & Orcutt, 1996). In addition, high day and night temperature of 32 /26 °C during flowering results in stunted flowers, shorter stamens and longer pistils (Polowick & Sawhney, 1988). Similarly, in our study, HDT and HDNT exposure significantly altered the floral morphology, yielding abnormally shaped and shriveled petals and shrunken stamens (Figure 3.6). As the duration of HDT and HDNT severity increased, the stamens were one-half as long as the gynoecium. Further, a majority of the flowers failed to open but had their stigma protruded beyond the closed sepals, indicating clear signs of pistil hyperplasia (Figure 3.6). Severe stress of 35/15°C during flowering is also shown to progressively reduce the total number of flowers that opened during the stress period (Angadi et al., 2000), or resulted in premature termination of flowering (Faraji, 2012). Although, decreased photosynthetic assimilation during flowering can potentially lead to lower availability of sugars to support pollen viability and fertilization (Kirby, 1988), HDT and HDNT in our study appears to have had the most significant impact on the viability of reproductive organs.

During flowering, pollen viability, fertilization and embryo development are sensitive to heat beyond a certain temperature threshold (Prasad et al., 2017; Rieu et al., 2017; Jagadish, 2020) and this is particularly true for cool-climate crops, including canola. Young et al. (2004) observed that pollen taken from plants exposed to 4 d of high temperature (35^{o}C) stress had lower *in vitro* pollen germination (17.5%) and pollen tube growth (59.2%) than the control plants regardless of whether *in vitro* germination was carried out at 23°C or 35°C. In another study, Singh et al. (2008)

evaluated *in vitro* pollen germination and pollen tube growth responses of 12 canola cultivars to a range of temperatures (from 5 to 35^{o}C) and concluded that the temperatures below or above the optimum (23.6^{o}C) caused a significant linear reduction in both traits in all 12 canola cultivars. In our study, similar damage to fertilization related processes or inaccessibility of pollen for fertilization due to pistil hyperplasia would have led to reduced pod number, translating to lower pod yield with HDT and HDNT exposure during flowering.

Release of heat stress demonstrated significant plasticity in canola in overcoming damage

Several studies on canola and *B. napus* species have reduced yield and yield components when exposed to heat stress during reproductive stages (Angadi et al., 2000; Gan et al., 2004; Young et al., 2004). Our previous study on HNT has shown a significant negative impact on yield, yield components and seed quality in susceptible winter canola cultivars during flowering and pod-filling stages (Pokharel et al., 2020). However, McGregor (1981) mentioned the possibility of considerable recovery, compensated by additional branches or flowers on new inflorescence or more seeds per pod after release of heat stress. Previous studies have observed significantly greater number of inflorescences, flowers and pods after release of short period (7-10 days) of HDT (35/15°C and 35/18°C) stress during early flowering (Angadi et al., 2000; Gan et al., 2004). Although the release in stress resulted in an increase in pod numbers and seed yield in canola, both the studies have reported significant reduction in seed yield ranging from 53 to 58% (Gan et al., 2004; Angadi et al., 2000).

In contrast to the above studies, we observed a 100% recovery with many of the cultivars recording significantly higher yield and yield components after two weeks of heat stress was released. Similar to the yield components, oil concentration was also significantly increased or unaffected, while protein concentration was significantly decreased in all tested winter canola

cultivars (Table 3.9). It is known that the synthesis and storage of seed components including oil occurs between two to five weeks after first flowering in oilseeds (Deng and Scarth, 1998; Aksouh-Harradj et al., 2006). However, with a short two weeks heat stress exposure during flowering, all new branches and pods were formed after stress was released and developed under non-stress conditions, leading to a significant increase in final plant yield (Tables 3.6, 3.7 and 3.8) and oil concentration (Table 3.9). Post-stress developed pods had more and larger sized seeds per pod, wherein the plants possibly overcame the low to moderate impact of QY and chlorophyll index (Tables 3.1 and 3.2; Tables 3.3 and 3.4) to increase the assimilate pool to overcome any loss in yield or oil concentration. The differences in plasticity observed by Gan et al. (2004), Angadi et al. (2000) and this study indicate a wide genetic diversity for post-stress plasticity in canola cultivars that needs to be further explored. This trait could help develop canola as an ideal alternative crop under future climate which is predicted to become more variable with increased frequency of heat shock episodes coinciding with sensitive reproductive stages.

Long duration HDT negatively affects oil formation and alters fatty acids composition under field conditions

Prevailing air temperature during seed-filling period has shown to negatively affect oil concentration and positively impact protein concentration in both open pollinated and hybrid cultivars of canola (Faraji, 2012). Reduced seed oil and increased protein content with high temperature (30/20^{o}C day/night) exposure from first day of flowering to maturation is associated with downregulation of several genes associated with photosynthesis and lipid metabolism, and upregulation of the genes associated with protein biosynthesis (Zhu et al., 2012). Similar mechanisms combined with no opportunity to exercise its genetic potential for plasticity, as

observed with short episode of heat stress under controlled environments conditions, would have resulted in reduced oil concentration under field conditions.

With longer duration of heat stress (30/25°C) until 40 days after first flowering or short period of extreme heat stress (38/23°C for 5 days) from 25 to 29 DAF altered the fatty acids profiles in different canola cultivars (Deng & Scarth, 1998; Pritchard et al., 2000; Aksouh-Harradj et al., 2006). These studies recorded higher levels of saturated fatty acids [palmitic (16:0) and stearic (18:0) acids] and oleic acid (18:1) and lower levels of linoleic and linolenic acids. Similarly, in a very early study, reduction in both linoleic and linolenic acids and an increase in oleic acid content was observed under constant post-flowering temperatures above 27°C in zero-erucic acid winter oilseeds (Canvin, 1965). In summary, the fraction of saturated fatty acids and mono-unsaturated fatty acids were increased while the fraction of polyunsaturated fatty acids were reduced in above studies. A recent study by Elferjani & Soolanayakanahally (2018) has documented similar increase in saturated fatty acids but contrasting results with unsaturated fatty acids with a decrease in oleic acids and an increase in linoleic fatty acid under high temperature stress (29/18°C day/night) starting from flowering till pod maturation in canola cultivars. Our results showed partial similarity with above studies with an increase in the fractions of saturated fatty acids in some cultivars and the mono-unsaturated fatty acids [gadoleic and erucic acids] but a consistent decrease in linolenic acid under long duration HDT under field conditions. The above findings indicate that high temperature stress greatly influences the seed fatty acid composition, but responses vary depending on the intensity of stress, duration and the stages included under stress exposure and the genetic background of the canola cultivars.

3.5 Conclusions

Short episodes of HDT and HDNT stress coinciding with flowering in winter canola resulted in a significant negative impact on floral morphology and induced complete sterility in flowers that opened during the stress conditions. At maturity, aboveground biomass and yield were significantly increased or unchanged which demonstrated inherent plasticity in winter canola to overcome short episodes of HDT coinciding with flowering. Long duration heat stress under field conditions decreased yield, oil concertation and altered fatty acid composition. Winter canola cultivars shifted their peak flower opening time towards early cooler hours of the morning under HDT, HNT and HDNT regimens, which could be an important adaptive trait for maintaining productivity under warmer climates in the future.

3.6 References

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Table 3.1 Effective quantum yield of PSII on the 2th and 4th day under control, HNT, HDT and HDNT treatments under growth chamber conditions. Values presented are mean ± SE. **, *** significance at 1%, 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultivar

	Effective quantum yield of PSII (2nd)				Effective quantum yield of PSII (4th)			
Cultivars	Control	HNT	HDT	HDNT	Control	HNT	HDT	HDNT
Mercedes	0.725 ± 0.005	0.729 ± 0.002	0.717 ± 0.004	0.716 ± 0.005	0.732 ± 0.007	0.733 ± 0.004	0.715 ± 0.011	0.721 ± 0.005
Edimax CL	0.716 ± 0.005	0.748 ± 0.007	0.707 ± 0.008	0.717 ± 0.004	0.718 ± 0.005	0.735 ± 0.008	0.708 ± 0.009	0.708 ± 0.005
Popular	0.732 ± 0.009	0.727 ± 0.006	0.696 ± 0.008	0.721 ± 0.007	0.727 ± 0.01	0.733 ± 0.01	0.703 ± 0.011	0.719 ± 0.008
46W94	0.723 ± 0.001	0.74 ± 0.004	0.716 ± 0.005	0.708 ± 0.004	0.727 ± 0.002	0.732 ± 0.003	0.718 ± 0.003	0.712 ± 0.007
DKW46-15	0.711 ± 0.002	0.721 ± 0.009	0.69 ± 0.003	0.707 ± 0.003	0.738 ± 0.003	0.731 ± 0.004	0.693 ± 0.003	0.715 ± 0.004
DKW44-10	0.727 ± 0.004	0.737 ± 0.008	0.708 ± 0.005	0.716 ± 0.005	0.734 ± 0.005	0.729 ± 0.005	0.705 ± 0.003	0.715 ± 0.002
T			***				***	
C			***				NS	
T x C			**				NS	

Table 3.2 Effective quantum yield on the 7th and 14th day under control, HNT, HDT and HDNT treatments under growth chamber conditions. Values presented are mean ± SE. **, *** significance at 1%, 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultiva

	Effective quantum yield of PSII (7th)				Effective quantum yield of PSII (14th)			
Cultivars	Control	HNT	HDT	HDNT	Control	HNT	HDT	HDNT
Mercedes	0.741 ± 0.007	0.728 ± 0.007	0.721 ± 0.008	0.718 ± 0.006	0.759 ± 0.005	0.763 ± 0.003	0.739 ± 0.004	0.755 ± 0.005
Edimax CL	0.715 ± 0.004	0.729 ± 0.003	0.719 ± 0.008	0.686 ± 0.004	0.725 ± 0.002	0.759 ± 0.004	0.723 ± 0.006	0.724 ± 0.006
Popular	0.729 ± 0.007	0.733 ± 0.005	0.712 ± 0.008	0.709 ± 0.008	0.762 ± 0.004	0.766 ± 0.003	0.724 ± 0.011	0.739 ± 0.008
46W94	0.732 ± 0.003	0.724 ± 0.005	0.709 ± 0.005	0.703 ± 0.005	0.753 ± 0.004	0.749 ± 0.002	0.73 ± 0.005	0.728 ± 0.004
DKW46-15	0.74 ± 0.004	0.729 ± 0.011	0.698 ± 0.004	0.712 ± 0.003	0.759 ± 0.005	0.737 ± 0.007	0.722 ± 0.011	0.733 ± 0.002
DKW44-10	0.732 ± 0.007	0.737 ± 0.002	0.694 ± 0.003	0.7 ± 0.004	0.763 ± 0.004	0.744 ± 0.002	0.724 ± 0.001	0.719 ± 0.006
T			***				***	
C			**				***	
T x C			**				***	

Table 3.3 Chlorophyll index on the 2th and 4th day under control, HNT, HDT and HDNT treatments under growth chamber conditions. Values presented are mean ± SE. *** significance at 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultivar

	Chlorophyll index (2nd)				Chlorophyll index (4th)			
Cultivars	Control	HNT	HDT	HDNT	Control	HNT	HDT	HDNT
Mercedes	61.34 ± 1.79	59.60 ± 2.10	61.64 ± 1.11	57.16 ± 3.23	59.56 ± 1.05	58.44 ± 1.68	59.10 ± 2.04	51.94 ± 2.54
Edimax CL	60.96 ± 1.47	57.60 ± 1.40	63.06 ± 2.40	58.48 ± 1.64	54.03 ± 1.32	57.30 ± 1.30	60.08 ± 2.25	53.50 ± 1.36
Popular	66.76 ± 1.74	63.62 ± 1.98	65.47 ± 1.49	63.72 ± 1.81	63.76 ± 2.42	64.80 ± 2.08	64.50 ± 1.57	63.68 ± 0.82
46w 94	62.80 ± 0.66	63.60 ± 0.40	66.23 ± 1.02	65.78 ± 1.63	61.50 ± 1.69	65.30 ± 1.16	65.75 ± 1.39	60.75 ± 1.28
DKW46-15	67.68 ± 2.21	68.20 ± 0.86	69.83 ± 1.23	64.38 ± 3.18	62.14 ± 1.78	68.75 ± 0.97	65.13 ± 1.89	59.80 ± 2.33
DKW44-10	72.44 ± 1.37	69.33 ± 1.23	70.73 ± 1.81	62.25 ± 1.70	69.48 ± 2.52	68.33 ± 0.59	65.25 ± 0.49	64.00 ± 1.58
T		***				***		
C		***				***		
T x C		NS				NS		

Table 3.4 Chlorophyll index on the 7th and 14th day under control, HNT, HDT and HDNT treatments. Values presented are mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultivar

	Chlorophyll index (7th)				Chlorophyll index (14th)			
Cultivars	Control	HNT	HDT	HDNT	Control	HNT	HDT	HDNT
Mercedes	56.00 ± 1.55	57.20 ± 1.71	57.20 ± 1.20	55.60 ± 0.87	54.2 ± 1.85	58.40 ± 1.57	57.20 ± 1.66	51.80 ± 1.07
Edimax CL	52.20 ± 2.99	53.40 ± 1.63	58.00 ± 1.26	54.75 ± 0.66	50.75 ± 2.08	53.80 ± 1.24	57.20 ± 3.02	51.25 ± 0.58
Popular	64.20 ± 0.92	60.80 ± 1.24	57.75 ± 1.02	59.20 ± 0.58	58.00 ± 1.82	59.20 ± 1.28	56.75 ± 1.46	58.60 ± 1.17
46w 94	56.07 ± 1.87	55.10 ± 1.82	62.00 ± 0.32	56.25 ± 0.37	59.40 ± 0.75	61.50 ± 0.50	60.00 ± 1.58	57.75 ± 0.86
DKW46-15	61.00 ± 2.00	60.60 ± 0.60	61.00 ± 1.00	56.20 ± 0.73	58.00 ± 2.28	59.40 ± 1.17	66.75 ± 2.71	56.60 ± 3.08
DKW44-10	62.60 ± 0.68	61.00 ± 0.55	62.50 ± 1.69	57.75 ± 0.73	63.00 ± 1.38	61.87 ± 0.67	61.50 ± 1.94	60.00 ± 1.82
T		***				***		
C		***				***		
T x C		***				***		

Table 3.5 Aboveground biomass per plant (g) under control, HNT, HDT and HDNT treatments under controlled environment chamber conditions. Values presented are mean ± SE. *** significance at 0.1%; T - Treatment, C – Cultivar

	Above ground biomass (g)			
Cultivars	Control	HNT	HDT	HDNT
Mercedes	70.14 ± 2.02	87.70 ± 3.87	114.16 ± 4.60	106.18 ± 10.04
Edimax CL	90.51 ± 3.22	83.39 ± 3.41	129.21 ± 4.73	109.45 ± 08.26
Popular	86.72 ± 5.14	84.87 ± 4.42	97.38 ± 2.60	91.03 ± 03.48
46W94	94.87 ± 5.11	90.85 ± 4.57	140.36 ± 4.38	116.80 ± 00.42
DKW46-15	77.80 ± 6.12	70.75 ± 4.61	112.93 ± 5.91	114.01 ± 04.59
DKW44-10	60.93 ± 2.41	73.71 ± 2.64	86.35 ± 4.98	75.07 ± 02.99
T			***	
C			***	
T x C			***	

Table 3.6 Pod number from flowers coinciding with stress period and total pod number per plant under control, HNT, HDT and HDNT treatments under growth chamber conditions. Variation in traits is given as mean ± SE. **, *** significance at 1%, 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultivar

	Pod numbers within stress				Total pod numbers per plant			
Cultivars	Control	HNT	HDT	HDNT	Control	HNT	HDT	HDNT
Mercedes	321.20 ± 13.04	346.20 ± 14.80	48.00 ± 11.76	06.00 ± 3.02	486.80 ± 27.53	552.60 ± 22.57	662.80 ± 28.62	640.40 ± 22.70
Edimax CL	317.60 ± 09.97	295.25 ± 10.67	14.20 ± 08.55	00.60 ± 0.60	479.60 ± 25.59	443.00 ± 11.15	532.00 ± 47.58	553.00 ± 29.02
Popular	394.00 ± 22.20	365.75 ± 17.18	25.00 ± 13.53	02.80 ± 1.96	554.40 ± 32.18	541.00 ± 30.40	633.00 ± 33.78	658.40 ± 28.33
46W94	339.30 ± 32.16	340.50 ± 17.94	26.00 ± 09.98	16.50 ± 3.01	675.75 ± 69.93	573.75 ± 38.53	653.00 ± 13.47	691.00 ± 44.55
DKW46-15	314.20 ± 19.20	279.80 ± 27.89	16.75 ± 05.02	13.75 ± 6.20	591.00 ± 81.32	532.20 ± 22.40	591.75 ± 32.55	741.75 ± 41.25
DKW44-10	224.40 ± 24.79	189.5 0± 06.34	12.50 ± 03.49	04.77 ± 2.41	371.40 ± 29.74	567.50 ± 56.34	443.00 ± 29.25	451.47 ± 05.94
T		***				***		
C		***				***		
T x C		***				**		

Table 3.7 Pod weight (g) from flowers coinciding with stress period and total pod weight per plant (g) under control, HNT, HDT and HDNT treatments under growth chamber conditions. Variation in traits is given as mean ± SE. **, *** significance at 1%, 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultivar

	Pod weight within heats tress (g)				Total pod weight per plant (g)			
Cultivars	Control	HNT	HDT	HDNT	Control	HNT	HDT	HDNT
Mercedes	35.23 ± 2.31	40.79 ± 2.89	2.31 ± 0.56	0.22 ± 0.11	49.91 ± 2.04	61.51 ± 2.18	73.16 ± 3.52	68.76 ± 7.08
Edimax CL	42.22 ± 1.28	41.28 ± 1.90	0.55 ± 0.35	0.02 ± 0.02	61.88 ± 2.00	57.32 ± 1.97	65.11 ± 4.62	60.51 ± 6.12
Popular	48.18 ± 2.82	43.26 ± 1.69	1.02 ± 0.60	0.12 ± 0.08	63.34 ± 4.06	60.58 ± 3.36	60.40 ± 2.21	57.47 ± 3.86
46W94	34.07 ± 5.25	36.89 ± 4.09	1.30 ± 0.56	0.86 ± 0.23	60.34 ± 4.93	57.83 ± 3.01	77.44 ± 1.38	66.40 ± 0.85
DKW46-15	32.40 ± 1.35	28.39 ± 3.70	0.98 ± 0.13	0.74 ± 0.31	52.47 ± 3.87	50.07 ± 3.04	61.78 ± 4.13	69.11 ± 3.19
DKW44-10	20.10 ± 1.91	16.77 ± 1.19	0.92 ± 0.32	0.16 ± 0.08	32.02 ± 1.89	37.76 ± 1.36	46.45 ± 3.78	38.34 ± 1.39
T		***				***		
C		***				***		
T x C		***				**		

Table 3.8 Seed weight (g) from flowers coinciding with stress period and total seed weight per plant (g) under control, HNT, HDT and HDNT treatments under growth chamber conditions. Variation in traits is given as mean ± SE. *** significance at 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultivar

	Seed weight within heat stress (g)				Total seed weight per plant (g)			
Cultivars	Control	HNT	HDT	HDNT	Control	HNT	HDT	HDNT
Mercedes	20.10 ± 1.58	23.30 ± 1.74	0.9 ± 0.24	0.09 ± 0.04	28.17 ± 1.42	34.44 ± 1.15	41.25 ± 1.86	40.84 ± 3.98
Edimax CL	23.78 ± 0.95	22.88 ± 0.99	0.14 ± 0.09	0.01 ± 0.01	34.60 ± 1.03	32.17 ± 0.82	30.49 ± 2.48	32.23 ± 3.09
Popular	27.61 ± 1.87	24.57 ± 0.99	0.41 ± 0.24	0.03 ± 0.02	35.30 ± 2.38	34.22 ± 1.81	35.82 ± 1.27	32.83 ± 2.80
46W94	18.67 ± 3.17	19.27 ± 2.22	0.38 ± 0.21	0.25 ± 0.05	30.70 ± 2.64	28.91 ± 1.51	39.93 ± 0.82	34.99 ± 1.55
DKW46-15	18.28 ± 0.61	15.53 ± 2.14	0.27 ± 0.05	0.15 ± 0.08	28.73 ± 2.87	27.59 ± 1.71	30.86 ± 2.24	36.36 ± 1.68
DKW44-10	10.28 ± 1.08	08.34 ± 0.74	0.40 ± 0.13	0.04 ± 0.02	15.78 ± 1.08	16.63 ± 1.28	24.27 ± 1.96	21.07 ± 0.68
T			***				***	
C			***				***	
T x C			***				***	

Table 3.9 Oil concentration per plant (%) under control, HNT, HDT and HDNT treatments under growth chamber conditions. Values presented are mean ± SE. *, **, *** significant at 5%, 1% and 0.1%. T - Treatment, C – Cultivar

	Oil content (%)				Protein content (%)			
Cultivars	Control	HNT	HDT	HDNT	Control	HNT	HDT	HDNT
Mercedes	39.68 ± 1.31	41.41 ± 0.80	46.32 ± 0.48	44.68 ± 1.47	25.92 ± 0.43	23.38 ± 0.59	19.84 ± 0.29	20.76 ± 0.97
Edimax CL	40.22 ± 0.26	39.60 ± 0.99	40.26 ± 0.84	40.98 ± 1.43	23.89 ± 0.39	23.79 ± 0.55	21.80 ± 0.56	21.65 ± 0.71
Popular	40.25 ± 0.48	41.01 ± 0.61	44.51 ± 0.47	41.28 ± 1.07	25.11 ± 0.52	25.28 ± 0.92	21.84 ± 0.41	23.12 ± 0.69
46W94	39.20 ± 1.52	38.68 ± 0.80	45.49 ± 0.35	41.73 ± 1.35	24.08 ± 0.69	23.69 ± 0.22	19.90 ± 0.41	20.62 ± 0.04
DKW46-15	39.72 ± 1.39	42.94 ± 0.91	43.16 ± 1.64	44.20 ± 0.20	25.64 ± 0.76	24.69 ± 0.36	22.36 ± 1.17	21.26 ± 0.31
DKW44-10	34.32 ± 0.35	33.26 ± 0.60	38.88 ± 0.43	39.47 ± 1.15	27.39 ± 0.20	26.00 ± 0.12	24.69 ± 0.29	24.56 ± 0.94
T			***				***	
C			***				***	
T x C			**				*	

Table 3.10 Effective quantum yield and chlorophyll index after 2 and 4 weeks of stress under control and HDT treatments under field conditions. Values presented are mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; (NS) non-significant based on ANOVA.T - Treatment, C – Cultivar

	Quantum yield of PSII (2 weeks)		Quantum yield of PSII (4 weeks)		Chlorophyll index (2 weeks)		Chlorophyll index (4 weeks)	
Cultivars	Control	HDT	Control	HDT	Control	HDT	Control	HDT
Mercedes	0.656 ± 0.005	0.655 ± 0.015	0.670± 0.001	0.666 ± 0.014	54.88 ± 1.83	54.14 ± 0.55	50.65 ± 2.43	50.97 ± 1.46
Edimax CL	0.658 ± 0.019	0.676 ± 0.007	0.647 ± 0.005	0.674 ± 0.008	54.44 ± 1.06	55.38 ± 0.95	47.07 ± 0.45	47.72 ± 1.52
Popular	0.680 ± 0.006	0.673 ± 0.011	0.660 ± 0.007	0.670 ± 0.009	54.84 ± 1.55	54.38 ± 2.99	50.97 ± 0.32	51.43 ± 3.18
DKW46-15	0.667 ± 0.003	0.622 ± 0.023	0.671 ± 0.012	0.636 ± 0.001	56.68 ± 0.14	53.59 ± 1.53	53.53 ± 0.33	50.65 ± 1.53
DKW44-10	0.652 ± 0.012	0.664 ± 0.018	0.671 ± 0.009	0.655 ± 0.003	66.07 ± 2.15	63.68 ± 1.56	58.63 ± 0.39	53.80 ± 2.15
T	NS		NS		NS		NS	
C	NS		***		NS		***	
T x C	NS		NS		*		NS	

Table 3.11 Aboveground biomass per plant (g) under control and HDT treatments under field conditions. Values presented are mean ± SE. **, *** significance at 1% and 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultivar

	Above ground biomass	
Cultivars	Control	HDT
Mercedes	61.45 ± 2.11	59.48 ± 1.13
Edimax CL	61.31 ± 3.21	56.54 ± 4.36
Popular	52.77 ± 2.99	53.66 ± 3.66
DKW46-15	38.72 ± 0.6	32.87 ± 1.26
DKW44-10	42.22 ± 0.4	31.78 ± 0.85
T	**	
C	***	
T x C	NS	

Table 3.12 Total pod number, total pod weight (g) and total seed weight per plant (g) under control and HDT treatments under field experiment. *, **, *** significance at 5%, 1%, 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultivar

	Total pod number per plant		Total pod weight per plant (g)		Total seed weight per plant (g)	
Cultivars	Control	HDT	Control	HDT	Control	HDT
Mercedes	266.22 ± 7.91	213.44 ± 16.78	43.69 ± 1.60	37.76 ± 0.83	22.62 ± 0.75	19.44 ± 0.87
Edimax CL	306.00 ± 8.31	240.56 ± 17.22	42.37 ± 1.33	35.34 ± 0.85	21.42 ± 0.12	17.10 ± 0.10
Popular	274.14 ± 22.12	234 ± 11.07	39.08 ± 1.85	35.71 ± 1.85	21.37 ± 1.05	19.33 ± 0.49
DKW46-15	224.22 ± 10.56	162.75 ± 16.55	28.94 ± 0.36	20.92 ± 0.48	14.74 ± 0.06	08.86 ± 0.15
DKW44-10	217.25 ± 19.69	130.33 ± 3.49	29.64 ± 0.66	19.54 ± 0.56	15.47 ± 0.10	09.35 ± 0.19
T	***		***		***	
C	***		***		***	
T x C	NS		*		**	

Table 3.13 Oil and protein concentration per plant (%) under control and HDT treatments under field conditions. *, **, *** significance at 5%, 1%, 0.1%; (NS) non-significant based on ANOVA. T - Treatment, C – Cultivar

Cultivars	Oil concentration (%)		Protein concentration (%)	
	Control	HDT	Control	HDT
Mercedes	39.84 ± 0.44	37.83 ± 0.85	25.29 ± 0.34	28.81 ± 0.38
Edimax CL	38.85 ± 0.41	35.65 ± 0.25	24.14 ± 0.60	27.97 ± 0.35
Popular	39.51 ± 0.90	37.93 ± 1.01	24.69 ± 0.62	28.12 ± 0.46
DKW46-15	40.27 ± 0.53	35.09 ± 0.54	25.65 ± 0.09	29.19 ± 0.34
DKW44-10	37.33 ± 0.08	33.61 ± 0.14	26.11 ± 0.36	30.27 ± 0.39
T	***		***	
C	***		**	
T x C	*		NS	

Table 3.14 Saturated seed fatty acid composition (%) under control and HDT treatments under field conditions. Values presented are mean ± SE. *, **, *** significance at 5%, 1%, 0.1%; T - Treatment; C – Cultivar

	Palmitic acid (16:0)		Stearic acid (18:0)	
Cultivars	Control	HDT	Control	HDT
Mercedes	4.30 ± 0.03	4.18 ± 0.03	1.40 ± 0.03	1.47 ± 0.04
Edimax CL	4.45 ± 0.03	4.38 ± 0.04	1.33 ± 0.03	1.30 ± 0.05
Popular	4.65 ± 0.03	4.28 ± 0.04	1.73 ± 0.06	1.65 ± 0.00
DKW46-15	4.52 ± 0.06	4.65 ± 0.05	1.65 ± 0.03	2.05 ± 0.09
DKW44-10	4.30 ± 0.13	4.10 ± 0.06	1.52 ± 0.02	1.58 ± 0.08
T	**		*	
C	***		***	
T x C	**		***	

Table 3.15 Unsaturated seed fatty acid composition (%) under control and HNT treatments. Values presented are mean ± SE. *, *** significance at 5%, 0.1%; NS = Non-significant based on ANOVA, T - Treatment; C - Cultivar

	Oleic acid (18:1)		Linoleic acid (18:2)		Linolenic acid (18:3)		Gadoleic acid (20:1)		Erucic acid (22:1)	
Cultivars	Control	HDT	Control	HDT	Control	HDT	Control	HDT	Control	HDT
Mercedes	67.50 ± 0.26	66.70 ± 0.65	17.95 ± 0.10	18.55 ± 0.46	6.65 ± 0.10	6.60 ± 0.15	1.20 ± 0.00	1.20 ± 0.00	0.23 ± 0.03	0.30 ± 0.00
Edimax CL	64.62 ± 0.75	64.37 ± 0.57	19.18 ± 0.42	19.53 ± 0.22	7.90 ± 0.40	7.82 ± 0.33	1.23 ± 0.03	1.25 ± 0.00	0.23 ± 0.03	0.30 ± 0.00
Popular	67.60 ± 0.61	68.85 ± 0.05	17.00 ± 0.28	16.30 ± 0.08	6.38 ± 0.22	6.10 ± 0.00	1.17 ± 0.07	1.27 ± 0.02	0.30 ± 0.00	0.37 ± 0.02
DKW46-15	64.88 ± 0.46	64.32 ± 0.82	19.17 ± 0.37	18.72 ± 0.77	7.32 ± 0.07	6.38 ± 0.23	1.13 ± 0.02	1.52 ± 0.06	0.27 ± 0.03	0.43 ± 0.03
DKW44-10	65.32 ± 0.96	64.50 ± 0.54	19.37 ± 0.80	19.90 ± 0.31	7.23 ± 0.42	6.78 ± 0.24	1.22 ± 0.03	1.45 ± 0.00	0.22 ± 0.04	0.37 ± 0.02
T	NS		NS		*		***		***	
C	***		***		***		***		*	
T x C	NS		NS		NS		***		NS	

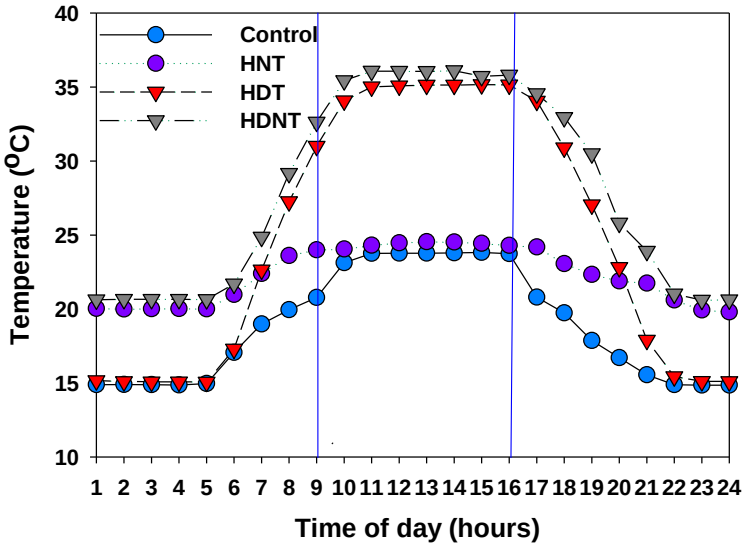

Figure 3.1 Variation in temperatures (°C) during heat stress imposition between Control (blue), HNT (purple), HDT (red) and HDNT (grey) in controlled environment chambers.

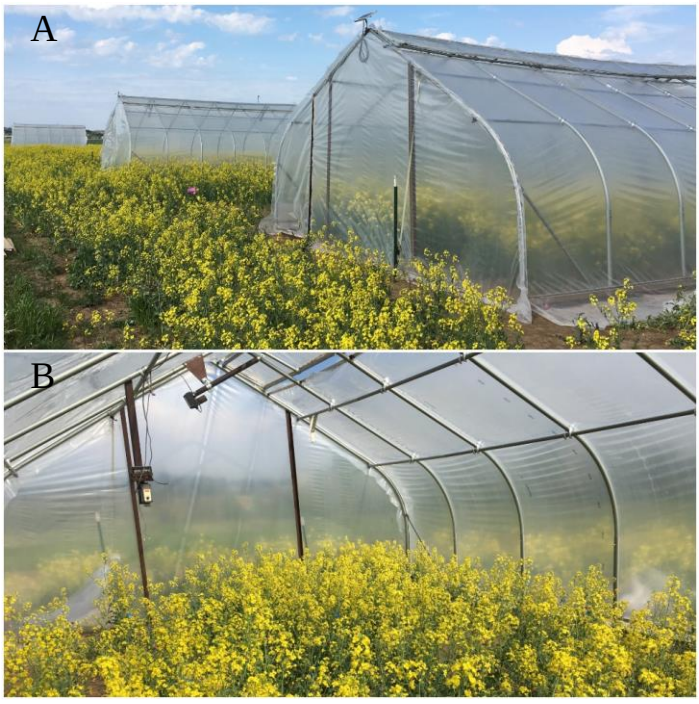

Figure 3.2 Unique field-based heat tents placed over canola cultivars to impose heat stress during flowering and pod-filling stages (A) and canola cultivars inside the heat tent (B).

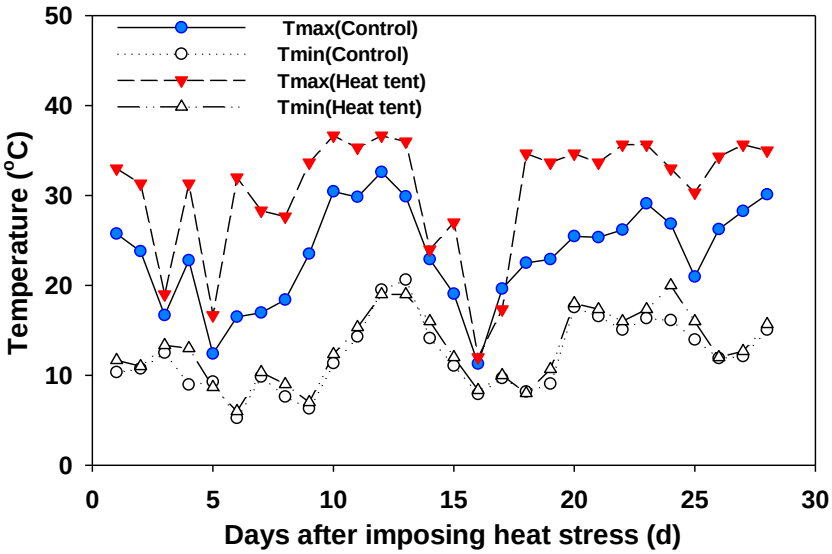

Figure 3.3 Figure 3.3 Daily average maximum day time and minimum night temperatures (°C) inside and outside the heat tents starting from the day of heat stress imposition until physiological maturity in the field experiment.

Figure 3.4 Marking of flowering branches. First red arrow points to black marking which indicates the start of stress exposure (A) and second red arrow points to red marking, depicting the end of stress imposition (B).

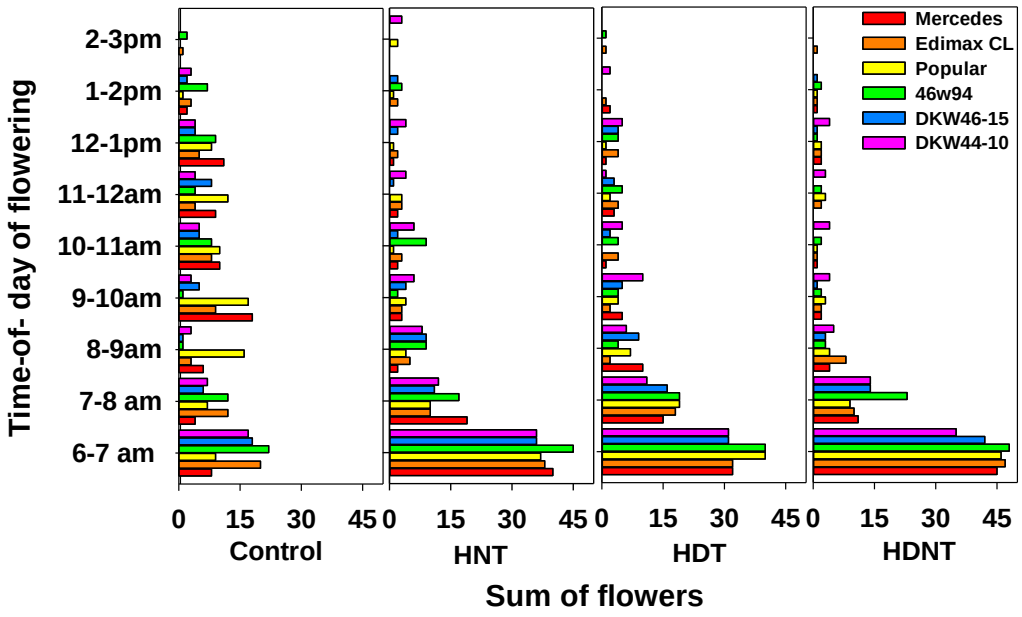

Figure 3.5 Time-of-day of flower opening in different canola cultivars under different temperature treatments- Control, HNT (High night temperature), HDT (High day temperature) and HDNT (High day and night temperature) in growth chambers experiment.

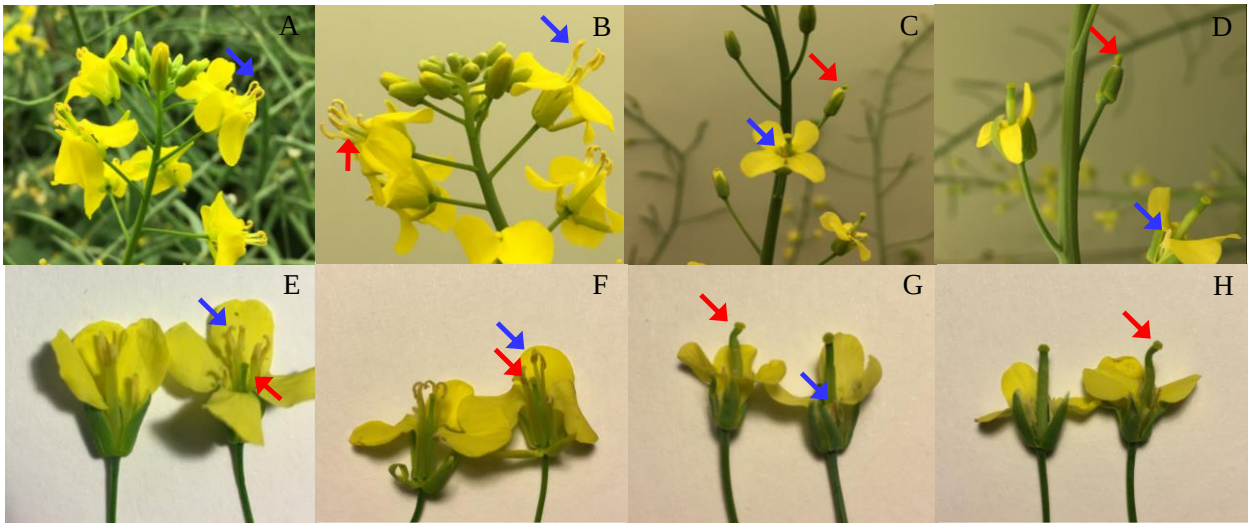

Figure 3.6 Changes in floral morphology after exposure to different temperatures for a week during flowering in canola- A and E (Control), B and F (HNT), C and G (HDT) and D and H (HDNT). Red arrows point to stigma and blue arrows to anthers.

Chapter 4 - Exploring the impact of drought stress on physiological and yield parameters during flowering and pod-filling stages in winter canola

Abstract

Canola (*Brassica napus* L.), also known as oilseed rape or double-low rapeseed, is an economically valuable oilseed crop. Winter-grown canola is adversely affected by several environmental factors including drought stress. Global climate models forecast an unpredictable and erratic rainfall pattern in the future which can induce both yield and quality losses in winter canola. Hence, this study was conducted to investigate the impact of drought stress during flowering and pod-filling stages on physiological and yield parameters in winter canola. A field experiment involving five canola cultivars was conducted using field-based rain-out shelters following a split-plot design. The cultivars were exposed to drought stress by covering the plots using rain-out shelters during the rainy days during the treatment period, starting from early flowering till physiological maturity. Effective quantum yield of PSII and chlorophyll index were recorded after two and four weeks of drought stress imposition. At physiological maturity, above ground biomass (leaves, stems and pods) and total seed weight were recorded. The effective quantum yield of photosystem II was significantly decreased in DKW46-15 (9%), Edimax CL (8%) and DKW44-10 (6%) under drought stress. Similarly, the same set of canola cultivars, on average, recorded 32% lower aboveground biomass. No significant reduction in total seed weight was observed in Mercedes while other cultivars, DKW44-10, DKW46-15, Edimax CL and Popular recorded 54, 46, 34 and 25% reduction under drought stress exposure, respectively. In conclusion, drought stress had a significant negative impact on effective quantum yield of PSII, above ground biomass and seed yield in winter canola.

4.1 Introduction

Canola (*Brassica napus* L.), also known as oilseed rape or double-low rapeseed, is an important agricultural crop grown primarily for oil production (Zhang & Flottmann, 2016). Globally, canola production has increased to 68.9 million metric tons in 2014 compared to 35 million metric tons in 2000 (FAO, 2017). Increasing water scarcity, caused by global climate change and increasing competition for available water resources, is a major constraint for crop production and global food security (Rosegrant et al., 2009). Drought stress is considered the most damaging abiotic stress affecting crops and its increased occurrence could result in significantly greater yield reductions than any other abiotic stressor. (Farooq et al., 2009).

Canola is poorly adapted to drought conditions (Wright et al., 1998). Drought stress has deleterious effects during flowering (Faraji et al., 2009; Daneshmand et al., 2008; Ghobadi et al., 2006) and pod-filling stages in canola cultivars (Zirgoli & Kahrizi, 2015; Rad & Abbasian, 2011; Gan et al., 2004). Seed yield can be limited even by a relatively short period of soil moisture shortage during reproductive development (Chaghakaboodi et al., 2012). Within the reproductive stages, Faraji et al. (2009) revealed flowering to be the most sensitive stage to drought stress due to susceptibility to pollen development, pollen viability and fertilization leading to lower seed yield in canola.

Photo-assimilate supply during fertilization determines seeds per pod, whereas seed weight depends on the continued supply of photosynthates after fertilization until maturity. Previous studies have indicated that drought stress during early flowering and pod filling reduced the photosynthetic rate in leaves of rapeseed/spring canola cultivars due to a higher production of reactive oxygen species (ROS) as a result of increased electron leakage during photosynthetic

processes (Moghadam et al., 2009), dramatic loss of pigments and disorganization of thylakoid membranes (Ladjal et al., 2000).

Canola yield is determined by biomass accumulation and is associated with more pods, increased seeds per pod, and greater seed weight (Zhang & Flottmann, 2016). Maximum dry matter accumulation in leaves occurs at the start of flowering and in stems at the end of flowering (Faraji, 2012). Taken together, this indicates that higher above ground dry matter around flowering is extremely important to support production of flowers, pods, and seed yield. Previous studies have shown that drought during flowering and pod-development stages caused significant reductions in the number of pods per plant, number of seeds per pod, seed size and weight, and seed oil content in rapeseed/canola cultivars (Din et al., 2011; Rahnema et al., 2006). These negative impacts of drought include insufficient fertility and flower abscission (Daneshmand et al., 2008; Rad & Zandi, 2012), and a shortening of the flowering and pod-fill durations (Gan et al., 2004; Nasri et al., 2008; Zirgoli & Kahrizi, 2015). In addition, drought stress at the flowering and pod-development stages decreases plant height and assimilation supply, thus leading to reduced number of branches per plant and loss in grain yield (Darjani et al., 2013; Rad & Zandi, 2012; Naeemi et al., 2007). Nonetheless, current information mostly relates to rapeseed and spring canola cultivars obtained from climate-controlled facilities, with limited information on response of field-grown winter canola cultivars exposed to drought.

Winter canola cultivars produce 20 to 30% higher yield than spring canola in the U.S. southern Great Plains (Boyles et al., 2012). Increasing demand for canola products has expanded winter canola cultivation into much drier regions, exposing the crop to drought stress and resulting in frequent yield losses. Currently, the impact of drought stress on the physiological processes, yield and yield components of winter canola is not fully understood, with no report quantifying

the impact of stage specific controlled drought stress imposition on field grown winter canola. Hence, to fill this knowledge gap, a field study was conducted to investigate the impact of drought stress on physiological responses, yield and its components during flowering and pod-filling stages in winter canola, using a rain-out shelter facility.

4.2 Material and methods

4.2.1 Plot establishment and experimental design

The experiment was conducted in the 2018-2019 growing season using a field-based, rain-out shelter facility at the Agronomy North Farm (39 11'N, 96 35'W), Department of Agronomy, Kansas State University, Manhattan, KS, USA. The experiment included four canola hybrids; 46W94, Edimax CL, Mercedes and Popular and two open-pollinated canola cultivars; DKW44-10, DKW46-15. Seedlings of the 46W94 hybrid were killed due to winter frost and hence no data was collected on this cultivar.

The field experiment was laid out in a randomized complete block design in a split-plot arrangement with 4 replications of each cultivar in each treatment. Drought and well-watered treatments were the main plot and the cultivars were considered as subplots. Plot preparation prior to planting included multiple tillage passes of a disc, cultivator, and harrow in the summer/fall of 2018. Ammonium polyphosphate (10-34-0) was applied at a rate of 15 liters/hectare and ammonium thiosulfate (10-0-0-22) was applied at a rate of 18 liters/hectare at or before planting. Weed control in the canola plots was managed using the pre-emergence herbicide, trifluralin, (0.4 liters/hectare) along with hand weeding as necessary to minimize weed pressure throughout the growing season. The recommended rate of 13 kg N/hectare was top dressed as urea (46-0-0) on the canola plots on 28- Mar-2018 using a variable rate drop spreader (Gandy Company, Owatonna, MN).

For this study, two rain-out shelters were used with an effective planting area of 111 m^2 per shelter. Two replications of the six canola cultivars were seeded under rain-out shelter one and two replications were seeded under rain-out shelter two. Four replicates of the six canola cultivars were seeded as a control adjacent to the rain-out shelters. The planting date was 13-Sep-2018 and a research plot drill equipped with Great Plains row openers (Great Plains Mfg., Salina, KS) was used. Plot size was 1.5m wide x 2m long with 6 rows spaced 0.25m apart, and the seeding rate was 6.7 kg ha^{-1}.

4.2.2 Drought Stress imposition

Drought stress was imposed using rain-out shelters after approximately 50% of flowering within the plots in all tested cultivars. The days to reach 50% flowering across the five cultivars ranged from 217 to 221 days after planting. To avoid rain falling on the experimental plots, drought stress was imposed by closing the roofs of rain-out shelters based on signs of a rain event and this process was continued until physiological maturity (Figures 4.1 and 4.2). Soil moisture content was monitored by calculating gravimetric soil moisture content by taking representative soil samples at a 30-cm depth in the rain-out shelters and control plots after approximately two and four weeks of drought stress imposition.

4.2.3 Observations

4.2.3.1 Effective quantum yield and chlorophyll index

Effective Quantum Yield (QY) of photosystem II in the light adapted state was measured using FluorPen (Photon System Instruments, Ltd., Brno, Czech Republic) and chlorophyll index was measured using a self-calibrating chlorophyll meter (Soil Plant Analyzer Development [SPAD], Model 502, Spectrum Technologies, Plainfield, IL, USA). Both the quantum yield and the

chlorophyll index were recorded two and four weeks after drought imposition. Three representative plants were selected from each sub plot (replicate) to measure the traits. Both measurements were taken at three different places on the fourth leaf from the top of the main stem and averaged to get a single replicate value for a plant.

4.2.3.2 Yield and yield components

At physiological maturity, four representative plants were selected from each sub plot to measure the impact of drought on yield and yield components. Plants were hand harvested by cutting at the base of the stem. Vegetative parts (leaves still attached to the plant and stems) and pods were dried at 40°C for two weeks inside a greenhouse. Above ground biomass was determined as the weight of leaves and stem and pods per plant. Pods were threshed using an ALMACO belt thresher (ALMACO, Nevada, IA). Total seed weight per plant was recorded for control and drought stress conditions.

4.2.4 Data analysis

Both drought and control treatments were laid out in a randomized complete block design with a split-plot treatment structure. Temperature was the main plot and cultivar was the subplot. Analysis of variance (ANOVA) was performed using the Proc GLM procedure in SAS 9.4 (SAS Institute, 2013). Means were separated using least significant difference (LSD) at probability level of 0.05 ($p = 0.05$). Graphs were created using SigmaPlot 12.5 (Systat Software, 2013).

4.3 Results

4.3.1 Drought stress severity

Significant differences in the soil water content were observed at both time points comparing drought stressed and control plots (Table 4.1). Two weeks of drought exposure resulted in a

reduction of 36 and 29% in soil water content at 15 cm and 30 cm depth from soil surface, respectively, in the drought stressed plots. After four weeks of drought stress, a similar effect was documented with a reduction of 44% and 24% at 15 cm and 30 cm soil depth, respectively, in drought stressed plots (Table 4.1). We observed a gradual decreased in the soil water content with 13% and 20% reductions at 15 and 30 cm soil layers, respectively, from two to four weeks after starting drought exposure.

4.3.2 Effective quantum yield and chlorophyll index

Effective quantum yield (QY) was significantly affected by cultivar and treatment but not treatment by cultivar interaction after two weeks and four weeks of drought stress treatments (Tables 4.2 and 4.3). After two weeks of drought stress exposure, the highest reductions in QY were observed for DKW46-15 (10%) followed by Edimax CL (8%) (Table 4.2). Likewise, a significant decrease in QY was recorded in DKW46-15 (9%) and DKW44-10 (6%) cultivars after four weeks of drought stress treatment (Tables 4.3). The cultivars Popular and Mercedes showed the lowest reduction of (1%) and (3%) in QY, respectively, under drought stress exposure (Tables 4.2 and 4.3).

Chlorophyll index was not significantly affected by treatment, cultivar or treatment by cultivar interaction after two weeks and four weeks of drought treatment exposure, except for cultivar after two weeks of treatment (Tables 4.2 and 4.3).

4.3.3 Yield and yield components

4.3.3.1 Above ground biomass

Above ground biomass differed significantly between treatment and cultivar but not for the treatment by cultivar interaction. Drought stress exposure under field conditions resulted in a

significant reduction in above ground biomass for all the cultivars except Mercedes and Popular (Figure 4.3). DKW44-10 recorded the highest reduction of 40% and Mercedes the lowest reduction of 9% in above ground biomass under drought stress (Figure 4.3).

4.3.3.2 Seed weight per plant

Seed weight per plant was significantly affected by treatment and cultivar but not by their interaction. Across all cultivars, DKW44-10, DKW46-15, Edimax CL and Popular recorded significant reductions (54, 46, 34 and 25%) in total seed weight under drought stress, respectively (Figure 4.4). Mercedes had lowest reduction (11%) in seed weight per plant under drought stress exposure (Figure 4.4).

4.4 Discussion

Drought reduces effective quantum yield of PSII

Our findings demonstrate that effective quantum yield of PSII was reduced under drought exposure for many of the winter canola cultivars tested. Similar reductions in maximum quantum yield of photosystem II have been reported by Norouzi et al. (2008) and Qaderi et al. (2006) under drought stress during the flowering stage in rapeseed and spring canola. A decrease in the QY due to drought stress during flowering and pod filling indicates decreased photochemical efficiency of PSII. Lower PSII activity indicates decline in photosynthetic rate (Maxwell & Johnson, 2000; Tang et al., 2018), which is the key process that contributes toward final yield (Raza et al., 2017). Previous studies reported decreased photosynthetic rate in the leaves of canola cultivars when drought stress was imposed during the vegetative stage under greenhouse conditions (Gao et al., 2018) and reproductive growth stages under field conditions (Moghadam et al., 2009). This decrease was due to the higher production of ROS species caused by increased electron leakage during photosynthetic processes. Similarly, reduction in molecular oxygen, generation of ROS and

disintegration of thylakoid membranes have been reported in rice (Dalal & Tripathy, 2018) and bent grass species under drought exposure (Dacosta & Huang, 2007).

The decrease in leaf water potential, leaf relative water content and stomatal conductance were recorded for rapeseed genotypes with drought imposed during flowering period (Norouzi et al., 2008). A decrease in photosynthesis in cotton plants under water stress was associated with a decrease in the activity of Rubisco (Silva et al., 2012), activity of adenosine triphosphate (ATP)-synthase (Tezara et al., 1999). Chlorophyll concentration is used as an indicator for source capacity and the photosynthetic potential of the plant (Zhang et al., 2007). In the present study, chlorophyll index was not statistically reduced under drought stress among the tested canola cultivars, however a decrease in chlorophyll (*a* & *b)* content has been reported in previous studies under drought stress exposure during the flowering stage in canola plants (Din et al., 2011; Gibon et al., 2000). This decrease can be attributed to a decrease in the activity of chlorophyllase and an increase in ROS concentration that damages the chloroplast membranes (Gill & Tuteja, 2010). In our study, similar physiological changes mentioned above would have led to reduced photochemical efficiency of PSII, disturbing the normal photosynthetic process during flowering and pod-filling stages, and ultimately resulting in yield loss in winter canola.

Drought negatively affects the above ground biomass and seed weight in winter canola

Our findings revealed a significant reduction in above ground biomass and total seed weight of winter canola as a consequence of water stress during the flowering and pod-filling stages. These results are in agreement with previous studies in *Brassica* species (Tesfamariam et al., 2010; Sinaki et al., 2009). In addition, water stress during the vegetative stage as a result of decline in leaf area index, wilting and senescence of leaves, and the abortion and abscission of pods, can lead to reduced biomass accumulation (Tesfamariam et al., 2010). The other reasons leading to reduce

above ground biomass are associated with limited leaf area development that in turn reduced radiation interception in other *Brassica* species (Kumar et al., 1994) and reduced stomatal conductance leading to reduced carbon assimilation under water-deficit conditions (Issarakraisila et al., 2007).

Seed yield of canola is primarily determined by the number of pods, seeds per pod and seed weight (Angadi et al., 2000). These components are highly influenced by the environmental conditions, physiology and the genetics of the cultivars. Among yield components, number of pods per plant was reported to be highly sensitive to drought stress than others (Norouzi et al., 2008). Further, the total number of flowers and their conversion to pods depends on the plant biomass and the intensity of stress (Bhattacharya, 2019). In our study, the significant impact of drought stress on the aboveground biomass would have resulted in an negative impact on the number of pods and ultimately seed yield. A strong negative correlation has been documented between seed weight and number of pods per plant during the flowering and pod-set stages, as a consequence of water stress imposed on rapeseed and spring canola (Sinaki et al., 2009; Jensen et al., 1996). Further, along with the number of pods per plant, applying drought stress during early stages of flowering and pod-filling stages caused a significant reduction in the number of seeds per pod, 1000-seed weight, seed size and ultimately, seed yield in rapeseed/canola cultivars (Din et al., 2011; Daneshmand et al., 2008; Rahnema et al., 2006). These changes were attributed to insufficient fertility and flower abscission (Rad & Zandi, 2012), shortening the flowering period and the reproductive growth duration in rapeseed cultivars (Zirgoli & Kahrizi, 2015; Nasri et al., 2008; Wright et al., 1995). Additionally, the number of seeds per pod depends on the photosynthetic supply during fertilization and seed size and weight depends on the continued supply of photosynthates until maturity (Faraji, 2012). This was in agreement with our results

wherein, seed yield was reduced in cultivars that recorded a significant reduction in quantum yield of photosystem II under drought stress. Similar results were observed under drought stress conditions for chickpea (Ghassemi-Golazani et al., 2008), soybean (Demirates et al., 2010). Faraji et al., (2009) also concluded that the flowering and early pod set to be the most sensitive stages to drought stress due to susceptibility of pollen development and the fertilization leading to lower seed yield in canola. Darjani et al. (2013) demonstrated that interrupting irrigation at pod-development stage and beyond significantly reduced the number of branches per plant, pod number per plant, number of seeds per pod and ultimately grain yield. In the present study, limitations of photo-assimilates seen through reduced QY, abortion of pods, and shortened grain fill duration could be major contributing factors leading to lower grain yield reduction under drought stress in winter canola cultivars.

4.5 Conclusions

Our findings revealed a negative impact of water stress on yield and yield components by reducing the effective quantum yield and above ground biomass during reproductive growth stages in winter canola. This study also demonstrated cultivar differences in response to drought stress. Among the tested cultivars, Mercedes maintained above ground biomass accumulation and total seed weight without a significant penalty. Identifying cultivars similar to Mercedes will help support ongoing breeding programs to develop canola cultivars with increased post flowering drought tolerance.

4.6 References

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Table 4.1 Gravimetric soil water content (GSMC) (%) at two soil depths (0-15 and 15-30 cm) after two and four weeks of drought imposition under stress and control treatments. Values presented are mean ± SE. *** significance at 0.1% based on ANOVA, T – Treatment

Soil depth (cm)	GSMC (%) - 2 weeks		GSMC (%) - 4 weeks	
	Control	Drought	Control	Drought
0-15	23.09 ± 0.31	14.72 ± 0.35	22.98 ± 0.31	12.79 ± 0.57
15-30	25.94 ± 0.24	18.35 ± 0.51	19.43 ± 0.20	14.68 ± 0.21
T	***		***	

Table 4.2 Effective quantum yield of PSII and chlorophyll index after two weeks of stress imposition under control and drought treatments. Values presented are mean ± SE. *, ** significance at 5%, 1%; NS - non-significant based on ANOVA, T – Treatment, C – Cultivar

Cultivars	Effective quantum yield of PSII		Chlorophyll index	
	Control	Drought	Control	Drought
Mercedes	0.696 ± 0.012	0.669 ± 0.013	55.19 ± 3.34	51.53 ± 1.32
Edimax CL	0.684 ± 0.008	0.631 ± 0.012	52.97 ± 1.57	55.28 ± 2.25
Popular	0.687 ± 0.005	0.694 ± 0.022	55.03 ± 1.45	56.03 ± 1.56
DKW44-10	0.700 ± 0.010	0.668 ± 0.024	60.50 ± 2.50	58.75 ± 2.88
DKW46-15	0.720 ± 0.008	0.649 ± 0.025	53.39 ± 0.63	54.71 ± 1.15
T	**		NS	
C	*		*	
T x C	NS		NS	

Table 4.3 Effective quantum yield of PSII and chlorophyll index after four weeks of stress imposition under control and drought treatments. Values presented are mean ± SE. *, significance at 5%; NS - non-significant based on ANOVA, T – Treatment, C – Cultivar

Cultivars	Effective quantum yield of PSII		Chlorophyll index	
	Control	Drought	Control	Drought
Mercedes	0.686 ± 0.015	0.682 ± 0.011	49.93 ± 1.02	49.88 ± 1.33
Edimax CL	0.659 ± 0.023	0.631 ± 0.010	47.91 ± 2.68	53.08 ± 1.76
Popular	0.680 ± 0.011	0.664 ± 0.010	50.85 ± 1.41	50.23 ± 0.84
DKW44-10	0.670 ± 0.010	0.627 ± 0.032	52.32 ± 0.70	57.06 ± 2.40
DKW46-15	0.693 ± 0.023	0.628 ± 0.009	51.80 ± 0.30	50.41 ± 1.58
T	*		NS	
C	*		NS	
T x C	NS		NS	

Figure 4.1 Field based rain-out shelters (opened)

Figure 4.2 Imposing drought stress by avoiding rainfall using field based rain-out shelter facilities (closed)

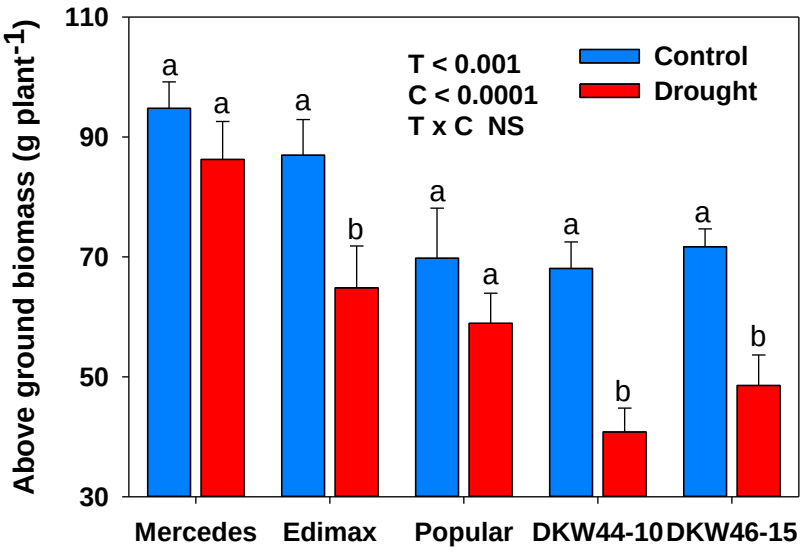

Figure 4.3 Above ground biomass per plant (g) under drought and control treatments in five canola cultivars under field conditions.

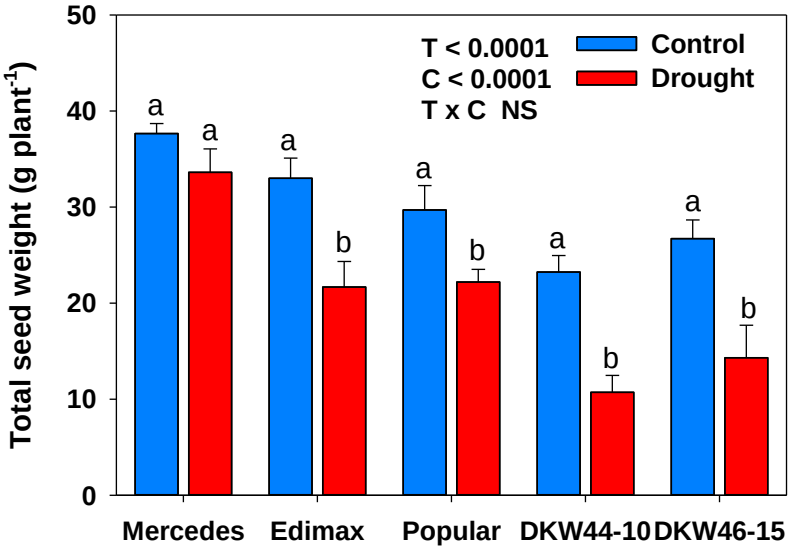

Figure 4. 4 Total seed weight per plant (g) under drought and control treatments in five canola cultivars under field conditions.

Chapter 5 - General discussion and future line of work

Canola (*Brassica napus* L.), also known as oilseed rape or double-low rapeseed, is becoming one of the most important oilseed and potential bio-energy crops grown globally and in the United States. It contains high oil concentration and high unsaturated/saturated fatty acid ratio among vegetable oils, providing significant health benefits. With increasing demand and rotational benefits to wheat, canola cultivation has expanded to much warmer and drier regions, increasing canola's exposure to heat and drought conditions. Day and night air temperatures and erratic rainfall patterns are increasing and are forecasted to continue with higher frequency and intensity due to climate change (Sillmann et al., 2013; Screen, 2014; IPCC, 2014). Winter canola grown in the Southern Great Plains is normally planted in September and harvested in June. Given the phenology of canola in this geography, the flowering and pod development periods potentially overlap with the warmer temperatures and drier weather during the year. This concurrence of flowering and pod filling with high temperature and dry period present a challenge as winter canola has been shown to be highly sensitive to increased temperatures and water deficit during these stages (Weymann et al., 2015; Zirgoli & Kahrizi, 2015). This demonstrates that it is increasingly important to enhance or explore both the heat and drought stress tolerance in winter canola to maintain its productivity under current and impending threat posed by predicted harsher climates in the future.

High temperature stress during reproductive stages has been reported to reduce seed yield and oil quality in *Brassica* species (Angadi et al., 2000, Gan et al., 2004). All previous studies related to heat stress have focused on high day temperature (HDT); however, the impact of high night temperature (HNT) on the physiology, yield and yield components, and quality of canola is not currently available. Hence, in the first study, we quantified the impact of HNT exposure from

gametogenesis to maturity and from post-flowering to maturity in six hybrids and four open-pollinated cultivars. Our study documented a significant negative impact on physiological traits (photochemical efficiency of PSII and thylakoid membrane damage), yield components (pod number and seed yield) and oil and protein concentration in the susceptible cultivars. We also quantified the impact of HNT on fatty acid composition, showing significant increases in saturated fatty acid levels but no impact on unsaturated fatty acids in both the hybrids and the open-pollinated cultivars. This is the first report to record the impact of HNT stress exposure in winter canola and the work has been published in the *Journal of Agronomy and Crop Science* (JAC-11-2019-0519.R2). Although our study initiated quantifying HNT implications in winter canola, important physiological processes such as gametogenesis, anther dehiscence, and pollen germination during flowering under HNT are unexplored and should be systematically investigated under controlled environment chambers and field experiments.

Limited information exists on short episode, independent high day (HDT), high night temperature (HNT) and their combination (HDNT) during flowering. Thus, our second study ascertained their impact on physiology, agronomic and oil quality in winter canola using controlled environment studies. The results demonstrated that both HDT and HDNT induced floral sterility, flower abortion and complete loss of yield in all winter canola cultivars within the two weeks of stress imposition. However, after removal of heat stress, yield components and oil formation were not significantly decrease and in some cases increased, which demonstrated the significant plasticity in winter canola to overcome short episodes of HDT and HDNT damage. In the second part of the study, we documented a significant decrease in pod numbers, grain yield and the oil concentration in the canola cultivars due to the longer duration heat stress under field conditions, similar to HDT and HDNT under controlled environment chambers. Taken together, the findings

provided evidence for increased sensitivity of winter canola to direct heat stress during flowering and at the same time, the extent of recovery with release of stress. In the third study, impact of drought stress in winter canola during flowering and pod-filling stages on physiological and yield parameters were assessed using field-based rain-out shelters. The results documented that drought stress had a significant negative impact on effective quantum yield of PSII, above ground biomass and yield components in winter canola.

Prevailing air temperature during flower opening has been closely linked to reproductive success or failure (Jagadish et al., 2007; Bheemanahalli et al., 2017). We attempted to record time of flower opening under HNT, HDT and HDNT stress exposure in winter canola. The results from these experiments demonstrated a significant shift in the peak flower opening towards earlier, cooler hours. This could be an important adaptive trait to escape key physiological processes such as pollen germination and pollination from late-morning and early-afternoon heat stress. Similar phenomenon under heat stress have been reported in previous studies in rice (Hirabayashi et al., 2014) and wheat (Aiqing et al., 2018), and have been shown to minimize yield losses under warmer day temperature. The mechanisms behind this interesting phenomenon are not known in crops including canola, hence further investigation is justified.

Greater number of inflorescences, flowers and pods after release of short episodes of HDT stress at early flowering in canola has been documented by Angadi et al. (2002) and Gan et al. (2004), although, these studies reported 53 to 58% reduction in the final seed yield. In contrast, we observed a 100% recovery for all cultivars, and with some cultivars recording significantly greater yield and yield components after release of stress. These results indicate a large diversity in plasticity across different genetic backgrounds. This warrants the need for additional research to

effectively capture this diversity to develop canola cultivars having enhanced plasticity to future warmer climates to sustain canola yield and quality.

Many of the previous heat and drought stress studies on rapeseed/canola have been limited to climate-controlled chambers due to a lack of field-based phenotyping facilities (Faraji et al., 2012; Singh et al., 2008; Gan et al., 2004). There were no previous efforts to quantify the impact of abiotic stresses on winter canola cultivars under field conditions, using field-based heat tents, rain-out shelters or other high-throughput phenotyping facilities. It is becoming increasingly important to undertake such efforts to allow timely and accurate testing of physiological and agronomic responses of winter canola exposed to heat and drought stress under realistic field conditions and to build upon the knowledge generated using controlled environment studies. The findings presented in this dissertation have attempted to achieve this by connecting across scales i.e. controlled environment chambers and field conditions, which could be further strengthened in the future by integrating with sensor based high throughput phenotyping approaches.

The selection or developing tolerant canola cultivars would be one of the ideal strategies to maintain yield and quality in current and future warming and dry environments. Our results identified tolerant canola hybrids that showed the ability to minimize the negative effect of heat stress and drought on the seed yield and quality, e.g., Mercedes. In addition, early flowering and shorter maturation periods may enhance heat and drought avoidance in canola by completing flowering and seed development before the onset of heat and drought stressors.

In conclusion, across the heat and drought experiments, we have discovered a novel heat escaping early-morning-flowering behavior in winter canola, which would be helpful to minimize heat stress impact during flowering. We have also identified heat and drought tolerant cultivars with inherent plasticity to overcome short episodes of heat stress at early flowering. We

have quantified the impact of abiotic stresses on important physiological processes, yield and yield components and oil quality in winter canola exposed to high day and night, and drought stress at sensitive flowering and pod-filling stages. We are confident that these findings will be useful in the context of designing breeding strategies and supporting ongoing canola breeding programs to develop future winter canola cultivars with enhanced abiotic stress resilience.

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